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A Comprehensive and Broad Approach to Resting-State Functional Connectivity in Adult Patients with Mild Traumatic Brain Injury

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ABSTRACT

BACKGROUND AND PURPOSE: Several recent works using resting-state fMRI suggest possible alterations of resting-state functional connectivity after mild traumatic brain injury. However, the literature is plagued by various analysis approaches and small study cohorts, resulting in an inconsistent array of reported findings. In this study, we aimed to investigate differences in whole-brain resting-state functional connectivity between adult patients with mild traumatic brain injury within 1 month of injury and healthy control subjects using several comprehensive resting-state functional connectivity measurement methods and analyses.

MATERIALS AND METHODS: A total of 123 subjects (72 patients with mild traumatic brain injury and 51 healthy controls) were included. A standard fMRI preprocessing pipeline was used. ROI/seed-based analyses were conducted using 4 standard brain parcellation methods, and the independent component analysis method was applied to measure resting-state functional connectivity. The fractional amplitude of low-frequency fluctuations was also measured. Group comparisons were performed on all measurements with appropriate whole-brain multilevel statistical analysis and correction.

RESULTS: There were no significant differences in age, sex, education, and hand preference between groups as well as no significant correlation between all measurements and these potential confounders. We found that each resting-state functional connectivity measurement revealed various regions or connections that were different between groups. However, after we corrected for multiple comparisons, the results showed no statistically significant differences between groups in terms of resting-state functional connectivity across methods and analyses.

CONCLUSIONS: Although previous studies point to multiple regions and networks as possible mild traumatic brain injury biomarkers, this study shows that the effect of mild injury on brain resting-state functional connectivity has not survived after rigorous statistical correction. A further study using subject-level connectivity analyses may be necessary due to both subtle and variable effects of mild traumatic brain injury on brain functional connectivity across individuals.

ABBREVIATIONS: BOLD = blood oxygen level–dependent; fALFF = fractional amplitude of low-frequency fluctuations; FDR = false discovery rate; ICA = independent component analysis; IFCN = intrinsic functional connectivity networks; mTBI = mild traumatic brain injury; NBS = network-based statistics; rs-FC = resting-state functional connectivity; rs-fMRI = resting-state fMRI; SCAT3 = Sport Concussion Assessment Tool, 3rd edition; Spatial-GICs = Spatial Group Independent Components; Temporal-GICs = Temporal Group Independent Components; TFCE = threshold-free cluster enhancement

Traumatic brain injury is a significant cause of death and disability worldwide,[1] with up to 50–60 million new cases a year globally[2] and 2.5 million in the United States.[3] Most of these cases are caused by mild head impacts, known as mild TBI (mTBI) or concussion.[4] According to the committee of the Head Injury Interdisciplinary Special Interest Group of American Congress of Rehabilitation Medicine, a person with an mTBI is identified by a trauma-induced disruption of brain function, evidenced by ≥1 of the following: a period of loss of consciousness, memory loss for events around the accident, altered mental state at the time of the accident (like feeling dazed or confused), and possible focal neurologic deficits. However, this condition is considered mild if the

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loss of consciousness is <30 minutes, the initial Glasgow Coma Scale score is between 13 and 15, and posttraumatic amnesia lasts no more than 24 hours. This definition encompasses situations such as the head being struck, the head hitting an object, and the brain experiencing whiplash-like acceleration/deceleration movements, even when there is no direct external trauma to the head.[5] Annually, >1 million emergency department visits in the United States are related to mTBI,[6] resulting in a more than $22.5 billion economic burden on the health care system and society.[7] Moreover, up to 15% of patients have prolonged postconcussive symptoms,[8] which include a broad range of somatic, behavioral, and emotional issues[9] and can significantly impact the quality of life.[10] Attempting to unpack the underlying pathophysiology of mTBI remains central to a better understanding of this injury. Specifically, relating structural injuries to functional deficits remains a challenge. It is known that WM injury can occur after mTBI;[11] thus, fMRI has naturally been used to investigate potential related disruption of coordinated neural activity.[12]

Several recent works using resting-state fMRI (rs-fMRI) suggest that there may be some alterations of resting-state functional connectivity (rs-FC) after mTBI. However, a variety of differing rs-fMRI analysis approaches, differences across the patient population, and small study cohorts add variance to results and contribute to a broad and somewhat confusing array of reported findings that range across many different functional networks and regions. For example, using a seed-based method, Mayer et al[13] reported a decreased rs-FC within the default mode network and increased connectivity between the default mode network and lateral prefrontal cortex in mTBI participants (mTBI, 27; control, 26; mean time since injury = 11.5 days). However, their later study showed no significant differences using an independent component analysis (ICA) approach in a larger cohort (mTBI, 51; controls, 51; mean time since injury = 14 days).[14] Similarly, Amir et al[15] found decreased connectivity between the right lateral parietal and precuneus region of the default mode network using a seed-to-voxel method, while their ROI-to-ROI analysis failed to reveal significant group differences in the default mode, task-positive, or salience network (mTBI, 27; controls, 26; mean time since injury = 3 months). Many different regions (eg, dorsolateral prefrontal cortex, hippocampus, precuneus, thalamus) and different functional networks (eg, default mode, dorsal attention, frontoparietal, salience, visual) have all been implicated previously.[16-24] Many individual studies have limited cohort sizes (sometimes as few as 13 subjects). In addition, the characteristics of the cohorts vary (eg, time since injury, civilian versus sport-related) as well as the steps and parameters used in data preprocessing, and the methods to measure rs-FC vary.

In this study, an unbiased, broad, and comprehensive approach was used to investigate rs-FC changes for individuals with mTBI compared to healthy normals using a sizable prospective study cohort, refraining from any a priori assumptions, and using rigorous, multilevel statistical analyses. Standard fMRI preprocessing pipelines were performed using 3 popular analysis approaches, including ROI/seed-based analyses, ICA, and fractional amplitude of low-frequency fluctuations (fALFF). These techniques are among prominent choices in the field, each with its unique interpretation and limitations, yet they offer complementary perspectives in rs-FC analysis.[25] Our aim was to integrate these methods in a singular study, providing a benchmark for evaluating both past and forthcoming research, with a focus on uncovering consistent findings and reliable rs-fMRI biomarkers through a comparative approach.

MATERIALS AND METHODS

Study Population

This prospective study was approved by our institutional review board at New York University Langone Health, and subjects provided written, informed consent before participation. Adult subjects (18–65 years of age) within 1 month of documented mTBI by the American Congress of Rehabilitation Medicine criteria[26] were included in this cohort. Participants with a history of prior TBI or other neurologic disorders, a history of participation in organized contact sports, and imaging contraindications were excluded. The cohort included 82 individuals with mTBI and 53 age- and sex-matched healthy controls. Five with mTBI did not complete imaging, and 7 other subjects (5 with mTBI and 2 controls) had fMRI quality issues. A total of 72 individuals with mTBI (mean age, 30 [SD, 11.66] years, time since injury = 18 [SD, 7.7] days; 47 women) and 51 controls (31 [SD, 12.09] years; 30 women) were finally included in this study (Table and Online Supplemental Data). Age, sex, educational background (as measured by years of education beyond high school), and time since injury for the mTBI group were included in a generalized linear model to investigate any correlation between measurements and these covariates. This investigation was conducted separately for each covariate and then combined as independent variables. Fifty-one patients with mTBI completed the Sport Concussion Assessment Tool, 3rd edition (SCAT3) questionnaire[27] to measure symptom severity. The number of symptoms (total of 22 symptoms) and a total symptom severity score (maximum possible score, 132)[28] were collected (Table). Individuals with mTBI who had SCAT3 symptom severity scores exceeding 39 (the median score) were categorized as in the more-severe subgroup, resulting in 26 subjects being identified as high SCAT3 subjects.

MR Imaging Acquisition

Imaging was performed on 3T MR imaging scanners (Magnetom Skyra/Prisma; Siemens, 60/63 subjects) using a 64-channel head coil. Closed-eye resting-state blood oxygen level–dependent (BOLD) images were acquired using an EPI sequence with the following imaging parameters: FOV = 220 × 220 mm, matrix = 74 × 74 × 38, in-plane resolution = 3 × 3 mm^2, slice thickness = 3 mm, TR/TE = 2000/25 ms, number of volumes = 153 (5.1 minutes), flip angle = 70°, generalized autocalibrating partially parallel acquisition factor = 2, bandwidth/pixel = 1826 Hz. For coregistration and segmentation, the MPRAGE sequence (TR/TE = 2100/3.19 ms, flip angle = 8°, image resolution = 1 × 1 × 1 mm^3, matrix = 256 × 256 × 192) was also performed.

Image-Preprocessing Pipeline

We used a standard preprocessing pipeline implemented in the CONN toolbox (www.nitrc.org/projects/conn, Version 21.a).[29] Specifically, the pipeline included the following: motion correction using rigid registration on the first volume, slice-time

Demographics and injury characteristics of studied participants

Variables	mTBI	Control	Statistics
Initial recruitment (No.)	82	53	NA
Excluded (No.)			
Incomplete session	5	0	NA
Image quality issues	5	2	NA
Final inclusion (No.)	72	51	NA
Age (range) (mean) (yr)	18–65 (30 [SD, 11.66])	19–65 (31 [SD, 12.09])	$t = -0.82$, $P = .41$
Sex, M/F	25:47	21:30	$\chi^2 = 0.017$, $P = .89$
Education (range) (mean) (yr)	11–20 (16.08 [SD, 1.67])	12–20 (16.46 [SD, 1.81])	$t = -1.10$, $P = .27$
Hand preference, right (No.) (%)	44 (86.27%)	51 (70.33%)	$\chi^2 = 0.017$, $P = .89$
Cause of injury (No.) (%)			
Fall	21 (29.16%)	NA	NA
Hit by object	27 (37.5%)	NA	NA
Motor-vehicle accident	9 (12.5%)	NA	NA
Assault	4 (5.56%)	NA	NA
Sport-related	4 (5.56%)	NA	NA
Other	7 (9.72%)	NA	NA
Time since injury (range) (mean) (day)	3–31 (18 [SD, 7.7])	NA	NA
SCAT3			
No. of symptoms (range) (mean)	0–22 (14.25 [SD, 6.30])	NA	NA
Symptom severity score (range) (mean)	0–103 (43.53 [SD, 29.98])	NA	NA

Note:—NA indicates not applicable.

correction using the Siemens interleaved slice-timing pattern, unwrapping using a unified segmentation and normalization procedure to register the functional scan on the standard space (Montreal Neurological Institute 152_2mm),[30] and spatial smoothing with an 8-mm Gaussian full width at half maximum kernel. Outlier frames were identified and scrubbed using motion parameters (in-plane motion of >0.5 mm) and global mean intensity (SD, >3). Structural scans were also normalized and segmented into WM, GM, and CSF using direct nonlinear registration to the atlas (Montreal Neurological Institute 152_1 mm). The anatomic component correction denoising method[31] was applied to decrease the effect of biologic noise. The hyperparameters (the number of principal components) were set to maintain a global mean BOLD signal of zero and a voxel-to-voxel correlation distribution with a zero mean. The motion-related, scrubbing, and rest-confounding regressors were also included in the anatomic component correction method. Then, a temporal bandpass filter with a frequency range of 0.008 to 0.09 Hz, linear detrending, and despiking were used to remove the remaining potential confounders.

Functional Connectivity Measures

Functional connectivity, characterized by synchronized low-frequency fluctuations of the BOLD signal across distinct brain regions, was measured to investigate group differences in rs-FC using 2 methods: 1) a model-based approach to measure the correlation of the signal between ROIs or seeds, and 2) a data-driven method, ICA, which eliminates the need for defining ROIs. These methods were selected on the basis of their general popularity, the established application of these techniques in mTBI rs-FC studies, and the complementary perspectives on rs-FC.

ROI/Seed-Based Connectivity. In this study, we used a total of 4 popular parcellation methods: 1) 8 functional networks (32 ROIs, available in the CONN toolbox), 2) 7 functional networks (7 ROIs),[32] 3) 14 functional networks (264 seeds),[33] and 4) 22 functional networks (132 anatomic ROIs, available in the CONN toolbox). The first and second parcellations are created on the basis of rs-fMRI data (using ICA and surface-level clustering methods, respectively). The third was based on a combination of task-mediated and rs-fMRI data, and the last was based on an anatomic atlas using a combination of supratentorial cortical and subcortical ROIs from the Harvard-Oxford atlas[34] and cerebellar parcellation from the automated anatomic labelling atlas[35] (Online Supplemental Data). The selection of ROI/seed locations influences the ROI/seed-based connectivity analysis.[25] Therefore, we opted for these specific parcellations to gain a comprehensive understanding and enable comparison with results from other studies.

Temporal Pearson correlation coefficients between each pair of ROIs were calculated as the rs-FC measure. Fisher z transformation was applied to account for the normality assumption in statistical analysis methods. Accordingly, corresponding connectivity matrices were generated for both groups. The Pearson correlation coefficient between group-level connectivity matrices was calculated as a measure of similarity. We applied 2 group-level comparisons: 1) a binary comparison between significantly connected ROIs uniquely present in mTBI and control groups as well as those present in both groups or neither group, and 2) correlation coefficient distributions examined over all connections to investigate differences in the amplitude of connectivity between groups.

We also calculated ROI-to-voxel correlations, which measured the connectivity between the ROI and all voxels in the brain. The outcome was a whole-brain correlational contrast map for each ROI. This method limits prior assumptions of connectivity among specific brain regions.

The impact of spatial smoothing on global and regional rs-FC measurements has been debated.[36] To address this concern, similar ROI-based comparisons were conducted using unsmoothed data (before applying the Gaussian full width at half maximum kernel). Furthermore, assuming that an rs-FC alteration may be seen only in patients with more severe symptoms, subsequent analyses were

conducted solely between the high SCAT3 mTBI subset and the control group.

Group ICA. A group ICA was applied, avoiding all a priori assumptions dependent on the selection of ROIs. The parameters were set to the default generally used in many ICA rs-FC studies including G1/tanh Fast ICA and group ICA 3 back-projection methods, dimensionality reduction of 64, and 40 total independent components. The correlational spatial match technique was used, and major intrinsic functional connectivity networks (IFCN) were identified according to the standard functional parcellation used in the ROI/seed-based method. For group-level analysis, subject-level activation maps, known as the β map for each spatial component, were compiled to extract statistically significant differences between groups (spatial-GICs analysis). Additionally, subject-level temporal components were cross-correlated to create a connectivity matrix between IFCNs (temporal-GICs analysis), and similar connectivity matrices, such as the ROI/seed-based method, were generated.

Fractional Amplitude of Low-Frequency Fluctuations. Voxelwise fractional amplitude of low-frequency fluctuations (fALFF)[37] was used to calculate the power ratio of band-passed (range, 0.008–0.09 Hz) frequencies to the full BOLD signal frequency spectrum (range, 0–0.25 Hz). Groups were compared with respect to BOLD power ratios to identify any potential ROIs of interest.

Statistical Analysis

Here, we strove for a high level of statistical rigor. First, we applied the Shapiro-Wilk test to assess the normality assumption over the group-level distribution of correlation coefficients ($P > .05$). Following this step, significantly connected ROIs/seeds/temporal-GICs within groups was determined using a 1-sample t test or Wilcoxon signed-rank test, depending on whether the normality assumption held for that specific connection. We then performed between-group comparisons using the Welch t test if both the mTBI and control groups' correlation coefficients were found to be normally distributed; otherwise, the Mann-Whitney U test was used. The significance level was set at $P < .05$ for both within-group and between-group comparisons; then, per each analysis, we corrected P values ($p_{corrected}$) for false discoveries using the Benjamini-Hochberg false discovery rate correction (FDR, $\alpha = 5\%$) method of multiple comparisons at the whole-brain level, rather than the ROI/network level. To account for the high number of hypothesis tests associated with the number of ROIs for a given parcellation, we used the method of network-based statistics[38] (NBS; https://www.nitrc.org/projects/nbs/) as an alternative to whole-brain multiple comparisons (t-statistic threshold = 1.6, with 10,000 permutations and a significance level of $P < .05$). A linear regression analysis was performed to investigate the relationship between symptom-severity scores within the mTBI group and the connectivity amplitude, focusing on connections identified as different before multiple comparison correction. For between-group comparisons on spatial contrasts such as the outcomes of ROI-to-voxel, spatial-GICs, and fALFF, the nonparametric method of threshold-free cluster enhancement (TFCE)[39] was applied to correct cluster size in addition to the FDR correction. Due to many contrasts, the TFCE was used on between-source contrasts using any effect test (F-test). The power analysis for this study revealed that with the sample size used, it is feasible to achieve a statistical power of 80% at an .05 significance level, assuming an approximate effect size of 0.5 before multiple comparison correction. The statistical analyses were performed by the CONN toolbox and in-house scripts written in Python (using SciPy [https://scipy.org/] and Statsmodels [https://pypi.org/project/statsmodels/] libraries).

RESULTS

Demographics

We found no differences between subject cohorts in terms of demographics: age, sex, educational background, and hand preference (Table). There were no associations determined between covariates and connectivity measures for both separated and combined schemes ($P_{corrected} > .05$).

ROI/Seed-Based Connectivity

The 1-sample t test (or Wilcoxon signed-rank test) for within-group analysis revealed 164 positively and 115 negatively correlated ROIs of 496 investigated connections in the control group ($P_{corrected} < .05$, Fig 1*A*), whereas 164 positively and 129 negatively connected ROIs were found in the mTBI group (Fig 1*B*). The Pearson correlation coefficient of connectivity matrices between controls and the mTBI groups (Fig 1*A*, -*B*) is 0.97 ($P < .001$). We identified 239 statistically significant connections common to the mTBI and control groups, 40 unique to the control group and 54 unique to the mTBI group (Fig 1*C*). While group comparisons of connectivity distributions revealed no statistically significant connections between pairs of ROIs after multiple comparison correction (Fig 1*E*), there were 19 connections between pairs of ROIs that showed hyperconnectivity (mTBI > control), and 20 showed hypoconnectivity (mTBI < control), with the cerebellar network being most affected (10 hyperconnected links to ROIs in visual, sensorimotor, and dorsal attention networks; and 6 hypoconnected links to ROIs in the language and frontoparietal networks) ($P_{uncorrected} < .05$; Fig 1*D*). Among the 39 identified connections, 3 specific pathways—linking the posterior cerebellum to the left visual lateral region, the anterior cerebellum to the left inferior frontal gyrus associated with language, and the anterior cerebellum to the left posterior superior temporal gyrus related to language—demonstrated significant predictive capability for the SCAT3 symptom-severity scores (Online Supplemental Data). Use of the NBS approach for multiple comparison correction did not yield a consistent network showing a statistically significant difference ($P > .05$). No significant difference in connectivity amplitude was found using the other 3 brain-parcellations after multiple comparison correction. The detailed results are summarized in the Online Supplemental Data. Similar results emerged without smoothing, albeit with fewer connected ROIs within each group due to, possibly, the increased noise effect (Online Supplemental Data).

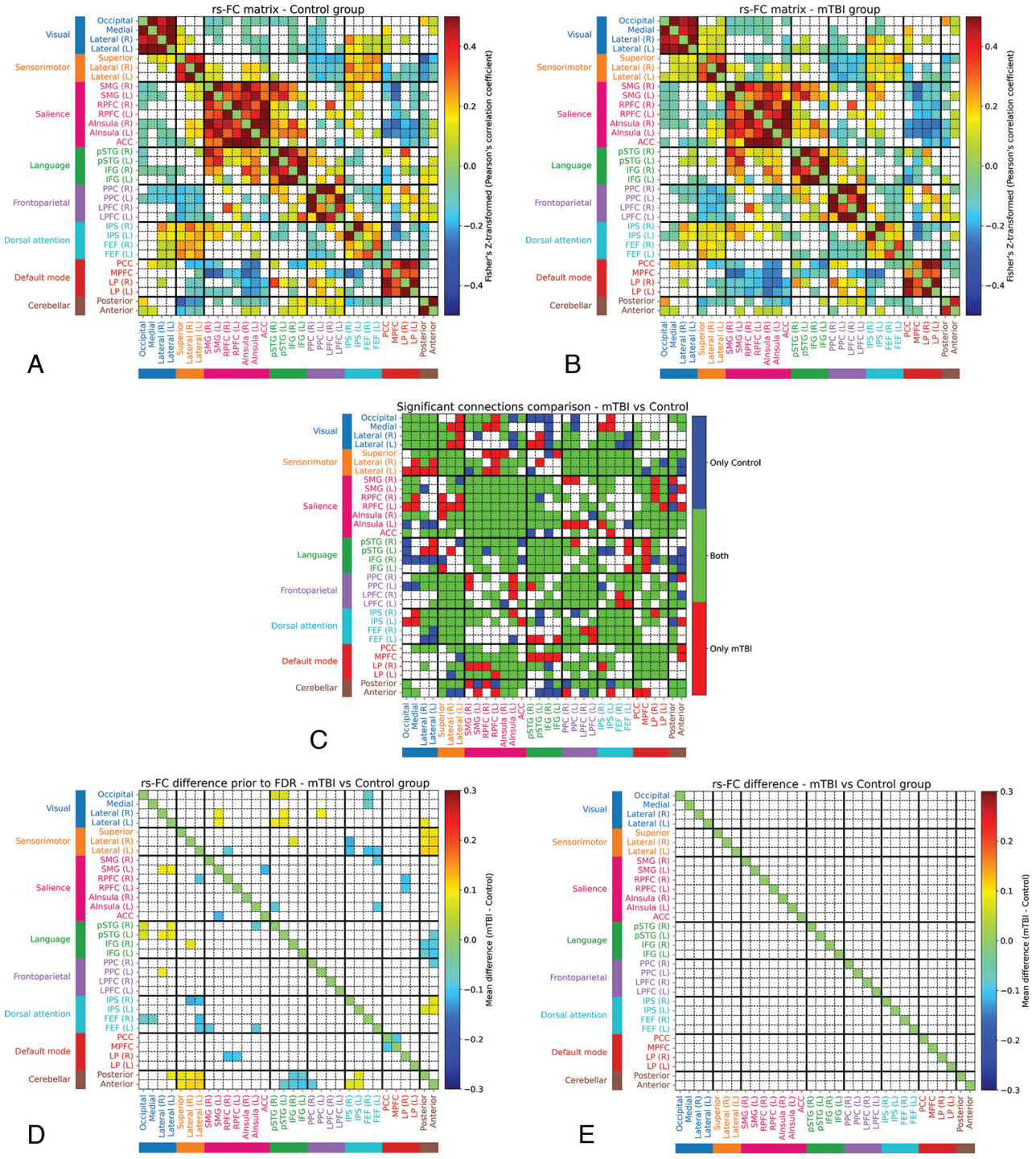

FIG 1. ROI-based analysis results using the CONN toolbox standard functional parcellation. *A* and *B*, The connectivity matrix for control and mTBI groups. The color for each pair of ROIs reflects statistically significant connectivity after FDR correction ($p_{corrected} < .05$) measured by the average Fisher *z*-transformation of the Pearson correlation coefficient. *C*, Matrix of significant connections, comparing *A* and *B*: Red indicates a significant connection unique to the mTBI group; blue indicates significant connections unique to the control group; and green indicates significance in both mTBI and control groups. *D*, Matrix of the mean difference (mTBI versus controls) for the connections that satisfy $P_{uncorrected} < .05$ using the Welch *t* test when comparing mTBI with controls. Highlighted connections involve all networks and nearly all ROIs, and the magnitude of differences is small. *E*, After multiple comparison correction (FDR) to account for the number of connections examined, no difference remains significant. Note that diagonal values (self-connection) are set to zero. A full list of ROIs is provided in the Online Supplemental Data. Results from other parcellation methods yield analogous results, whose matrices are presented in the Online Supplemental Data. L indicates left; R, right; SMG, Supramarginal gyrus; RPFC, Rostral prefrontal cortex; pSTG, Posterior superior temporal gyrus; MPFC, Medial prefrontal cortex; IFG, Inferior frontal gyrus; LP, Lateral parietal; PPC, Posterior cingulate cortex; LPFC, Lateral prefrontal cortex; FEF, Frontal eye fields; IPS, Intraparietal sulcus.

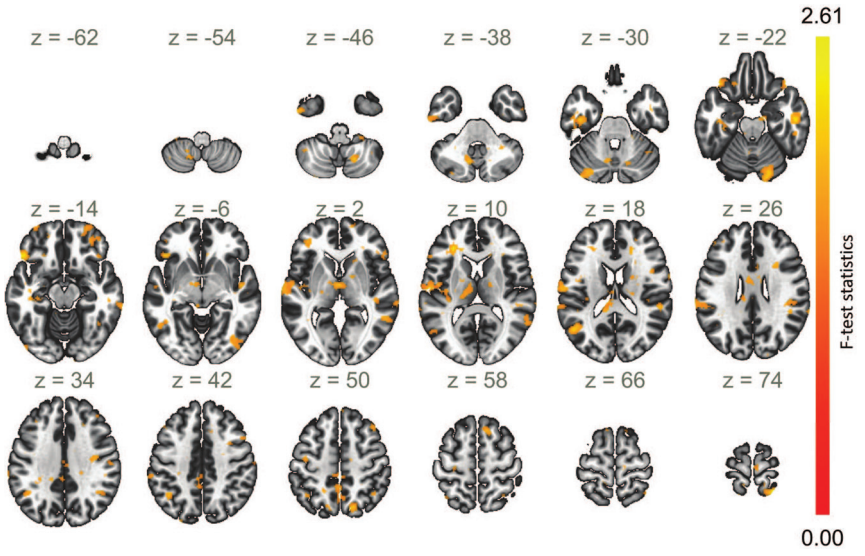

FIG 2. ROI-to-voxel results showing voxels with $P_{uncorrected}$ <.05 based on any effect *F*-test statistics. All 32 ROIs from the standard functional parcellation of CONN are included here to highlight possible areas of connectivity difference between mTBI and control groups. The highlighted voxels are seen to be scattered across the brain, and no cluster survives after FDR and TFCE correction.

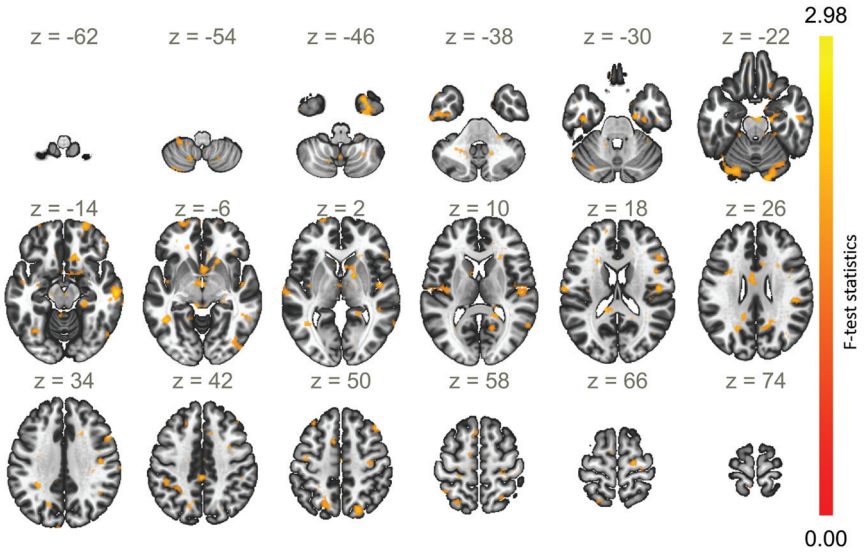

FIG 3. Similar to ROI-to-voxel analysis, this figure illustrates voxels with $P_{uncorrected}$ <.05 according to any effect *F*-test on all 20 spatial-GICs. As in the analysis in Fig 2, no regions survive FDR and TFCE.

In the high SCAT3 mTBI subset, neither whole-brain analyses nor NBS multiple comparison procedures were able to identify statistically significant variations in connection or a consistent network. Before multiple comparisons, 27 connections (17 hyperconnectivity and 10 hypoconnectivity) scattered in all 8 functional networks were observed, while the only within-network difference was between the cerebellum anterior and the posterior ROIs (Online Supplemental Data).

Figure 2 shows the result of any effect (*F*-test) among sources for all 32 ROI-to-voxel contrast maps. The 3 largest clusters were found in the cerebellum, left temporal lobe, and left angular gyrus. Nonetheless, no cluster remained significant after applying the FDR and TFCE. Because spatial smoothing reduces the sensitivity of TFCE,[39] the analysis was also executed on unsmoothed data, showing no statistically significant clusters of difference (differences before correcting for multiple comparison are depicted in the Online Supplemental Data).

Group ICA

Forty spatially independent components were produced from which we identified 20 components (spatial-GICs) to represent 9 IFCNs: auditory (1 component), cerebellar (2), default mode (2), dorsal attention (3), frontoparietal (2), language (2), sensorimotor (3), salience (1), and visual (4) (Online Supplemental Data). Evaluation of the spatial-GICs revealed no significant clusters after using FDR and TFCE. The results using the *F*-test before FDR and TFCE on all 20 spatial-GICs are depicted in Fig 3. The 3 largest clusters overlie the right accumbens, right central and parietal operculum cortex, and left cerebellum based on the standard anatomic atlas.

Among a total of 190 investigated connections, the subject-level temporal components (temporal-GICs) of the 20 spatial-GICs revealed 54 positively and 43 negatively connected components in the control group ($P_{corrected}$ <.05, Fig 4*A*). In the mTBI group, 63 positively and 44 negatively correlated components were found ($P_{corrected}$ <.05, Fig 4*B*). Connectivity matrices between the control and mTBI groups were significantly correlated ($r = 0.94$, $P < .001$). The binary comparison between connected or disconnected components (positively or negatively) is depicted in Fig 4*C*. Before correction for multiple comparisons, there were 1 hyperconnectivity and 1 hypoconnectivity for components within the visual network, 2 hyperconnectivities for components of visual-auditory and salience-language, as well as 2 hypoconnectivities for components of visual-cerebellar, and salience-default mode networks (Fig 4*D*). None survived FDR correction (Fig 4*E*).

fALFF

The voxelwise *t* test showed significant fALFF differences in the lateral occipital cortex (inferior and superior divisions), right cerebellum, left superior and medial frontal gyri, and precuneus anatomic regions (P <.05, Fig 5). However, no clusters remained significant after applying FDR and TFCE corrections.

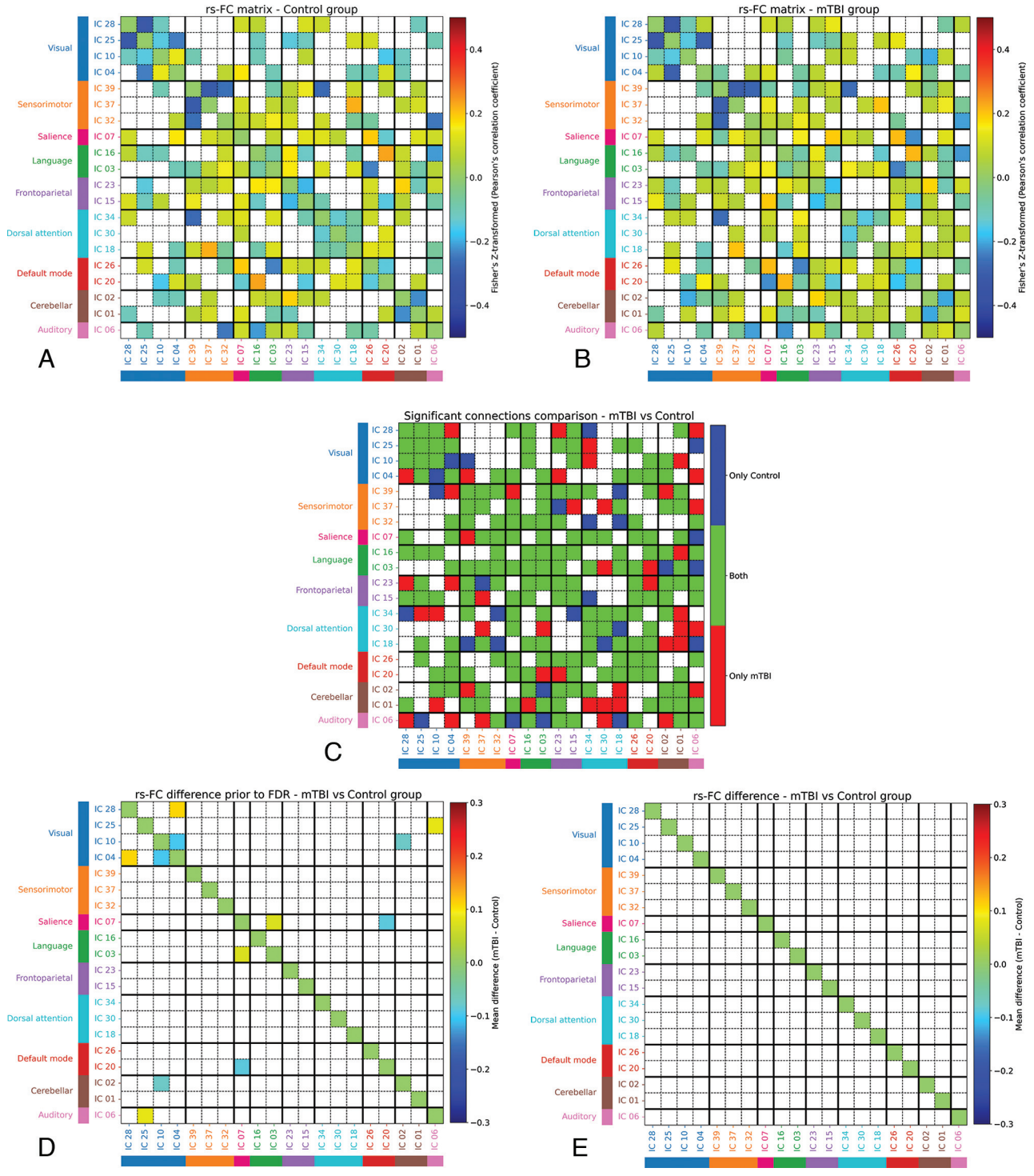

FIG 4. Temporal-GICs analysis results. *A* and *B*, The connectivity matrix for control and mTBI groups. The color for each pair of temporal-GICs reflects statistically significant connectivity after FDR correction ($P_{corrected}$ <.05) measured by average Fisher *z*-transformation of the Pearson correlation coefficient. *C*, Matrix of significant connections, comparing *A* and *B*: Red indicates significant connections unique to the mTBI group; blue indicates significant connections unique to the control group; and green indicates significance in both the mTBI and control groups. *D*, Matrix of mean difference (mTBI and controls) for the temporal-GICs connections that satisfy $P_{uncorrected}$ <.05 using the Welch *t* test comparing those with mTBI with controls. Highlighted connections involve temporal-GICs of 6 IFCNs, excepting sensorimotor, frontoparietal, and dorsal attention; however, the magnitude of differences is small. *E*, After multiple comparison correction (FDR) to account for the number of connections examined, no difference remains significant. IC indicates independent component.

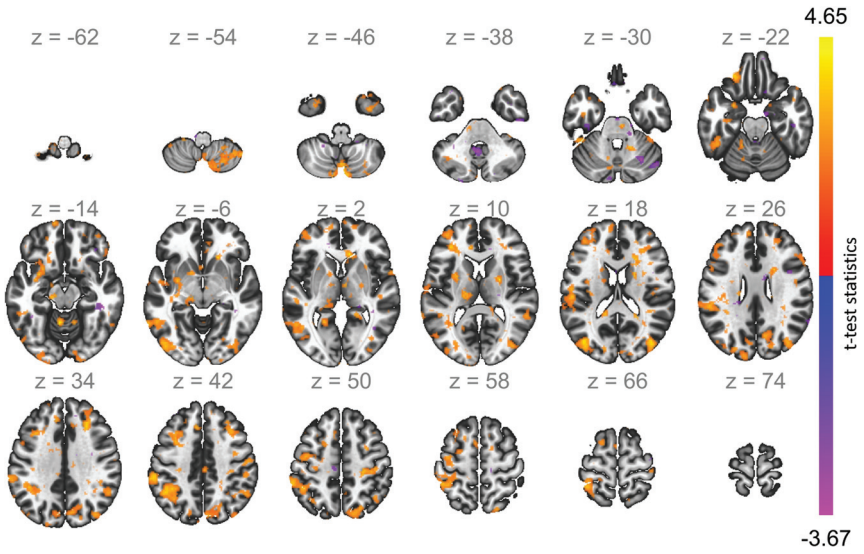

FIG 5. Voxelwise *t* test on fALFF measures comparing mTBI with healthy control groups with $P_{uncorrected} < .05$. Because fALFF is a voxelwise scalar measure, any effect *F*-test was not used, and the contrast shows *t*-values. No clusters survive FDR and TFCE correction.

DISCUSSION

By means of a broad approach using several popular and generally used methods, the results of this study show no statistically significant differences between subjects with mTBI and matched healthy controls measured by whole-brain rs-FC after multiple comparison corrections. The findings are consistent across multiple functional connectivity measures and methods applied to our well-characterized cohort of subjects with mTBI using rigorous statistical analysis. A few differences could be seen before correction for multiple comparisons, though these are only variably aligned with what has been previously reported in the literature.

During the past 2 decades, many studies have investigated connectivity differences between those with mTBI and control subjects using a whole family of measurements collectively referred to as rs-FC, reporting various ranges of results. Here, we focus on the explicit study of rs-FC without extending to other analysis methods such as dynamic rs-FC, graph theory measures, regional homogeneity, or other acquisition methods such as task-mediated fMRI and structural connectivity. These other methods are not explored here though they do represent additional and valid approaches that may shed light on brain connectivity in individuals with mTBI.[14,19,40-47] Furthermore, though we explore the popular primary methods of investigating rs-FC, we recognize that no study can be genuinely exhaustive because there are innumerable ways to combine optimization of parameters, preprocessing methods, and brain parcellations. In this study, we have taken a conservative statistical approach in applying multistep corrections for multiple comparisons. This level of statistical rigor would not necessarily be required for research focusing on only a very limited number of regions rather than taking a whole-brain survey approach; this methodologic difference may contribute to some of the differences between the results of the current study and those of prior works. Despite using NBS as an alternative to whole-brain multiple comparison methods to mitigate the elevated risk of false-negatives due to numerous hypothesis tests, the results were inconclusive and failed to identify any subnetwork of differences. This finding leads us to acknowledge that hypothesis-driven methodologies, similar to those reported in earlier studies, may yield more robust and replicable rs-FC biomarkers in mTBI populations. However, the objective of the current study was to discern stable whole-brain differences, a goal that was not achieved by applying stringent-yet-rigorous statistical correction.

This study raises a critical issue regarding the statistical power of its analysis. The small effect sizes derived from the measurements lead to underpowered conclusions, a problem that is exacerbated when considering the multiple comparison issue, which significantly lowers the threshold for statistical significance.[48] For instance, the largest effect size observed was 0.5723 for the ACC-SMG (L) connection within the salience network, using the ROI/seed-based method and the 8 functional networks parcellation. A sample size of 49 is required, which is marginally met by our current cohort, to confirm this finding with a pre-multiple comparison framework. However, this requirement applies only to the finding with the largest effect size; confirming that other connection differences would require a much larger sample size. The need for increased sample sizes becomes even more pronounced when accounting for multiple comparisons. This reliance on conventional statistical methods introduces a degree of uncertainty in establishing consistent and reproducible biomarkers for rs-FC differences in the literature regarding patients with mTBI. To overcome this challenge, we propose 3 potential solutions: 1) increasing the participant count, 2) using hypothesis-driven and prior-based investigations, and 3) developing new rs-FC measurements and statistical analyses that are less sensitive to these parameters. Each of these options has its advantages and drawbacks. For instance, acquiring a large sample size may not be practical, and hypothesis-driven studies risk confirmation bias. Consequently, the existence of rs-FC differences in subjects with mTBI remains a complex and somewhat unresolved question. Nonetheless, our study strives to address this issue as thoroughly as possible by using rigorous and standard methodologies, setting a benchmark for future research in this field.

Before multiple comparison correction, the findings point to the cerebellum as an area of interest in rs-FC after injury. Specifically, there is some reduced connectivity between cerebellar ROIs and language networks and increased connectivity between the cerebellum and sensorimotor regions, as well as the cerebellum and intraparietal sulcus ROIs of the dorsal attention network. This observation is supported by knowing that only connections to and from the cerebellum can predict the symptom-severity score. In addition, on the basis of ROI-to-voxel analysis, >50% of the largest differences cluster overlaps with 20% of the right cerebellum. Spatial-GICs and fALFF analyses also

highlight sizable clusters within the right cerebellum. Although there is limited evidence in the literature discussing cerebellar rs-FC in mTBI,[40,41,49,50] further studies are warranted to investigate changes in this region after injury. Our findings also show that several clusters of differences throughout the entire supratentorial brain are peripherally located (Figs 2, 3, and 5). Nonetheless, all these observations are made before using rigorous correction for multiple comparisons, making it overall difficult to speculate very much. Additionally, a common assumption is a lower rs-FC in patients with severe mTBI, but our analysis of participants with high SCAT3 and mTBI demonstrated a smaller number of potential injured connections compared with the analysis including all participants with mTBI ($27 < 39$); this unusual observation signals that the rs-FC differences without multiple comparisons are possibly false discoveries.

This study has several limitations. First, the increased number of hypothesis tests involved in whole-brain statistical correction could impede the true discoveries, potentially leading to the oversight of significant results,[51] a challenge stemming principally from the currently predominant strategies used for multiple comparisons. We endeavored to temper this issue by leveraging NBS and undertaking preliminary analysis before applying corrections; however, we identified only a restricted set of conclusive biomarkers, localized mainly in cerebellum regions, which have been underexplored in the existing literature. Furthermore, while FDR-based multiple comparison correction methods do not necessitate independent test statistics, their effectiveness is greatly influenced by the correlations among these statistics.[52] This issue is a significant consideration because test statistics in practical scenarios are often not completely independent. Second, the effect of mTBI on human brain connectivity manifests with a considerable degree of heterogeneity, requiring a more detailed stratification that accounts for the variable nature of symptoms, both acute and persistent, alongside correlations with neuropsychological evaluations and the diverse kinematics of the injuries sustained. This nuanced approach is imperative in developing reliable imaging biomarkers through rs-FC. Last, the potential influence of chronic diffuse axonal injury on prolonged postconcussion symptoms[53] suggests that studying only the acute stages of injury, as pursued in this research, might not reveal significant distinctions. Future research, adopting a longitudinal approach with follow-up assessments, stands to better facilitate more pronounced discoveries, enhancing our comprehension of the complexities involved.

CONCLUSIONS

By using group-level statistical analyses after applying a range of standard, state-of-the-art methods to study rs-FC, we did not observe statistically significant group-level rs-FC differences between patients with mTBI within 1 month of injury and healthy controls after multiple comparison correction. It is likely that due to the kinematic properties of mTBI and other subject-specific features, functional injuries are challenging to probe using group-based analyses such as those used here. Current conventional rs-FC methods and whole-brain statistical analysis frameworks may be insufficient for amplifying subtle changes in neural activity as may occur in patients with mTBI, and overall variance may be high. There is a need to examine all sensitive features of the BOLD signal, develop subject-level rs-FC measurements to consider injury individually, and explore new ways to study rs-FC in this population.

Disclosure forms provided by the authors are available with the full text and PDF of this article at www.ajnr.org.

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