Deficits in Functional Connectivity of Hippocampal and Frontal Lobe Circuits After Traumatic Axonal Injury

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Objective: To examine the functional connectivity of hippocampal and selected frontal lobe circuits in patients with traumatic axonal injury (TAI).

Design: Observational study.

Setting: An inpatient traumatic brain injury unit. Imaging and neurocognitive assessments were conducted in an outpatient research facility.

Participants: Twenty-five consecutive patients with brain injuries consistent with TAI and acute subcortical white matter abnormalities were studied as well as 16 healthy volunteers of similar age and sex.

Interventions: Echo-planar and high-resolution T1-weighted images were acquired using 3-T scanners. Regions of interest (ROI) were drawn bilaterally for the hippocampus, anterior cingulate cortex (ACC), and dorsolateral prefrontal cortex and were used to extract time series data. Blood oxygenation level–dependent data from each ROI were used as reference functions for correlating with all other brain voxels. Interhemispheric functional connectivity was assessed for each participant by correlating homologous regions using a Pearson correlation coefficient. Patient functional and neurocognitive outcomes were assessed approximately 6 months after injury.

Main Outcome Measures: Interhemispheric functional connectivity, spatial patterns of functional connectivity, and associations of connectivity measures with functional and neurocognitive outcomes.

Results: Patients showed significantly lower interhemispheric functional connectivity for the hippocampus and ACC. Controls demonstrated stronger and more focused functional connectivity for the hippocampi and ACC, and a more focused recruitment of the default mode network for the dorsolateral prefrontal cortex ROI. The interhemispheric functional connectivity for the hippocampus was correlated with delayed recall of verbal information.

Conclusions: Traumatic axonal injury may affect interhemispheric neural activity, as patients with TAI show disrupted interhemispheric functional connectivity. More careful investigation of interhemispheric connectivity is warranted, as it demonstrated a modest association with outcome in chronic TBI.

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Functional-connectivity magnetic resonance imaging (MRI) is a technique for analyzing functional MRI data to determine the functional relatedness of selected brain regions. It is based on determining brain regions that demonstrate temporally correlated blood oxygenation level-dependent (BOLD) signal. This technique demonstrates differential patterns of functional hippocampal connectivity during the resting state between patients with Alzheimer disease and healthy volunteers, as controls showed diffuse cortical and subcortical connectivity and patients demonstrated reduced connectivity including absence of connectivity with the frontal lobes. While patients with Alzheimer disease demonstrate a reduction in hippocampal functional connectivity, the association between functional connectivity and behavioral measures is not well understood, as the aforementioned studies examined patients documented to have poorer performance on tasks of memory ability than controls but did not correlate their memory performance to connectivity measures.

The goal of the present study is to examine whether hippocampal and frontal lobe circuits of patients with suspected TAI differ from those of healthy individuals during the resting state. Given that hippocampal and frontal lobe injuries are common after TAI and subsequent memory and executive function deficits are frequently reported, we hypothesize that patients with TAI will demonstrate distinct hippocampal and frontal lobe connectivity patterns and weaker bilateral connectivity than controls. Furthermore, we will examine whether the degree of functional connectivity in these regions correlate with test performance in their respective neurocognitive domains.

METHOD

PARTICIPANTS

Twenty-five patients with TBI were recruited from Parkland Memorial Hospital, Dallas, Texas. Inclusion criteria required that patients (1) sustain closed-head traumatic brain injury through a mechanism consistent with TAI (such as high-speed motor vehicle collision) and (2) were at least 16 years old. Exclusion criteria were (1) preexisting neurologic disorders or history of TBI; (2) presence of local lesions (including contusion, extraaxial hematoma, and/or intraparenchymal hemorrhages) with volume greater than 10 mL visible on cranial computed tomography; (3) conditions that may result in abnormal MRI findings and compromise cognitive functions (ie, prior brain tumor, epilepsy, multiple sclerosis, encephalitis/meningitis, Parkinson disease, Alzheimer disease/mild cognitive impairment, human immunodeficiency virus encephalopathy, vascular malformation, and psychiatric disease); or (4) being a prisoner, homeless patient, or pregnant woman. All patients demonstrated subcortical white matter lesions visible on T2 fluid-attenuated inversion recovery MRI. Sixteen healthy volunteers of similar age and sex were recruited as controls. All controls had good general health and no known neurocognitive disorders. Informed consent was obtained from all participants or their legally authorized representative.

IMAGE ACQUISITION AND PROCESSING

Functional and anatomical magnetic resonance images were obtained for each participant using either a Siemens Trio 3T (Siemens AG, Erlangen, Germany) or a General Electric Signa Excite 3T (General Electric Healthcare, Milwaukee, Wisconsin) scanner. A time series of 128 echo-planar image volumes was acquired at 36 axial slice locations throughout the whole brain. Participants were asked to direct their attention to crosshairs projected onto a screen during image acquisition and not think of anything. Each scanner acquired images from controls and patients. The Siemens scanner acquired images from 7 controls and 9 patients, and the GE scanner acquired images from 9 controls and 16 patients. The echo-planar image data acquired by the Siemens scanner were obtained with single-shot gradient-recalled pulse sequence with time to repetition (TR) of 2 seconds; echo time (TE), 25 milliseconds; flip angle, 90°; matrix, 64 × 64; field of view (FOV), 210 mm; and 3.5-mm slice thickness. High-resolution T1-weighted structural images acquired by the Siemens scanner were acquired using magnetization prepared rapid acquisition gradient echo with slice thickness of 1.0 mm; FOV, 240 mm; TR, 4 milliseconds; inversion time, 900 milliseconds; TE, 2250 milliseconds; flip angle, 9°; and number of excitations, 1. Echo-planar image data acquired by the GE scanner were obtained with single-shot gradient-recalled pulse sequence with TR, 2 seconds; TE, 25 milliseconds; flip angle, 90°; matrix, 64 × 64; field of view, 210 mm; and slice thickness, 3.5 mm. High-resolution T1-weighted structural images acquired by the GE scanner were acquired using fast spoiled gradient recall with slice thickness, 1.3 mm; FOV, 240 to 280 mm; TR, 8 milliseconds; TE, 2.4 milliseconds; flip angle, 25°; and number of excitations, 2. Patients’ neuroimaging data were acquired between 6 and 10 months after injury, which coincides with the day their outcome evaluations were conducted.

PREPROCESSING OF FUNCTIONAL IMAGING DATA

The images were first converted from DICOM (digital imaging and communications in medicine) to an Analysis of Functional NeuroImages (AFNI; National Institutes of Mental Health, Bethesda, Maryland)–readable format. The AFNI software was used for selected preprocessing steps. Slice-time correction was performed to adjust for varying acquisition time for slices. Time series data were corrected for motion and linear drift artifacts. The amount of movement observed on a frame-by-frame basis did not exceed 1 voxel in size for any participant in this study. Given that coherence in BOLD signal fluctuations occurs at low frequencies, high-frequency components were removed prior to analysis of functional connectivity by setting a low-pass filter at 0.12 Hz. The signal to noise ratio was then increased by spatially smoothing the data with a 3-dimensional Gaussian tapering function (5 mm full-width at half maximum). Functional images were coregistered to high-resolution T1 images for each participant, and masks of regions containing cerebrospinal fluid was created using FSL (FMRIB Software Library) 4.1.4. These masks were used to obtain averaged time series data from regions unlikely to contribute variance of neuronal origin. The time series from these regions were later regressed out from functional data of interest.

SEED REGIONS OF INTEREST

Six anatomical regions of interest (ROI) corresponding to the hippocampus, anterior cingulate, and dorsolateral prefrontal cortex were hand drawn bilaterally using AFNI’s graphical user interface by trained research assistants who used a human brain atlas for reference. These ROIs were used as seed volumes to extract average time series data in subjects’ native space (Figure 1). Owing to low spatial resolution involved in func-
tional MRI, seeding the anterior cingulate unilaterally may include signal from both left and right hemispheres and ultimately make interpreting interhemispheric connectivity difficult. The anterior cingulate cortex seed ROI was drawn by excluding slices on either side of the midline.

OUTCOME MEASURES

Functional Outcomes

Functional and neurocognitive outcomes were assessed the same day the neuroimaging scans were obtained (ie, at least 6 months after injury). Functional outcome was assessed using the Glasgow Outcome Scale–Extended (GOSE). The GOSE is a commonly used structured interview that assesses functional abilities in multiple domains following a head injury. Total GOSE scores range from 1 to 8, with higher scores associated with better outcome.

Neurocognitive Outcome

Information processing speed, learning and memory, and executive function deficits are common after TBI. Memory functioning is commonly affected after TBI and was also assessed approximately 6 months after injury using the California Verbal Learning Test–II. Total items learned across 5 trials were used to measure learning, while short and long delay-free recall trials were used to measure memory.

Various neurocognitive tests were used to evaluate executive functions, which are largely influenced by the frontal lobes. Trail Making Test B was used to measure patients' ability to shift mental sets efficiently. The Dodrill-Stroop Color-Naming condition was used to measure ability to selectively attend to meaningful information while inhibiting a prepotent response. The Controlled Word Association Test was used to assess verbal generativity.

STATISTICAL ANALYSIS

Demography

Between-group differences in age and interhemispheric functional connectivity were examined using an independent-samples t test, as these data were suitable for analyzing with parametric tests. Group differences for sex were examined using a \( \chi^2 \) test for independence.

Functional Connectivity Analyses

For each ROI, the averaged time courses of BOLD signal were used as the seed reference time series for calculating correlation with all other brain voxels' time series. A false discovery rate–corrected \( P < .05 \) was considered statistically significant to reduce the occurrence of multiple comparison-related false-positive results. The result is a spatial map of correlation coefficients for every voxel in the brain representing an individual's pattern of functional connectivity with the seed region. Fisher \( z \) transformation was applied to the individual correlation maps to adjust the variance of correlation coefficients for subsequent group-level comparisons. The results were then transformed into Talairach space, and separate group maps were generated for patients and controls. A significant cluster of correlation was defined as a group of at least 200 adjacent voxels. Spatial statistical analyses were conducted using AFNI.

Interhemispheric Connectivity Analysis

Interhemispheric functional connectivity examined synchronicity of BOLD signal fluctuations of bilateral regions over time. This analysis used the BOLD data extracted from the ROIs from each participant. Averaged left and right BOLD fluctuations from each ROI across 124 time points (excluded first 4 frames) were tested for significant associations using a Pearson correlation coefficient. The Fisher \( z \) transformation was applied to the correlation coefficients to allow for group comparisons between patients and controls. Between-group \( t \) tests were used to detect significant differences in degree of interhemispheric connectedness between the respective groups for each ROI. Amplitude of BOLD fluctuation was inspected and not determined to be statistically different between groups.
Outcomes Analysis

Spearman correlations were used to test the association between connectivity measures and functional outcome, as the GOSE is an ordinal measure. Pearson correlations were used to examine associations between connectivity measures and neuropsychological outcome. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS v11.0; SPSS Inc, Chicago, Illinois).

RESULTS

DEMOGRAPHY

As expected, no differences were found between patients and controls in age (mean [SD], 30 [14] and 37 [14] years, respectively) or sex (80% and 63% male, respectively). Patients had traumatic brain injuries ranging in severity from complicated mild to severe, as the mean (SD) Glasgow Coma Scale score was 8 (5). Patients’ average GOSE scores were in the upper moderate recovery range (Table 1). Additionally, their neurocognitive outcomes ranged from mildly impaired to low average impairment, with the lowest scores on the Digit Symbol subtest, Controlled Oral Word Association Test, Stroop word reading condition, and California Verbal Learning Test-II short delay recall.

INTERSCANNER VARIABILITY

To investigate whether there is significant variability between the two scanners used, we examined the interhemispheric connectivity measures among controls in a between-scanner fashion. Nine controls were scanned using the GE magnet and 7 using the Siemens magnet. The results of 2-sample independent t tests showed that all 3 interhemispheric connectivity measures were similar across scanners (hippocampi, \( P = .06 \); DLFPC, \( P = .82 \); ACC, \( P = .13 \)), suggesting that the data from the 2 scanners are comparable.

HIPPOCAMPAL CONNECTIVITY

Connectivity Between Hippocampi

Figure 2 illustrates the fluctuation of BOLD signal in the bilateral hippocampi for a representative control relative to a representative patient with TAI. The degree of interhemispheric hippocampal connectivity was significantly greater for controls than for patients with TAI (\( P = .04 \)) (Table 2). The degree of interhemispheric hippocampal connectivity among patients was negatively correlated with delayed recall of verbal information (Table 3). A scatterplot of this association is displayed in Figure 3.

Whole-Brain Connectivity

Generally, the spatial distribution of hippocampal connectivity among healthy controls showed a strong, focused signal bilaterally within the body of the hippocampi (Figure 4A). Controls also demonstrated connectivity in the septal and subthalamic nuclei. While the hippocampus appeared to have connectivity with bilateral parahippocampal gyri, most of the correlated signal among controls occurred in the anterior medial temporal lobes. In contrast, patients demonstrated more abundant connectivity with the parahippocampal gyrus and posterior cingulate, as well as more diffuse connectivity in the tempo-

Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n=16)</th>
<th>Median</th>
<th>Patients With TAI (n=25)</th>
<th>Median</th>
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<tr>
<td>Age, y</td>
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<td>24.00</td>
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<td>Education, y b</td>
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<td>12.00</td>
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<td>Days in hospital</td>
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<td>48.50</td>
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<td>35.47 (13.36)</td>
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<tr>
<td>TMT B T score</td>
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<td>45.00</td>
<td>43.78 (13.27)</td>
<td>46.67</td>
</tr>
</tbody>
</table>

Abbreviations: COWAT, Controlled Oral Word Association Test; CVLT-II, California Verbal Learning Test–Second Edition; GCS, Glasgow Coma Scale; GOSE, Glasgow Outcome Scale Extended; ICU, intensive care unit; TAI, traumatic axonal injury; TMT A, Trail Making Test A; TMT B, Trail Making Test B.

aNo significant differences between groups in age, sex, handedness, or ethnicity.
bDenotes a significant difference between group difference in education, \( P < .05 \).
nal and frontal lobes, basal forebrain, and subthalamic nuclei than controls. Furthermore, in areas of bilateral hippocampal connectivity, patients demonstrated weaker contralateral connectivity than controls (Figure 4B). Healthy right hippocampal connectivity showed a similar pattern of connectivity as the left hippocampus.

ANTERIOR CINGULATE CONNECTIVITY

Connectivity Between Bilateral Anterior Cingulate

Interhemispheric connectivity for the anterior cingulate was significantly different between healthy and injured brains, as the average strength of bilateral anterior cingulate interconnectivity was greater for controls than for patients with TAI ($P = .02$) (Table 2). The degree of interhemispheric anterior cingulate connectivity among patients was not significantly associated with outcome but showed a trend toward significance with the GOSE ($P = .10$) (Table 3).

Whole-Brain Connectivity

Anterior cingulate connectivity was similar for both left and right seeds. The pattern of anterior cingulate cortex connectivity for controls is displayed in Figure 5A. Among controls, synchronous areas include focused signal in the anterior cingulate bilaterally, bilateral ventral posterior cingulate cortex, and bilateral caudate. Anterior cingulate connectivity for patients showed diffuse correlations surrounding the anterior cingulate bilaterally, bilateral caudate and thalamus, bilateral dorsal posterior cingulate cortex, and cingulate cortex connecting the anterior and posterior cingulate cortices (Figure 5B). Patients also demonstrated negatively correlated signal in the occipital-temporal gyrus.

DORSOLATERAL PREFRONTAL CORTEX CONNECTIVITY

Connectivity Between Bilateral Dorsolateral Prefrontal Cortex

There was no significant difference in connectivity for bilateral dorsolateral prefrontal cortex (DLPFC) between controls and patients with TAI ($P = .35$) (Table 2). Additionally, the degree of bilateral DLPFC connectivity among patients was not significantly associated with outcome but demonstrated a trend toward significance for 2 outcome measures ($P = .10$) (Table 3).

Whole-Brain Connectivity

Left DLPFC connectivity for healthy individuals (Figure 6A) includes the ipsilateral inferior frontal gyrus, middle temporal gyrus, anterior cingulate, posterior cingulate, bilateral angular gyrus, dorsal aspect of superior and middle frontal gyri, and contralateral precuneus. The pattern of left DLPFC is similar between healthy volunteers and patients, but patients demonstrate stronger correlations in bilateral angular gyri, and more diffuse correlations in contralateral frontal and temporal lobes, occipito-temporal region, and parahippocampal gyri. Additionally, while negative correlations are found in the contralateral precuneus in patients and controls, patients also demonstrate a relatively greater number of negatively correlated voxels in the ipsilateral precuneus (Figure 6B). The pattern of right DLPFC is similar to that of the left DLPFC within patients and controls.

COMMENT

The synchronicity of BOLD signal fluctuations throughout the brain has been useful in understanding function-
ally related brain regions/networks. Functional connectivity patterns in healthy individuals demonstrate a functional link between various regions known to communicate during various tasks and at rest. In contrast, connectivity patterns among clinical populations deviate considerably from those observed in healthy brains. For example, patients with Alzheimer disease have demonstrated disrupted hippocampal and frontal lobe connectivity throughout the brain compared with healthy peers. Furthermore, significant functional connectivity between hemispheres is found in healthy individuals, whereas interhemispheric functional connectivity is significantly reduced in clinical populations with compromise in the corpus callosum (CC). The relationship between CC integrity and functional connectivity was demonstrated by Quigley and colleagues, who found that patients with agenesis of the CC showed significantly reduced interhemispheric connectivity compared with controls. Likewise, Johnston et al demonstrated dramatic reductions in interhemispheric functional connectivity of various functional systems after a complete callosotomy, while intrahemispheric connectivity was relatively preserved. These results implicate the CC as having a significant role in the degree of interhemispheric functional connectivity observed using func-

Table 3. Association Between Functional Connectivity and Outcome Measures

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Hippocampus</th>
<th>ACC</th>
<th>DLPFC</th>
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<tr>
<td>r</td>
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Abbreviations: ACC, anterior cingulate cortex; COWAT, Controlled Oral Word Association Test; CVLT-II, California Verbal Learning Test–Second Edition; DLPFC, dorsolateral prefrontal cortex; GOSE, Glasgow Outcome Scale Extended; TMT A, Trail Making Test A; TMT B, Trail Making Test B.

<sup>a</sup>Pearson correlations between measures of interhemispheric functional connectivity and neurocognitive outcome. A Spearman correlation was used for the GOSE, as it is an ordinal measure.

<sup>b</sup>P < .05.

Figure 3. Association between interhemispheric hippocampal connectivity and verbal memory. CVLT-II indicates California Verbal Learning Test–Second Edition.
tional MRI and suggest investigation of the influence of corpus callosum damage in functional connectivity in other clinical populations with axonal damage.

Given that the CC is the most commonly injured white matter structure in traumatic axonal injury, and that compromise to the integrity of the CC results in reduced interhemispheric functional connectivity in other clinical populations, patients with TAI should demonstrate reduced functional connectivity as well. Indeed, the interhemispheric connectivity results in this study are commensurate with findings in other clinical populations, as patients with TAI demonstrated significantly reduced interhemispheric functional connectivity in the bilateral hippocampi and anterior cingulate relative to controls. The results are consistent with a those of a case study MacDonald et al that demonstrated compromised hippocampal connectivity in a patient with a TBI. To our knowledge, this investigation is the first to examine functional connectivity differences between a group of patients with TAI and healthy controls.

The general pattern of hippocampal functional connectivity in controls included stronger bilateral hippocampal and greater connectivity in the septal nuclei near the anterior commissure than the septal nuclei compared with patients. In contrast, the pattern of hippocampal connectivity in patients with TAI demonstrated reduced contralateral strength of correlation, but generally preserved correlation ipsilaterally, and greater correlation in the subthalamic nuclei near the posterior commissure, parahippocampal gyrus, and posterior cingulate.
cortex than controls. Although contralateral hippocampal connectivity was significantly reduced in patients, it was not absent. This is consistent with a prior study of functional connectivity before and after a complete surgical corpus callosotomy. Their observation of limited interhemispheric connectivity despite the complete transection of the main commissural fiber suggests that interhemispheric connectivity also occurs through other commissural fibers such as the anterior and/or the posterior commissures. Patients in our study undoubtedly underwent varying degrees of subcortical white matter injury including injury to the CC, as evidenced by fluid-attenuated inversion recovery MRI; therefore, they presumably have varying degrees of healthy callosal axons allowing some (albeit reduced) functional connectivity with the contralateral hemisphere through this most parsimonious route. However, it is also possible that patients in this study demonstrate some interhemispheric connectivity via the use of the anterior or posterior commissures or the dorsal hippocampal commissure in lieu of the CC. Studies of both animals and humans have suggested that these smaller commissures play a role in interhemispheric hippocampal connectivity owing to their proximity to the hippocampus. Our data show that interhemispheric connectivity occurs near both the anterior and posterior commissures in healthy brains. In contrast, interhemispheric connectivity in injured brains occurs more posteriorly and may rely on posterior commissural fibers (ie, posterior commissure and/or dorsal hippocampal commissure) to a greater degree than healthy brains. Additionally, patients demonstrated more diffuse but weaker correlations throughout the medial temporal and frontal lobes and posterior cingulate cortex compared with controls. This appears to be evidence of neural inefficiency and may represent the brain’s attempt to restore interhemispheric hippocampal connectivity by using less direct connections in light of injured subcortical white matter.

The functional connectivity pattern of the anterior cingulate cortex demonstrated interesting spatial differences between groups, as healthy brains showed greater correlation with the ventral posterior cingulate cortex compared with injured brains, and injured brains had greater connectivity with most of the cingulate cortex, particularly the dorsal aspect of the posterior cingulate cortex, than healthy brains. An altered connectivity pattern within the cingulate cortex has been found in clinical populations. Castellanos et al described compromised connectivity between the precuneus and posterior cingulate and areas of the default mode network including the anterior cingulate. Furthermore, Wang et al found that patients with early-stage Alzheimer disease showed compromised resting state connectivity between the posterior cingulate and the hippocampus, areas involved in the default mode network. Taken together, these studies and the results from the present investigation suggest that the connectivity between anterior and posterior cingulate may be sensitive to compromise in clinical populations with functional or neurocognitive deficits. Furthermore, the results of this study support examining the functional connectivity between various brain regions involved in the default mode network, as their connectedness may be a marker of cerebral integrity or compromise.

Interestingly, functional connectivity of the DLPFC for patients and controls demonstrate a similar pattern observed in the default network (ie, medial superior frontal lobe, posterior cingulate and bilateral inferior parietal lobes,
parahippocampal gyrus). Furthermore, patients demonstrate significantly greater negative connectivity in occipitotemporal and parahippocampal gyrri than controls, which may suggest that patients are suppressing brain activity to a greater degree than controls. While it is unclear whether the DLPFC is suppressing brain activity in the aforementioned regions or if these regions are suppressing brain activity in the DLPFC and other regions involved in the default network, the fact that the frontal lobes play a role in modulating and coordinating complex behaviors suggests that the DLPFC is more likely modulating activity in other regions. A greater amount of negative correlations observed when using the DLPFC as a seed among patients may suggest that they are less efficient in quieting their minds during rest, demonstrating that the default mode network is sensitive to changes after TAI. Subsequent investigation into this matter should use a time-lag analysis of connectivity, as this may help determine whether there is a causal relationship between the DLPFC and the negatively correlated brain regions.

Relatively few studies have examined the association between measures of functional connectivity and cognitive ability. Their findings generally suggest that functional connectivity in various brain regions have a significant relationship with certain cognitive abilities. In this study, the degree of functional connectedness between hippocampi is negatively associated with performance on an auditory verbal memory task, such that patients with less bilateral connectivity recalled more words after a delay than patients with greater interhemispheric connectivity. Given the role of the hippocampus in learning and memory, we hypothesized that interhemispheric hippocampal connectivity is associated with performance in this cognitive domain. Interestingly, the observed negative association between hippocampal connectivity and memory suggests that cognitive functioning is not completely dependent on the integrity of structural connections. Additionally, these results hint at neural plasticity, as the data suggest that hippocampal signal is rerouted to reach its homologous contralateral region through more indirect posterior connections (ie, posterior commissure/thalamic nuclei) after TAI, and the degree of inefficiency (ie, diffuse connectivity) seen in the pattern of hippocampal connectivity in patients may be evidence of this plasticity. However, while this plasticity eventually results in neuronal signal reaching its contralateral destination and a relative increase in interhemispheric hippocampal connectivity, it occurs at the expense of memory ability.

While the interhemispheric connectivity for the anterior cingulate cortex and the DLPFC was significantly lower in patients than controls, the correlation between degree of connectivity in these regions and outcome only trended toward significance with outcome. Though the associations between interhemispheric functional connectivity and outcome observed in our study do not fully support the hypothesis that frontal lobe functional brain synchronicity is associated with executive functions after TBI, the results are not entirely surprising, as resting state interhemispheric connectivity should not be assumed to have a strong association with functional or neurocognitive outcome in this clinical population. Patients with TBI or TAI present with heterogeneous injury profiles including mechanism of injury, injury severity, and most importantly, location of brain lesions. Although every patient in this sample was selected based on a head injury consistent with traumatic axonal injury, the degree of injury to particular white matter structures undoubtedly varied widely. For example, Benson et al examined the integrity of whole-brain white matter of 20 patients with TAI using a histogramic analysis and demonstrated that the distribution of white matter fractional anisotropy (ie, measure of the directionality of water diffusion along axons) for individual patients was significantly more variable than the distribution for controls. Variability of white matter integrity in various interhemispheric structures may, in part, explain how interhemispheric connectivity can be reduced without reducing cognitive or functional ability, as it is possible that certain patients had damage to the CC and subsequently rerouted neuronal signal between hippocampi through less direct but more intact commissural fibers (ie, posterior cingulate, hippocampal commissure), thereby lowering their degree of interhemispheric hippocampal connectivity but maintaining enough contralateral connectivity to approximate the desired behavior.

A limitation of our study is that the degree of compromise to interhemispheric white matter is not described. Decrease in interhemispheric structural connectivity may account for degree of functional connectivity in patient populations, but DTI studies must be performed to measure the degree of structural compromise. It is highly recommended that diffusion tensor tractography (or similar analysis of diffusion tensor imaging data) be incorporated into the research design to more directly examine the association between the integrity of certain white matter structures, functional connectivity, and outcome. Another limitation of the current study, and any neuroimaging study of TBI, has to do with heterogeneity inherent in TBI, as injury profiles including mechanism of injury, injury severity, and most importantly, location of brain lesions can vary from patient to patient. Therefore, the results of this investigation may not be generalizable across traumatic brain injuries with different profiles.

Given that the functional connectivity measures for this study are obtained from a resting-state fMRI paradigm, it is not known whether patterns of connectivity during rest should correlate to functional or neurocognitive tasks. Consequently, it is possible that the association between functional connectivity measures and outcome could be significantly different had synchronicity of BOLD signal between regions been measured during a cognitive task rather than during rest. Future studies may benefit from incorporating both resting state and task-related functional connectivity measures in their design. It is also important to note that the results of this study are specific to functional connectivity patterns present 6 months after injury, as results may be different in a more acutely or more chronically brain-injured sample.

The use of MRI scanners from different manufacturers in this study may be perceived as a limitation despite demonstrating equivalence in interhemispheric connectivity in controls between scanners. However, novel neuroimaging modalities must show robust differences between control and clinical populations across scanners.
to be useful as a clinical biomarker, as hospitals/medical centers use scanners from various manufacturers. While attempts were made to limit the influence of multiple comparisons on the results of the statistical analyses (ie, using false discovery rate correction and cluster thresholding), this study may still be affected by false positives owing to the large number of voxel × voxel comparisons.

This study provides support for the use of functional connectivity MRI in clinical populations, including patients with compromised anatomical connectivity such as TAI. The results support the hypothesis that the hippocampus and frontal lobe circuits of patients with TAI have distinct patterns of interconnectedness and less connectivity with their contralateral homologue compared with those of healthy individuals. Additionally, the degree of bilateral connectivity in hippocampal circuits appears to correlate with patients' memory-related outcome after TAI.

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