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## Alterations of triple network dynamic connectivity and repetitive behaviors after minibasketball training program in children with autism spectrum disorder

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Physical exercise has been demonstrated to effectively mitigate repetitive behaviors in children with autism spectrum disorder (ASD), but the underlying dynamic brain network mechanisms are poorly understood. The triple network model consists of three brain networks that jointly regulate cognitive and emotional processes and is considered to be the core network underlying the aberrant manifestations of ASD. This study investigated whether a mini-basketball training program (MBTP) could alter repetitive behaviors and the dynamic connectivity of the triple network. 28 male children with ASD were scanned twice with resting-state functional MRI and assessed for repetitive behaviors using the repetitive behavior scale (RBS-R). 15 children in the exercise group participated in a 12week MBTP, while 13 in the control group maintained their regular routines. The feature of Dynamic independent component analysis (dyn-ICA) is its ability to capture the rate of change in connectivity between brain regions. In this study, it was specifically employed to examine the triple network dynamic connectivity in both groups. Compared to the control group, the exercise group exhibited distinct dynamic connectivity patterns in two networks: Network 1 involved cross-network dynamic connectivity changes within the triple network, and Network 2 pertained to dynamic connectivity alterations within the default mode network. Furthermore, a reduction in the RBS-R Total score was observed in the exercise group, reflecting improvements in self-injurious behavior and restricted behavior. Correlation analysis revealed that the amelioration of repetitive behaviors was associated with enhanced dynamic connectivity in parts of the triple network. These findings suggest that MBTP can improve repetitive behaviors in ASD children and is linked to changes in triple network dynamic connectivity.

**Keywords** Physical exercise, Autism spectrum disorder, Dynamic independent component analysis, Triple network

### Background

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that typically manifests in early childhood<sup>1</sup>. Over the past decade, the global prevalence of ASD has risen significantly, currently estimated to affect 1 in 36 individuals. This alarming trend underscores its emergence as a major public health concern worldwide<sup>2</sup>. According to the DSM-5, the core impairments of ASD include: (1) difficulties with communication and

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Physical activity interventions have been shown to have a significant impact on improving symptoms of ASD. A meta-analysis highlighted the positive influence of physical exercise on repetitive stereotypic behaviors<sup>4</sup> The types of physical activity interventions are diverse, with Kata techniques training<sup>5</sup>, therapeutic horseback riding<sup>6</sup>, and mini-basketball training program<sup>7</sup>, all demonstrating effectiveness in reducing repetitive stereotypic behaviors in children with ASD. Among these, mini-basketball training program (MBTP) are considered to have broad prospects The ease of implementation, enjoyable nature, and effectiveness of MBTP have contributed to their growing adoption in autism rehabilitation in recent years. A 12-week MBTP has been shown to have positive effects on the physical fitness and social communication of children with ASD<sup>8</sup>. MBTP includes numerous balls passing and catching exercises that require direct collaboration with peers, stimulating the perceptual system of individuals with ASD. Additionally, MBTP involves learning complex motor skills, which places demands on higher cognitive functions such as attention and executive function in individuals with ASD. In recent years, researchers have explored the mechanisms underlying the positive effects of MBTP on repetitive stereotypic behaviors. Neuroimaging studies have revealed that these improvements in behavior are associated with enhanced functional connectivity within central executive networks and an increase in gray matter volume<sup>7,9</sup>. Research on exercise and its influence on brain plasticity has demonstrated that sports-related changes in the brain are multifaceted<sup>10</sup>, making it challenging to elucidate dynamic brain plasticity changes in specific networks or regions through individual analysis. A study employing resting-state functional imaging techniques in adolescents with ASD found that abnormalities in dynamic changes within the salience network, default mode network, and central executive network are linked to restricted and repetitive behaviors<sup>11</sup>

The triple network model, introduced by Menon in 2011, encompasses the Salience Network (SN), Default Mode Network (DMN), and the Central Executive Network (CEN)<sup>12,13</sup>. These networks play pivotal roles in cognitive functioning and emotional processing by facilitating real-time information integration across brain regions. The triple network model is widely employed in ASD research and is considered a fundamental network for understanding ASD symptomatology<sup>13,14</sup>. Prior studies have identified connectivity irregularities within the SN, DMN, and  $CEN^{15-18}$ . Notably, recent research has increasingly recognized the connection between repetitive behavioral performance and abnormal connections within the triple network<sup>11,19-22</sup>. Many contemporary neuroimaging studies have operated under the assumption that functional connectivity remains static over time, inadvertently neglecting the time-varying attributes of brain networks, which are vital indicators of the impact of restricted and repetitive behaviors in children with ASD<sup>23-25</sup>. Moreover, the plasticity changes associated with physical exercise are extensive and systematic<sup>26-29</sup>, and overlooking these dynamic network properties due to movement-related alterations could result in misconceptions. Dynamic connectivity analysis, which captures time-varying features based on functional connectivity, has not yet clarified the current dynamic connectivity relationships associated with exercise interventions aimed at improving restricted and repetitive behaviors. Hence, this study sought to explore dynamic connectivity changes among brain regions within the triple network following a 12-week MBTP designed to ameliorate repetitive behavior in children with ASD. These findings provide valuable insights into how exercise interventions influence the dynamics of the core symptom network in children with ASD.

### Methods Participants

The present study originally recruited a total of 57 preschool children, ranging in age from 3 to 6 years, from two educational institutions. However, the study was completed by a total of 28 individuals. The criteria for inclusion in the study were as follows: (1) Han Chinese ethnicity; (2) Children between the ages of 3 and 6 years who satisfied the diagnostic criteria outlined in the DSM-V and received a clinical diagnosis of moderate to severe ASD; (3) male; (4) consent from the guardians for the subjects to participate in the study (5) cases meeting the MRI scan criteria. The study's exclusion criteria encompassed several factors: (1) a documented record of head trauma; (2) a documented history of neurological disease and/or psychotic disorders; (3) documented impairments in hearing or vision; (4) recent usage of any medication that affects the central nervous system within the preceding 6 months; (5) engagement in basketball training or regular physical exercise within the preceding 6 months; and (6) the presence of any documented extremity disability.

Children with ASD from two institutions were assigned to the control group and the exercise group using a cluster grouping method. The control group followed a standard rehabilitation program based on the Applied Behavior Analysis (ABA), with specific lesson plans jointly developed by institutional teachers and hospital physicians. To maintain consistency, the control group was instructed to avoid participating in similar physical activities during the study period. The exercise group, in addition to following the standard rehabilitation program, engaged in a 12-week MBTP.

Of the 28 subjects included in the final analysis, 15 were in the exercise group, and 13 were in the control group. Cases were excluded for the following reasons: (1) incomplete post-test questionnaires for subjects, as their parents did not complete the relevant forms (n=27); (2) missing T1-MPRAGE data (n=2). Please refer to the CONSORT diagram in Fig. 1 for a visual representation of participant flow and exclusions.

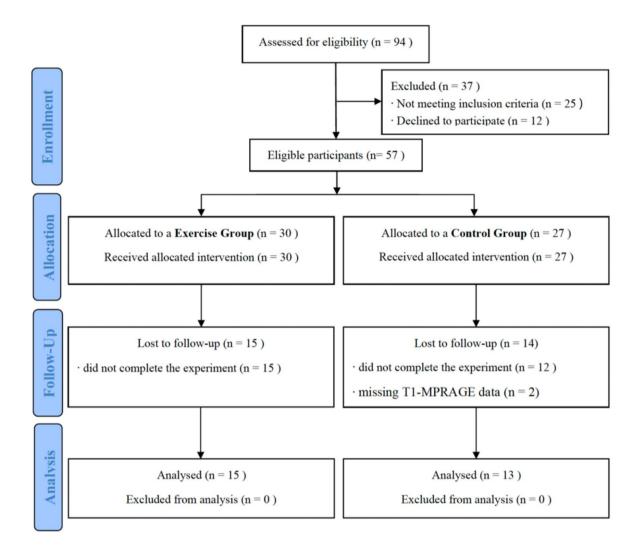


Fig. 1. CONSORT diagram.

### Study design

The present investigation was undertaken as a cluster randomized controlled trial employing a  $2 \times 2$  mixed experimental design. In this design, the factor of "time" was manipulated within-subjects, while the factor of "group" was manipulated between-subjects.

### Mini-basketball training program

For this experiment, we implemented a mini-basketball training program as a rehabilitation intervention for children with ASD, following the methods described in previous studies<sup>7,8,30,31</sup>. The MBTP is comprised of three distinct stages, as outlined in Fig. 2<sup>32</sup>. A typical exercise session has four sequential components, including introduction, warm-up activity, mini-basketball training program (MBTP), and relaxation. The MBTP intervention was implemented on a frequency of five sessions per week over a duration of 12 weeks. Each session had a duration of 40 min, with the exercise activity level classified as moderate.

### **Behavioral assessment**

The repetitive behavior scale (RBS-R) revised by Bodfish and colleagues was used to assess the repetitive behaviors of preschool ASD children<sup>33</sup>. This scale, which is completed by the subject's parents or guardians, is known for its strong reliability and validity. The scale consists of 43 items divided into six subdimensions (stereotyped behavior, self-injurious behavior, compulsive behavior, ritualistic behavior, monotonous behavior, and restricted behavior), and the total score is obtained, with higher scores indicating more severe repetitive behaviors.

Furthermore, the impact of illness severity, sleep disturbances, and eating patterns on ASD has been observed, the Childhood Autism Rating Scale (CARS)<sup>34</sup>, the Children's Sleep Habits Questionnaire (CSHQ)<sup>35</sup> and Child

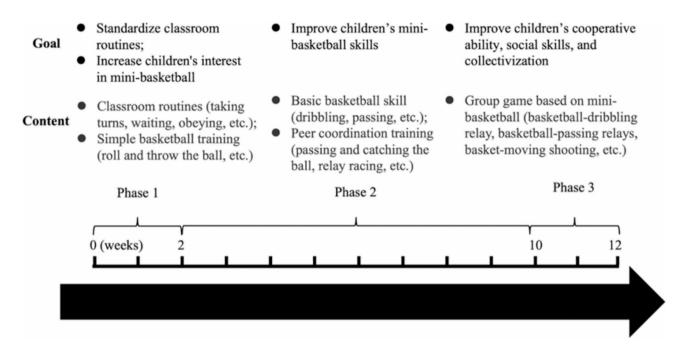


Fig. 2. Mini-basketball training program.

Eating Behavior Questionnaire (CEBQ)<sup>36</sup> were used to control confounding variables. The CARS scale was administered by the chief physician of the Child Autism Clinic, who assessed the clinical manifestations of the children. The evaluating doctors ensured consistency in their assessments. The CSHQ and CEBQ scales were completed by the parents based on their observations of their children's specific behaviors and characteristics in daily life. The parents also maintained consistency in their responses before and after filling out the scales.

### fMRI data acquisition

With the approval of family members or guardians, the participants underwent sleep deprivation, and a childsafe method involving the administration of 10% chloral hydrate sedation was used before the scan to prevent excessive head movement<sup>37,38</sup>. All participants underwent scanning procedures within a time frame of three days before to and following the administration of the MBTP. Additionally, the presence of a guardian was ensured during the completion of the procedures. Neuroimaging data were acquired using a 3.0 T GE scanner (GE Discovery MR750w 3.0 T, Chicago, United States).

T1-weighted (T1W) images were acquired using a magnetization-prepared, rapid gradient-echo sequence with the following parameters: repetition time (TR)=1900 ms; echo time (TE)=2.52 ms; flip angle=9 °; matrix= $256 \times 256$ ; slice thickness=1.0 mm; field of view (FOV)= $250 \times 250$  mm. A total of 240 vol of resting-state background echo-planar imaging (EPI) were obtained. The parameters were as follows: TR=2000 ms; TE=30 ms; flip angle=60; 28 axial slices; matrix= $64 \times 64$ ; voxel size= $3.5 \text{ mm} \times 3.5 \text{ mm} \times 4 \text{ mm}$ ; FOV= $224 \times 224 \text{ mm}^2$ .

### fMRI data pre-processing and Dyn-ICA analysis

Data were preprocessed and analyzed using the default preprocessing program in the CONN toolbox v21a software<sup>39</sup>. The current functional images were labeled as the original data as part of the preprocessing steps. Functional images underwent realignment and unwarping. The functional volumes have been transformed to coordinates of x, y, z (0 0 0). Slice time correction, outlier detection, segmentation, and spatial normalization came next. Structural imaging volumes were translated to (0 0 0) coordinates, followed by segmentation and spatial normalization. Lastly, spatial convolution using a Gaussian kernel with a full width at half maximum (FWHM) of 6 mm was used for functional smoothing<sup>40</sup>. In addition, fMRI data were denoised using a CONN default denoising pipeline<sup>41</sup> including the regression of potential confounding effects characterized by white matter timeseries, CSF timeseries, motion parameters and their first order derivatives, outlier scans, session and task effects and their first order derivatives, and linear trends within each functional run, followed by bandpass frequency filtering of the BOLD timeseries<sup>42</sup> between 0.008 Hz and 0.09 Hz.

The independent component analysis (ICA) approach is widely recognized for its ability to effectively separate signals from noise, thereby improving the sensitivity in identifying inter-individual variations<sup>43,44</sup>. The present study employed dynamic connectivity analysis to investigate the temporal modulation of functional connectivity within a matrix of regions of interest (ROIs)<sup>45</sup>. To this end, dynamic independent component analysis (dyn-ICA) was performed using the CONN toolbox v21a<sup>41</sup>.

The analysis began by performing ICA on the connectivity time series, which represents the functional interactions between each pair of ROIs across the entire dataset. This process computed the connectivity strength at each time point, capturing dynamic changes in brain network interactions. Unlike static ICA, which assumes

fixed connectivity patterns, dyn-ICA identifies time-varying connectivity changes, allowing for a more nuanced understanding of how functional connectivity fluctuates over time. Specifically, dyn-ICA extracts independent components representing different brain networks, and these components are further analyzed to reveal the temporal fluctuations in connectivity strength between ROIs<sup>41</sup>. By examining these dynamic components, we are able to capture the evolving nature of brain network interactions across different time scales, providing insights into the temporal organization of large-scale brain networks.

These ROIs were a set of functional brain networks described in numerous previous studies<sup>46</sup>. These ROIs can be accessed through the Greicius Lab resources page (https://greiciuslab.stanford.edu/resources), with permission requests available via Google Drive (https://drive.google.com/drive/u/1/folders/1TH5Fa6dQe2hg jKJt3MrhBghlFm12ht31). In the present study, the SN, DMN, and CEN were classified as ROIs for analysis according to the definition of the previous triple network, and the number of components was set to 20 and the smoothing kernel was set to 30 s (15TRs) FWHM, as described in the literature<sup>11,39,47,48</sup>.

### **Statistical analysis**

The demographic variables (age and BMI), as well as the CARS, CSHQ scores, and CEBQ scores of both the exercise and control groups, were subjected to a homogeneity test. The accomplishment of this task involved the utilization of an independent sample t-test, using the Jamovi program (The Jamovi Project, Jamovi Version 2.3.19.0). The findings were presented in the form of mean  $\pm$  standard deviation (M  $\pm$  SD).

Subsequently, the repetitive behavior scale data was subjected to repeated measures analysis of variance (ANOVA), with effect sizes reported as partial eta-squared (partial  $\eta^2$ ). Once a significant interaction was identified, a basic effects analysis was performed.

During the second-level analysis in the CONN Toolbox, the connectivity threshold was set to p < 0.01 (uncorrected), cluster threshold was set to p < 0.05 (FDR corrected). After calculating the difference between pre-test and post-test functional connectivity values for the significant regions, these values were imported into Jamovi software. Pearson correlation analyses were then conducted between these differences and the differences in RBS-R scores between the pretest and posttest assessments.

### Results

### **Demographic characteristics**

The demographic characteristics of the patients are summarized in Table 1. Our analysis revealed no significant differences among participants in terms of age [t (26) = -0.99, p=0.33>0.05], Body Mass Index (BMI) [t (26) = -0.01, p=0.99>0.05], CARS [t (26)=1.18, p=0.25>0.05], CSHQ [t (26)=0.32, p=0.75>0.05], and CEBQ [t (26)=0.30, p=0.77>0.05] during the study period.

### Repetitive behavior performance

In this study, we utilized the method of repetitive measure analysis of variance to examine the influence of MBTP on the RBS-R scores in preschool children diagnosed with ASD. The results of the interaction between groups and time periods shed light on whether the MBTP led to changes in the RBS-R scores, including the total score and its six subdimensions (i.e., Restricted Behavior, Routine Behavior, Sameness Behavior, Compulsive Behavior, Self-injurious Behavior, Stereotyped Behavior). Our findings revealed a significant interaction between groups and time periods for the total RBS-R score [F (1, 26) = 7.65, p = 0.01 < 0.05, pp = 0.227], restricted behavior [F (1, 26) = 5.71, p = 0.024 < 0.05, pp = 0.21], and self-injurious behavior [F (1, 26) = 7.88, p = 0.009 < 0.05, pp = 0.180]. Additional scrutiny using simple effects analysis, as delineated in Table 2, indicated that the pre-test scores in all dimensions of the RBS-R were similar between the exercise and control groups (p > 0.05). However, significant differences were observed for the total RBS-R score (p = 0.004), the score for restricted behavior (p = 0.009), and the score for self-injurious behavior (p = 0.054) between the pre-test and post-test assessments in the control group. Specifically, the post-test score in the control group was higher than the pre-test score, indicating a worsening of symptoms. In contrast, in the exercise group, the post-test score was lower compared to the pre-test score. Importantly, a higher score on the RBS-R indicated more severe symptoms.

### Dyn-ICA

To investigate the alterations in dynamic connectivity within the triple network induced by the MBTP in individuals with ASD, we conducted a second-order analysis employing the CONN toolbox. This analysis aimed to examine the 20 factors identified by dyn-ICA. Our findings revealed two distinct networks exhibiting different patterns of dynamic functional connectivity following MBTP.

	Exercise group	Control group	Р
Ν	15	13	
Age(years)	$4.90\pm0.66$	$4.77\pm0.70$	0.614
BMI (weight/ height2)	$16 \pm 1.18$	$15.7 \pm 1.67$	0.622
CARS	$37.7 \pm 4.57$	39.9±5.65	0.254
CSHQ	$57.4 \pm 5.25$	57.8±13.0	0.904
CEBQ	$52.4 \pm 9.91$	$55.2 \pm 20.8$	0.642

**Table 1**. Baseline characteristics of participants  $(M \pm SD)$ .

	Exercise Group (N=15)		Control group (N=13)		
	Pre-test	Post-test	Pre-test	Post-test	F
RBS-R Total score	13±6.3	$7.93 \pm 4.93$	$21 \pm 11.7$	$22.5 \pm 13.6$	7.65*
Stereotyped behavior	3.73±1.39	$2.33 \pm 1.4$	$4.54 \pm 2.82$	$4.15\pm3.05$	2.57
Self-injurious behavior	$1.13 \pm 1.81$	0.8±1.37	$1.31 \pm 0.947$	$2.23 \pm 1.42$	7.88*
Compulsive behavior	$2.73 \pm 2.02$	$1.47 \pm 1.3$	$4.38 \pm 3.33$	$4.92\pm5.02$	4.15
Sameness behavior	$1.4 \pm 1.18$	$0.733 \pm 0.961$	$3.85 \pm 3.78$	4±3.83	0.64
Routine behavior	$2.27 \pm 1.79$	$1.67 \pm 1.54$	$3.92 \pm 2.9$	$3.54 \pm 3.45$	0.03
Restricted behavior	$1.73 \pm 1.91$	$0.933 \pm 1.1$	$3 \pm 1.87$	$3.62 \pm 1.71$	5.71*

**Table 2**. Pre- and post-test RBS-R scores of both groups ( $M \pm SD$ ). \* denotes a significant interaction effect between time and group, with *P* < 0.05; the F value represents the interaction between time and group.

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Network 1 (kurtosis = 9.773, skewness = 1.764, variability = 0.037, and frequency = 0.008) included the following region of interest: Left supramarginal gyrus (SMG.L) showed increased functional connectivity with Left anterior cingulate cortex (ACG.L) [t(26) = 3.32, p = 0.003]; Right thalamus (THA.R) exhibited increased functional connectivity with Left supramarginal gyrus (SMG.L) [t(26) = 3.47, p = 0.002]; Right thalamus (THA.R) displayed decreased functional connectivity with Right middle frontal gyrus (MFG.R) [t(26) = -3.14, p = 0.004]; Left thalamus (THA.L) showed increased functional connectivity with Right middle frontal gyrus (MFG.R) [t(26) = -3.14, p = 0.004]; Left thalamus (THA.L) showed increased functional connectivity with Right supramarginal gyrus (SMG.R) [t(26) = 2.88, p = 0.008]; Left fusiform gyrus (FFG.L) displayed decreased functional connectivity with Right inferior parietal lobule (IPL.R) [t(26) = -2.98, p = 0.006]; Left thalamus (THA.L) exhibited increased functional connectivity with Right middle frontal gyrus (MFG.R) [t(26) = 2.82, p = 0.009], and Right fusiform gyrus (FFG.R) showed decreased functional connectivity with Left angular gyrus (ANG.L) [t(26) = -2.91, p = 0.007]. Network 1 findings are depicted in Fig. 3 above.

Network 2 (kurtosis = 5.249, skewness = 0.356, variability = 0.030, and frequency = 0.008) encompassed the following region of interest: Right fusiform gyrus (FFG.R) showed increased functional connectivity with Right calcarine cortex (CAL.R) [t(26) = 3.57, p = 0.001]; Right superior frontal gyrus (SFGdor.R) exhibited decreased functional connectivity with Right middle cingulate cortex (DCG.R) [t(26) = -3.29, p = 0.003]. Network 2 findings are depicted in Fig. 3 below.

### Correlation between brain regions and repetitive behavior performance

Pearson correlation analysis was used to explore the relationship between alterations in dynamic functional connectivity within Networks 1 and 2 (posttest-pretest) and shifts in behavioral performance (posttest-pretest) concerning the total score of the RBS-R and its two sub-dimensions (self-injurious and restrictive behaviors), among preschool children with ASD following participation in the MBTP. There were no significant differences between the exercise group and control group in the pretest of the RBS-R scale, indicating their homogeneity.

The results revealed that the change in the total score of RBS-R was negatively correlated with the change in the dynamic connection between Left supramarginal gyrus (SMG.L) and Left anterior cingulate cortex (ACG.L) in Network 1 (rs = -0.517, p = 0.005), the change in self-injurious behavior score was negatively correlated with the change in dynamic connectivity of Left supramarginal gyrus (SMG.L) and Left anterior cingulate cortex (ACG.L) in Network 1 (rs = -0.497, p = 0.001) and the change in the score of restricted behavior was negatively correlated with the change in the dynamic connection between Right thalamus (THA.R) and Left supramarginal gyrus (SMG.L) in Network 1 (rs = -0.685, p < 0.001). In Network 2, the change in the score of restricted behavior was negatively correlated with the change in dynamic connectivity of Right fusiform gyrus (FFG.R) and Right calcarine cortex (CAL.R) (rs = -0.578, p = 0.001).

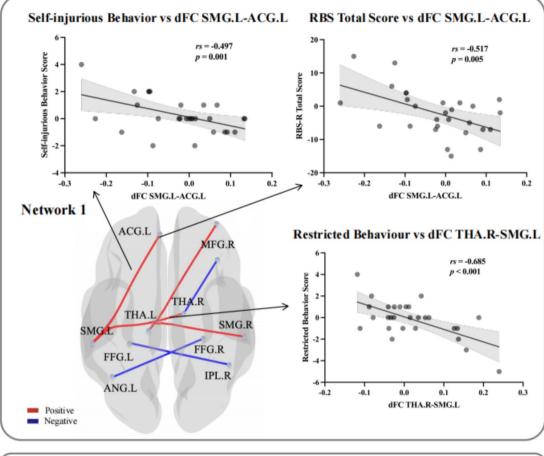
### Discussion

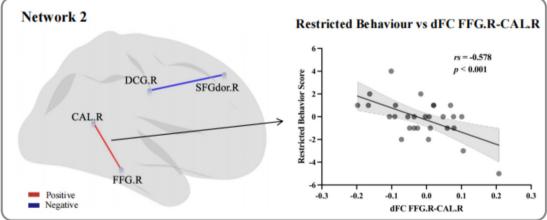
Herein, we investigated the dynamic connectivity mechanisms underlying the therapeutic effects of MBTP on restricted and repetitive behaviors in children with ASD. A cluster randomized controlled trial was used, focusing on the SN, DMN, and CEN, three networks that are extensively involved in cognitive control and considered to be central to the symptomatology of individuals with ASD<sup>11</sup>. As expected, the MBTP effectively improved repetitive behavioral performance and corrected abnormalities in the dynamic connectivity of the SN, DMN, and CEN triple network. Importantly, these results remained robust even after accounting for potential confounding factors (BMI, CSHQ, CEBQ, and CARS). Our findings suggest that MBTP improves repetitive stereotypic behaviors and abnormal dynamic connectivity in the three core networks in children with ASD. Furthermore, this study unveils the neural mechanisms through which exercise intervention influences improvements in repetitive stereotypic behaviors, emphasizing the role of dynamic connectivity.

### MBTP improves repetitive behavior performance in children with ASD

Our primary focus was to investigate whether MBTP could lead to improvements in repetitive stereotypic behaviors among children with ASD<sup>7</sup>. Our results demonstrated that MBTP effectively reduced both total scores related to repetitive stereotypic behaviors and scores within the restricted interest sub-dimension for children with ASD. Additionally, MBTP successfully mitigated the increase in self-injurious behavior scores, indicative of an overall improvement in behavioral performance. Importantly, the MBTP not only improved repetitive

## Triple Network Dynamic Connectivity Changes after MBTP Exercise Group < Controls Group





**Fig. 3**. Changes in Triple Network Dynamic Connectivity of Network 1 and Network 2 Between the Exercise Group and Control Group. Correlations with RBS-R Total and Sub-dimensions Score (Self-injurious Behavior and Restrictive Behavior) are Presented in the Figure.

stereotypic behaviors but also broadened the scope of interests and attentional focus for children with ASD. It also played a crucial role in restraining behaviors such as self-directed aggression and self-harm.

Consistent with our previous research, the present study replicated the beneficial effects of mini-basketball training on RBS-R total scores, self-injurious behavior, and restricted behavior<sup>7</sup>. These findings are supported by previous research, which indicates that children with ASD exhibit RBS while seeking sensory stimulation, often

accompanied by self-injurious and stereotyped behaviors<sup>49</sup>. Physical exercise, which requires intensive cognitive engagement and features regularity, entertainment, and feasibility, can exert positive effects on RBS. Moreover, the sensory stimulation provided by physical activities resembles the self-stimulatory nature of RBS, potentially fulfilling the self-stimulatory needs of individuals with ASD<sup>5–7</sup>.

Ball sports encompass multiple elements including equipment interaction, peer communication, and environmental adaptation, providing more comprehensive stimulation for addressing RBS. MBTP incorporates basic basketball skills such as throwing, touching, dribbling, and passing, along with sports games and physical fitness exercises as fundamental training components. The technical movements in MBTP share similarities with certain repetitive behaviors, while common self-injurious behaviors in ASD manifest as head-banging against walls or floors, self-hitting, hand-biting or arm-biting, and self-injury with sharp objects<sup>50</sup>.

In MBTP, the soft mini-basketball serves as an interactive medium, continuously stimulating hand-eye sensory systems through throwing, touching, dribbling, and passing activities, thereby fulfilling certain self-stimulatory needs and alleviating sensory overload<sup>51</sup>. Additionally, MBTP encompasses rich peer interaction and environmental engagement through sports games and physical exercises, which positively impact restricted behavior<sup>52,53</sup>.

### MBTP improves abnormalities in triple network dynamic connectivity in children with ASD

Next, we sought to ascertain whether MBTP could rectify abnormalities in dynamic connectivity within the triple network among children with ASD. We identified substantial alterations in dynamic connectivity occurring within the SN, DMN, and CEN among children with ASD in the exercise group compared to those in the control group. Within the context of the triple network model, we observed widespread changes in dynamic connectivity patterns within Network 1 and Network 2.

These findings align with existing research suggesting that physical exercise can modify brain functional networks in children with ASD. Current evidence indicates that physical exercise can induce functional connectivity changes in various brain networks, including the default mode network<sup>54</sup>, central executive network<sup>31</sup>, and salience network<sup>55</sup>, which our study has similarly demonstrated. However, existing evidence has been limited to static changes in localized brain regions, failing to capture dynamic alterations. The brain functions as a dynamically collaborative system, with various higher cognitive functions during exercise involving dynamic brain cooperation<sup>26–29</sup>.

This study is the first to demonstrate that MBTP can modify dynamic connectivity within the triple network in children with ASD. We hypothesize that MBTP's exercises requiring proprioceptive coordination control (such as head-around ball handling and behind-the-back catches) and cooperative sports games (like dribbling and passing) may enhance cognitive control abilities in ASD individuals, promoting attention switching and facilitating dynamic collaboration among triple networks.

Furthermore, physical exercise promotes neuroplasticity in children and adolescents. The moderate-intensity physical training included in MBTP can facilitate structural and functional changes in the ASD brain<sup>10</sup>.

In conclusion, this study provides novel evidence that MBTP can effectively modulate dynamic connectivity patterns within the triple network system in children with ASD. These findings extend beyond previous static connectivity observations, highlighting the potential of structured exercise interventions in promoting neural plasticity and improving brain network dynamics. The combination of coordinated exercises and interactive sports activities in MBTP appears to create a comprehensive framework for enhancing both cognitive control and neural network integration in children with ASD.

### Triple network dynamic connectivity mechanism of MBTP to improve repetitive behaviors

Our findings contribute to the development of a theoretical model encompassing the triple network and its role in understanding repetitive stereotypic behavior in children with ASD, as well as how sports can ameliorate these behaviors. Within the triple network model, SN plays a crucial role in attentional shifting and cognitive control, regulating both the CEN and the DMN in the management of external attention and internal mental processes. Notably, dynamic connectivity abnormalities, as elucidated by the triple network model, are closely linked to repetitive stereotypic behavior in children with ASD<sup>11</sup>. Our findings suggest that alterations in the dynamic connectivity of the triple network could serve as a mechanism through which sports lead to an improvement in repetitive stereotypic behavior in children with ASD.

Within the context of the triple network model, we observed widespread changes in dynamic connectivity patterns within Network 1. These shifts encompassed augmented dynamic connectivity within the SN, coupled with diminished dynamic connectivity within the CEN. Furthermore, we detected an increase in dynamic connectivity between nodes of the SN and nodes of the DMN, along with weakened dynamic connectivity to nodes within the CEN. Concurrently, we noted a decrease in dynamic connectivity between nodes of the DMN and nodes of the CEN. These outcomes strongly suggest that our implementation of MBTP led to extensive dynamic connectivity modifications within the framework of the triple network model. In Network 2, we uncovered alterations in dynamic connectivity occurring between nodes within the DMN. These changes serve as a foundation for further exploration into the dynamic interactions spanning various networks.

Specifically, within Network 1, we detected enhanced dynamic connectivity in the SN, particularly between the right thalamus (THA.R) and left supramarginal gyrus (SMG.L). This strengthened connectivity demonstrates a negative correlation with restricted interest scores. These alterations appear to compensate for differences observed between children with ASD and typically developing children, correlating with improvements in repetitive stereotypic behaviors<sup>56–59</sup>. Current evidence suggests that THA.R plays a crucial role in perceptual attention integration, while SMG.L is involved in language and emotional processing<sup>60,61</sup>.

Moreover, in Network 2, we identified enhanced dynamic connectivity between the right fusiform gyrus (FFG.R) and right calcarine cortex (CAL.R) within the DMN. Our results indicate that this enhanced dynamic

connectivity correlates with decreased restricted behavior scores. These findings align with previous research suggesting compensation mechanisms for ASD<sup>59,62</sup>. FFG.R is known to be engaged in visual information processing and face recognition, while CAL.R plays a critical role in regulating emotional responses and environmental adaptation<sup>63</sup>.

These findings suggest that MBTP's impact on restricted interests operates through multiple pathways, potentially producing beneficial effects through various mechanisms. Additionally, we observed that decreased dynamic connectivity between SMG.L and anterior cingulate gyrus (ACG.L) in Network 1 correlates with improvements in RBS-R total scores and self-injurious behavior. SMG.L is associated with emotional and language processing, while ACG.L is linked to primary sensory and motor functions<sup>64</sup>, showing negative correlations with RBS scores<sup>65</sup>. This research supports our findings, notably that MBTP effectively reduces RRBs and their self-injurious behavior subdimension. Using soft mini-basketballs as a medium, our study designed diverse sensory stimulations to meet ASD individuals' self-stimulatory needs, with the mechanism potentially involving deactivation of SMG.L-ACG.L dynamic connectivity.

All observed negative correlations in this study suggest a potential compensatory mechanism where decreased connectivity reduces these maladaptive processes, thereby facilitating behavioral improvement. The design of MBTP follows the multi-pathway theory of sports<sup>53</sup>, which includes scenario interaction and mental state pathways. At the scenario interaction level, MBTP creates a rich environment with diverse interactions involving people and objects, fostering engagement with teachers and parents and incorporating a variety of teaching formats and content. At the mental state level, MBTP revolves around the concept of enjoyable sports and incorporates rich and engaging structured sports games.

Taken together, our observations suggest that dynamic connectivity changes, as explained by the triple network model, may serve as the underlying mechanism by which MBTP effectively improves restricted behaviors in children with ASD. Moreover, our findings may suggest that the MBTP not only modulates the strength of network connectivity but also promotes a more balanced dynamic interaction between networks. This hypothesis aligns with the broader notion that optimal cognitive functioning depends on flexible and context-appropriate network dynamics, rather than consistently high or low connectivity levels<sup>66</sup>.

### Limitation

In the present study, we investigated the effects of MBTP on repetitive stereotypic behaviors and dynamic connectivity within the triple network among children with ASD. However, several limitations should be acknowledged. Firstly, the small sample size of this study was primarily due to the low completion rate of MRI scans during the pre-test and post-test, a common challenge in the field of ASD brain imaging<sup>67,68</sup>. Secondly, our study exclusively included male subjects to avoid gender-related heterogeneity. Future research should encompass larger and more diverse samples to explore potential gender-based differences in the effects of physical activity on individuals with ASD<sup>21,69</sup>.

Thirdly, most participants in this study were individuals with severe symptoms of ASD, and the lack of intelligence and language information limited the generalizability of our findings. Future research should include comprehensive assessments of these impairments to better understand their impact on interventions like MBTP.

Fourth, despite the absence of statistical significance in pre-test homogeneity checks, the small sample size and considerable standard deviations between groups limited the interpretation of our results. Future studies should employ larger sample sizes and ensure consistency between samples to enhance generalizability.

Lastly, while our study concentrated on dynamic connectivity changes within various pairs of regions of interest, future research should delve into whether network-level dynamic alterations, offer further evidence of MBTP's effectiveness in enhancing repetitive stereotypic behavioral performance<sup>29</sup>.

**Ethics Approval and Consent to Participate** The study protocol received approval from the Ethics and Human Protection Committee of The Affiliated Hospital of Yangzhou University and was registered in the Chinese Clinical Trial Registry on 18/12/2018 (first registered on 18/12/2018, registration number: ChiCTR1900024973). Written informed consent was obtained from the parents or legal guardians of the study participants prior to their participation in the study. All methods were performed in accordance with the relevant guidelines and regulations. For research involving human participants, the study was conducted in accordance with the Declaration of Helsinki. Identifying information has been removed to ensure privacy.

### Conclusion

Our results demonstrate the efficacy of a 12-week MBTP in improving repetitive stereotypic behaviors and inducing dynamic connectivity changes within the triple network in children with ASD. Our results shed new light on the underlying brain mechanisms through which MBTP ameliorates repetitive stereotypic behaviors in this population. Specifically, we observed enhancements in total repetitive stereotypic behavior scores, as well as improvements in two sub-dimensions related to restricted interests and self-injurious behavior in children with ASD. These positive outcomes were associated with increased dynamic connectivity within the SN and the DMN. In summary, our study addresses a critical gap in understanding the dynamic changes within functional networks resulting from motor interventions aimed at improving repetitive stereotypic behaviors in children with ASD.

### Data availability

The anonymized dataset used for analysis will be made available from the corresponding author upon reasonable request.

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### Author contributions

Conceptualization and original idea generation were primarily carried out by Weike Zhang and Aiguo Chen. The methodology for this research was developed collaboratively by Weike Zhang. Software tools were employed and curated by Lina Zhu, Kelong Cai, and Xuan Xiong. Data curation was overseen by Sixin Yang and Wei Cheng. The initial draft of the manuscript was prepared by Weike Zhang, and the subsequent review and editing stages involved the contributions of Lina Zhu, Zhiyuan Sun, Xuan Xiong, Kelong Cai, and Aiguo Chen. Visualization elements, including figures and charts, were created by Weike Zhang Haiyong Mao, Zhiyuan Sun, and Aiguo Chen. The project was supervised by Lina Zhu, Kelong Cai, and Xuan Xiong. Project administration responsibilities were managed by Kelong Cai. Funding for the research was acquired by Aiguo Chen. All authors have reviewed and agreed upon the final version of the manuscript.

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### Declarations

### **Competing interests**

The authors declare no competing interests.

### Additional information

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