White matter integrity related to functional working memory networks in traumatic brain injury

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ABSTRACT

Objective: This study explores the functional and structural patterns of connectivity underlying working memory impairment after severe traumatic axonal injury.

Methods: We performed an fMRI n-back task and acquired diffusion tensor images (DTI) in a group of 19 chronic-stage patients with severe traumatic brain injury (TBI) and evidence of traumatic axonal injury and 19 matched healthy controls. We performed image analyses with FSL software and fMRI data were analyzed using probabilistic independent component analysis. Fractional anisotropy (FA) maps from DTI images were analyzed with FMRIB’s Diffusion Toolbox.

Results: We identified working memory and default mode networks. Global FA values correlated with both networks and FA whole-brain analysis revealed correlations in several tracts associated with the functional activation. Furthermore, working memory performance in the patient group correlated with the functional activation patterns and with the FA values of the associative fasciculi.

Conclusion: Combining structural and functional neuroimaging data, we were able to describe structural white matter changes related to functional network alterations and to lower performance in working memory in chronic TBI. Neurology® 2012;78:1-1

GLOSSARY

BOLD = blood oxygenation level–dependent; DTI = diffusion tensor image; EPI = echoplanar imaging; FA = fractional anisotropy; FOV = field of view; FWE = family-wise error; IC = independent component; ICA = independent component analysis; MNI = Montreal Neurological Institute; TBI = traumatic brain injury; TE = echo time; TFCE = threshold-free cluster enhancement; TI = inversion time; TR = repetition time.

Multimodal integration studies of brain connectivity allow the investigation of the relationship between brain structure and function. Areas with strongly correlated activity are more likely to be anatomically connected1 although increased functional connectivity does not necessarily predict increased structural connectivity.2 Traumatic axonal injury causes multifocal damage, disrupting critical cortical–subcortical circuitry leading to significant functional consequences, and cognitive dysfunction after traumatic brain injury (TBI).3,4 Working memory is frequently impaired following TBI. fMRI studies reported patterns of increased or decreased cerebral activations in the regions that form part of the working memory network,5–8 and white matter alterations measured by diffusion tensor imaging (DTI) correlated with working memory impairment in severe TBI.9

However, no previous studies with TBI patients have combined these techniques to investigate the relationship between the functional and structural substrates underlying working memory impairment. In this study, we assessed white matter integrity and functional brain connectivity using DTI and independent component analyses (ICA), respectively. The ICA method extracts patterns or independent components (IC) that reflect brain functional connectivity. Each IC consists of a spatial map that shows a pattern of synchronized activation over time of different brain regions in response to stimuli.10,11 We hypothesized that 1) white matter alteration would affect functional...
working memory and default mode networks; and 2) alterations in both white matter integrity and functional networks would explain working memory deficits.

**METHODS**

**Subjects.** We performed a cross-sectional study of 38 subjects. We recruited 19 patients with TBI from a database of 366 chronic patients at the Head Injury Unit of the Institut de Neurorehabilitació Guttmann. After excluding patients living outside the area of Barcelona (n = 175), we applied the following inclusion criteria: 1) severe closed-head injury and severe TBI defined as Glasgow Coma Scale score ≤8; 2) adults aged ≥40 years; 3) chronic stage of recovery ≥2 years since the TBI; 4) possible diffuse pathology reported in the MRI scans in the subacute stage without macroscopic lesions. The exclusion criteria were 1) visual, sensorial, or visuo-perceptual deficits; 2) previous history of TBI, drug intake, neurologic, or psychiatric disorders; 3) injury requiring craniectomy or craniotomy. Sixty-seven patients met these criteria and were phoned consecutively until a sample of 44 participants were obtained, who were then included in a study of the long-term consequences of severe TBI. Since we were interested in diffuse white matter injury after TBI, the neuroradiologist (N.B.) described the chronic brain lesions seen in the current MRI. Patients with large lesions were excluded, similarly to previous studies in the traumatic axonal injury literature.13 This left us with 27 subjects. We excluded 3 patients because of motion artifacts in the MRI, and 5 for lack of collaboration. The final patient group comprised 19 patients. Table 1 shows their clinical and neuroimaging characteristics. The etiology of TBI was traffic accident in all cases.

Nineteen healthy volunteers matched by age, sex, education, and handedness were recruited as the control group (table 2). None had a previous history of neurologic or psychiatric diseases and brain scans were reported as normal. In patients, we evaluated handedness by premorbid writing hand preference.

**Standard protocol approvals and patient consent.** The Research Ethics Committees of the Institut Universitari de Neu­rorehabilitació Guttmann and the University of Barcelona approved the study. All participants gave written informed consent.

**Neuropsychological assessment.** To evaluate the current neurocognitive status of the subjects, a trained neuropsychologist blinded to the clinical data administered the tests assessing executive function, verbal and visual memory, visuo-perception, and processing speed, all common deficits after TBI. We administered the neuropsychological assessment 1 week before the fMRI in order to identify possible motor, sensorial, or perceptual deficits that might interfere with the fMRI task. The assessment included Letter-Number Sequencing and Digit Span Forward and Backwards tests,13 Trail-Making Test A and B, Rey-Osterrieth complex figure, Rey Auditory Verbal Learning Test, Stroop reading and color-naming conditions, and verbal fluency.14 Results are summarized in table 2.

**Image acquisition.** The fMRI data were acquired on a Siemens Magnetom TrioTim syngo 3-Tesla at the Centre de Diagnostic per la Imatge de l’Hospital de la Cerdanya (CDIC), Barcelona. We acquired a high-resolution T1-weighted structural image scan for each subject with a magnetization-prepared rapid gradient echo 3-dimensional protocol (repetition time [TR] = 2,300 msec; echo time [TE] = 3 msec; inversion time [TI] = 900 msec; field of view [FOV] = 244 mm; 1 mm isotropic voxel) and a single shot gradient-echo echoplanar imaging (EPI) sequence (TR = 2,000 msec; TE = 16 msec; flip angle = 90 grad; FOV = 220 mm; voxel size = 1.7 × 1.7 × 3.0 mm) for fMRI. Visual stimuli were projected on a screen seen through a mirror mounted on the head coil. Diffusion-weighted images were sensitized in 30 noncollinear directions with a b-value = 1,000 s/mm², using an EPI sequence (TR = 9,300 msec; TE = 94 msec; slice thickness = 2.0 mm; voxel size = 2.0 × 2.0 × 2.0 mm).
Table 2 Demographic and neuropsychological characteristics of patients and controls

<table>
<thead>
<tr>
<th></th>
<th>TBI group, mean (SD)</th>
<th>Control group, mean (SD)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>26.78 (5.55)</td>
<td>27.47 (6.04)</td>
<td>0.72</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>Secondary education</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>College and university</td>
<td>4</td>
<td>4</td>
<td>0.86</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>7</td>
<td>9</td>
<td>0.52</td>
</tr>
<tr>
<td>Handedness</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>15</td>
<td>15</td>
<td>0.73</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Neuropsychological performance</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TMT A</td>
<td>31.68 (10.9)</td>
<td>22.72 (6.23)</td>
<td>0.05</td>
</tr>
<tr>
<td>TMT B</td>
<td>79.16 (22.7)</td>
<td>57.28 (14.15)</td>
<td>0.001</td>
</tr>
<tr>
<td>TMT A &amp; B</td>
<td>47.47 (21.43)</td>
<td>35.11 (16.1)</td>
<td>0.056</td>
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<tr>
<td>Digit forward</td>
<td>9.37 (1.9)</td>
<td>9.90 (2.32)</td>
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<tr>
<td>Digit backward</td>
<td>6.59 (1.12)</td>
<td>7.61 (1.6)</td>
<td>0.03</td>
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<tr>
<td>Symbol digit</td>
<td>69.74 (13.25)</td>
<td>86 (14.4)</td>
<td>0.001</td>
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<td>Stroop reading</td>
<td>98 (14.67)</td>
<td>109.61 (13.28)</td>
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<tr>
<td>Stroop color naming</td>
<td>64.58 (8.46)</td>
<td>77.72 (9.12)</td>
<td>&lt;0.001</td>
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<tr>
<td>Stroop interference</td>
<td>6.86 (6.18)</td>
<td>7.62 (4.36)</td>
<td>0.66</td>
</tr>
<tr>
<td>LNS</td>
<td>10.37 (2.06)</td>
<td>12.50 (1.6)</td>
<td>0.001</td>
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<tr>
<td>Fluencies</td>
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<tr>
<td>Semantic</td>
<td>22.44 (11.68)</td>
<td>24 (4.85)</td>
<td>0.62</td>
</tr>
<tr>
<td>Phonemic</td>
<td>34.53 (12.4)</td>
<td>45.5 (9.4)</td>
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<td>RAVLT (DR)</td>
<td>8.32 (3.44)</td>
<td>13.56 (2.15)</td>
<td>&lt;0.001</td>
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<tr>
<td>ROCF (DR)</td>
<td>10.05 (5.9)</td>
<td>23.44 (4.44)</td>
<td>0.015</td>
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<tr>
<td>0-back (RT)</td>
<td>0.55 (0.08)</td>
<td>0.49 (0.07)</td>
<td>0.06</td>
</tr>
<tr>
<td>0-back d’</td>
<td>3.20 (1.24)</td>
<td>4.12 (0.18)</td>
<td>0.005</td>
</tr>
<tr>
<td>2-back (RT)</td>
<td>0.59 (0.1)</td>
<td>0.54 (0.08)</td>
<td>0.03</td>
</tr>
<tr>
<td>2-back d’</td>
<td>2.47 (0.85)</td>
<td>3.32 (0.66)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: d’ = accuracy measure; DR = delayed recall; LNS = Letter-Number Sequencing [Wechsler Adult Intelligence Scale–III]; RAVLT = Rey Auditory Verbal Learning Test; ROCF = Rey-Osterrieth Complex Figure; RT = reaction time (s); TBI = traumatic brain injury; TMT = Trail-Making Test.

mm; FOV = 240 mm; no gap). Subjects were scanned twice. In the first scan we acquired the structural sequences and in the second scan performed a week later we acquired the fMRI data.

fMRI paradigm. We administered a visual facial33 n-back task. The n-back paradigm is widely used to investigate the neural basis of working memory.24 The experimental conditions were 0-back (control task) and 2-back (working memory task). In the 0-back condition, we asked individuals to decide whether the stimuli matched a single target stimulus specified before the epoch began. During the 2-back condition, they were asked to decide whether the stimuli currently presented matched the stimuli that had been presented 2 trials previously. Each n-back condition involved a 30-second epoch, preceded by a 2-second short written instruction. The design alternated 0-back and 2-back conditions starting with 0-back. We used a block design with 16 cycles of alternation between conditions, presented in the course of the 8-minute experiment. There was no period of control fixation between each condition. Each stimulus appeared on the screen for 1 second against a black background, followed by a fixation cross for another 1 second. Participants had to press a button only when the currently presented stimulus was a target. We registered hits, misses, correct rejects, false alarms, and reaction times. Prior to the scan, participants rehearsed a shorter familiarization session of the task, reaching an accuracy plateau of 80%. We recorded reaction times and calculated the d’ measure, a bias-free measure that takes both correct answers and errors into account, to determine the accuracy of performance. Higher accuracy measures (d’) indicate higher performance.

RESULTS Behavioral functional data. Table 2 summarizes the mean RT and accuracy measure ($d'$) for TBI patients and healthy controls for the 2 task conditions. TBI differed from controls in both accuracy and RT measures.
Identification of functional brain activation patterns. Decomposition of the fMRI dataset resulted in 36 different activation patterns of connectivity (ICs). We selected the first 2 components as having the highest Z value and highest correspondence with the temporality of the stimuli, and avoided the ones representing known artifacts such as noise, motion, or venous pulsation.

The first activation pattern, IC1, was associated with the 0-back >2-back contrast ($p < 0.001$) and its spatial activation maps corresponded to the default mode network (figure 1A). The brain regions that presented activations included the precuneus, frontal pole, medial temporal gyrus, cingulate gyrus, temporal fusiform cortex, and bilateral inferior parietal cortex. This component also included deactivations involving the superior frontal gyrus, superior parietal lobe, frontal pole, lingual gyrus, insular cortex, and the paracingulate gyrus.

The second pattern of activation, IC2, was related to the 2-back >0-back contrast ($p < 0.001$). The distribution of the topographic activation corresponded to the working memory network (figure 1B). Activated brain regions included the superior parietal lobes, middle frontal gyrus, insular cortex, temporal fusiform gyrus, middle temporal gyrus, and anterior paracingulate gyrus. Deactivations for this condition were observed in the following areas: cingulate gyrus, frontal pole, middle temporal gyrus, bilateral inferior parietal cortex, orbitofrontal cortex, left superior temporal gyrus, and precentral gyrus. Table e-1 summarizes the cerebral areas, Montreal Neurological Institute (MNI) maximum coordinates, and cluster size of the spatial maps.

We found a significant correlation between the activation of the working memory network and the deactivation of the default mode network for both the TBI group ($r = 0.753$, $p < 0.001$) and the control group ($r = 0.877$, $p < 0.001$).

TBI patients showed reduced default mode network activity in group comparison. We found statistically significant differences between groups for the activation pattern IC1. Patients showed decreased activation compared to the control group in the cerebral regions that corresponded to the default mode network ($p < 0.009$). For the working memory activation pattern IC2, we found a trend toward significance, suggesting that patients also had reduced activation, especially in the parietal lobes, in comparison with the control group ($p < 0.06$).

To control for the effects of diffuse white matter damage on brain activation, we selected the pattern corresponding to the visual system. There were no group differences in the activation of this component (patients, mean = 1.76; controls, mean = 1.24; $p = 0.45$).

White matter integrity correlated with functional activity in TBI patients. Global FA measures correlated with the default mode network deactivations and the activations of the working memory network ($r = 0.41$, $p = 0.04$ and $r = 0.57$, $p = 0.006$), respectively. Patients with reduced white matter integrity showed a lower level of activation in these functional networks for both default mode network and working memory.

In TBI patients, but not in controls, FA maps correlated with individual functional activation. Patients with better white matter integrity had higher activation in both networks. The fasciculi that achieved significant correlations were intrahemispheric association fibers of the inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculi, and the cingulum bundle; interhemispheric fibers of the corpus callosum (genu and splenium) and projection fibers of the corticospinal tracts as well as in the anterior thalamic radiations, anterior limb of the internal capsule, and anterior corona radiata. Figure 2 illustrates FWE-corrected maps $p < 0.05$ (TFCE).

Impairment of specific fasciculi explained working memory performance. FA values correlated with the 2-back accuracy measure in the TBI patients, FWE-corrected $p < 0.05$. Patients with better white matter integrity in specific fasciculi performed better in the working memory task. Figure 3 summarizes the fasciculi that achieved significant correlations. We did not find significant correlations for the control group.

Working memory performance correlated with functional and structural measures. We found significant correlations for the TBI patients in the default mode and working memory networks with the accuracy measure $d’$ ($r = 0.53$, $p = 0.009$). These results indicate that patients with better performance had higher activation in the working memory network and increased deactivation of the default mode network.

No correlations were found between the gray matter volume and working memory performance ($r = 0.31$; $p = 0.10$).

DISCUSSION We combined fMRI and DTI techniques to obtain a better understanding of the connectivity alterations underlying working memory impairment after severe traumatic axonal injury. We demonstrate that alterations in the patterns of functional activity can be explained by structural connectivity damage in TBI patients. Moreover, these alterations correlated with poorer performance on the working memory fMRI task.

In our study, we found 2 different patterns of activation. One of these patterns corresponded to the
Figure 1  Default mode and working memory networks identified in the fMRI analysis of the groups

(A) Spatial map of IC1 (0-back vs. 2-back) corresponding to the default mode network and its associated time series. (B) Spatial map of IC2 (2-back vs. 0-back) corresponding to working memory network and its associated time series. Hot and cold colors are used to represent activations and deactivations respectively. In the time-series plots, red line represents the independent component time course and green line the task time design. Images are in radiologic convention.
default mode network, which is known to be active during rest and deactivated during externally oriented tasks.\textsuperscript{21,22} The other pattern of activation corresponded to the task-related condition with activation of core areas of working memory circuitry. We observed a relationship between the activation of the working memory network and the deactivation of the default mode network. This finding is in agreement with a previous report which concluded that people who require more cerebral resources to perform a cognitive task will show greater deactivation of the default mode network.\textsuperscript{23} Group comparison showed that patients had decreased brain activity in the default mode network as reported in TBI patients with different severity of injury.\textsuperscript{24–26}

We observed widespread patterns of correlation of several white matter fasciculi, the default mode, and working memory networks. Patients with lower fractional anisotropy values had lower brain activation for both default mode and working memory networks. The lack of white matter specificity of these correlations suggests that the decrease in fractional anisotropy values due to the diffuse axonal injury explains the decrease in functional activation. In this case, FA might be reflecting the microbleed effects due to traumatic axonal injury pathology.\textsuperscript{27}

When we focused on the working memory performance, only the interhemispheric and intrahemispheric associative fasciculi correlated with working memory function. Whole brain DTI maps revealed

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Figure 2  Correlation between white matter damage and functional activations during the task in traumatic brain injury patients

The relationship between fractional anisotropy (FA) values and functional activation scores is represented in green on the standard Montreal Neurological Institute brain (in A for default mode network, and in B for working memory network). Maps are thresholded at \( p < 0.05 \), family-wise error corrected. The fasciculi that achieved significance are described in the main text. Scatterplots show individual values for mean FA within the fasciculi and mean blood oxygenation level-dependent (BOLD) response.
that structural damage in specific tracts such as the inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculi, cingulum, uncinate, and corpus callosum explained the alteration of the lower performance. The superior longitudinal fasciculus is the main fasciculus linking the parietal and frontal lobes and hence it probably plays an important role in working memory. Relations between the superior longitudinal fasciculi and working memory deficits have been reported in multiple sclerosis and in TBI. The inferior longitudinal fasciculus also correlated with working memory performance. We expected this finding since in our working memory task the fusiform gyrus activation was recruited and this structure is domain-specific for face recognition. The structural integrity of corpus callosum also correlated with working memory performance. The corpus callosum is the largest white matter fasciculus and is especially vulnerable after traumatic brain injury. As it connects both hemispheres, its damage interrupts the transfer of information that is crucial for efficiency in cognitive performance. In summary, all these fasciculi link cerebral regions that form part of the working memory network as identified in the fMRI analyses and damage to them is associated with working memory deficits. These results are in agreement with previous reports which found that specific patterns of FA decreases correlated with different cognitive domains such as declarative memory and executive functions. In aged subjects, global FA decreases correlated with speed of mental processing, probably indicating a widespread white matter integrity loss.

We found that patients who performed better had higher activations of the working memory network. Similarly, patients who presented greater deactivation of the default mode network also had better performance. The relationship between the reduction of brain activity in the regions forming part of the default mode network and poorer performance supports the concept that dysfunction in this network is associated with cognitive impairment, as has been reported in other neurologic diseases. Our results indicate that working memory impairment may be a result of a deficit in activated task-related areas together with deficits in deactivating the default mode network during task processing.

The use of an approach that combines different imaging modalities offers an excellent opportunity to elucidate the underlying structural and functional substrates of cognitive deficits. The present study provides strong evidence of the role of structural damage in dysfunctional patterns of working memory and default mode networks in TBI patients. Both structural and functional alterations contribute to working memory deficits.

**AUTHOR CONTRIBUTIONS**

E.P., R.S.L.L., and C.J. made substantial contributions to the conception and design of the study, the interpretation of data, as well as to the preparation of the first draft and further revisions of the manuscript.
aging data were analyzed by E.P. and R.S.L.L. Neuroimaging sequence acquisitions and neuroradiologic evaluations of the MRI were performed by N.B. T.R. participated in the collection of neuropsychological acute clinical data. J.T. and P.V. made a critical revision of the manuscript for important intellectual content, providing additional comments and contributions. C.J. supervised the study. All authors contributed in the discussion and approved the final version of the manuscript.

ACKNOWLEDGMENT
The authors thank Cesar Garrido from the Centre de Diagnostic per la Imatge of the Hospital Clinic (CDIC) for assistance with data recollection.

DISCLOSURE
E.M. Palacios was supported by a fellowship from the Institute of Biomedical Research August Pi i Sunyer (IDIBAPS) and research support from the MAPFRE Foundation. R. Sala-Llonch was supported by a fellowship from the Institute of Biomedical Research August Pi i Sunyer (IDIBAPS). Dr. Junque, Dr. Vendrell, Dr. Tormos, Dr. Roig, and Dr. Bargallo report no disclosures.

Received May 18, 2011. Accepted in final form August 16, 2011.

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