Mapping brain function underlying naturalistic motor observation and imitation using high-density diffuse optical tomography

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Abstract

Background: While autism spectrum disorder (ASD) is defined by deficits in social communication with restricted interests and repetitive behaviors, autistic individuals often show early impairments in motor imitation that persist through childhood and into adulthood. High-density diffuse optical tomography (HD-DOT) overcomes logistical challenges of functional magnetic resonance imaging (fMRI) to provide an open scanning environment conducive to neuroimaging during naturalistic motor imitation. Additionally, the mirror neuron system (MNS) is crucial for understanding and imitating actions, and its dysfunction is hypothesized to underlie key ASD features.

Objective: We aim to investigate brain function underlying motor observation and motor imitation in adult autistic and non-autistic individuals (NAI). We hypothesize that HD-DOT will reveal greater MNS activity during motor imitation than motor observation, and that MNS activity will exhibit a negative correlation with autistic traits.

Methods: We imaged brain function using HD-DOT in N=100 participants as they passively observed videos of an actor completing sequences of meaningless arm movements. Additionally, while being simultaneously recorded with both HD-DOT and Kinect 3D cameras for computer-vision-based assessment of motor imitation (CAMI), participants imitated different videos of an actor completing similar arm movements. Responses to the tasks were estimated using general linear models, and multiple regression was used to investigate brain-behavior associations with autistic traits, using the Social Responsiveness Scale, and imitation fidelity as measured with CAMI.

Results: Both motor observation and imitation tasks elicited significant activations in visual, temporal, and MNS areas, with imitation showing stronger activation in motor regions. Notably, MNS regions exhibited greater activation during observation than imitation. Additionally, activity during observation in the right parietal lobe correlated with autistic traits assessed with the SRS.

Conclusions: Our findings provide robust evidence of shared and task-specific neural mechanisms underlying motor observation and imitation, emphasizing the differential engagement of MNS regions during motor observation and imitation.

Key words: Autism spectrum disorder (ASD); High-density diffuse optical tomography (HD-DOT); Motor observation (OBS), Motor imitation (IM), and Computer-vision-based assessment of motor imitation (CAMI)

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition traditionally characterized by deficits in social communication and enhanced presentation of restricted interests and repetitive behaviors, with characteristically heterogeneous traits across the population[1,2]. Difficulty in motor skills is highly prevalent, with recent estimates suggesting that up to 88% of children with ASD experience motor challenges[3-8]. Impairments in motor imitation are particularly notable, as imitation is essential for early prosocial and language development[9,10]. Imitating across a range of behaviors, e.g., vocalizations, arm gestures, facial expressions, and other non-verbal cues, is a key component for effective social interaction[11]. Thus, early deficits in imitation can hinder social development, contributing to difficulties in social engagement and communication[12,13]. Additionally, impaired motor imitation may be related to disrupted visual-motor integration in the brain [14], and further may be related to the observation that ASD individuals often rely more on proprioceptive inputs than visual cues compared to their typically developing peers[15,16]. While motor imitation deficits in ASD are well-documented, their underlying neural mechanisms remain poorly understood [17], in part due to the physical constraints and limitations of neuroimaging modalities like functional magnetic resonance imaging (fMRI) [18,19]. Specifically, the MRI environment requires participants to lay supine in a small bore that inherently restricts studies to small-scale movements, such as those of fingers or hands, and fail to capture brain function during more general and naturalistic motor behaviors. In contrast, high-density diffuse optical tomography (HD-DOT) provides a naturalistic and open scanning environment that enables the assessment of brain function across the lifespan, such as healthy adults, infants, and school-age autistic children[20-24], and even in bedside clinical settings such as the intensive care unit[24]. Moreover, HD-DOT is less sensitive to motion artifact, and provides fMRI comparable spatial resolution and anatomical specificity over a broad field of view, capturing activity in sensorimotor systems implicated in viewing and performing motor movements[25-27].

Extant neuroimaging studies suggest that the mirror neuron system (MNS) plays a crucial role in observational learning and the promotion of imitation in the general population[28-31]. This system becomes activated not only when an individual performs a motor action, but also when they observe someone else performing the same action[32-35], highlighting the importance of the MNS in bridging the gap between observation and imitation, enabling individuals to learn and replicate actions by simply watching others[28,29,31,32,36-38]. The MNS has been shown to include brain regions such as the premotor cortex, inferior parietal lobule, and inferior frontal gyrus[32,37-41], as well as the pre-supplementary and primary motor area[34,42]. Additionally, differential activity within the MNS between typically developing individuals and those with ASD have been observed during motor imitation and action observation[31,40,43-49]. Variability in MNS activations has been shown to correlate with autistic traits[17], suggesting that the MNS may underlie key behavioral variability associated with ASD. Furthermore, dynamic interactions between the ventral attention network (VAN) and dorsal attention network (DAN) support sensory processing and action planning [50-54] - critical for imitation and motor coordination. Therefore, impairments in the MNS,

VAN, and DAN may help explain the deficits in imitation and related behaviors seen in individuals with ASD.

Early detection of challenges with motor imitation could bolster early autism diagnostic assessments[55]. However, human-observation coding (HOC) schemes to assess motor imitation fidelity are highly time consuming and require extensive training. To enable automated computer-driven assessment of the three-dimensional motor output of motor imitation, members of our team have developed an automatic, continuous, computerized assessment of motor imitation (CAMI) that utilizes metric learning and dynamic time warping to assess motor imitation fidelity[56]. This method uses dynamic time warping (DTW) to assess the spatiotemporal differences between the movements of a participant and of a gold standard[57]. The CAMI method has been validated as a reliable method for assessing motor imitation in children [56,58]. This work has shown that CAMI-generated scores were highly correlated with those generated using traditional labor-intensive, subjective HOC methods. Additionally, the CAMI had better discriminative ability to classify the children into ASD vs non-autistic groups than did HOC, and CAMI scores were significantly correlated with autism trait severity, as measured using the Social Responsiveness Scale, Version 2 (SRS-2)[59], a well-established clinical metric of quantitative autistic traits. While these CAMI methods show promise in detecting group differences in motor imitation fidelity, the relationships between variability in brain function and motor imitation fidelity remain unknown.

Herein, we aim to use HD-DOT and simultaneous CAMI to investigate how variability in brain function relates to both observation of and imitation of purposeless gross motor movements in a sample of adults, including those with ASD. We hypothesize that both observing and imitating will engage visual, motor, and temporal brain regions. Additionally, we hypothesize that regions within the MNS, DAN, and VAN will be activated when observing and imitating motor movements, with stronger brain activation within key regions of the DAN and MNS during motor imitation. Last, we hypothesize that brain response strength within the MNS will be negatively correlated with dimensional measures of social responsiveness (i.e., Social Responsiveness Scale, SRS-2) during either observation or imitation, and positively correlated with imitation fidelity (i.e., CAMI scores) during motor imitation.

Material and Methods

Participants

We recruited N=100 participants (age 30.15 ± 12.97 years, 45 male/55 female, **Table 1**) from the St. Louis, Missouri metropolitan community. All participants gave written informed consent to participate in this study, which was approved and carried out in accordance with the Human Research Protection Office at Washington University School of Medicine. All participants with ASD had a prior community diagnosis.

HD-DOT System

The continuous-wave HD-DOT system has been previously described [60] and was comprised of 128 dual-wavelength optical sources at wavelengths of 685 nm and 830 nm, and 125 avalanche-photodiode detectors. The imaging cap supported multiple source-detector distances (i.e., 11, 24, 33, and 40 mm), providing over 3,500 measurement pairs per wavelength for image reconstruction at a 10 Hz frame rate. The HD-DOT field-of-view covered continuous aspects of temporal, visual, parietal, and prefrontal cortical areas (**Figure 1A,B**).

Stimuli

The motor observation and motor imitation paradigms each consisted of six different movement sequences separated by 30 second inter-stimulus-intervals (ISIs; **Figure 1C)**. The movement sequences lasted 16-21 seconds and were comprised of 14-18 individual movement types, which were unfamiliar, did not have an end goal, and required moving both arms simultaneously. These movements were chosen based on prior research indicating difficulties in these movements for autistic participants[56]. All movements were performed by the same woman and the stimuli were displayed on a monitor placed 110 cm from the nasion in front of the participant. For the observation task, participants were instructed to passively watch the movement sequences and to remain as still as possible. For the motor imitation task, participants were instructed to mirror the actor's arm movements as accurately as possible and limit head motion (**Figure 1D**). Stimulus paradigms were presented via Psychophysics toolbox 3 for MATLAB using in-house custom-built scripts. Participant brain function was recorded with HD-DOT during all experimental runs. During the motor imitation runs, three-dimensional spatial tracking of participants' movements were also simultaneously recorded using an Xbox Kinect 3D camera located on top of the stimulus presentation monitor.

Computerized Assessment of Motor Imitation (CAMI)

The general CAMI algorithm has been previously described[56]. The CAMI algorithm was modified for a seated imitation task to allow for the imitation task to be used while participants were in our fiber-based HD-DOT system. The seated CAMI algorithm only considering joints from the upper body as there was limited involvement of the lower limbs. The spatial coordinates of 20 joints were extracted from the Xbox Kinect 3D motion sensor depth recordings using iPi Motion Capture Software (iPiSoft, Moscow, Russia). Calculation of CAMI scores consisted of five main steps: (1) preprocessing, (2) automatic joint importance estimation, (3) computation of the distance feature, (4) computation of the time features, and (5) computation of the CAMI score. Preprocessing consisted of defining the hip position as the origin, mapping the sequence of movements to a skeleton of fixed size, correcting the initial orientation of the participants to match that of the avatar (**Figure 1E**), the average position of the participants' joints associated with their spine (Hip, Lower Spine, Middle Spine, and Neck) were corrected to match those of the average position of the gold standard (**Figure 1F**). This alignment step was completed to avoid penalizing participants for their initial posture or sitting position in the cap, as this could vary between

participants. Dynamic time warping was utilized to assess the spatial-temporal differences between 20 joints of the participant and of a gold standard expert imitator. The features were linearly combined to compute the overall CAMI score using previously published weights [56].

Behavioral Measurements

The Social Responsiveness Scale, 2nd Edition (SRS-2)[61], provided a measure of social reciprocity, with higher scores correspondent to higher levels of autism traits that have been shown to be continuously distributed throughout the general population. The SRS raw scores were converted to standardized SRS t-scores for analyses across a general population to avoid bias for age or sex. Participant handedness was determined by the Edinburgh Handedness Inventory (EHI) [62]. Measures of verbal and non-verbal intelligence quotients (IQ) were assessed with the Kaufman Brief Intelligence Test- 2 (KBIT-2)[63]. All participants had a full–scale or verbal IQ > 80.

Data pre-processing and Image Reconstruction

HD-DOT data were pre-processed using the NeuroDOT toolbox in MATLAB (https://www.nitrc.org/projects/neurodot). Each of the raw source-detector pair light level measurements were converted to optical density by calculating the log-ratio of the raw data to its temporal mean. Measurements with greater than a 7.5% temporal standard deviation in the least noisy (lowest mean motion) 60s of each run were removed from further analyses. Data were bandpass-filtered between 0.02 and 1.0 Hz using 9-pole Butterworth filters. Then, the superficial signal for each wavelength was estimated as the average across the closest nearest neighbor measurements (11 mm S-D pair separation) and regressed from all measurement channels. The optical density time-traces were then low pass filtered with a cutoff frequency of 0.5 Hz and temporally down sampled from to 1 Hz. As previously described[23], a wavelength-dependent sensitivity matrix was computed and inverted to calculate relative changes in absorption at the two wavelengths via reconstruction using a Tikhonov regularization parameter of 0.01 and spatially variant regularization parameter of 0.1. Relative changes in the concentrations of oxygenated, deoxygenated, and total hemoglobin were obtained from the absorption coefficient changes by the spectral decomposition of the extinction coefficients of oxygenated and deoxygenated hemoglobin at the two wavelengths (i.e., 685 and 830 nm). All data were resampled to a $1\times1\times1$ mm^3 standard MNI [64,65] atlas using a linear affine transformation for statistical analyses.

Data Quality Assessment and Motion Censoring

Quantitative raw data quality assessments included calculating the percentage of good measurements (GM) retained[23] and the median pulse band signal-to-noise ratio (SNR)[23,66]. As previously described, runs with GM < 80% and/or SNR < 1.0 and were excluded from further analysis [23,66,67] to ensure robust data reliability. Global variance in the temporal derivative (GVTD) was used to detect and censor time points with motion-driven artifact in the data[23,27,68]. Briefly, GVTD is calculated as the RMS of the first temporal derivative across the set of measurements for

each time point. Higher GVTD values indicate higher global variance in the optical time course and are exquisitely sensitive to motion-based changes in optode-scalp coupling. Motion censoring with GVTD excludes the time points that exceed the GVTD noise threshold. We used a stringent GVTD threshold of 0.1% based on previous studies utilizing GVTD motion based censoring[23,66,67].

Statistical Analyses

After pre-processing and data quality assessment, 78 participants were identified as having high-quality motor observation runs, while 64 participants had high-quality imitation runs that met the data quality assessment thresholds described in the previous section. Additionally, one participant was excluded due to technical issues with the stimulus presentation. Further, nine participants did not return SRS scores and 21 participants were unable to finalize K-BIT testing due to scheduling difficulties. As a result, the final sample consisted of 77 participants for motor observation analysis and 63 participants for motor imitation analysis.

We used general linear modeling (GLM) to estimate the brain response to each task (task vs. rest) using a canonical hemodynamic responses function (HRF) based on HD-DOT data[69]. We present the HbO results in the main figures as HbO signals exhibits a higher contrast-to-noise ratio compared to HbR or HbT [20,68,70].

We used the Gordon cortical parcellation [71] to provide an unbiased spatial sampling for hypothesis driven analyses. The functionally defined Gordon parcellation provided a framework to couch our analyses within functionally identified parcels of interest. The intersection of each Gordon parcel with the HD-DOT field of view provided 192 parcels associated with nine functional networks (i.e., auditory, frontal-parietal, default mode, dorsal-attention, somatosensory hand, somatosensory mouth, ventral attention, cingulo-opercular, and visual functional networks; **Figure 1B**). Small parcels of size $< 500\ mm^3$ were excluded from analyses, providing a final parcel count of 118. Based on the Euclidean mean MNI coordinate of each parcel location and its associated Brodmann area (BA)[72,73], we selected 58 parcels located in premotor cortex (BA6), inferior parietal lobule (BA 39 and 40), inferior frontal gyrus (BA 44 and 45), pre-supplementary (BA6) and primary motor area (BA4) to represent the spatially distributed mirror neuron system (MNS) [29,32,38].

For the within-task statistics, we used paired t-tests to compare the task effect compared to rest, with the $p < 0.01$ for each tail with a false discovery rate (FDR) correction. We used two-sample t-tests to compare contrast effects between motor observation and motor imitation, with $p < 0.05$ for each tail with an FDR correction. We calculated Pearson correlations between the parcel-specific responses to motor observation and motor imitation with the SRS-2 to investigate brain-behavior correlations relative to autism traits. We also calculated the Pearson correlation between the motor imitation responses and the CAMI scores to assess brain-behavior relationships relative to motor imitation fidelity.

Results

Behavioral Measures

First, autistic participants exhibited significantly higher scores on the Social Responsiveness Scale (SRS-2), indicating elevated autism-related traits compared to NAI participants (p = 0.0003, t = 4.437, NAI N = 74, ASD N = 17, **Figure 1G (i, ii)**). Second, there were no significant differences between the NAI and ASD groups in verbal or non-verbal IQ **Figure 1G (iii)**. Third, in line with previous CAMI studies[74,75], the NAI group exhibited significant higher CAMI scores compared to the ASD participants (p = 0.004, t = 2.96, NAI N = 74, ASD N = 15, **Figure 1G (iv)**); indicating that the NAI individuals had the better motor imitation performance than the ASD group.

Neural Correlates of Motor Observation and Imitation

Whole-brain analyses indicated widespread activation in the visual and temporal cortices during both observation and imitation tasks (**Figure 2**), and additional motor brain activation during the motor imitation task. Additionally, the visual and temporal brain area exhibit significant the significant greater activation during motor observation task instead of the motor imitation task. Moreover, We observed overlapping brain activation in the temporal, visual, and bilateral inferior frontal gyrus (IFG) regions during both the motor observation and motor imitation tasks (**Figure 3**). Similarly, additional activation in the motor cortex was observed during the IM task, highlighting enhanced engagement of motor-related regions during active imitation compared to passive observation (**Figure 4**). Significant activations are apparent in MNS in response to both observing and imitating motor movements, specifically the bilateral IFG and IPL. Notably, compared to observing, imitating led to significantly greater activation in extended MNS areas, including M1 and SMA (**Supplementary Figure 1B, C**). Moreover, we observed that the MNS area exhibit the greater activation during observing compared to imitating **(Figure 5**, **Supplementary Figure 1B, C**).

To further investigate the activations from a functional network perspective, we quantified the number of cortical parcels activated within each network for each task (**Supplementary Figure 2**). Most brain networks were dynamically engaged during motor observation and imitation tasks. The dorsal attention network (DAN) and the somatosensory hand network exhibited larger involvement during imitation than observation (**Supplementary Figure 2D, Supplementary Figure 3**). In contrast, the visual and ventral attention network (VAN) exhibited greater recruitment during observation than imitation (**Supplementary Figure 2D, Supplementary Figure 4**).

Associations with SRS

During the observation task, a positive correlation was identified between SRS scores and brain activation in the left secondary visual cortex, right premotor cortex, right somatosensory cortex, and right inferior parietal lobule (r-IPL). Notably, the correlation in the r-IPL remained significant after FDR correction (FDR corrected $p < 0.001$, $r = 0.472$). Moreover, activation magnitude in the right angular gyrus (r-AG), left secondary visual cortex, and left visual association cortex (l-VAC) exhibited a negative correlation with SRS score ($p < 0.05$, **Figure 6**). This negative correlation indicates that individuals with higher SRS scores exhibited reduced activation in these cortical regions. A similar pattern of negative correlations in left primary motor and secondary visual cortex was observed during imitation. Further, we conducted hypothesis driven brain behavioral analyses for parcels within the MNS, DAN, and VAN. We found negative correlations between parcels within MNS (i.e., **Supplementary Figure 5**) and SRS scores, suggesting autistic individuals with stronger autistic characteristics exhibit lower activity in parcels within the MNS. No significant correlations were observed between SRS scores and brain function during imitation in parcels in either the DAN or VAN.

Associations with CAMI

Positive correlations between cortical responses during imitation and CAMI scores were observed in primary motor cortex, indicating that higher CAMI scores are associated with increased activation in these areas (**Figure 7**). Conversely, negative correlations were identified in left IFG areas. Additionally, we ran hypothesis driven analyses using the brain activity in the MNS, VAN, and DAN areas (**Supplementary Figure 6**). Core regions of the MNS (i.e., particularly the IFG and IPL), exhibited negative correlations between activation and CAMI scores. Within the VAN and DAN, significant negative correlations with CAMI scores were observed in areas proximal to the IFG.

Discussion

Our results confirm that both observation and imitation of motor tasks engage a spatially and functionally distributed set of brain regions, with significant involvement throughout the MNS. Additionally, we observed task-specific differences, highlighting variability in brain activity that correlate with motor imitation fidelity and social responsiveness. These findings contribute to understanding how variability in MNS may contribute to the motor imitation deficits and their association with autistic traits.

The current study provides compelling evidence for the role of the MNS during both motor imitation and observation. Specifically, our results demonstrate significant activation in the posterior superior temporal sulcus (STS), inferior parietal lobule (IPL), and inferior frontal gyrus (IFG), key regions of the MNS [32,33,76]. As hypothesized, motor imitation elicited greater activation in motor-related areas, including the primary motor cortex (M1) and supplementary motor area (SMA), which aligns with the functional role of the MNS in encoding observed actions and translating them into motor commands [32,39,77]- processes integral to motor imitation. Notably, the

negative correlation observed between MNS activation and SRS scores further underscores the association between MNS variability and autistic traits, suggesting that lower MNS activation may contribute to disrupted social engagement and communication[17].

The VAN is primarily involved in stimulus-driven attention, as it responds to salient stimuli in the environment [51,52,54,78]. In the context of motor imitation, we observed engagement of VAN regions during both motor observation and imitation, albeit to a lesser extent compared to the MNS and DAN regions. Although no significant brain-behavior correlations were found between VAN activation and SRS t-scores, the role of VAN in sensory processing and attention shifting remains crucial for imitation. Our findings suggest that dysfunction in the VAN could potentially impair how individuals with ASD attend to social stimuli, affecting their ability to replicate accurately observed actions. Future studies with larger sample sizes may provide more conclusive evidence regarding the contribution of VAN in imitation deficits associated with ASD. Similarly, the DAN, known for supporting goal-directed attention and motor planning [51,79-81], showed robust activation during both motor observation and imitation. As hypothesized, greater involvement of DAN was observed during the IM task, indicating that motor planning and goal-directed attention processes are more critical during active imitation[52,54,82]. Interestingly, we observed a significant negative correlation between DAN activation and CAMI scores, which suggests that deficits in the DAN may contribute to reduced motor adaptability and difficulties in motor execution[51,79].

The analysis of brain-behavior correlations between brain activation and CAMI scores revealed significant associations in MNS regions implicated in motor planning and execution. Positive correlations were observed in motor regions such as the right primary motor cortex, suggesting that greater motor imitation fidelity (higher CAMI scores) is linked to enhanced activation in these areas. This aligns with the hypothesis that stronger motor system engagement supports better imitation performance. An fMRI study found a positive correlation in the primary motor cortex with participants motor performance[83]. Additionally, we found brain activation in IFG negatively correlated with CAMI scores. Another study found inferior parietal lobe (IPL; also implicated as part of the MNS) activity was negatively correlated with imitation accuracy. Overall, this suggests that reduced activation in the core MNS regions may be associated with difficulties in motor adaptability [31,84,85]. These findings provide insight into how deficits in neural regions critical for motor control and imitation may contribute to reduced imitation fidelity in individuals with ASD.

The relationship between brain activation and social responsiveness was further investigated for both motor observation and imitation. Negative correlations between SRS scores and brain activation were observed in regions such as the right angular gyrus (r-AG) and left secondary visual cortex (l-SVC), particularly during observation. Although these correlations did not reach statistical significance during motor imitation, they provide preliminary evidence that impaired brain function in regions involved in action understanding and social cognition may underlie the social difficulties observed in ASD. Interestingly, our results show a positive

correlation between SRS and brain activation in right parietal lobe. This finding may indicate that individuals with higher SRS scores rely more heavily on this region to process and understand observed actions or social cues, potentially compensating for difficulties in other aspects of social interaction [86,87].

Limitations

While this study provides valuable insights into the neural correlates of motor imitation deficits in ASD, several limitations should be addressed in future research. First, the relatively small sample size of the ASD group may limit the generalizability of the findings. Future studies with larger and more diverse populations are needed to confirm these results. Additionally, although HD-DOT offers significant advantages over traditional neuroimaging methods, its field of view is less extensive than that of fMRI, which limits sensitivity to neural activity in deeper regions such as cerebellum and sub-cortical regions previously implicated in ASD functional neuroimaging studies[88,89]. Finally, while the current study focused on adults, investigating neural mechanisms of motor imitation in children with ASD will provide critical developmental insights. This study provides essential feasibility for future studies on motor imitation in adolescent, school-age, and even infant and toddler autistic and typically developing children using combined CAMI and HD-DOT methods.

Conclusions

We used simultaneous HD-DOT functional neuroimaging with CAMI methods to investigate how brain function covaries with observing and imitation motor movements. We observed significant activation in the MNS during both motor observation and imitation tasks. Notably, MNS regions demonstrated greater activation during motor observation compared to imitation, while motor areas exhibited higher activation during imitation. These findings suggest distinct yet overlapping neural mechanisms for action observation and execution, with shared engagement of visual, temporal, and bilateral IFG regions. Additionally, task-specific activation was observed in the DAN and the extended MNS, underscoring their roles in motor planning and goal-directed attention during imitation. Brain-behavior analyses revealed that reduced MNS activation was associated with higher autism-related traits and lower motor imitation fidelity. Overall, these findings highlight the neural correlates of action observation/execution and their association with social reciprocity and motor imitation fidelity. Finally, these studies lay essential foundation for future investigations using simultaneous methods for naturalistic functional neuroimaging and quantitative CAMI that may be applied during early childhood development when presentation of autistic traits become apparent and interventions may hold the greatest promise to improve outcome.

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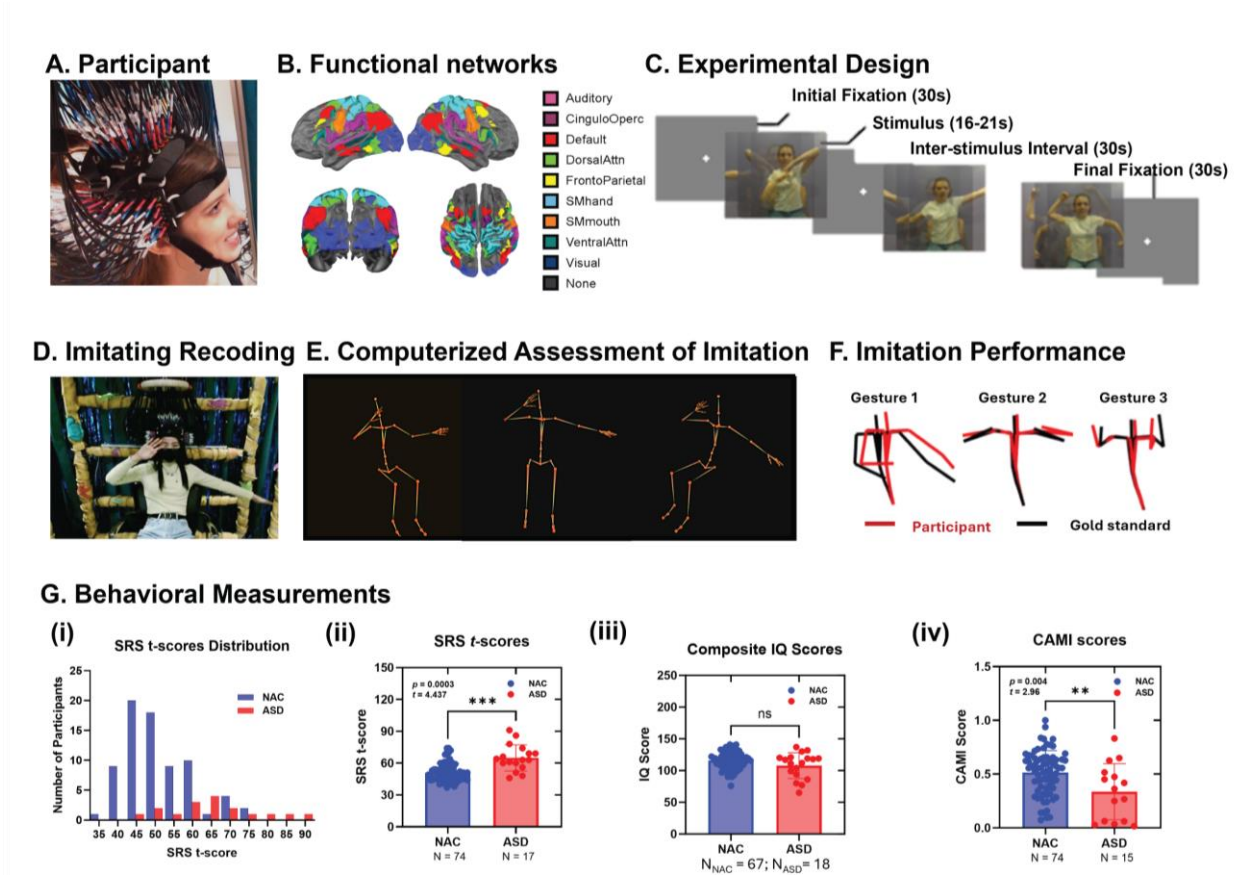

Figure 1: Study Design and Behavioral Assessments. A Participant wearing the DOT cap. **B** Field of view from dorsal, posterior, and lateral perspectives and overlapping Gordon parcels colored by functional networks assignment. **C** Experimental design for the motor observation and imitation tasks. **D** Participant performing imitation of movements. **E** Three examples of extracted skeleton of moving participant for CAMI analyses. **F** Comparison of imitation performance with a gold-standard skeleton positions during the movements. **G** Behavioral assessments including SRS scores, IQ, and CAMI scores.

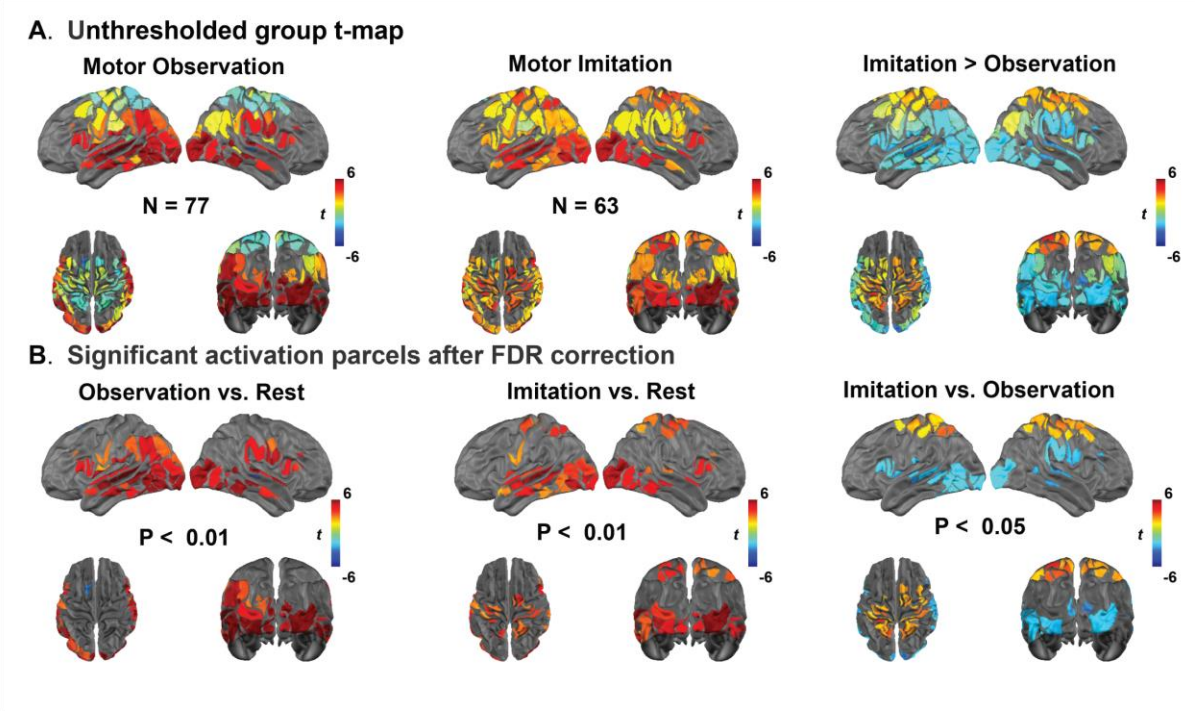

Figure 2: Neural correlates of observation and imitation. A. Group-level t-maps for motor observation (N = 77), motor imitation (N = 63), and their contrast. **B.** FDR-corrected t-maps highlighting statistically significant regions, with false discovery rate correction applied to account for multiple comparisons.

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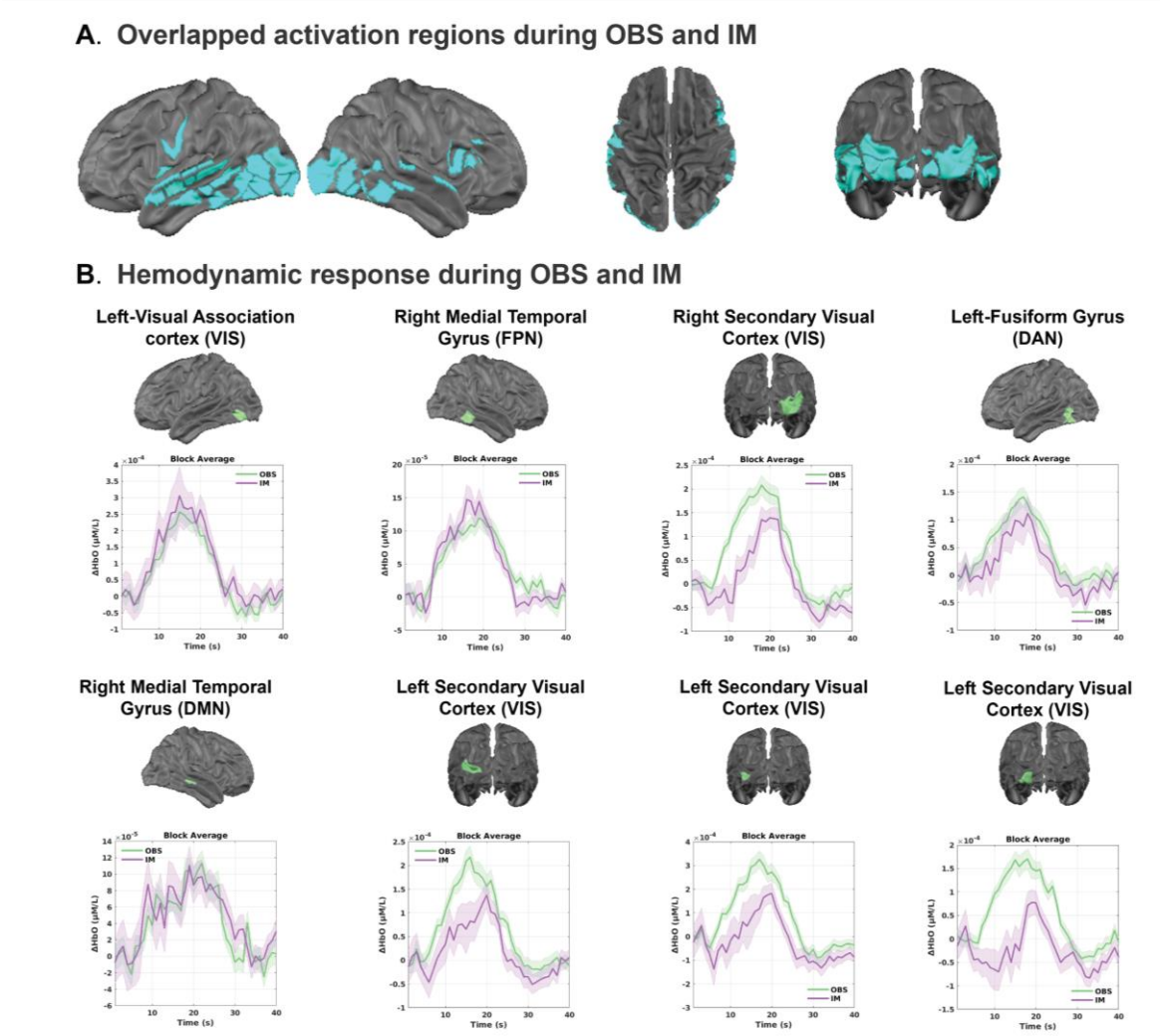

Figure 3: Brain regions exhibiting significant responses to both motor observation and imitation. **A.** Brain regions with overlapping significant activation identified through conjunction analysis. **B.** Temporal profiles of the ΔHbO_2 (oxygenated hemoglobin) hemodynamic response for each task, with shaded areas indicating the standard error of the mean across participants. The selected parcels represent the significant regions with the eight smallest FDR-corrected p-values, showing the greatest activation. The abbreviations correspond to their respective functional networks: VIS refers to the Visual Network, FPN to the Frontoparietal Network, DAN to the Dorsal Attention Network, and DMN to the Default Mode Network.

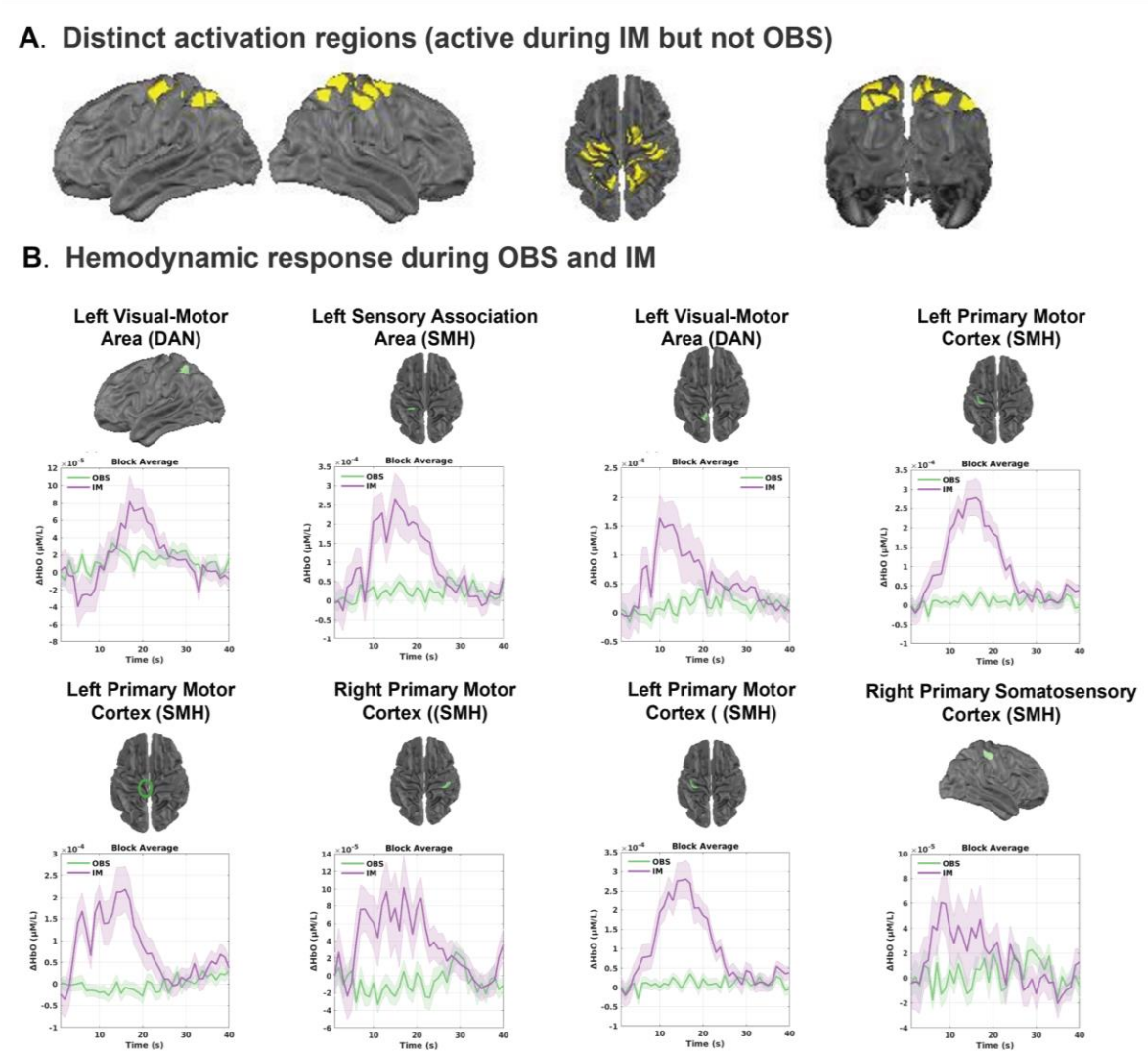

Figure 4: Brain regions exhibiting significant responses during motor imitation but not motor observation. A. Brain regions with distinct significant activation during motor imitation that are not significantly active during motor observation. **B.** Temporal profiles of the ΔHbO_2 (oxygenated hemoglobin) hemodynamic response for each task, with shaded areas representing the standard error of the mean across participants. The selected parcels correspond to the significant regions with the eight smallest FDR-corrected p-values, demonstrating the great activation. The abbreviations correspond to their respective functional networks: DAN refer to the Dorsal Attention Network and SMH to the Somatomotor Hand Network.

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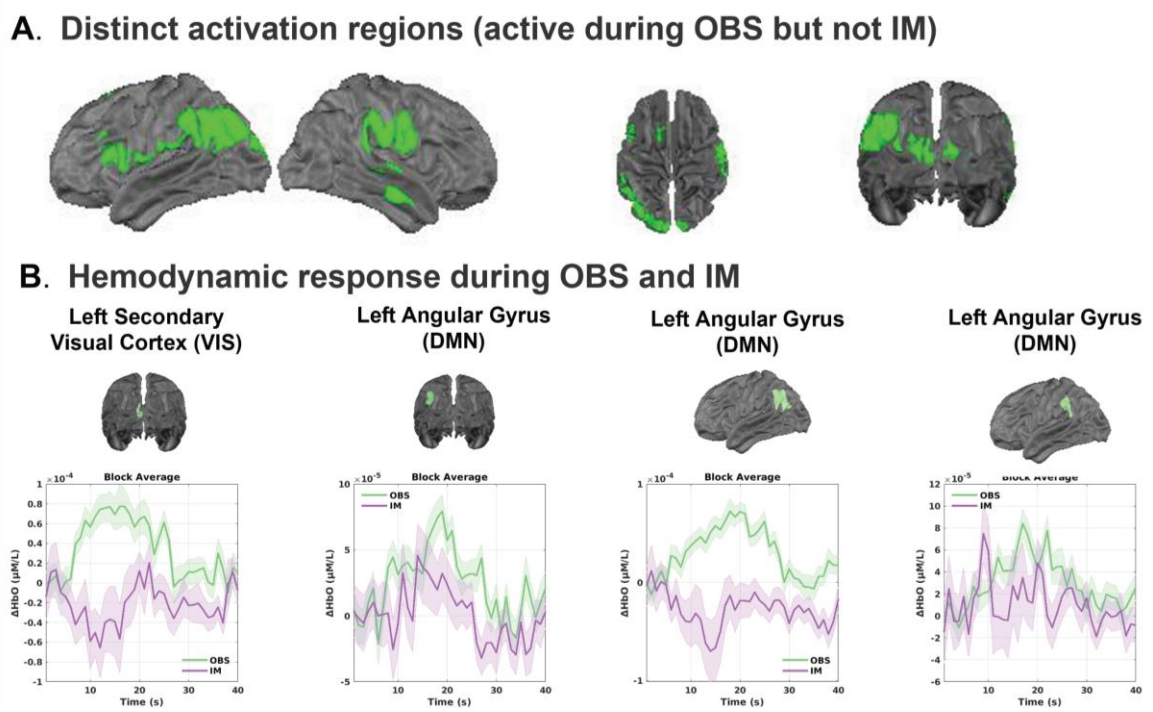

Figure 5: Brain regions exhibiting significant responses during motor observation but not motor imitation. A. Brain regions with distinct significant activation during motor observation task that are not significantly active during motor imitation. **B.** Temporal profiles of the ΔHbO_2 (oxygenated hemoglobin) hemodynamic response for each task, with shaded areas representing the standard error of the mean across participants. The selected parcels correspond to the significant regions with the four smallest FDR-corrected p-values, demonstrating the great activation. The abbreviations correspond to their respective functional networks: VIS refers to the Visual Network and DMN to the Default Mode Network.

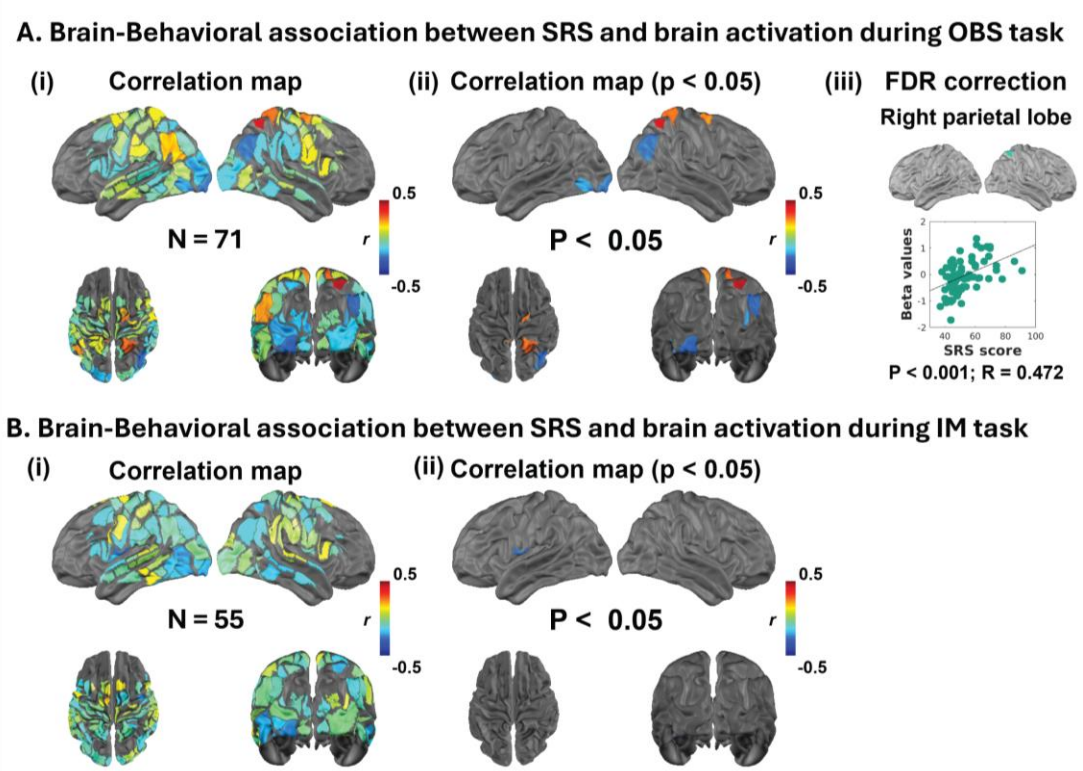

Figure 6. Brain-behavioral association between SRS scores and brain activation. **A (i)** Correlation map of brain activation during motor observation with SRS in 71 participants**,** **(ii)** the brain regions which correlation $p < 0.05$, and **(iii)** the correlation passed the FDR correction (FDR corrected $p < 0.001$, and $R = 0.472$)**. B (i)** Correlation map of brain activation during motor imitation with SRS in 55 participants. **(ii)** the brain regions which correlation $p < 0.05$. There is no correlation passed the FDR correction.

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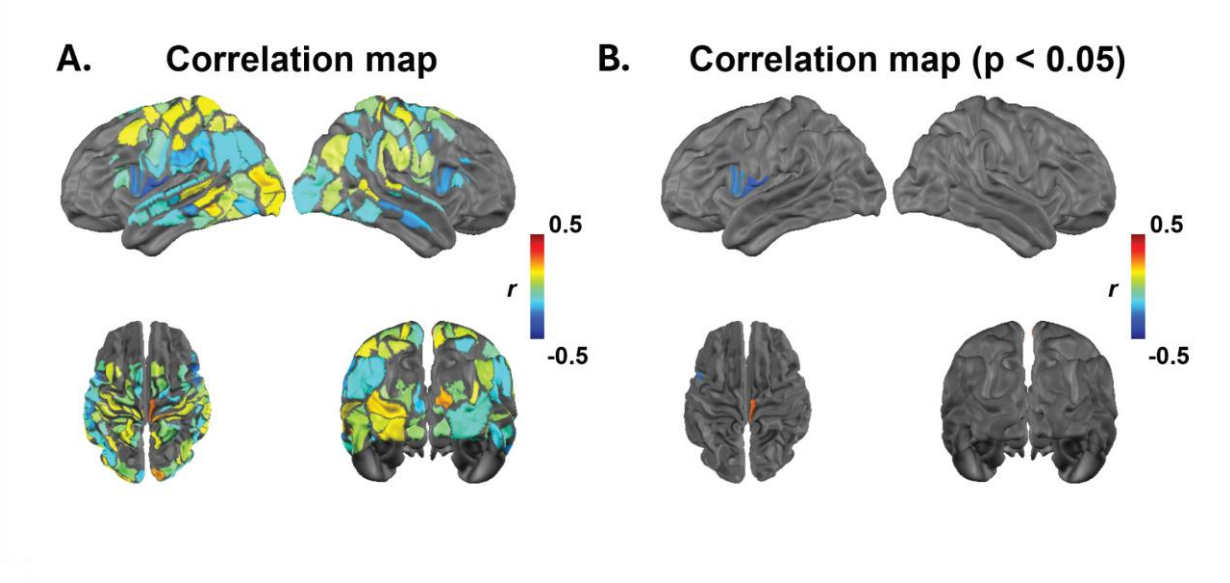
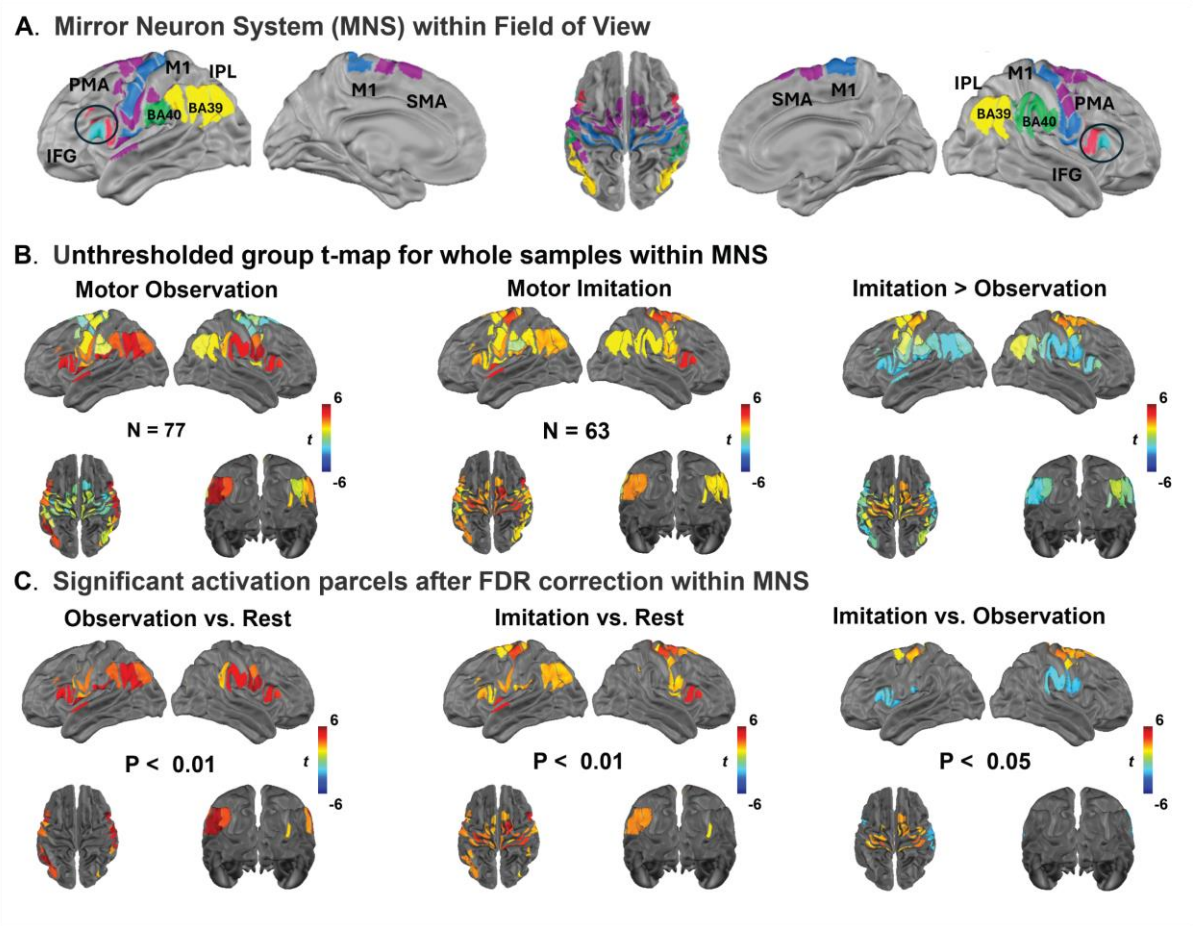


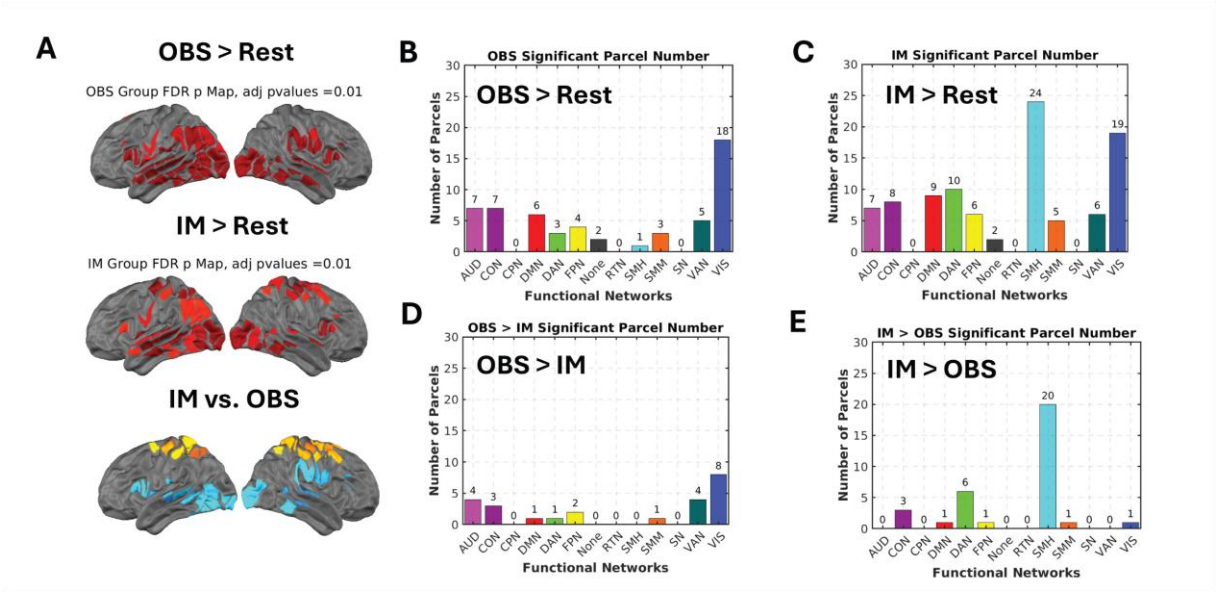
Figure 6. Brain-behavioral association between CAMI and brain activation during motor imitation task. A The correlation map for motor imitation activity against SRS scores for 57 participants. **B** The same map thresholded ($p < 0.05$).

Table 1 Participant demographics and group characteristic

Measure		**Full Sample NAC n = 81**			**Full Sample ASD n = 19**		
Sex, Males:Females			33:48			12:7	
		Means	*S.D.*	*n*	*Means*	*S.D.*	*n*
Age, years, [range]		27.56	9.36	81	31.47	16.73	19
SRS	Raw score	43.61	24.68	74	82.94	33.72	17
	T score	51.07	8.67	74	65.00	11.89	17
KBIT 2 IQ	Verbal	113.38	17.22	61	103.39	20.04	18
	Nonverbal	117.61	11.97	61	110.67	16.82	18
	Full scale	117.75	13.69	61	107.83	19.77	18
CAMI	Scores	0.52	0.21	75	0.31	0.26	18



Supplementary Figure 1: Neural correlates of observation and imitation within the mirror neuron system. A. Parcel-based analysis of the MNS within the field of view, highlighting regions activated during both motor observation and imitation. **B.** Group-level t-maps depicting the areas of the MNS that are significantly activated during motor observation and imitation. **C.** FDR-corrected t-map showing the statistically significant regions within the MNS, with false discovery rate correction applied to control for multiple comparisons.



Supplementary Figure 2: Functional network involvement during motor observation and imitation. A Task specific significant brain parcels under of three different conditions (OBS, IM, and their contrast). **B** Functional networks involved during the OBS task. **C** Functional networks involved during the IM task. **D** Functional networks more active during the OBS task than the IM task. **E** Functional networks more active during the IM task than the OBS task.

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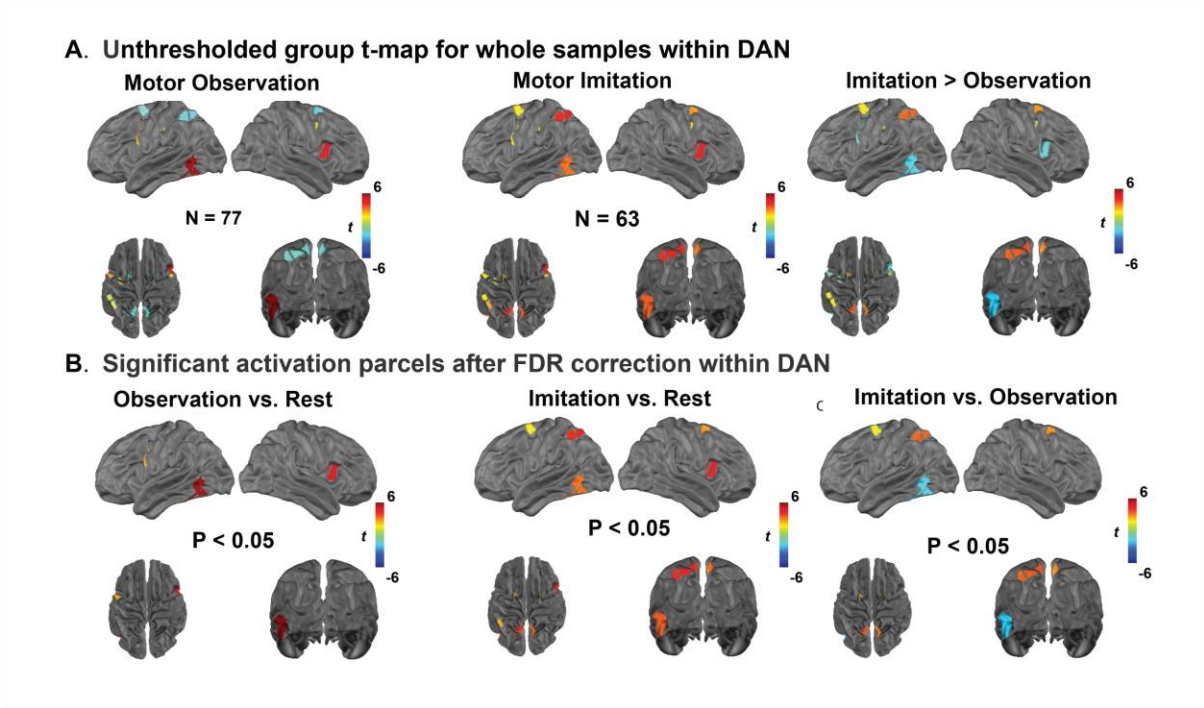
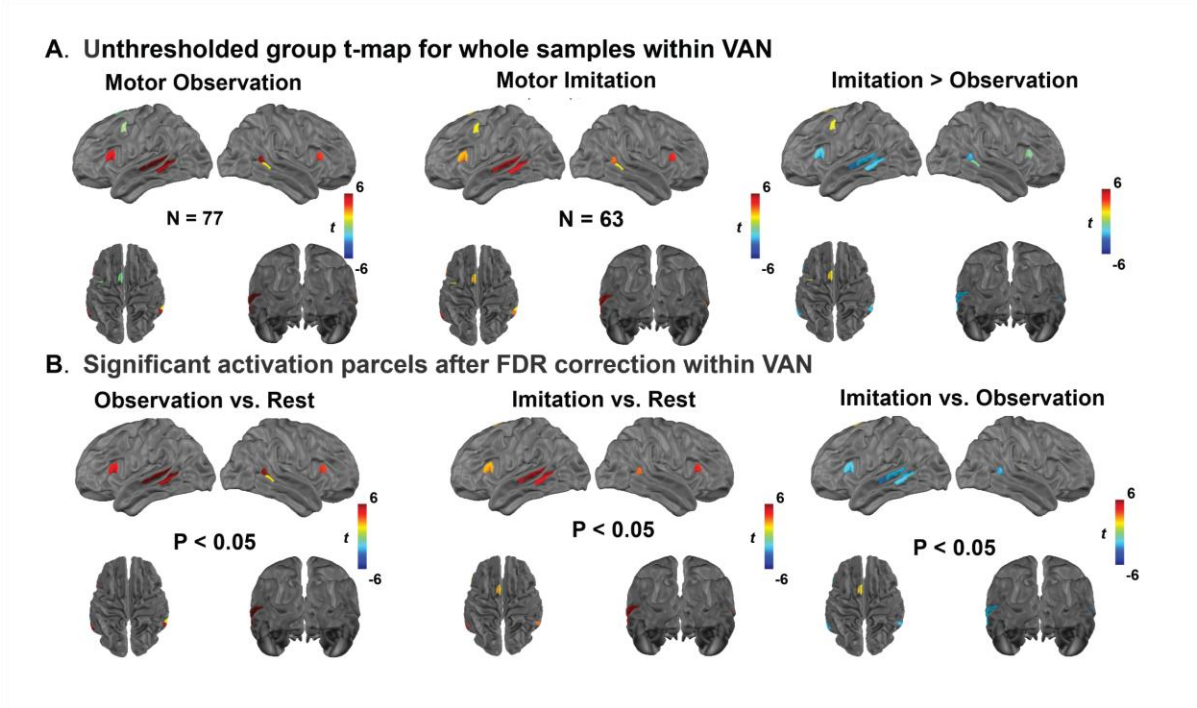
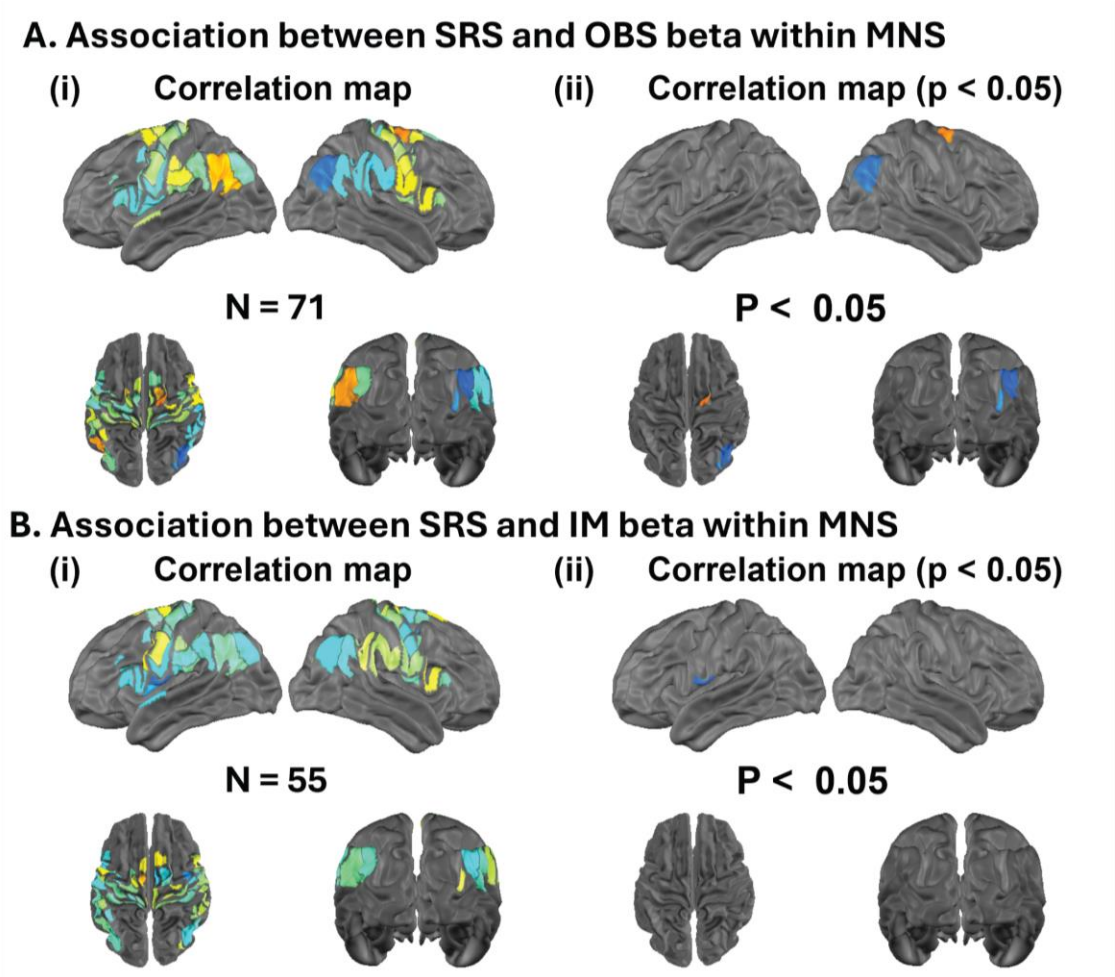


Supplementary Figure 3: Neural correlates of OBS and IM within dorsal attention network. A. Group-level t-maps for motor observation (N = 77), motor imitation (N = 63), and their contrast (red indicates brain regions where OBS > IM, and blue indicates brain regions where IM > OBS). **B.** FDR-corrected t-map highlighting statistically significant regions, with false discovery rate correction applied to account for multiple comparisons.



Supplementary Figure 4: Neural correlates of OBS and IM within ventral attention network. A. Group-level t-maps for motor observation (N = 77), motor imitation (N = 63), and their contrast (red indicates brain regions where OBS > IM, and blue indicates brain regions where IM > OBS). **B.** FDR-corrected t-map highlighting statistically significant regions, with false discovery rate correction applied to account for multiple comparisons.



Supplementary Figure 5: Brain behavioral association (SRS vs beta) within MNS. A Correlation map of OBS brain activation with SRS in 71 participants and the brain regions which correlation $p < 0.05$. **B (i)** Correlation map of IM brain activation with SRS in 55 participants and brain regions which correlation $p < 0.05$. There is no correlation passed the FDR correction.

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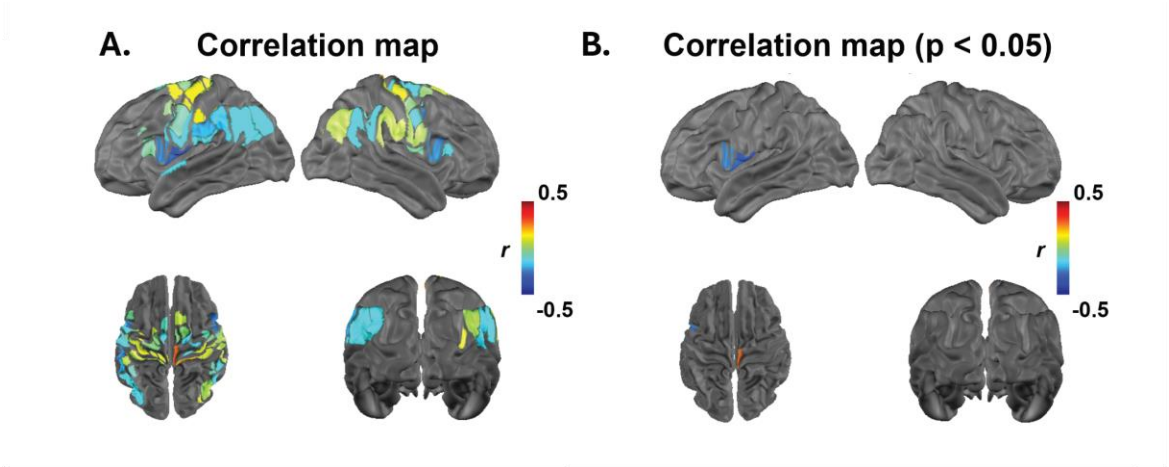

Supplementary Figure 6. brain-behavioral association between CAMI and brain activation during motor imitation task within MNS brain areas. A The correlation map of the IM beta values with the SRS scores for 57 participants. **B**. presents the R map, highlighting the brain parcels that show significant correlations with CAMI ($p < 0.05$). **C.** provides the p-value maps, emphasizing the significant parcels with a correlation to CAMI ($p < 0.05$).

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