Structural And Functional Connectivity Alterations And Their Relationship With Cognitive Impairment In Traumatic Brain Injury

Eva M. Palacios

UNIVERSITAT DE BARCELONA

Structural And Functional Connectivity Alterations And Their Relationship With Cognitive Impairment In Traumatic Brain Injury

Thesis presented by

Eva M. Palacios

to obtain the degree of doctor
from the University of Barcelona in accordance
with the requirements of the international
PhD diploma

Supervised by

Dr. Carme Junqué i Plaja

Department of Psychiatry and Clinical Psychobiology
Faculty of Medicine, University of Barcelona
Medicine Doctoral Programme

2013

Barcelona, 15 March 2013

Dr. Carme Junqué i Plaja, Professor at the University of Barcelona,

CERTIFIES that she has guided and supervised the doctoral thesis entitled "Structural And Functional Connectivity Alterations And Their Relationship With Cognitive Impairment After Traumatic Brain Injury" presented by Eva M. Palacios. She hereby asserts that this thesis fulfils the requirements to present her defence to be awarded the title of doctor.

Signature,

Dr. Carme Junqué i Plaja

This thesis has been undertaken in the Neuropsychology Group of the Department of Psychiatry and Clinical Psychobiology, Faculty of Medicine, University of Barcelona. The group forms part of the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS).

The present work, including the studies that have previously been published, have been financially supported by a pre-doctoral grant from the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Financial support was also received from the MAPFRE Foundation for the neuroimaging work.

INDEX

FOREWORD

This thesis, presented for the degree of Doctor by the University of Barcelona, is the result of different studies carried out over a four-year period at the Department of Psychiatry and Clinical Psychobiology of the University of Barcelona. During this period, I have obtained the degree of Master of Neuroscience, which is linked to the Doctorate in Medicine Programme (Quality Mention MCD2008-00023; Mention to Excellence MEE2011-0316) at the University of Barcelona.

This thesis follows the published papers format. The global impact factor of the four published papers is 24.13 (ISI of Knowledge, Journal Citation Reports inferred from 2012):

- Palacios EM, Fernandez-Espejo D, Junque C, Sanchez-Carrion R, Roig T, Tormos JM, Bargallo N, Vendrell P. *Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury.* BMC Neurology 2011; 11:24. IF:2.16

- Palacios EM, Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *Long-term declarative memory deficits in diffuse TBI: Correlations of cortical thickness, white matter integrity and hippocampal volume.* Cortex 49 (3):646-57, 2012. IF: 6.08

- Palacios EM, Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *White matter integrity related to functional working memory networks in traumatic brain injury.* Neurology 20 (12):852-860, 2012. IF: 8.31

- *Palacios EM, *Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *Resting-State Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury*. 2013 (in press) *Equal Contribution. IF: 7.58

GLOSSARY OF ABBREVIATIONS

ACC: anterior cingulate cortex

AD: axial diffusivity

ADC: apparent diffusion coefficient

ALIC: anterior limb of the internal capsule

BPV: brain parenchymal volume

BVMT: brief visuospatial memory test

CC: corpus callosum

COWAT: controlled word association

CP: cerebral peduncle

CPT: Conners' continuous performance

CSO: centrum semiovale

Cth: cortical thickness

CVLT: california verbal learning test

DAI: diffuse axonal injury

DLPFC: dorsolateral prefrontal cortex

DMN: default mode network

DTI: diffusion tensor imaging

FA: fractional anisotropy

fMRI: functional magnetic resonance

GCS: Glasgow coma scale

GOS/E: Glasgow outcome scale/extended

H-DAI: haemorrhagic diffuse axonal injury

ICA: independent component analysis

IPG: inferior parietal gyrus

MD: mean diffusivity

MRI: magnetic resonance imaging

MTBI: moderate traumatic brain injury

Nh-DAI: non-haemorrhagic diffuse axonal injury

PASAT: paced auditory serial auditory test

PCC: precuneus

PFC: prefrontal cortex

PLIC: posterior limb of internal capsule

PPI: psychophysiological interactions

PTA: postraumatic amnesia

PUT: putamen

RD: radial diffusivity

IFG: inferior frontal gyrus

IPG: inferior parietal gyrus

ROI: region of interest

RT: reaction time

RUFF: figural fluency test

SLF: superior longitudinal fasciculus

SST: stop signal task

STBI: severe traumatic brain injury

TAI: traumatic axonal injury

TMT: trail making test

TOL: tower of London

VBM: voxel-based morphometry

VPFMC: ventromedial prefrontal cortex

BACKGROUND

The main aim of the current PhD research was to identify possible connectivity alterations in the brain of patients suffering from diffuse axonal injury and to attempt to relate them with cognitive impairment by applying the most up-to-date neuroimaging techniques.

The concept that each cognitive function is carried out by one brain area or structure is nowadays considered outdated. It is true that single brain structures may play a major role in specific cognitive functions (i.e. declarative memory and hippocampus). However, single structures alone cannot conduct all the complex functional components that are needed to process and/or inhibit processing of a stimulus in order to respond efficiently to our environment. As a result of advances in neuroimaging techniques that allow us to observe the brain in vivo, we now know that the brain is a complex network that processes and transports information between structurally connected areas in response to environmental demands.

Traumatic axonal injury is widespread damage to axons throughout the white matter in the brain, caused by sustained acceleration and deceleration forces at the moment of injury. These phenomena produce shear and strain deformation of the axons and microscopic changes may result in progressive axonal disconnection. The consequences of the damage are usually manifested as cerebral atrophy. This evolves over months and even years after the injury and is the cause of the most devastating

disabilities following brain trauma. Magnetic resonance imaging is the most widely-used technique for detecting structural brain abnormalities resulting from injury. However, standard clinical magnetic resonance is insufficient for detecting subtle brain changes caused by traumatic axonal injury. There have been many important developments in this field recently, the most interesting of which are related to connectivity, the study of alterations in the connections between cerebral regions. The study of connectivity provides a unique opportunity for the exploration of the brain substrates of cognitive and behavioural changes caused by brain injuries.

Patterns of brain network connectivity are usually disrupted as a result of traumatic brain injury and this disruption may result in cognitive deficits. Our knowledge of the mechanisms underlying cognitive dysfunction after traumatic brain injury is limited and there are still many unanswered questions: Is grey matter damage the main cause of cognitive dysfunction? Are the underlying white matter connections responsible for the alterations in functional connectivity? How does disruption in connectivity result in cognitive deficits? Do networks reorganise after damage? If so, which is the neurobiological mechanism that supports reorganisation? Are there behavioural metrics that we can use to establish a better prognosis that may allow us to improve outcome? The work I have been working on these four years in my doctoral studies have attempted to provide answers to some of these questions.

As traumatic axonal injury causes broad white matter damage, it provides a unique insight into the loss of connectivity in the brain networks compromised by traumatic brain injury. Two of the articles stemming from my doctoral work have focused on the study of structural white matter/grey matter changes in patients with diffuse injury and memory impairment. Combining volumetric techniques and diffusion tensor imaging magnetic resonance, which is very sensitive for the detection of white matter damage, we have demonstrated that memory impairment can be primarily explained as being due to alterations in cortico-subcortical connectivity rather than localized damage in the hippocampus, the main structure that sustains memory functions. These studies have provided an insight into the neural basis of memory function/dysfunction in diffuse and chronic traumatic brain injury.

These findings were from structural imaging and so could not tell us the extent to which the structural connectivity alterations affect the brain while in action responding to external stimuli. The cortical brain networks involved in the performance of a cognitive task can be measured by functional magnetic resonance. Functional connectivity is defined as the temporal dependence of neuronal activity patterns of anatomically distinct brain regions. Recently, temporal synchrony within distributed brain regions has been demonstrated even when the brain is at rest i.e. not responding to external stimuli. These resting state networks are an important discovery since they have been shown to exist while awake, during sleep, and under general anaesthesia, suggesting that spontaneous neuronal activity plays a fundamental role in brain functions. Resting-state functional connectivity is becoming a crucial tool since it can be used to examine disconnection effects following brain disease. In this thesis, the combination of resting functional magnetic resonance and diffusion tensor imaging resulted in the first multimodal study of working memory dysfunction in a chronic stage traumatic brain injury. We related the ability to deactivate one of the most important resting-state networks following cognitive dysfunction and identified the main white matter structures involved in working memory deficits. Furthermore, the frontal activity measured from the resting-state differences between patients and controls was found to be an indicator of the global cognitive outcome of traumatic brain injury patients.

It is my hope that the research work carried out so far may be seen as contributing to the state of knowledge of the area of cognitive impairment after traumatic brain injury with diffuse pathology by establishing the neuroanatomical bases that may contribute to improving clinical management and rehabilitation programmes.

1. INTRODUCTION

Traumatic Brain Injury is defined as an alteration in brain function,
or other evidence of brain pathology,
caused by an external force
(Menon et al., 2010)

Traumatic brain injury (TBI) is the leading cause of death and disability in young adults around the world. On average, 39% of patients with severe TBI die from their injury and 60% have an unfavourable outcome. Survivors face prolonged care and rehabilitation, and have long-term physical, cognitive and psychological disorders that affect their independence (Rosenfeld et al., 2012), contributing to poor social and vocational integration. The main aetiology of traumatic injuries is road traffic accidents and falls and TBI is considered as a "silent epidemic" since society is largely unaware of the magnitude of this problem (Langlois and Sattin, 2005) and standardised monitoring of TBI in Europe is deficient. The variability in both diagnostic criteria and case ascertainment contributes to the inconsistency of incidence and estimation and confounds comparison between studies (Roozenbeek et al., 2013). Thus, more standardised research is needed. Advances in TBI research are few and the slow rate of research progress can be attributed to the heterogeneity of TBI and poor understanding of its pathology and prognosis, which leads to potential therapies not always being tested in the people most likely to benefit.

While it is to be hoped that prevention efforts will lead to a reduction in the incidence of TBI, it will always be necessary to understand these injuries when they do occur and so it is essential to deepen our knowledge of their brain neural substrates and its relation with cognitive and behavioural outcome so as to be able to improve the efficacy of clinical interventions.

1.1. THEORETICAL FRAMEWORK OF TRAUMATIC BRAIN INJURY

1.1.1. Classification

Clinical severity

TBI is an alteration in brain function when the head is hit by an external mechanical force. Loss or alteration of the conscious state is the cardinal feature of TBI. The duration and degree of alteration is of major significance in indicating the severity of the injury. The Glasgow Coma Scale (GCS; Teasdale and Jennett, 1974), is the most widely-used physiological measure for the classification of TBI severity at the initial assessment stage. GCS uses a scoring system to evaluate the best ocular, verbal and motor responses. A normal healthy person will obtain, by adding up the eye opening, verbal and motor scores, a GCS score of 15. This scale permits the following classification: mild head injury (GCS= 13-15); moderate head injury (GCS= 9-12); severe head injury (GCS=3-8). This measure gives no indication of the underlying structural or functional basis of impairment. Thus, patients with the same GCS may have different underlying pathological lesions altering different functions.

The severity of TBI may be also assessed according to the duration of loss of consciousness and post-traumatic amnesia (PTA), a temporary stage of confusion or memory loss that occurs immediately following traumatic brain injury. Mild TBI is defined as loss of consciousness less than 1h or amnesia of less than 24h; in moderate TBI there is a loss of consciousness of 1-24 hours or post-traumatic amnesia for 1-7 days; in severe TBI there is loss of consciousness for more than 24 hours or post-traumatic amnesia for more than a week (Blumbergs et al., 2008).

Mechanism and structural damage

PENETRATING HEAD TRAUMA is a wound in which a projectile breaches the cranium. These injuries can be induced by either non-missile, low-velocity objects or by high velocity objects such as bullets. The outcome is typically better in the case of penetration by low-velocity objects as the primary injuries are more localized. High velocity objects, on the other hand, result in more complex injuries and a high rate of mortality.

CLOSED HEAD INJURY is a trauma in which the brain is injured as a result of a blow to the head, or a sudden violent motion that causes the brain to impact against the vault of the skull. After the impact, shock waves propagate throughout the skull from the point of impact, as well as directly through the brain. These shock waves cause local changes in tissue pressure and if sufficient brain distortion, then small localised intraparenchimal petechial haemorrhages result (Gennarelli and Graham, 1998; Graham et al., 1995).

1.1.2. Neuropathology of brain damage

Brain damage resulting in head injury is highly complex due to the heterogeneous nature of the pathology. The process of head injury can be separated into: primary injury, secondary or progressive injury.

Types of brain damage

PRIMARY DAMAGE occurs at the moment of the injury and is caused directly as a consequence of the direct impact either by mechanical or external forces producing tissue deformation. These deformations may directly damage blood vessels, axons, neurons and glia in a focal, multifocal or diffuse pattern. Diffuse injuries (axonal, DAI; vascular, DVI), contusions, laceration, and intracerebral, subarachnoid, subdural, extradural (epidural) haemorrhages are included as primary damage. The nature, intensity and duration of the dynamic and evolving processes of the primary damage determine the pattern and extent of the secondary damage (Maas et al., 2008).

SECONDARY DAMAGE occurs as a consequence of the primary damage and includes ischemic and hypoxic damage, cerebral swelling, consequences of raised intracraneal pressure (ICP), hydrocephalus, and infection. A cascade of biological reactions occurs:

1) Microcirculatory derangements involving stenosis and loss of microvasculature.
2) Reversal of glutamate uptake.
3) Neuronal depolarization through excitotoxic mechanisms.
4) Generation of free radicals, mitochondrial dysfunction and postsynaptic receptor modifications.
5) Calcium influx in injuries to white and grey matter.
6) Calcium influx into axons initiating a series of protein degradation cascades that result in axonal disconnection.
7) Proliferation of astrocytes.
8) Inflammatory cells also mediate secondary injury contributing to the activation of cell-death cascades or postsynaptic receptor modifications.

(See Park et al., 2008; Büki and Povlishock, 2006)

The delayed consequences of secondary injury evolve up to years' post-injury cumulatively interacting with the evolving consequences of primary injuries. Delayed consequences include processes such as atrophy, gliosis, neural deafferentation and reinnervation, Wallerian degeneration, trans-synaptic degeneration immune reactions, synaptic plasticity, and neurogenesis (Blumbergs et al., 2008)

Pathology of brain damage

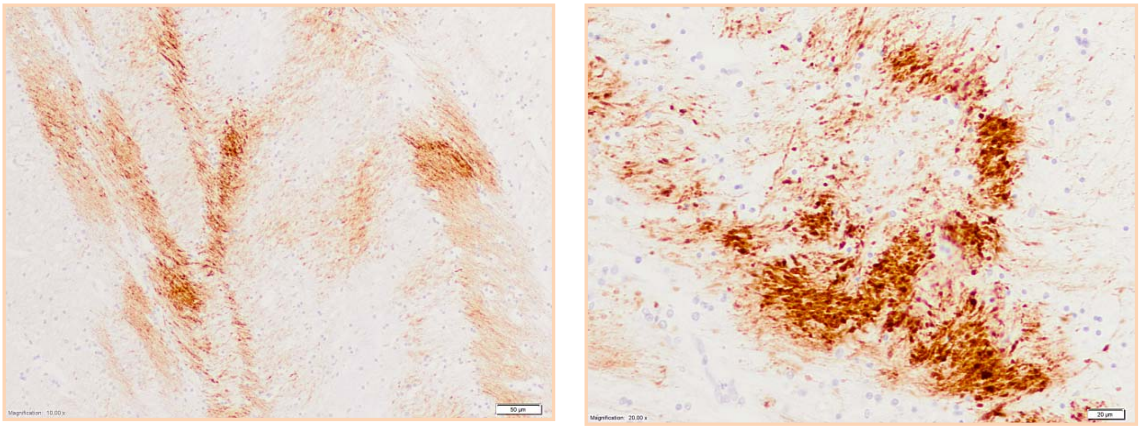
FOCAL DAMAGE includes contusions on the surface of the brain, including haemorrhages and the formation of haematomas in the extradural, subdural, subarachnoid, and intracerebral areas (Gennarelli, 1993). The impact of the brain against the skull results in contusions being typically observed in the inferior aspect of the frontal lobes, inferolateral part and poles of the temporal lobes and the cortex above and below the Sylvian fissure given the characteristic morphology that the skull has. The occipital lobes and cerebellum are rarely contused in the absence of skull fractures (Adams et al., 1989b, 1980).

In general focal lesions provoke a more direct dysfunction by direct local damage of the neural tissue and only induce coma when they are large enough to cause brain shifts, herniation or brainstem compression. The effect *coup-contrecoup*, is the most common contusion type injury also known as an acceleration/deceleration injury. Coup impacts are more common when the motion of the head is accelerated causing damage beneath the site of impact in the absence of fracture. Afterwards, *countercoup* contusions occur opposite or away from the site of the impact as the brain strains and bounces back into the opposite side of the skull (Gennarelli and Graham, 1998; Ommaya et al., 1971).

DIFFUSE DAMAGE, also known as diffuse axonal injury (DAI), is estimated to be the predominant mechanism of injury in up to 50% of TBI patients (Meythaler et al., 2001). It is characterised by multiple focal small haemorrhages throughout the brain. It was initially defined as widespread damage to axons throughout the white matter evoked by intense shear and strain forces resulting from rapid acceleration and deceleration of the brain with or without impact after TBI. DAI is frequently related with post-traumatic coma alterations in the absence of mass lesions (Adams, 1982; Gennarelli et al., 1982). Damage of white matter, especially damage anatomically concentrated in the brain stem, interrupts the parallel distributed neuronal networks for arousal and awareness producing coma. The most common locations of DAI as determined by pathological studies are cerebral hemispheres, corpus callosum (CC), and brainstem. However, other regions, such as the thalamus, basal ganglia or fornices are susceptible to diffuse damage. The severity of DAI has been graded according to the location of the lesion: Grade I, DAI only involving encephalic of the grey-white matter junction; Grade II, which also involves the corpus callosum in addition to the grey-white junction; and, Type III, which involves the rostral brainstem in addition to the two previous regions (Adams et al., 1989a). Diffuse damage makes the main contribution to neurological dysfunction in TBI patients and typically associated with poor prognosis (Smith and Meaney, 2000).

In the absence of significant focal contusions, diffuse brain damage may be not detected on neuroimaging MRI initial scans and macroscopical brain examination, and only may be apparent on microscopic evaluation.

Histopathologically, DAI is characterised by axonal swellings and retraction bulbs scattered throughout the white matter (Fig.1) .



Neurological Tissue Bank - Biobanc, Hospital Clínic de Barcelona- IDIBAPS. Courtesy of Dr. Gelpí

Fig1. Left. Zigzag pattern of amyloid precursor protein (APP)- immunoreactive axonal injury; Right. APP axonal swellings and axonal retraction bulbs.

Moreover, DAI is characterized by Wallerian-type axonal degeneration. Once the axon is disrupted, retrograde or anterograde degeneration evolves causing white matter atrophy and structural changes in the brainstem, pyramidal tract, lobar white matter, compensatory enlargement of the ventricles and thinning of the corpus callosum (D I Graham et al., 1995). However, as originally believed, the majority of traumatically injured axons, are not mechanically severed at the time of the impact (Adams et al., 1982), instead they show progressive changes that progress gradually to axon disconnection (Maxwell et al., 1997; Povlishock and Christman, 1995). Thus, immediate primary axotomy may occur due to DAI, but often disruption is delayed. Delayed secondary axotomy processes develop over hours and days after injury. In a quantitative histopathological study (Maxwell et al., 2010) of the cerebral cortex of forty-eight patients that suffered TBI, greater loss of large pyramidal and large non-pyramidal neurons correlated with a more severe score on GSC. It should be noted that loss of pyramidal neurons was greatest in the prefrontal cortex of the patients with diffuse axonal injury.

More recently, **TRAUMATIC AXONAL INJURY** (TAI) has been suggested as a more appropriate term for describing diffuse axonal damage. This term encompasses not only the primary axonal damage specifically caused by shear/strain injury but also the secondary injury, including cytoskeletal disorganization, protein accumulation (amyloid precursor protein), metabolic, hypoxic and microvascular damage, and excitotoxicity, which can provoke the abovementioned delayed axonal disconnection (Büki and Povlishock, 2006; Povlishock and Katz, 2005). Thus, TAI will be the term used in this thesis to refer to the global diffuse damage post- injury.

Focal contusions and diffuse lesions can occur after TBI and very often coexist. However, not all head traumas result in large lesions and neuropathological evolving mechanisms of injury between contusions and diffuse damage differ. The risk of persistent impairment is related to the initial severity and type of damage and is difficult to predict due to the heterogeneity of the pathology. Subsequently, in order to describe the impairment that is specifically due to white matter damage, patients with major focal lesions have been excluded from the studies of this thesis and the main focus of attention has been kept on specific diffuse white matter damage after TBI.

1.2. COGNITIVE OUTCOME

The recovery process after TBI includes three distinct phases from the acute to chronic stages (Katz and Alexander, 1994). The first phase, immediately after injury, is unconsciousness. The second phase occurs when patients regain consciousness or begin to emerge from the coma state. TBI patients go through a period of temporary confusion or memory loss. Retrograde amnesia appears which inhibits patients from being able to remember events that happened near the time to the accident. Furthermore, the patients suffer disorientation and are unable to remember events in the period immediately after the injury period, PTA. This period can last days or weeks and even months in severe cases. The resolution of confusion and PTA does not imply that the patient's memory has returned to normal. There follows a third phase during which cognitive functions are restored to a greater or lesser extent (Povlishock and Katz, 2005).

Traumatic axonal injury results in shear stress on axons and may result in reversible injury or complete disruption of critical white matter pathways between the cortex and deep grey matter structures (Smith et al., 2003; Warner et al., 2010). Thus it is likely that TAI makes a major contribution to cognitive deficits after TBI. Cognitive dysfunction is one of the most prevalent and troublesome sequelae after TBI (Whitnall et al., 2006; Dikmen et al., 2009).

The main cognitive domains altered after TBI are memory systems (Levin, 1995; Bigler, 2003; Scheid et al., 2006). Deficits in declarative memory - the capacity for conscious recollection of facts and events - are a common consequence of head trauma that is disproportionately suffered in comparison with other cognitive functions (Levin et al., 1988). The cause of memory disorders following TBI may be secondary to the relatively predictable pattern of diffuse and focal neuropathology sustained after TBI. The neuroanatomical areas that subserve memory storage, the temporal lobes, are frequently damaged after TBI (Simons and Spiers, 2003). The medial temporal lobe includes a system of anatomical structures that are essential for declarative memory. The system includes the hippocampus (CA fields, dentate gyrus, and sublicular complex) and the adjacent perirhinal, enthorinal, and parahippocampal cortices (Squire et al., 2004). The hippocampi may be damaged after TBI directly by contusions, raised intracranial pressure, seizures and hypoxia-ischaemia. Moreover, declarative memory also depends on the integrity of the hippocampus and its connections with the neocortex (Eichenbaum, 2000). Additionally, damage to the hippocampus and its connections may also be a result of neuroexcitotoxicity after the injury rather than purely an effect of the direct damage (Tate and Bigler, 2000).

Working memory is defined as the ability to maintain and manipulate information temporarily (Baddeley, 2003). There is considerable evidence that working memory depends on network activity including the frontal and parietal regions. A meta-analysis of functional neuroimaging studies conducted by Owen et al., (2005) provided strong evidence for the activation of frontal and parietal cortical regions by various versions of the n-back paradigm. Working memory arises in the dorsal premotor areas and supports the maintenance and manipulation of data. The superior frontal gyrus, in particular, is activated during working memory tasks and the lateral areas of the

prefrontal cortex are thought to reflect control operations involved in memory retrieval areas (D'Esposito et al., 1999; Boisgueheneuc et al., 2006; Mohr et al., 2006; Velanova et al., 2003). Impairment of this memory is frequent in moderate to severe TBI patients given that the frontal cortex is highly vulnerable in this type of injury (McAllister, 2008).

Attention deficits are also commonly altered after TBI (Stuss et al., 1989; Stuss et al., 2003). These deficits may not appear until sufficient cognitive load is placed as a demand on the patient's brain. Attention deficits include sustained, divided, and selective attention. Sustained attention refers to the ability to maintain concentration on a task during a period of time. Divided attention is the ability to attend to two or more tasks simultaneously and, selective attention is the ability to focus on a specific target despite the presence of other stimuli. The most common problems are related to multitasking attention where a component of working memory may also be involved. There are different brain networks supporting attention systems. One of the most important networks is the fronto–striato–thalamo–cortical loop. Neuroanatomically, neuroimaging studies have shown that the main areas involved in the different types of attention are frontoparietal regions particularly in the right hemisphere in conjunction with the anterior cingulate cortex, temporo-parietal junction, pulvinar, and the superior colliculus (Raz and Buhle, 2006; Umarova et al., 2010).

Slowed information processing speed is sensitive and well documented cognitive sequelae of head trauma. Information processing speed alterations are usually as a consequence of widespread diffuse white matter damage due to frontal lobe injury, damage in any of the circuits that support storage and retrieval of information, and damage to multiple sites in the brain (Granacher et al., 2008).

1.3. NEUROIMAGING AND NEUROPSYCHOLOGY STUDIES IN TRAUMATIC BRAIN INJURY.

There are several neuroimaging methods to assess brain injury after TBI:

VOLUMETRY allows the study of the white and grey matter and subcortical structures to be studied with a high level of precision and to relate them with clinical or behavioural measures. From high-resolution MRI acquisitions, it is possible to measure differences

on tissue concentration at a voxel-wise level, voxel-Based Morphology (VBM), as well as differences on whole-brain or regional volumes using region of interest (ROI) based approaches. By using such techniques it has been reported that grey and white matter degeneration are significant contributors to brain volume loss in the months following brain injury (Bigler and Maxwell, 2011; Farbota et al., 2012; Sidaros et al., 2009).

DIFFUSION TENSOR IMAGING (DTI) is a magnetic resonance based imaging modality that measures local microstructural characteristics of water diffusion within brain tissues. It has a unique capability to delineate axonal tracts within the white matter, which was not possible with previous non-invasive techniques (Mori and Zhang, 2006). Due to the presence of myelin sheaths and microstructural components of axons in white matter, water molecules move more freely along than perpendicularly to the long axis of the fibre, a phenomenon that is known as anisotropic diffusion. The anisotropy is higher than in less-organized brain tissues and this difference makes it possible to calculate the fractional anisotropy (FA). This allows to generate white matter fibre maps, which are believed to reveal the degree of myelination and axonal density and/or integrity (Arfanakis et al., 2002; Kraus et al., 2007). FA values range from 0 to 1 where 0 represents isotropic diffusion, or lack of directional organization, and 1 represents anisotropic diffusion, or organized tissues such as that which is found in white matter tracts (Le Bihan, 2003). Disruption of the structural integrity of white matter may alter both the magnitude and the anisotropy of the water diffusion, allowing us to investigate white matter abnormalities under pathological conditions. In chronic moderate to severe TBI, reduced FA is the most reported finding even without focal lesions due to TAI (Kraus et al., 2007; Marquez de la Plata et al., 2011b; Nakayama et al., 2006; Xu et al., 2007). However, the relationship between of white matter alteration integrity and cognitive function in TBI is not yet clear (See Table 1 for main TBI findings).

CORTICAL THICKNESS measurement of the human cerebral cortex in vivo could provide a powerful tool for diagnosing and studying a variety of neurological disorders. This is enabled by a procedure generating highly accurate models of both the white and pial surfaces. The distance between these two surfaces indicates the thickness of the cortical grey matter at any given point (Fischl and Dale, 2000).

In MRI studies of TBI, grey matter atrophy has mainly been investigated by means of VBM. However, grey matter atrophy measured by VBM includes the local cortical surface and cortical folding, and depends on the overall brain size (Hutton et al., 2009). Thus, estimation of cortical thickness may be a better alternative for measuring grey matter atrophy. Cortical thinning has been reported in paediatric samples after TBI. Merkley et al., 2008 described a diffuse pattern of cortical atrophy involving cortical regions in all lobes, while the pattern found by McCauley et al., 2010 showed frontal predominance. However, it is noteworthy that studies based on children are influenced by neurodevelopmental brain changes and cannot be extrapolated to an adult TBI population (Shaw et al., 2008). Thus, adult studies are needed in order to better describe the patterns of cortical thinning after TBI.

FUNCTIONAL MRI ANALYSIS (fMRI) measures the hemodynamic response or blood-oxygen-level-dependent (BOLD signal) related to neural activity in the human brain. It is a well suited technique to investigate neural responses due to a specific action or external stimulus and to examine spontaneous brain activity fluctuations during resting-state. Moreover, novel imaging analysis techniques allow the identification of brain systems or functional networks formed by spatially remote areas showing temporally correlated activity, a term commonly defined as functional connectivity. Interactions between remote brain regions produce distributed brain networks with distinct functions (Ham and Sharp, 2012). Furthermore, nodes of these networks show highly consistent interactions that can reflect the underlying structure of white matter connections within the brain (van den Heuvel and Pol, 2010).

After TBI, the white matter damage alters structural connectivity which, in turn, can affect functional connectivity, contributing to cognitive dysfunctions in TBI (Marquez de la Plata et al., 2011a; Sharp et al., 2011). Task-related fMRI techniques have been widely used to study functional abnormalities in TBI. Findings showed patterns of increased or decreased activations in the task-associated areas (Newsome et al., 2007; Sánchez-Carrión et al., 2008; Turner et al., 2011), as well as disrupted connectivity within functional networks related to task execution (Bonnelle et al., 2011; Sharp et al., 2011). Furthermore, resting-state fMRI studies have recently emerged as a useful tool to investigate brain functional connectivity after TBI (Bonnelle et al., 2012; Mayer et al., 2011). These studies can provide information about brain activity and connectivity of

spontaneous brain oscillations in the absence of task performance, a condition that allows researchers to investigate patient populations with broader degrees of severities since no specific cognitive ability is required. Different functional brain resting connectivity networks have been identified and proved to be consistent across subjects (Damosieaux et al., 2006). However, one of the most widely studied networks that should be mentioned is the default mode network (DMN). The spatial pattern of the DMN includes the ventromedial prefrontal cortex, the posterior cingulate cortex, the lateral parietal cortex, and the precuneus. This network is reported to be affected in a broad range of brain disorders and is commonly related to cognitive processes (Buckner et al., 2008). In TBI, it has been reported that the failure of the DMN to deactivate this network when a task is presented is associated with inefficient cognitive function (See Table 1 for functional connectivity findings of DMN after TBI). Another measure derived from resting-state fMRI acquisition is the Amplitude of Low Frequency Fluctuations (ALFF) which refer to the Spectral Power of the BOLD signal within the frequencies of interest and that can be considered as a measure of regional spontaneous neuronal activity (Zang et al., 2007). This measure has not been previously explored after TBI and is presented as a novelty in the fourth study of this thesis.

Table 1. Recent and related clinical and cognitive findings in adult moderate-severe TBI from neuroimaging studies.

Study	**TBI Sample**	**Summary of the main findings**
Reference and title	*N(px/control), $\bar{x}$ age px, severity* *MRI analysis* *Neuroimaging metrics* *Cognitive or Clinical function assessed (Questionnaire / Test)*	
Kraus et al., 2007 *White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study.*	N=45; $\bar{x}$ years(MildTBI)=35.85 , $\bar{x}$ years 34.8 (M/STBI); DTI: ROI Analysis, White matter load (DTI-Studio, SPM); Executive functions (TOL, Stroop, PASAT, TrailB, CPT, COWAT, RUFF, Digit/Spatial Span backward), Attention (Digit/Spatial Scan forward, TrailA, CPT-omission), Memory (CVLT, BVMT).	M/STBI ↓FA than controls and MTBI. ROI-FA: M/STBI < controls in all ROIs; Mild TBI > controls in SLF, sagital stratum, and CST. White matter load: controls < MildTBI <M/STBI. ↑AD/RD in S/MTBI. RD less affected in MildTBI → high axonal damage but less myelin damage. Whole brain FA correlated with executive and memory functions more than ROIs (widespread damage of networks).
Newcombe et al., 2007 *Analysis of acute traumatic axonal injury using diffusion tensor imaging.*	N =61; $\bar{x}$ years=34.6; $\bar{x}$ GCS=6.12 (Mild/M/STBI); DTI: whole brain white matter ROI and focal ROI in the splenium CC (SPM); GOS.	↓FA, ↑ADC in the whole brain white matter and CC ROIs. Increase in RD. No changes in AD. No correlations with outcome or severity.
Wang et al., 2008 *Diffusion tensor tractography of traumatic brain injury.*	N=12; $\bar{x}$ years=26; $\bar{x}$ GCS=4.4; DTI: fibre tracking multiple ROI approach; FA, 6 fibre variables: CC, fornix, peduncular projections (DTI-Studio, FSL); GOSE.	TAI associated alterations for each ROI tract (↓FA). CC fibre association with GOSE.
Sidaros et al., 2008 *Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study.*	N= 37; $\bar{x}$ years=32.1; GCS ≤8, STBI; Visit 1 : ~8 weeks post-injury Visit 2: ~12 months post-injury; DTI: Manual ROIs: PCC, PLIC, CSO, CP, PUT (SPM); FA, MD, RD, AD; Functional outcome at 12 months (Glasgow	First scan: ↓FA in all ROIs, no difference in MD, caused by decreased AD and increased RD. Longitudinal: FA increase in PLIC and CSO, and decreased in PCC. AD increased in all ROIs, RD increased in PCC and CP. Higher FA in CP related to higher GOS. Favourable outcome group: FA returned to normal in PLIC and CSO.

	Outcome Scale, GOS).	Correlation between individual RD in PLIC and GOS. Unfavourable outcome group: lower FA in PCC and CP. Microstructure reorganization during the scan interval.
Sidaros et al., 2009 *Long-term global and regional brain volume changes following severe traumatic brain injury: A longitudinal study with clinical correlates.*	N= 38; $\bar{x}$ years=33.2; GCS ≤8, STBI; Visit 1: ~8 weeks post-injury Visit 2: ~12 months post-injury; T1: Global brain volume change (SIENA), BPV (SIENAX); Regional volume change (SPM-TBM); Functional outcome at 12 months (GOS). Functional independence measure(FIM).	Reduced whole brain volume at 8 weeks and volume loss between scans. Regional: volume loss in brain stem, cerebellar peduncles, thalamus, internal capsule, external capsule, putamen, inferior/superior longitudinal fasciculus, CC, corona radiata. Correlation between first scan-BPV and PTA. FIM correlated with first scan-BPV and with % change. Volume change differences between GOS groups.
Wang et al., 2011 *Longitudinal changes of structural connectivity in traumatic axonal injury.*	N=48; $\bar{x}$ years=26; $\bar{x}$ GCS=4.4; DTI tractography (FSL, DTI Studio); FA, MD; Weshler Adult Processing Speed subtests, TMT, Stroop, COWAT, CVLT; GCS.	Longitudinal changes in white matter tracts in patients (↓FA, ↑MD) due to TAI. Changes in tractography measures predicted long-term outcomes. Acute tractography measurements predicted learning and memory performance; in chronic stage measurements they were related with speed processing and executive functions in the patients group. Outcome positive correlation in acute stage with MD in tracts that became negative in chronic stage.
Bendlin et al., 2008 *Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric analysis.*	N=71; $\bar{x}$ years=28.47; GCS =<13 (post-resuscitation GCS >=6); Visit 1: ~2 months post-injury Visit 2: ~1 year post-injury; DTI: FA and MD maps (FSL); T1: VBM (SPM); Interaction group x visit. Multiple regression (SPM): WM-vol, GM-vol, FA, MD, GCS, cognition (visit 2); CVLT, BVMT, COWAT, digit symbol, TMTB.	Cross-sectional: ↓FA, ↑MD. Little differences in WM volume. ↓GM volume. Longitudinal: ↓FA in frontal and temporal lobes. ↑MD is more diffuse and extended. WM and GM volume decline in TBI. GCS correlated positively with FA, GM volume and WM volume and negatively with MD. Memory negative correlation with MD. Executive function: higher correlation with WM volume. DTI greater cross-sectional changes and white matter volume over the long term.
Nakamura et al., 2009 *Resting network plasticity following brain injury.*	N=14; $\bar{x}$ years=19-55 years; STBI (GCS: 3-8); Visit 1(V1): ~3 months after PTA Visit 2(V2): ~6 months after PTA; Resting-fMRI from off-task blocks;	Same number of connections. Reduced strength of connectivity between V1 and V2 in TBI. At V1: TBI have greater local efficiency and clustering

	Partial correlations -> graph theory; Path length / Clustering /Global and local efficiency /degree distribution Weighted networks.	than control. TBI have larger number of high interconnected regions than V2 and control. Global and local efficiency reduced between V1 and V2. Increased path length.
Kumar et al., 2009a *Serial changes in the white matter diffusion tensor imaging metrics in moderate traumatic brain injury and correlation with neuro-cognitive function.*	N= 68; $\bar{x}$ years=30; $\bar{x}$ GCS = 10.8, MTBI: 3 groups: H-DAI / Nh-DAI DAI /no DAI; Visit 1: ~9.6 days Visit 2: ~6 months; DTI: ROI analysis (in-house-developed software) FA and MD; Neuropsychological assessment: memory, executive functions, processing speed.	Baseline: ↓FA in the genu in H-DAI compared with Nh-DAI and control. ↓FA in ALIC and PLIC for all patients compared to controls. No change in MD. Follow-up: ↓FA and ↑MD in rostrum of CC. Genu: ↓FA in all patients and MD increase only in Nh-DAI. Splenium: ↓FA, no MD change. ↓FA in ALIC and PLIC. Correlations with cognitive functions.
Kumar et al., 2009b *Serial changes in diffusion tensor imaging metrics of corpus callosum in moderate traumatic brain injury patients and their correlation with neuropsychometric tests: a 2-year follow-up study.*	N= 33. $\bar{x}$ years=30. $\bar{x}$ GCS = 10.8, MTBI; 3 groups: H-DAI / Nh-DAI Visit 1 (V1): ~9.6 days Visit 2 (V2): ~6 months Visit 3 (V3): ~24 months DTI: ROI analysis. 7 segments of CC. Metrics: FA, MD, RD, AD (in-house-developed software); Neuropsychological assessment: memory, executive functions, processing speed.	FA-findings: ↓FA in genu in patients at 3 timepoints. ↑FA in the genu at V2 and V3 compared with V1 in TBI, ↓FA in the midbody from V2 to V3. AD-findings: no change. RD-findings: TBI ↑RD in genu at V1. Decrease in ↓FA at V3 compared with V1. Correlations of FA and MD with neuropsychological tests at 6 months. Recovery of FA and RD in some regions of CC associate with neuropsychological improvement.
Warner et al., 2010b *Regionally selective atrophy after traumatic axonal injury.*	N=47; $\bar{x}$ years=26.8; $\bar{x}$ GCS=6.2; Visit 1: ~1 week postinjury Visit 2: ~8 months postinjury; Global and regional brain volumes (FreeSurfer morphometry); GOSE	Selective vulnerability to atrophy of some brain regions after TAI. ↓Volumes in amygdala, hippocampus, thalamus, CC, PUT, PCC, post-central gyrus, paracentral lobule, and parietal and frontal cortices. ↓Whole brain parenchymal and some specific cortical regions volumes predictive of GOSE.
Warner et al., 2010a *Assessing spatial relationships between axonal integrity, regional brain volumes, and neuropsychological outcomes after traumatic brain injury.*	N=24; $\bar{x}$ years=27.2; $\bar{x}$ GCS=4.5; Visit 1: ~1 week postinjury Visit 2: ~8 months postinjury; DTI: tractography ROI, Regional Brain Volumes: amygdala, hippocampus, thalamus, CC; inferior/superior parietal,	↓FA related with volumes to their related grey matter structures. Volume of the thalamus related with information speed. Amygdala and hippocampal volumes associated with learning and memory performance. TAI as a primary measure of post-traumatic grey/white

	superior frontal and precuneus cortical regions; (FSL, DTI studio, FreeSurfer); FA, MD, volumes; Digit Symbol; Symbol search; TMT A/B; COWAT; Stroop; CVLT.	matter atrophy and results in cognitive deficits.
Marquez de la Plata et al., 2011a *Deficits in functional connectivity of functional hippocampal and frontal lobe circuits after traumatic axonal injury.*	N=41; $\bar{x}$ years=30.28; $\bar{x}$ GCS=8.1, Mild/M/STBI; Interhemispheric functional connectivity, resting state connectivity (AFNI, FSL); ROI: hippocampus, ACC and DLPC; Digit Symbol; Symbol search; TMT A/B; COWAT; Stroop; CVLT.	Disrupted interhemispheric functional connectivity for the hippocampus and ACC in patients with TAI. Pattern of weaker diffuse functional connectivity for patients in contrast to the stronger and more focused functional connectivity of the control group in the hippocampi, and ACC and a more focused recruitment of the DMN in the dorsal prefrontal ROI.
Kinnunen et al., 2011 *White matter damage and cognitive impairment after traumatic brain Injury.*	N=54; $\bar{x}$ years=38.9; Mayo classification severity system=moderate, severe, mild; DTI (FSL: TBSS); WAIS similarities, WAIS matrix, TMTA/B, Delis-Kaplan colour, word reading, and inhibitions tasks, verbal fluencies, Choice reaction task.	Group-differences in ↓FA and ↑MD. Time since injury correlated with ↑AD and ↑MD. DTI indices in the fornices with associative memory and learning in the patients and control group. Relationship between frontal lobe connections and executive functions.
Sharp et al., 2011 *Default mode network functional and structural connectivity after traumatic brain injury.*	N=41; $\bar{x}$ years=38.9; Mayo classification severity system=moderate, severe, mild. DTI: ROI white matter tracts CC, corticospinal tract, SLF (FSL-FEAT); fMRI-rest, (FSL-ICA, ROI); fMRI-choice reaction task.	fMRI task: Patients showed greater deactivation of the DMN in the VMPFC. fMRI rest: Greater functional connectivity of the PCC associated with faster reaction times: adaptive response to impairment.Functional connectivity at rest predicted brain activation associated with choice-reaction task. MD in the splenium correlated with DMN connectivity in PCC.
Bonnelle et al., 2011 *Default mode network connectivity predicts sustained attention deficits after traumatic brain injury.*	n=58px̄; years=36.7; Mayo classification severity system=moderate, severe, mild; fMRI-task (activity/connectivity), DTI, VBM (FSL); WAIS similarities, WAIS matrix, verbal fluency, Stroop, TMTA/B, Digit Span subtest and logical memory I II, Doors and People test battery for delayed verbal recall.	Increased activation within the DMN, specifically in the PCC and posterior cingulate cortex associated to sustained attention deficits (shift away from sustained and focused goal-directed behaviour). The interaction between the PCC and the DMN network at the start of the task was predictive of those patients with impairment in the attention task. ↓FA right cingulum bundle associated with attention impairment.

Hillary et al., 2011b *Changes in resting connectivity during recovery from severe traumatic brain injury.*	N= 20; $\bar{x}$ years=29.4; $\bar{x}$ GCS=5.4; Visit 1: ~3 months after PTA Visit 2: ~6 months after PTA; Resting-fMRI from off-task blocks; Connectivity of 2 networks: goal directed (DLPFC-ACC) and DMN (PFC and PCC). Seed-based correlation maps.	TBI: increases in DMN and decreases in goal-directed tasks. Down-regulation of DLPFC to parietal cortex. Increased involvement of PCC connectivity. Increased connectivity to insula and middle temporal lobe.
Bonnelle et al., 2012 *Salience Network Integrity predicts Default Mode Network function after traumatic brain injury.*	n= 57px; $\bar{x}$ years=29.4; $\bar{x}$ GCS=5.4; fMRI(event-related analysis), DTI: tract connection from loci of activation functional task (FSL); SST task (RT).	No differences in task-activity. TBI patients showed failure on DMN deactivation associated with inhibitory control. Damage in one of the white matter tracts within the salience network predicted the deactivation of the DMN= integrity of Salience network is necessary for regulation of DMN activity, and consequently to inhibitory control.
Hillary et al., 2011a *Examining working memory task acquisition in a disrupted neural network.*	N= 24; $\bar{x}$ years=29.4; GCS=3.8, M/STBI; fMRI Task-activity (SPM) ROIs connectivity analysis; Letter N-back fMRI Digit Span of WAIS III; TMTA/B; Stroop.	Mild impairment on working memory tests. Expanded activity in right prefrontal cortex. ↑TBI connectivity within right hemisphere associated with faster response. Deficits in left hemisphere. Involvement of right-prefrontal cortex. Anterior to posterior shift in TBI
Newcombe et al., 2011a *Mapping traumatic axonal injury using diffusion tensor imaging: correlations with functional outcome.*	N=104; $\bar{x}$ years=32-39; DTI: FA/ADC maps(FSL); ROI: whole brain WM, supratentorial WM, right/left cerebellar peduncles, cerebellar cortex, pons, midbrain, thalamus, and CC (Analyze); GOS.	Gradations of DTI abnormality with patients with worse clinical outcomes (↓FA, ↑ADC): differences between all patients in all the ROIs investigated except for midbrain and pons which were only significantly different in the patients with poorest outcomes. Functional deficits observed in TBI may be a consequence of damage to integrated neuronal systems rather than lesions at focal injury sites.
Newcombe et al., 2011b *Parcellating neuroanatomical basis of impaired decision-making in traumatic brain injury.*	N=88; $\bar{x}$ years=36.5; $\bar{x}$ GCS=7; DTI: ADC maps (FSL) ROIs: medial prefrontal cortex, ventrolateral prefrontal cortex, DLPFC, superior frontal gyrus, orbitofrontal gyrus, frontal white matter, hippocampus, insular cortex, thalamus, striatum, parietal cortex,	↑ADC correlations with Cambridge gambling task variables: Risk adjustment: thalamus, dorsal striatum (R) Impulsivity: Orbitofrontal gyrus, insular cortex (R/L), caudate (R). Rational Choice: ventrolateral prefrontal cortex (R), DLPFC, superior frontal gyrus (R/L), hippocampus (L).

	posterior CC (Analyze); Cambridge Gambling Task (risk adjustment, impulsivity index, rational choices, deliberation time, amount bet).	Amount bet: non-significant regions. Deliberation time: ventrolateral prefrontal cortex, DLPFC, superior frontal gyrus, orbitofrontal, insular cortex (R/L), medial prefrontal cortex (L).
Kasahara et al., 2011 *Traumatic brain injury alters the functional brain network mediating working memory.*	N=18; $\bar{x}$ years=31.8; $\bar{x}$ GCS=7.2; fMRI: task activation & connectivity (PPI) analyses (SPM); N-Back task (verbal).	(L)IPG: reduced fMRI activity in the TBI and correlation with accuracy in controls. (R)IFG: incremented activity in TBI correlated with task accuracy. Task accuracy related to the IPG for control group. PPI analyses showed compromised connectivity between (L) IPG and (R) IFG in TBI patients that may mediate altered verbal working memory performance in TBI.
Farbota et al., 2012a *Longitudinal diffusion tensor imaging and neuropsychological correlates in traumatic brain injury patients.*	N=21; GCS =<13 (GCS >=6); Visit 1: ~2 months post-injury Visit 2: ~1 year post-injury Visit 3: ~3 year post-injury; DTI: FA, AD, RD (SPM); COWAT, WRAT-III, finger tapping, digit span, TMT-A/B.	Patient improvement in tests TBI: ↓FA with time in CC. No difference in AD, but ↑RD No FA and CGS correlations. Correlation between TMTA/B improvement and FA increases: SS, SLF, OR. ↓FA in the genu of CC during the first year.
Medaglia et al., 2012 *The less BOLD the wiser: support for the latent resource hypothesis after traumatic brain injury.*	N=24; $\bar{x}$ years=32.1; $\bar{x}$ GCS=4.7, M/STBI; At least 1 year post-injury Analysis of activity(SPM); ROI analysis; Letter N-back fMRI (1,2-back); Digit Span of WAIS III, TMT-A/-B, Verbal comprehension WAIS-III, Stroop.	No differences in PFC activity at 2Back. Extensive recruitment of bilateral DLPFC, parietal and cingulate during 1Back. Reduced activity in DLPFC after task practice in TBI -> increased neural efficiency. Higher response does not facilitate better performance. Contribution of right-DLPF to cognitive challenge.

These studies synthesized in Table 1 reported structural and/or functional results with regards to brain alterations after TBI and their relation with clinical and cognitive outcomes.

Diffusion tensor imaging studies showed that DTI can qualitatively and quantitatively demonstrate white matter alterations not detected by other modalities. There is agreement in the finding that low FA is a characteristic of TBI although in some case FA increases are found in the acute stage. Decreases in FA indicate that there has been a loss of white matter integrity. Structurally, white matter alterations together with grey matter reductions measured from VBM analyses result in a brain volume loss which may be seen in longitudinal studies. On the other hand, brain functional alterations observed during cognitive tasks and during resting state are well documented as disruptions in connectivity. However, as TBI is a complex pathology involving different types of injuries, there are discrepancies in the results of cerebral damage obtained by neuroimaging studies and its association with clinical and cognitive variables. The specific reasons for these discrepancies may include: a) the demographic characteristics of patients involving subjects with a wide range of ages, which may lead to the neurodegenerative changes resulting from ageing being a confounding factor; b) the different times of evolution, which vary considerably between studies, is a variable that must also be taken into account in study designs. Depending on whether the patient is in acute, sub-acute and chronic stage, there is a different process of structural neurodegeneration coexisting with functional brain reorganization. Thus the changes in the brain may be a representation of these different phases rather than the outcome itself; c) the heterogeneity of the brain damage suffered by patients (focal versus diffuse damage). Considering patients with a diversity of areas of focal damage together with diffuse lesions in the same sample can produce confounding effects on the functional and structural brain reorganization; d) the fact that some studies mix patients with varying degrees of severity mostly measured by GCS (mild to severe) can add additional difficulties to the interpretation of the results; e) the variety of MRI acquisition, and the data analyses and approaches used, make it difficult to compare the results obtained by different research groups. All these differences represent a significant obstacle to summarising and reviewing the results as a whole and impede a specific understanding of the effects of the different brain damage after TBI. More homogeneous samples are needed in order to understand the

cognitive effects of brain injuries and outcome. For this reason, in the present thesis we have focused on severe, chronic and diffuse TBI, as the main target sample for the study. We chose a homogenous sample taking into account all confounding variables mentioned above in order to allow us to investigate in an optimal manner the effect of white matter alterations due to head injury in cognitive functions.

2. HYPOTHESES AND OBJECTIVES

MAIN HYPOTHESES AND MAIN OBJECTIVE

HYPOTHESIS: White matter damage is responsible for the alterations in connectivity making the main contribution to cognitive deficits after diffuse, chronic and severe TBI.

OBJECTIVE: To identify possible structural and functional connectivity alterations in the brains of patients suffering from diffuse axonal injury and to relate them with cognitive impairment by applying neuropsychological assessment and neuroimaging techniques.

SPECIFIC HYPOTHESES

1. Patients with TAI will present diffuse widespread white damage alteration throughout the brain compared to controls. Specifically:

    1.1. FA decreases in the superior longitudinal fasciculi will correlate with working memory deficits.

    1.2. FA decreases in the fornix will correlate with declarative memory impairment.

2. TBI patients will show cortical thickness reductions compared to the control group and this thinning will depend in part on the white matter alterations.

3. Hippocampal volume will be reduced and shape will be altered in TBI patients.

4. Memory impairment will be explained by damage to the hippocampus and cortical grey matter regions, as well as by their structural connectivity alterations.

5. White matter alterations will affect functional working memory and default mode networks explaining working memory deficits.

6. Altered activity and connectivity in resting state will be altered after TBI and will be related with general cognitive outcome.

SPECIFIC OBJECTIVES

1. To investigate the role of structural white matter damage connectivity in declarative and working memory deficits after TBI using DTI and focusing on the main associative fasciculi.

2. To assess structural cortical thickness and hippocampal volume by MRI in subjects with TBI to study their relationship with declarative memory impairment.

3. To explore the functional patterns of connectivity underlying working memory impairment after TBI using fMRI and to assess its relation with the structural connectivity alterations measured by DTI.

4. To explore abnormalities in brain activity and connectivity during resting state MRI and their relationship with structural tractography of the cingulum and neuropsychological measures of cognitive outcome.

3. MATERIAL AND METHODS

List of studies:

STUDY I. Palacios EM, Fernandez-Espejo D, Junque C, Sanchez-Carrion R, Roig T, Tormos JM, Bargallo N, Vendrell P. *Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury.* BMC Neurology 2011; 11:24.

STUDY II. Palacios EM, Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *Long term declarative memory deficits in diffuse TBI: Correlations of cortical thickness, white matter integrity and hippocampal volume.* Cortex 49 (3):646-57, 2012.

STUDY III. Palacios EM, Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *White matter integrity related to functional working memory networks in traumatic brain injury.* Neurology 20 (12):852-860, 2012.

STUDY IV. *Palacios EM, *Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *Resting-State Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury.* Archives of Neurology. 2013 (in press)
*Equal Contribution

3.1. SUBJECTS AND MRI DATA ACQUISITION

The papers presented here in this thesis studied subjects from two different TBI samples:

A. Sample for study I

A cross-sectional study of thirty-one subjects was performed. Fifteen patients (eleven male) with severe TBI were recruited from the Head Injury Unit of the Institut de Neurorehabilitació Guttmann. Inclusion criteria were: a) age <40 years, b) diffuse axonal injury according to clinical MRI without focal cortical lesions or larger than 1.5cm^3, c) severe TBI: defined as a minimal Glasgow Coma Scale (GCS) score ≤8 assessed at the first contact with the emergency services, d) emergence from the post-traumatic amnesia phase at the moment of the enrolment according to the Galveston Orientation and Attention Test, defined as two consecutive scores >65, and f) no previous history of TBI, drug intake, and neurological or psychiatric disorders.

The aetiology of TBI was a traffic accident in all cases. Fourteen patients were involved in car collisions, and one was a pedestrian hit by a motor vehicle. All patients had closed head injury and had not received surgery for extra- or subdural haematoma; all structural MRI scans were suggestive of TAI.

MRI DATA sets were acquired on a 1.5 T Signa GE (*General Electric, Milwaukee, WI*) at the Centre de Diagnostic per la Imatge of the Hospital Clínic (CDIC), Barcelona. Diffusion weighted images were sensitized in 25 non-collinear directions with a b-value=1000 sec/mm^2, using an echo-planar (EPI) sequence (TR=9999.996 ms, TE= 85 ms, 20 axial slices with a resolution of 0.9375 x 0.9375 mm, slice thickness=5 mm, gap=2 mm matrix size=128x128, FOV=100mm).

B. Sample for studies II, III, and IV

We recruited 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 (GCS) 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 macroscopical lesions. The exclusion criteria were (1) visual, sensorial, or visuoperceptual deficits; (2) previous history of TBI, drug intake, neurological, 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 was obtained, who were then included in a study of the long-term consequences of severe TBI. The aetiology of TBI was traffic accident in all cases. Since we were interested in diffuse white matter injury after TBI, the neuroradiologist described the chronic brain lesions seen in the MRI. In line with previous studies of traumatic axonal injury, patients with large lesions were excluded as our interest were non-focal injuries. This left us with 26 subjects: all of whom had acquired structural imaging, 19 patients had fMRI acquisitions and 21 patients had resting state scans after inspection for motion artefacts.

Twenty two healthy volunteers matched by age, sex, education, and handedness were recruited as the control group. None had a previous history of neurological or psychiatric diseases and brain scans were reported as normal. Handedness in patients was evaluated by premorbid writing hand preference.

THE MRI DATA were acquired on a SIEMENS Magnetom TrioTim syngo 3-Tesla at the Diagnostic Imaging Centre of the Hospital Clinic (CDIC) in Barcelona. For each subject a high resolution T1-weighted structural image scan was obtained using an MPRAGE 3D protocol (TR=2300 ms; TE=3 ms; TI=900 ms; FOV=244mm; 1mm isotropic voxel). Diffusion weighted images were sensitized in 30 non-collinear directions with a b-value=1000 s/mm^2, using an echo-planar (EPI) sequence (TR=9300 ms, TE= 94 ms, slice thickness=2.0 mm, voxel size=2.0×2.0×2.0 mm, FOV=240 mm, no gap). A single shot gradient-echo EPI-sequence (TR=2000 ms, TE=16 ms; flip angle=90 grad; FOV=220 mm; voxel size=1.7×1.7×3.0 mm) was used for fMRI. Visual stimuli were projected on a screen seen through a mirror mounted on the head coil.

For the lesion description, the neuroradiologist (NB) took into account T1-weighted, FLAIR (TR=9000ms, TE=85ms, voxel size=3.0mm, voxel size= 1.3×0.9×3.0 mm, FOV=240 mm), and T2* GE (TR=518ms, TE=20ms, voxel size=3.0mm, voxel size= 0.9×0.8×3.0 mm, FOV=240 mm) sequences.

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

3.2. NEUROPSYCHOLOGICAL ASSESSMENT AND FMRI TASK

To evaluate the neurocognitive status of the subjects, a trained neuropsychologist administered the tests assessing executive function, verbal and visual memory, visuoperception and processing speed, all of which are common deficits after TBI.

The following neuropsychological tests were selected:

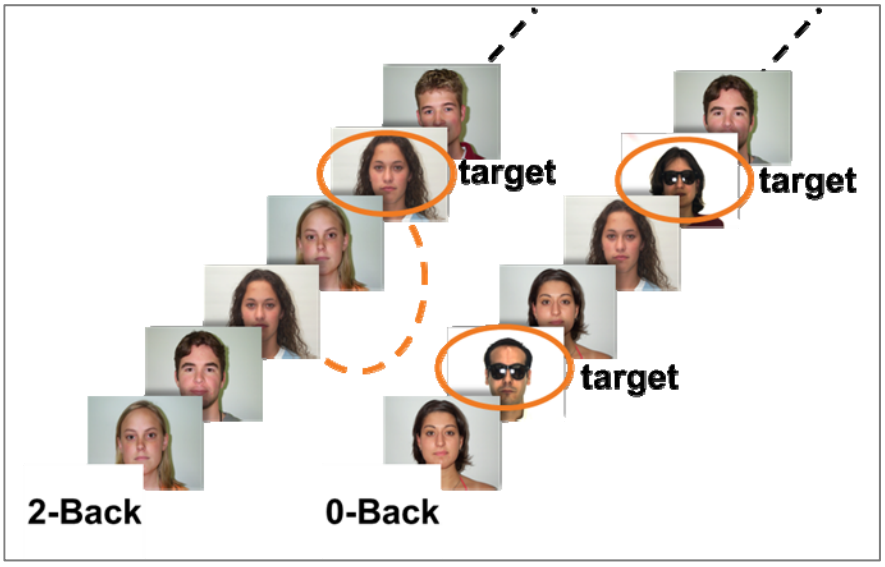
- Letter-number sequencing (Wechsler, WAIS-III)
- Digit span forward and backwards test (Wechsler, WAIS-III)
- Trail making test (A/B)
- Rey auditory verbal learning test
- Rey-Osterrieth complex figure
- Stroop reading and colour-naming conditions
- Phonemic verbal fluency
- Semantic verbal fluency

(See Lezak, 2004)

fMRI working mwmory task

The N-BACK task was used to test working memory. We administered a visual facial n-back task. The n-back paradigm is widely used to investigate the neural basis of working memory (Owen et al., 2005). 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 two trials previously. 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-prime 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.



(Images: Minear and Park, 2004)

3.3. GRAPHICAL REPRESENTATIONS OF THE NEUROIMAGING METHODOLOGY

Preprocessing and analysis for :

STUDY I. Palacios EM, Fernandez-Espejo D, Junque C, Sanchez-Carrion R, Roig T, Tormos JM, Bargallo N, Vendrell P. *Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury.* BMC Neurology 2011; 11:24.

STUDY I

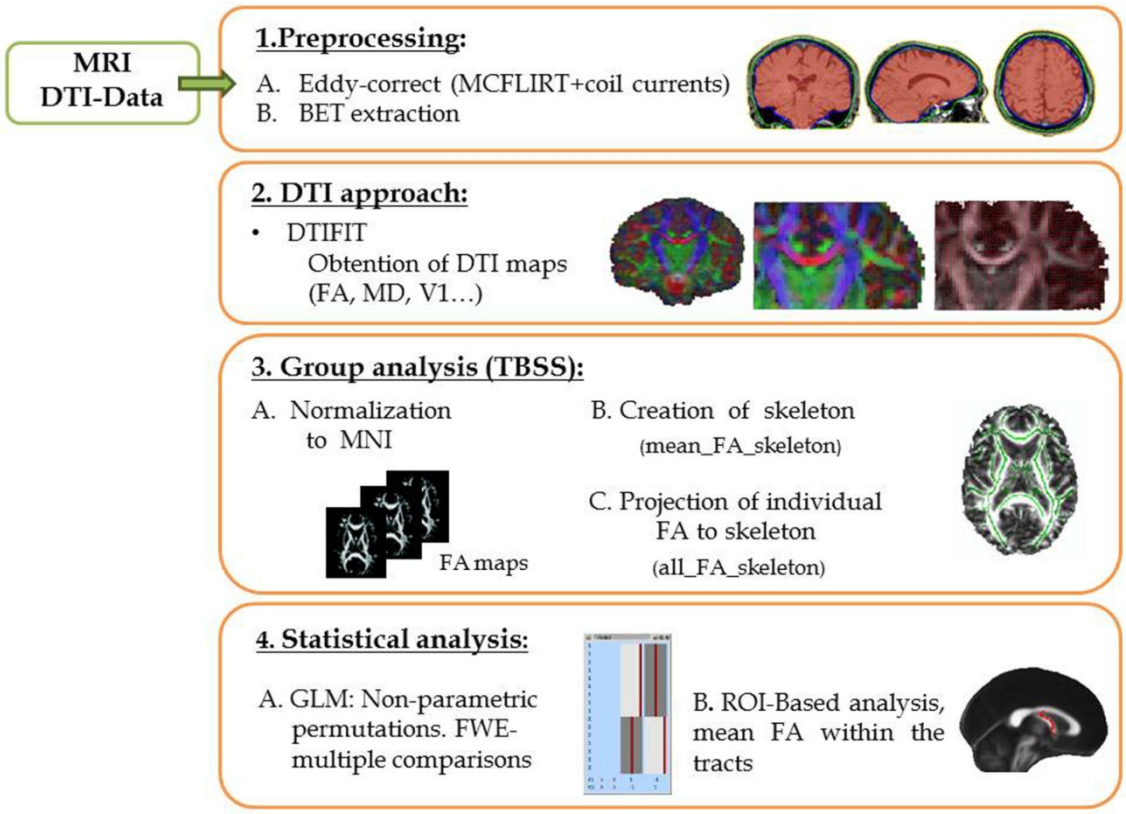


Image processing methods. FSL software was used (http://fsl.fmrib.ox.ac.uk/fsl/). 1) DTI images were pre-processed to correct for motion and coil eddy currents and non-brain voxels were removed; 2) A DTI approach was used to obtain individual fractional anisotropy (FA) maps; 3) A group-analysis (TBSS) was performed, where all individual FA maps were normalized together and projected into a mean FA tract skeleton, representing the centres of all tracts common to the group; and 4) A statistical analysis was performed on the skeletonised images obtained. This included a GLM-based testing of voxel-wise differences and correlations as well as the extraction of individual ROI-averaged FA values.

STUDY II. Palacios EM, Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *Long-term declarative memory deficits in diffuse TBI: Correlations of cortical thickness, white matter integrity and hippocampal volume.* Cortex 49 (3):646-57, 2012.

STUDY II

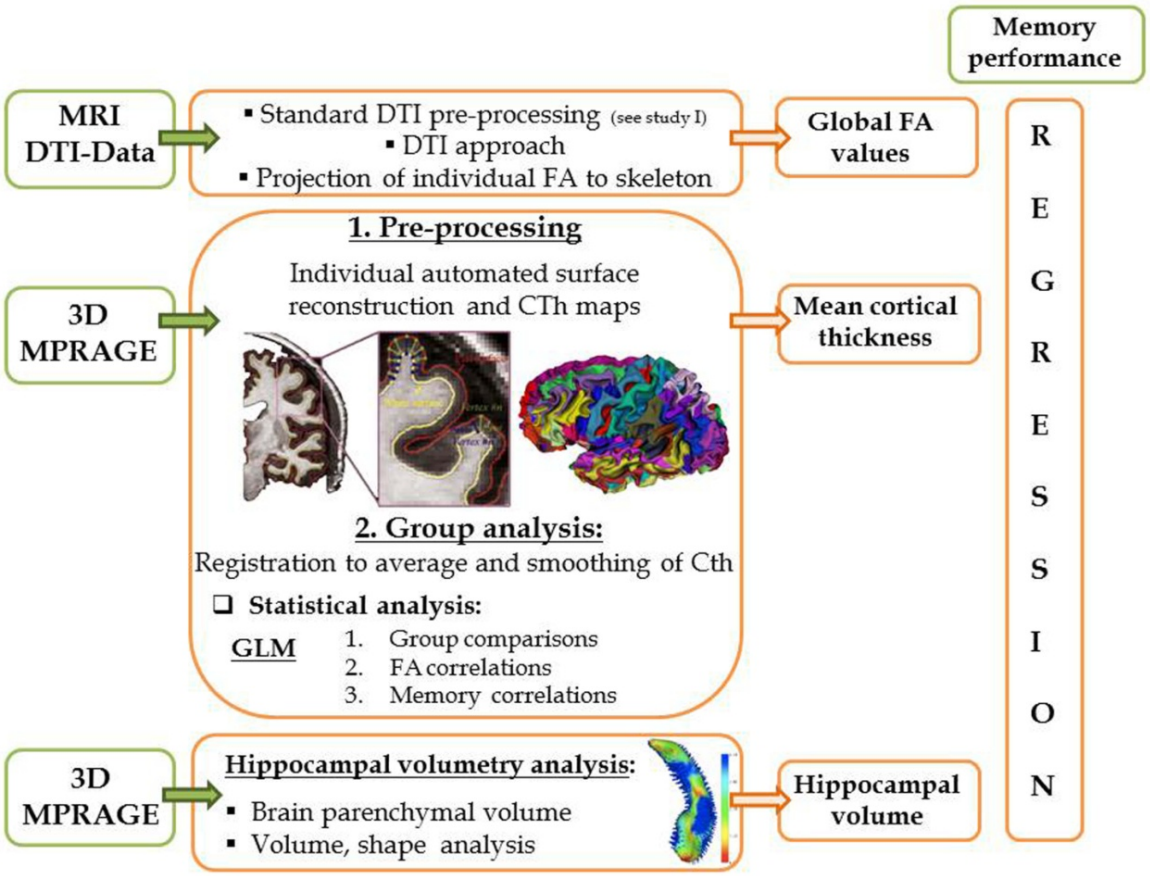


Image processing methods. FSL (http://fsl.fmrib.ox.ac.uk/fsl/) and FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) software were used. **1)** A group-based DTI analysis was performed (as in Study I) to obtain individual scores of global FA values (by averaging all FA values within the skeleton); **2)** 3D-MPRAGE acquisitions were used to reconstruct cortical surfaces and calculate cortical thickness (CTh) maps using FreeSurfer software. Individual CTh maps were registered to a common space and smoothed. A GLM-based group analysis was performed to study group differences and correlations with CTh and global FA and memory scores. The mean CTh of significant regions was extracted; **3)** A volumetric analysis was performed with FIRST and SIENAX tools of the FSL software. This allowed searching for group-differences on the shape of the hippocampus as well as extracting individual hippocampal and whole-brain volumes; and **4)** Global FA values, mean CTh and hippocampal volumes were introduced to a regression model in SPSS together with the individual scores obtained in neuropsychological tests to identify those which best predicted memory performance in TBI.

STUDY III. Palacios EM, Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *White matter integrity related to functional working memory networks in traumatic brain injury.* Neurology 20 (12):852-860, 2012.

STUDY III

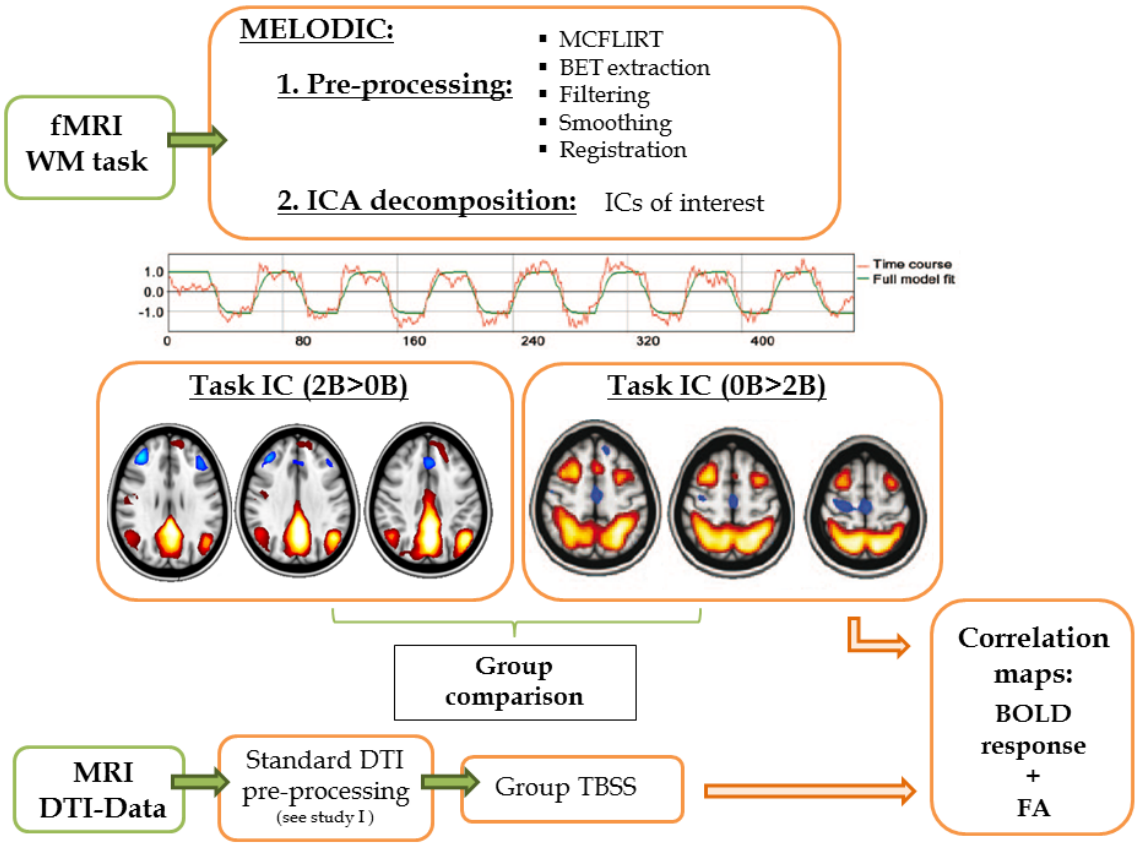


Image processing methods. FSL software was used (http://fsl.fmrib.ox.ac.uk/fsl/). **1)** Functional MRI acquired during the working-memory task was analyzed by independent component analysis (ICA), implemented in MELODIC. Before ICA, image pre-processing included motion correction, removal of non-brain voxels, temporal filtering, spatial smoothing and registration to the standard MNI template. A tensor-ICA approach then allowed the identification of common patterns of spatio-temporal brain oscillations (independent components, ICs). Two main ICs were selected as representing the main networks associated with working memory (2-back blocks) and with the brain default mode (0-back blocks). Between-group differences could be examined using a GLM design on the estimated ICs. Individual scores representing the strength of each IC were extracted to be used further in the FA analysis. **2)** DTI data were introduced in a group-based analysis (as in studies I, II). In this analysis, FA-skeletonised images were correlated with individual scores of brain activity obtained from the ICA as well as with individual performance scores of the functional task.

STUDY IV. *Palacios EM, *Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, Vendrell P. *Resting-State Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury.* Archives of Neurology. 2013 (in press)
*Equal Contribution.

STUDY IV

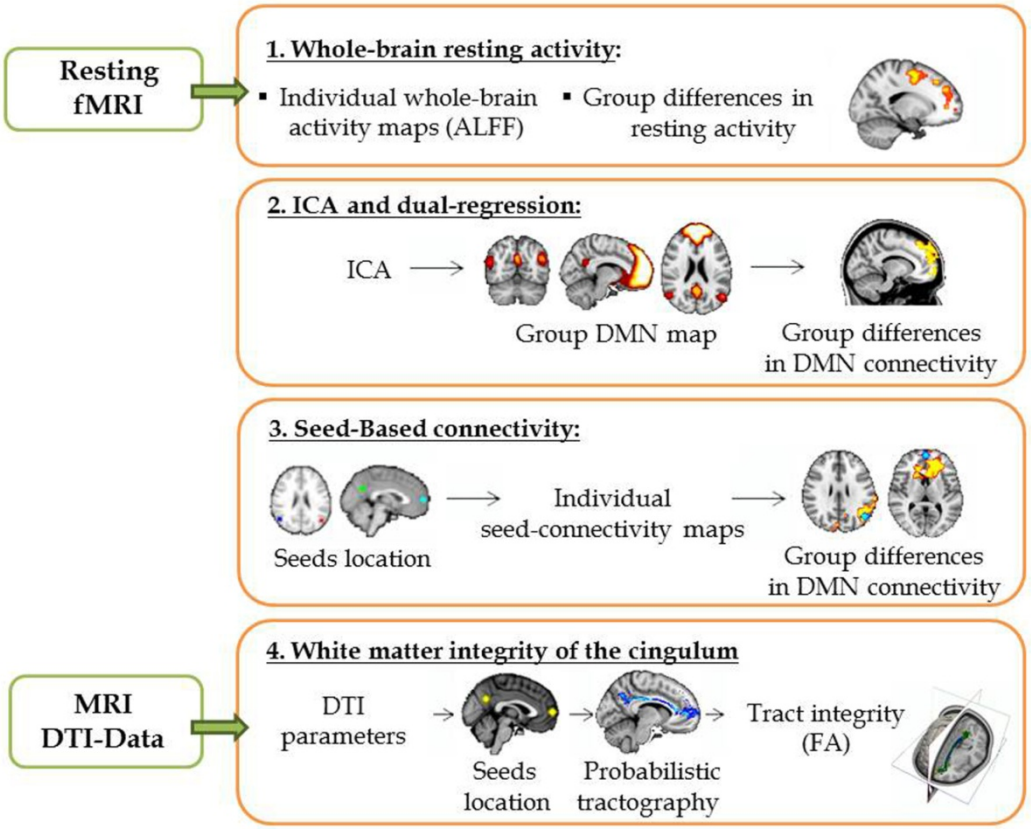


Image processing methods. FSL software was used (http://fsl.fmrib.ox.ac.uk/fsl/). **1)** Resting-fMRI data was pre-processed with motion correction, temporal filtering and spatial smoothing, and then entered into three different analyses: A whole brain analysis of brain activity measured as the amplitude of low-frequency BOLD fluctuations (ALFF); an ICA and dual regression analysis in order to study group differences in the spatial map of the default mode network (DMN); and a ROI-based connectivity analysis using 4 pre-defined DMN nodes as seed points; **2)** DTI data was pre-processed individually and then entered into a tractography analysis where two ROIs placed in the anterior cingulate and the precuneus were used to trace the trajectory of the cingulum for each subject. Mean FA values within the group-average cingulum tracts were used as a measure of tract integrity.

4. RESULTS

Palacios et al. BMC Neurology 2011, 11:24
http://www.biomedcentral.com/1471-2377/11/24

BMC Neurology

RESEARCH ARTICLE Open Access

Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury

Eva M Palacios[1,2], Davinia Fernandez-Espejo[1,2], Carme Junque[1,2*], Rocio Sanchez-Carrion[3], Teresa Roig[3], Jose M Tormos[3], Nuria Bargallo[4], Pere Vendrell[1,2]

Abstract

Background: Memory is one of the most impaired functions after traumatic brain injury (TBI). We used diffusion tensor imaging (DTI) to determine the structural basis of memory deficit. We correlated fractional anisotropy (FA) of the fasciculi connecting the main cerebral regions that are involved in declarative and working memory functions.

Methods: Fifteen patients with severe and diffuse TBI and sixteen healthy controls matched by age and years of education were scanned. The neuropsychological assessment included: Letter-number sequencing test (LNS), 2-back task, digit span (forwards and backwards) and the Rivermead profilet. DTI was analyzed by a tract-based spatial statics (TBSS) approach.

Results: Whole brain DTI analysis showed a global decrease in FA values that correlated with the 2-back d-prime index, but not with the Rivermead profile. ROI analysis revealed positive correlations between working memory performance assessed by 2-back d-prime and superior longitudinal fasciculi, corpus callosum, arcuate fasciculi and fornix. Declarative memory assessed by the Rivermead profile scores correlated with the fornix and the corpus callosum.

Conclusions: Diffuse TBI is associated with a general decrease of white matter integrity. Nevertheless deficits in specific memory domains are related to different patterns of white matter damage.

Background

Diffuse axonal injury (DAI) was initially defined as widespread damage to axons throughout the white matter, evoked by intense shear and strain forces resulting from rapid acceleration and deceleration of the brain with or without impact after traumatic brain injury (TBI) [1,2]. More recently, traumatic axonal injury (TAI) has been suggested as a more appropriate term for describing axonal damage because it encompasses not only the primary axonal damage specifically caused by shear/strain injury, but also secondary alterations of white matter such as metabolic, hypoxic and microvascular damage or excitotoxicity [3,4].

Although TAI has been described in neuropathological terms, magnetic resonance imaging (MRI) allows the detection of microhemorrhages and other indirect signs in regions commonly affected by this injury such as the subcortical white matter, the corpus callosum and the dorsolateral quadrant of the rostral brain-stem. It has recently been demonstrated that T2*-weighted MRI at high field strength is a useful tool for the identification of traumatic microbleeds even in the chronic stage of TBI [5]. However, diffusion tensor imaging (DTI) has been suggested as the best technique for the detection of subtle white matter changes [6] given that it can reveal significant abnormalities in white matter in patients with normal findings in conventional MRI [7,8].

DTI is a non-invasive MRI technique that identifies the microscopic physical properties of tissues directly through the observation of translational molecular movement of water [9]. Water diffusion in cerebral white matter tends to be anisotropic, because the highly linear organization of white matter fibers restricts movement in other directions [10,11]. Fractional anisotropy (FA), one of the main DTI-derived indices, provides information of the degree of directionality of water diffusion and on microstructural white matter changes. DTI has been shown to be an efficient technique for

* Correspondence: cjunque@ub.edu
[1]Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain
Full list of author information is available at the end of the article

determining white-matter integrity in several pathologies [12]. It has also been proposed as the most feasible biomarker of TAI and one of the best indicators of TBI severity [13,14]. Reductions in FA have been detected not only in moderate and severe TBI patients [15-18] but also in cases of mild TBI [19-24]. Moreover, DTI has proved to be an excellent tool for evaluating structural changes after TBI in longitudinal studies [16-18].

The advantages of DTI have resulted in a growing body of scientific evidence regarding the relationship between white matter damage and neuropsychological deficits in TBI. Studies conducted with pediatric samples have identified correlations between FA values and various cognitive functions, including cognitive processing speed and interference, executive functioning, IQ, verbal working memory, reading comprehension and letter naming speed [25-27]. Recently, Wu et al., [28] reported correlations between immediate recall and left cingulum bundles in adolescents after mild TBI. Some studies have related FA measurements with neuropsychological deficits in adults. Nakayama et al. [29] identified a positive correlation between the Mini-Mental State Examination (MMSE) and FA in the splenium of the corpus callosum. Salmond et al. [30] found a significant correlation between diffusivity and the impairment of learning and memory in the posterior cingulate, hippocampal formation and cortical areas. Kraus et al. [31] in a sample including all grade severities, found reduced FA in the ROIs analyzed and obtained a measure of the total regions of reduced FA that negatively correlated with the three cognitive domains evaluated. Furthermore, in a mild TBI sample, Niogi et al. [32] found a significant correlation between attentional control and FA within a ROI in the corona radiata and between memory performance and FA in the ROI placed in the uncinate both in the group of mild TBI patients and the control group. Kumar et al. [18] found correlations between the corpus callosum and neuropsychological tests involving processing speed as well as visuospatial and visuoperceptive tasks. Finally, Lipton et al., [33] and Miles et al. [34] found that reductions in FA in dorsolateral prefrontal cortex correlated significantly with tests of executive functions. In summary, DTI technique, in special FA measures, has been found sensitive to reflect cognitive deficits associate with TBI.

Memory is one of the functions that is most frequently impaired by TBI [35-37]. The concept of multiple memory systems and their different neuroanatomical substrates is currently accepted [38,39]. Declarative and working memory systems are significantly impaired after traumatic brain injury (TBI). Deficits in declarative memory - the capacity for conscious recollection of facts and events - are a common consequence of head trauma that are disproportionately suffered in comparison with other cognitive functions [35,40]. These memory difficulties improve slowly and although progress is made over the first and second year following injury, they remain apparent over time [40-42]. Neuroanatomically, declarative memory depends on the integrity of the hippocampus and its connections with the neocortex [43,44]. In neuroimaging studies with TBI patients, declarative memory has been found to correlate negatively with hippocampal [45,46] and fornix damage [47]. Working memory is defined as the ability to maintain and manipulate information temporarily [48]. Impairment of this memory is frequent in TBI patients given that implicated neural substrates, particularly the frontal cortex, are highly vulnerable in this type of injury. There is considerable evidence that working memory depends on network activity including the frontal and parietal regions and its connections. A meta-analysis of functional neuroimaging studies conducted by Owen et al. [49] provided strong evidence for the activation of frontal and parietal cortical regions by various versions of the n-back paradigm. The main fasciculus linking the parietal and frontal lobes is the superior longitudinal fasciculus (SLF) and hence it is likely that this has a role in working memory. Relations between the SLF and working memory deficits have been reported in multiple sclerosis [50] but not in TBI patients. To our knowledge there is no study investigating the impairment of white matter damage related to declarative and working memory deficits in a sample of severe and diffuse TBI.

The aim of this study was to investigate the role of white matter damage in declarative and working memory deficits after diffuse TBI, focusing on the main associative fasciculi [51] including those connecting the cerebral regions involved in the declarative memory and working memory networks.

Our study had two main hypotheses: firstly, that a decreased FA in the superior longitudinal fasciculi (SLF), which is presumably involved in working memory function since it links the parietal and prefrontal regions, would correlate with working memory deficits, and, secondly, that a decreased FA in the fornix, the main fasciculus interconnecting the hippocampus with the frontal lobe, would correlate with declarative memory impairment.

Methods

Subjects

A cross-sectional study of thirty-one subjects was performed. Fifteen patients (eleven male) with severe TBI were recruited from the Head Injury Unit of the Institut de Neurorehabilitació Guttmann. Inclusion criteria were: a) age < 40 years, b) diffuse axonal injury according to clinical MRI without focal cortical lesions or larger than 1.5 cm3, c) severe TBI: defined as a minimal Glasgow

Coma Scale (GCS) score ≤ 8 assessed at the first contact with the emergency services, d) emergence from post-traumatic amnesia (PTA) phase at the moment of the enrollment according to the Galveston Orientation and Attention Test (GOAT) [52], defined as two consecutive scores > 65, and f) no previous history of TBI, drug intake, neurological, or psychiatric disorders.

The etiology of TBI was a traffic accident in all cases. Fourteen patients were involved in car collisions, and one was a pedestrian hit by a motor vehicle. All patients had closed head injury and had not received surgery for extra- or subdural hematoma; all structural MRI scans were suggestive of TAI. The neuroradiologist (NB) took into account T1-weighted, FLAIR, and T2* GE sequences. The T2* GE sequences, which have a high level of sensitivity for detecting chronic hemosiderin, indicated evidence of TAI-related neuropathology. The method proposed by Gennarelli et al. [2] was used to classify the patients' TAI type. The grading system used was: type I, TAI only involving convexity gray-white matter junction; type II, also involving the corpus callosum in addition to the gray-white junction; and, type III, involving the rostral brainstem as well as the two previous criteria. Cases in which the midbrain was involved, but no corpus callosum lesions were apparent, were classified as type III (see Table 1).

A control group of sixteen healthy subjects (nine male) were recruited from relatives and friends of the TBI group. This control group was matched by age, years of education and premorbid intellectual function estimated using the Vocabulary subtest of the Wechsler Adult Intelligence Scale (WAIS-III) [53], recognized as an efficient method for estimating general intelligence [54] (see Table 2). All subjects were right-handed, Caucasian-Mediterranean, and none had a previous history of neurological or psychiatric diseases.

The study was approved by the Ethical and Research Committee of the Institut Universitari de Neurorehabilitacio Guttmann and all participants gave written informed consent.

Memory assessment

Working memory was evaluated by the Digit span and Letter-Number Sequencing (LNS) subtests of the WAIS-III [53] and a visual 2-back task [55]. Digit span was measured as the series length correctly reproduced at least once in the same order (forwards) and in reverse order (backwards). In the LNS, subjects heard lists of randomized numbers and letters (in alternating order) of increasing lengths, and were asked to reproduce the numbers and letters beginning with the lowest in each series, always with numbers first. The scores from the LNS were calculated by adding all correct items. In the 2-back task, numbers appeared on the screen for 500 ms against a black background, followed by a fixation cross for 1500 ms. The subjects were asked to decide whether the number they were looking at matched the one that they had seen two numbers earlier in the sequence. The numbers of correct responses as well as the reaction time were recorded. The d-prime index, a bias-free measure that takes both correct answers and errors into account, was also calculated to determine the accuracy of performance.

The Rivermead Behavioural Memory Test (RBMT) [56] was selected for its ability to explore declarative memory, and its ecological validity in assessing TBI patients. This test consists of 11 subtests including the following: remembering a name, a hidden belonging and an appointment; recognizing pictures and faces; recalling a prose passage; remembering a short route; remembering to deliver a message; and knowledge of some basic information such as the date, place and time. Those are designed as analogs of everyday tasks, reflecting the kinds of situations with which patients typically experience difficulty on a day-to-day basis. Two methods of standardizing scores across subtests allow for derivation of either a screening score, with subtest raw scores categorized on a scale of 0 ± 1 (maximum score 12 points), or a standardized profile score, with subtest raw scores categorized on a scale of 0 ± 2 (maximum score 24 points). The slightly more fine-grained standardized profile score provides a more sensitive analysis of performance [57] thus we use this score for our correlation analysis.

Image acquisition and analysis

MRI data sets were acquired on a 1.5 T Signa GE (*General Electric, Milwaukee, WI*) at the Centre de Diagnostic per la Imatge of the Hospital Clínic (CDIC), Barcelona. Diffusion weighted images were sensitized in 25 non-collinear directions with a b-value = 1000 sec/mm^2, using an echo-planar (EPI) sequence (TR = 9999.996 ms, TE = 85 ms, 20 axial slices with a resolution of 0.9375 × 0.9375 mm, slice thickness = 5 mm, gap = 2 mm matrix size = 128 × 128, FOV = 100).

Data preprocessing and analysis was performed using FMRIB's software library [FSL version 4.1; Oxford Centre for Functional MRI of the Brain (FMRIB), UK; http://www.fmrib.ox.ac.uk/fsl/]. Image artefacts due to eddy current distortions were minimized by registering the diffusion images to the b0 images. The registered images were skull-stripped using the Brain Extraction Tool (BET) [58]. Fractional anisotropy maps were calculated using the FMRIB's Diffusion Toolbox v.2.0 [FDT, [59]]. After calculation of the FA map for each subject, we implemented a voxel-wise statistical analysis of the FA data using Tract-Based Spatial Statistics v1.2 (TBSS) which aims to overcome the limitations of

Table 1 Clinical and neuroimaging characteristics of the TBI group

PT	GCS	PTA	Initial CT	MRI findings (T2*/FLAIR-Hemosiderin deposits)	tevol	TAI
1	8	150	SAH. Small hemorrhage in L thalamus	Microbleeds in L thalamus, R caudate, midbrain, frontal lobe, and CC	207	III
2	7	60	SAH. Small hemorrhagic lesion at the uncus	Microbleeds in R caudate, thalamus, pons, frontoparietal lobes and CC	285	III
3	3	125	Small frontobasal contusion and bilateral hemorrhagic foci in R frontal lobe and R thalamus	Microbleeds in R thalamus, R fronto-temporo parietal lobes, hippocampus and CC. Frontobasal contusion (< 1.5 cm)	315	II
4	5	45	No evidence of lesions	Microbleeds in dorsal midbrain and L frontal lobe	429	III
5	4	40	SAH. Hemorrhagic focus in L frontal white matter	Microbleeds in midbrain, R/L hippocampus, frontal and temporal lobes, and CC	550	III
6	7	51	SAH. Hemorrhagic focus in the R frontal white matter, and R intraventricular hemorrhage	Microbleeds in R/L hippocampus and R prefrontal region	146	I
7	4	45	Hyperdense lesion in the L medial temporal lobe	Microbleeds in L caudate, R/L hippocampus, midbrain, L parietal, R frontal lobes and CC	165	III
8	4	75	Multiple small bilateral subcortical hemorrhagic foci	Microbleeds in L thalamus, R midbrain, cerebellar peduncle, R/L frontal parietal and occipital lobes, R temporal and CC. Fonto-temporal deep white matter hyperintensities due to demyelination	86	III
9	3	70	Small hemorrhagic foci at R internal capsular and temporal region, and L CC. Intraventricular hemorrhage	Microbleeds in midbrain, cerebellum, R hippocampus, R internal capsule and thalamus, L fronto-parietal, and CC	443	III
10	3	60	SAH. Diffuse white matter alterations	Microbleeds in R thalamus, R midbrain, cerebellum, R/L frontal and CC. Parietal deep white matter hyperintensities due to demyelination.	114	III
11	7	120	Multiple puntiform hemorrhagic foci in both hemispheres	Microbleeds in L thalamus, R globus pallidus, R/L insula, R midbrain R/L frontal, parietal and temporal lobes and CC	306	III
12	4	171	Puntiform temporal contusion. L temporal subdural hematoma	Multiple subcortical microbleeds in pyramidal tract, centrum semiovale, pons, and CC. Deep white matter lesions. L temporal contusion (< 1 cm)	213	III
13	8	20	No evidence of lesions	Microbleeds in L insula, R frontal lobe and CC. Deep white matter lesions predominantly in the parietal lobe	115	II
14	4	105	Multiple hemorrhagic foci	Microbleeds in midbrain, fronto-parieto-occipital lobes and CC. Deep frontal white matter hyperintensities due to demyelination	143	III
15	6	120	Microhemorrhages in the L cerebellar hemisphere and R frontal lobe	Microbleeds in R thalamus, R temporal lobe and in fronto-parietal lobes. Contusion in R frontal gyrus and frontobasal (< 1.2 cm)	660	II

PT: Patient; GCS: Glasgow coma scale; PTA: posttraumatic amnesia; CT: computer tomography; MRI: magnetic resonance imaging; tevol: time of evolution since accident to the MRI evaluation; TAI: diffuse axonal injury; R/L: right/left; CC: corpus callosum; SHA: subarachnoidal hemorrhage.

the standard VBM-style analyses [60], particularly those regarding to its dependence on the goodness of the registration algorithm and on the choice of the spatial smoothing [61]. FA data were aligned into a common space using a non-linear registration algorithm (FNIRT) to register the images to the standard FMRIB58 FA template, which is in MNI152 standard space. Aligned FA maps were visually inspected after registration and we confirmed that the result of the previous step was correct. Next, a mean FA image was created from the images from all the subjects in this common space and narrowed to generate a mean FA skeleton that represented the center of all tracts common to the entire group. This was thresholded to FA 0.2 to include the major white matter pathways but to exclude peripheral tracts where there was significant inter-subject variability and partial volume effects with gray matter. This ensured that each subject's skeleton was in the group space while also representing the center of the subject's unique white matter bundles. The aligned FA image for each subject was then projected onto the skeleton by filling the skeleton with FA values from the nearest relevant tract centre. This is achieved for each skeleton voxel by searching perpendicular to the local skeleton structure for the maximum value in the FA image of the subject. The resulting skeletonised data was then fed into voxelwise cross-subject statistics.

Table 2 Demographic and clinical characteristics of TBI and control groups

	TBI group (n = 15)		Control group (n = 16)	
	Mean	SD (Range)	Mean	SD (Range)
Age	23.6	4.79 (18-32)	23.7	4.8 (18-32)
Education (years)	11.3	2.7 (8-16)	11.9	2.8 (8-16)
Vocabulary (WAIS-III)	9.9	2.0 (8-14)	10.3	1.9 (8-14)

TBI = traumatic brain injury.

Statistical analysis

Group comparisons and correlations with neuropsychological measures were performed using Randomise v2.1 from FSL [62,63]. As seen in table one, the time of evolution since injury was very heterogeneous. In order to control possible effects of this variable in the correlation results, time of evolution was entered as a non-interest variable in the matrix. The statistical threshold was set at $p < 0.05$ Family Wise Error (FWE) corrected, which is a conservative procedure that allows a high control of Type I error, being the probability of one or more false positives the same as the significance level. The Threshold-Free Cluster Enhancement (TFCE) method was used to define the clusters [64]. Correlation analyses were performed with the 2-back d-prime index and the Rivermead profile score using a region of interest (ROI) approach in the following associative fasciculi: corpus callosum, superior and inferior longitudinal, inferior fronto-occipital, uncinate, and cingulate as well as the fornix and arcuate fasciculi as the major pathways that connect associative cortical regions involved in working and declarative memory. ROI masks were obtained from the Jülich histological atlas [65,66] and the JHU white-matter tractography atlas [67-69]. Areas corresponding to significant clusters were identified using the JHU white-matter tractography atlas. Mean FA values were obtained from each subject's FA skeleton map and skeletonised SLF and fornix ROIs. Mean FA values were obtained from each subject's FA skeleton map and skeletonized for all the fasciculi ROIs mentioned above.

Statistical tests on non-imaging data were performed using SPSS (*Statistical Package for the Social Sciences) v.16* (SPSS Inc., Chicago Illinois). Group differences were examined using the Student t-test, since the data were normally distributed using a significance level of $p < 0.05$.

Partial correlation coefficients, controlling for the time of evolution, were used to explore the association between mean FA values and clinical variables and neuropsychological measures. Statistical significance was set at a two-tailed $p \leq 0.05$.

Results

Comparison between TBI patients and controls

Performance on the memory tests is described in Table 3. Statistical significance was obtained for the difference in scores in the LNS subtest (WAIS-III), d-prime index for the 2-back, and the RBMT profile. Forward and backward digits did not reach statistical significance.

Group comparison for FA skeleton maps revealed multiple areas of significant FA reductions in TBI patients as compared to controls. All the long associative fibers were affected, including the corpus callosum, the superior and inferior longitudinal fasciculi, and the inferior fronto-occipital fasciculi. Decreased FA was also observed in shorter fibers such as the uncinate fasciculus, cingulum, fornix and anterior thalamic radiation (Figure 1, Table 4). FA was not increased in the TBI group in any cerebral region.

Table 3 Neuropsychological performance for TBI and control groups

	TBI	group	Control	group	t (p values)
	Mean	SD	Mean	SD	
Digit forwards	6.1	1.0	6.6	1.1	-1.1 (ns)
Digit backwards	4.3	1.1	4.9	0.9	-1.6 (ns)
LNS	8.6	2.7	11.0	2.8	-2.4 (0.02)
2- back (d-prime)	2.7	1.0	3.4	0.4	-2.6 (0.01)
Goals 2-back	81.4	18.3	95.2	4.8	-2.7 (0.01)
Reaction time 2-back (ms)	693.4	200.0	475.7	88.9	3.7 (0.002)
RBMT (profile)	18.1	3.4	22.6	1.3	-4.85 (0.001)

TBI: traumatic brain injury; Letter-Number Sequencing (WAIS-III); Goals: number of targets correctly identified; RBMT: Rivermead Behavioral Memory Test; ns: not significant.

We obtained mean FA values of the whole skeletonized brain and all of the selected ROIs. Group comparisons for all these values reached statistical significance in all cases with $p < 0.001$ (Table 4).

Correlation analysis

Correlation with clinical variables

We observed significant negative correlations between FA and posttraumatic amnesia (PTA) in almost all the regions that showed significant FA decreases in the group analysis. Quantitative global mean FA values also showed a high correlation with this variable ($r = -0.903$ $p < 0.001$). However, no significant correlations were found in the FA maps analysis for the GCS ($r = 0.206$, $p = 0.499$).

Correlation with declarative and working memory performance

The mean global FA measure correlated significantly with 2-back d-prime index ($r = 0.584$, $p = 0.028$). The correlation of global FA with the RBMT profile score did not reach statistical significance.

The ROI procedure revealed a positive correlation between working memory performance assessed by the 2-back d- index and the FA skeletonized SLF, fornix, and corpus callosum ROIs (Figure 2, Table 5). 2-back d-prime index also correlated with the arcuate fascicle (Table 5). Declarative memory performance, assessed by RBMT, correlated with the fornix and the posterior part of the corpus callosum ROIs (Figure 3, Table 5).

No other correlation reached statistical significance in the TBI group. There were no significant correlations between FA and neuropsychological measures in the control group.

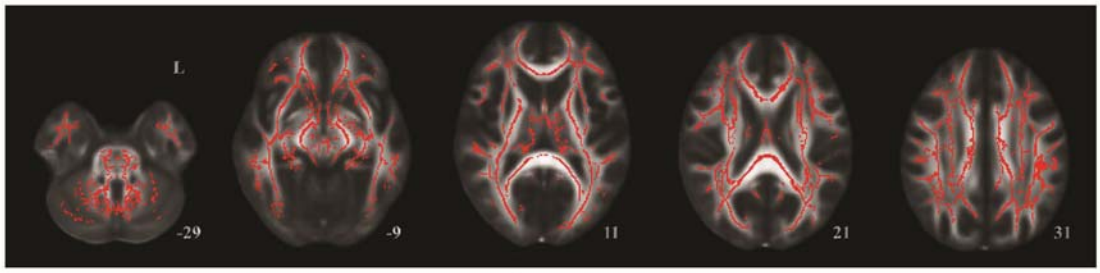


Figure 1 Results from TBSS analysis of FA maps showing the clusters of significantly reduced FA in TBI patients compared to controls in red (TFCE, p < 0.05 FWE-corrected). Widespread white matter affectation is observed.

Discussion

The present study provides evidence of the implications of TAI in declarative and working memory deficits in TBI. DTI group comparison revealed global whole brain reductions in mean FA values for patients and FA maps confirmed that almost all the major fibers were involved. Although our patients suffered global white matter integrity impairment, we found two different and restricted patterns of correlations with the FA and neuropsychological assessment. Whole brain DTI analysis showed that decreased FA throughout the brain correlated with 2-back measures but not with the Rivermead Test. Results from the ROI analyses of the main association fibers showed, as predicted, that working memory specifically correlated with the superior longitudinal fasciculi. However, it was also found to correlate with the corpus callosum, the arcuate fasciculi and with the fornix. On the other hand, declarative memory deficits only correlated with the fornix, as we had expected, and the corpus callosum. These results suggest that there are two different patterns of FA reduction related with two types of memory dysfunctions.

Table 4 Differences between groups in mean FA from the whole skeletonised brain and the ROIs

	TBI group		Control group		t (p values)
	Mean	SD	Mean	SD	
FA global	0.360	0.280	0.423	0.018	-7.43 (< 0.001)
FA CC	0.410	0.460	0.510	0.024	-7.04 (< 0.001)
FA SLF	0.364	0.026	0.421	0.018	-7.02 (< 0.001)
FA ILF	0.045	0.003	0.052	0.002	-7.23 (< 0.001)
FA IFO	0.390	0.031	0.456	0.018	-7.45 (< 0.001)
FA fornix	0.316	0.030	0.396	0.022	-8.05 (< 0.001)
FA cingulum	0.401	0.048	0.493	0.032	-6.16 (< 0.001)
FA arcuate	0.373	0.027	0.430	0.018	-6.05 (< 0.001)
FA uncinate	0.351	0.032	0.403	0.019	-5.34 (< 0.001)

TBI: Traumatic Brain Injury; FA: fractional anisotropy; CC: corpus callosum; SLF: superior longitudinal fasciculi; ILF: inferior longitudinal fasciculi; IFO: inferior fronto-occipital fasciculi.

We found that superior longitudinal fasciculi damage is related with working memory but not with declarative memory deficits. These correlations were expectable since the longitudinal fasciculi connect the associative frontal and parietal regions involved in working memory functions [70-72,49]. The correlation between working memory deficit and the superior longitudinal was also described in multiple sclerosis, pathology that also involves white matter damage [50].

In our sample, FA reductions of corpus callosum correlated with both working and declarative memory impairments. In declarative memory the correlations were seen in the posterior region whereas in working memory the correlations involved anterior and posterior regions, thus again these results point to differential patterns of correlations for both types of memory impairment.

According our results, declarative memory impairment did not depend on diffuse white matter damage since no correlations between FA maps or mean values and declarative memory values were seen. However, the ROI analysis revealed that the fornix FA impairment correlated with the Rivermead test. This result is in agreement with the role of the damage of the hippocampus and its connections in declarative memory deficits in TBI [45,46].

Our declarative memory results partially agree with those obtained by Salmond et al. [30]. Using a voxel-based analysis with SPM tools, these authors found a significant positive correlation between declarative memory and diffusivity in the left hippocampal formation, the left posterior cingulate, and the left frontal, temporal and occipital regions. The more widespread pattern of correlations observed in their study can be explained by the use of FDR correction, which is more liberal than the FWE correction used in ours [73]. Correlations between FA values in the fornix and declarative memory impairment have been also observed in patients with multiple sclerosis [74]. Other studies investigating FA correlations with declarative memory

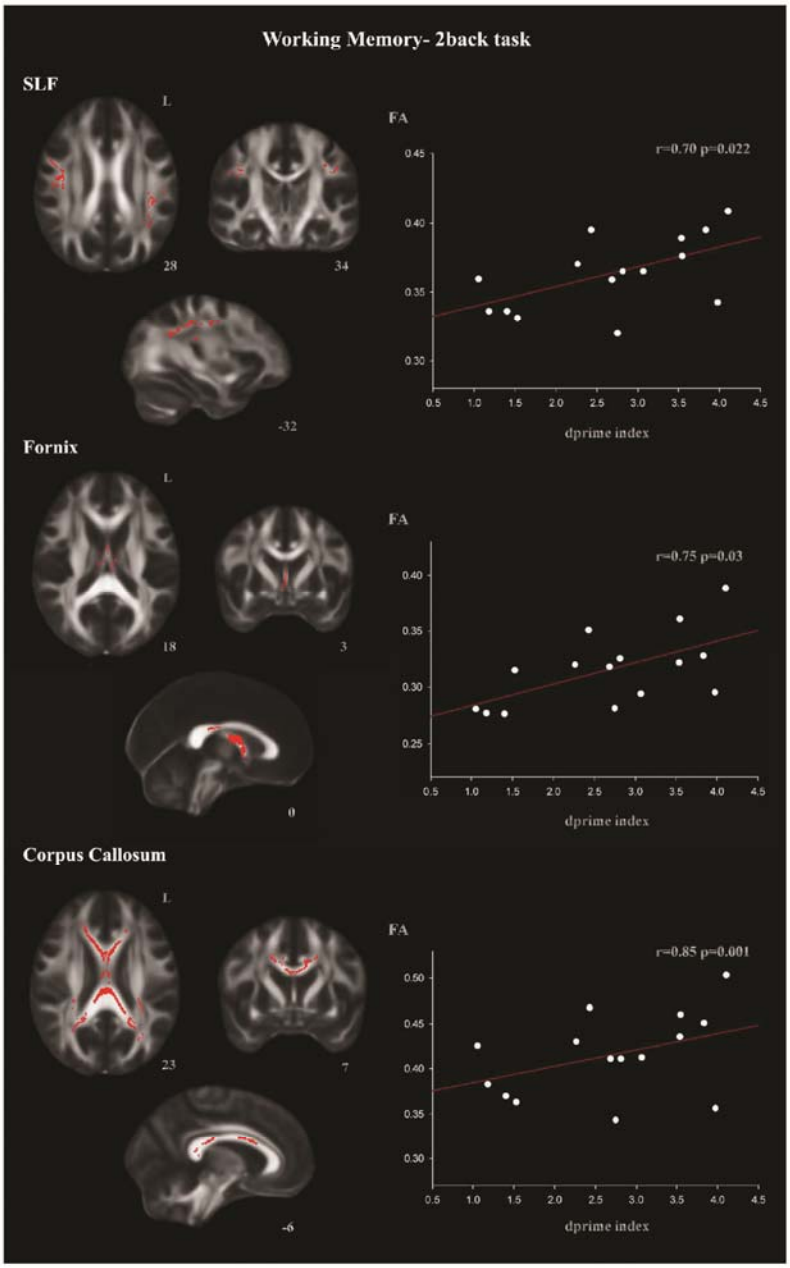


Figure 2 ROI correlations with d-prime 2-back index in the TBI group for the SLF, fornix, and corpus callosum ROIs (TFCE, p < 0.05 FWE-corrected). Correlation coefficient (*r*) was directly converted from t values of the TBSS output. The *t* and *r* values correspond to the most statistically significant voxel for each cluster.

functions in mild TBI samples have reported significant correlations with the uncinate fasciculi [30,32] and the cingulum [28]. Although we found decreased FA in these fasciculi, correlations did not reach statistical significance. These discrepancies may be explained by the varying grade of severity of the samples, the difference in the memory tests used, and DTI methodological differences.

In the present study, working memory deficits also correlated with the fornix in both the whole brain

Table 5 TBSS results. Correlation with working and declarative memory measures in the ROIs in the TBI group

2-back d-prime index	Cluster size	x	y	z	p	r
Superior longitudinal fasciculus	908	-38	-49	22	0.022	0.70
	440	51	2	24	0.025	0.71
	91	-29	-7	39	0.044	0.67
	27	-31	-14	40	0.045	0.69
	20	-46	-46	23	0.048	0.69
Arcuate fasciculus	121	50	3	27	0.030	0.75
	45	-34	-32	35	0.044	0.64
	30	-38	-49	23	0.045	0.73
	16	-36	-29	27	0.048	0.63
Corpus callosum	3310	-7	14	22	0.028	0.65
	2195	-13	-39	23	0.021	0.77
	211	-32	-41	16	0.043	0.67
Fornix	1206	-9	-14	13	0.001	0.85
RBMT	**Cluster size**	**x**	**y**	**z**	**p**	**r**
Corpus callosum	1008	-16	-40	27	0.033	0,71
	538	7	-12	25	0.042	0.68
Fornix	140	-2	-12	-24	0.034	0,54
	68	1	5	-4	0.042	0.60

All regions identified at family wise error corrected $p < 0.05$ with a cluster size of at least 10 voxels. Coordinates are given at peak voxel coordinate in MNI-152 standard space.

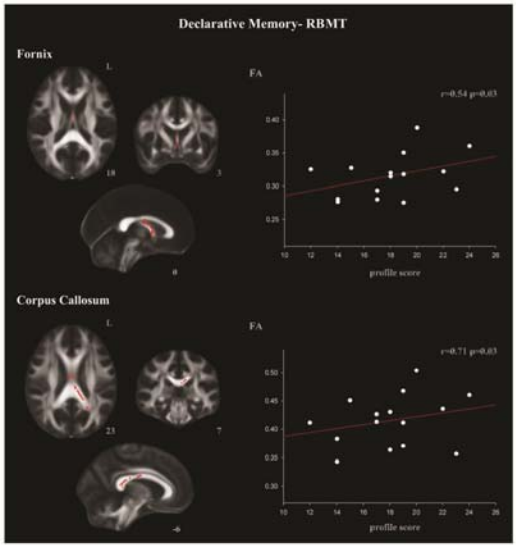


Figure 3 ROI correlations with RBMT in the TBI group: RBMT correlated with the fornix and the corpus callosum ROIs (TFCE, p < 0.05 FWE-corrected). Correlation coefficient *(r)* was directly converted from *t* values of the TBSS output. The *t* and *r* values correspond to the most statistically significant voxel for each cluster.

analysis and the ROI analyses. There is some evidence from fMRI studies that the hippocampus is involved in working memory functions in healthy subjects [75-78]. Moreover, several animal studies also suggest a role for the hippocampus in working memory [79-81]. Anatomically, prefrontal regions involved in working memory tasks receive projections from the hippocampus [82,83] and are connected directly to the ventral hippocampus and indirectly to the dorsal hippocampus via the thalamus [84-86]. This structural connectivity supports the idea that the hippocampus has a role in working memory functioning as suggested by our findings.

Finally, significant correlations were observed between the PTA variable and white matter integrity. Whole brain map analysis showed that PTA is an excellent index predictor of the degree of impairment of the major white matter tracts and association fibers. These results suggest that the recovery of memory functions is dependent on the integrity of the complex neocortical regions. Unlike previous studies [13,14,17], no correlations were found between GCS and FA maps or mean FA values. This result was to be expected as the fact that all our patients had severe TBI meant that GCS variability would not be sufficient to reach statistical significance.

Our study has certain limitations and our results should be regarded as preliminary. The small sample size and its specific diffuse characteristics may preclude the generalization of the results. The presence of mixed focal and diffuse pathology frequently observed in severe TBI may confound the mapping of neural and behavioral changes in these patients. As our study sample excluded significant cortical pathology, the cognitive impairment observed is more likely to be due to the diffuse pathology alone. Nevertheless, we cannot exclude the possibility that reductions in gray matter in several subcortical structures are also influencing memory deficits in TBI.

Conclusions

This DTI study suggests that declarative and working memory deficits in diffuse TBI patients are related to differential patterns of FA reduction. Working memory impairment reflects the diffuse white matter damage affecting large scale networks such as the superior longitudinal fasciculi, whereas declarative memory deficits seem to be the result of more local disruption of the cerebral circuitry.

Acknowledgements
This work was supported by a grant from the Spanish Ministry of Science and Innovation (SAF2007-66077) and a grant from the Generalitat de Catalunya (2009SGR941) to the Neuropsychology Research Group. Eva M.

Palacios was supported by a fellowship from the Institute of Biomedical Research August Pi i Sunyer (IDIBAPS) (89000313). Davinia Fernandez-Espejo was supported by a fellowship from the Spanish Ministry for Education (AP2006-00862).

Author details
[1]Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain. [2]Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, Spain. [3]Department of Neuropsychology, Institut Universitari de Neurorehabilitació Guttmann, Badalona, Spain. [4]Centre de Diagnòstic per la Imatge Hospital Clínic de Barcelona (CDIC), Hospital Clínic de Barcelona, Spain.

Authors' contributions
EP, DFE and CJ made substantial contribution to conception and design, interpretation of data, drafting and writing of manuscript and further revisions of the manuscript. Neuroimaging data were analyzed by EP and DFE. Neuroimaging sequence acquisitions, neurological description and classification of data by NB. RSC and TR participated in the collection of neuropsychological data and in the collection of acute clinical data. JT and PV made a critical revision of the manuscript for important intellectual content providing additional comments and contributions. CJ supervised the study. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 13 October 2010 Accepted: 23 February 2011
Published: 23 February 2011

References

1. Adams JH, Graham DI, Murray LS, Scott G: **Diffuse axonal injury due to non missile head injury in humans: An analysis of 45 cases.** *Ann Neurol* 1982, **12**:557-563.
2. Gennarelli TA, Thibault LE, Adams JH, Graham DI, Thompson CJ, Marcincin RP: **Diffuse axonal injury and traumatic coma in the primate.** *Ann Neurol* 1982, **12**:564-574.
3. Povlishock JT, Katz DI: **Update of neuropathology and neurological recovery after traumatic brain injury.** *J Head Trauma Rehabil* 2005, **20**:76-94.
4. Buki A, Povlishock JT: **All roads lead to disconnection?-Traumatic axonal injury revisited.** *Acta Neurochir* 2006, **148**:181-94.
5. Scheid R, Walther K, Guthke T, Preul C, von Cramon DY: **Cognitive sequelae of diffuse axonal injury.** *Arch Neurol* 2006, **63**:418-424.
6. Rugg-Gunn FJ, Symms MR, Barker GJ, Greenwood R, Duncan JS: **Diffusion imaging shows abnormalities after blunt head trauma when conventional magnetic resonance imaging is normal.** *J Neurol Neurosurg Psychiatry* 2001, **70**:530-533.
7. Rutgers DR, Fillard P, Paradot G, Tadie M, Lasjaunias P, Ducreux D: **Diffusion tensor imaging characteristics of the corpus callosum in mild, moderate, and severe traumatic brain injury.** *AJNR Am J Neuroradiol* 2008, **29**:1730-1735.
8. Miles L, Grossman RI, Johnson G, Babb JS, Diller L, Inglese M: **Short-term DTI predictors of cognitive dysfunction in mild traumatic brain injury.** *Brain Inj* 2008, **22**:115-22.
9. Le Bihan D: **Looking into the functional architecture of the brain with diffusion MRI.** *Nat Rev Neurosci* 2003, **4**:469-480.
10. Chenevert TL, Brunberg JA, Pipe JG: **Anisotropic diffusion in human white matter: Demonstration with MR techniques in vivo.** *Radiology* 1990, **177**:401-405.
11. Beaulieu C: **The basis of anisotropic water diffusion in the nervous system - a technical review.** *NMR in Biomed* 2002, **15**:435-455.
12. Ciccarelli O, Catani M, Johansen-Berg H, Clark C, Thompson A: **Diffusion-based tractography in neurological disorders: Concepts, applications, and future developments.** *Lancet Neurol* 2008, **7**:715-727.
13. Huisman TA, Schwamm LH, Schaefer PW, Koroshetz WJ, Shetty-Alva N, Ozsunar Y, Wu O, Sorensen AG: **Diffusion tensor imaging as potential biomarker of white matter injury in diffuse axonal injury.** *AJNR Am J Neuroradiol* 2004, **25**:370-376.
14. Benson RR, Meda SA, Vasudevan S, Kou Z, Govindarajan KA, Hanks RA, Millis SR, Makki M, Latif Z, Coplin W, Meythaler EJ, Haacke M: **Global white matter analysis of diffusion tensor images is predictive of injury severity in traumatic brain injury.** *J Neurotrauma* 2007, **24**:446-459.
15. Wilde EA, Chu Z, Bigler ED, Hunter JV, Fearing MA, Hanten G, Newsome MR, Scheibel RS, Li X, Levin HS: **Diffusion tensor imaging in the corpus callosum in children after moderate to severe traumatic brain injury.** *J Neurotrauma* 2006, **23**:1412-1426.
16. Sidaros A, Engberg AW, Sidaros K, Liptrot MG, Herning M, Petersen P, Paulson OB, Jernigan TL, Rostrup E: **Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: A longitudinal study.** *Brain* 2008, **131**:559-572.
17. Bendlin BB, Ries ML, Lazar M, Alexander AL, Dempsey RJ, Rowley HA, Sherman JE, Johnson SC: **Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging.** *Neuroimage* 2008, **42**:503-514.
18. Kumar R, Husain M, Gupta RK, Hasan KM, Haris M, Agarwal AK, Pandey CM, Narayana PA: **Serial changes in the white matter diffusion tensor imaging metrics in moderate traumatic brain injury and correlation with neuro-cognitive function.** *J Neurotrauma* 2009, **26**:1-16.
19. Arfanakis K, Haughton VM, Carew JD, Rogers BP, Dempsey RJ, Meyerand ME: **Diffusion tensor MR imaging in diffuse axonal injury.** *AJNR Am J Neuroradiol* 2002, **23**:794-802.
20. Inglese M, Makani S, Johnson G, Cohen BA, Silver JA, Gonen O, Grossman RI: **Diffuse axonal injury in mild traumatic brain injury: A diffusion tensor imaging study.** *J Neurosurg* 2005, **103**:298-303.
21. Bazarian JJ, Zhong J, Blyth B, Kavcic V, Peterson D: **Diffusion tensor imaging detects clinically important axonal damage after mild traumatic brain injury: A pilot study.** *J Neurotrauma* 2007, **24**:1447-1459.
22. Lipton ML, Gellella E, Lo C, Gold T, Ardekani BA, Shifteh K, Bello JA, Branch CA: **Multifocal white matter ultrastructural abnormalities in mild traumatic brain injury with cognitive disability: A voxel-wise analysis of diffusion tensor imaging.** *J Neurotrauma* 2008, **25**:1335-1342.
23. Niogi SN, Mukherjee P, Ghajar J, Johnson C, Kolster RA, Sarkar R, Lee H, Meeker M, Zimmerman RD, Manley GT, McCandliss BD: **Extent of microstructural white matter injury in postconcussive syndrome correlates with impaired cognitive reaction time: A 3T diffusion tensor imaging study of mild traumatic brain injury.** *AJNR Am J Neuroradiol* 2008, **29**:967-973.
24. Wilde EA, McCauley SR, Hunter JV, Bigler ED, Chu Z, Wang ZJ, Hanten GR, Troyanskaya M, Yallampalli R, Li X, Chia J, Levin HS: **Diffusion tensor imaging of acute mild traumatic brain injury in adolescents.** *Neurology* 2008, **70**:948-955.
25. Wozniak JR, Krach L, Ward E, Mueller BA, Muetzel R, Schnoebelen S, Kiragu A, Lim KO: **Neurocognitive and neuroimaging correlates of pediatric traumatic brain injury: A diffusion tensor imaging (DTI) study.** *Arch Clin Neuropsychol* 2007, **22**:555-568.
26. Ewing-Cobbs L, Prasad MR, Swank P, Kramer L, Cox CS Jr, Fletcher JM, Barnes M, Zhang X, Hasan KM: **Arrested development and disrupted callosal microstructure following pediatric traumatic brain injury: Relation to neurobehavioral outcomes.** *Neuroimage* 2008, **42**:1305-1315.
27. Levin HS, Wilde EA, Chu Z, Yallampalli R, Hanten GR, Li X, Chia J, Vasquez AC, Hunter JV: **Diffusion tensor imaging in relation to cognitive and functional outcome of traumatic brain injury in children.** *J Head Trauma Rehabil* 2008, **23**:197-208.
28. Wu TC, Wilde EA, Bigler ED, Yallampalli R, McCauley SR, Troyanskaya M, Chu Z, Li X, Hanten G, Hunter JV, Levin HS: **Evaluating the relationship between memory functioning and cingulum bundles in acute mild traumatic brain injury using diffusion tensor imaging.** *J Neurotrauma* 2010, **27**:303-7.
29. Nakayama N, Okumura A, Shinoda J, Yasokawa YT, Miwa K, Yoshimura SI, Iwama T: **Evidence for white matter disruption in traumatic brain injury without macroscopic lesions.** *J Neurol Neurosurg Psychiatry* 2006, **77**:850-855.
30. Salmond CH, Menon DK, Chatfield DA, Williams GB, Pena A, Sahakian BJ, Pickard JD: **Diffusion tensor imaging in chronic head injury survivors: Correlations with learning and memory indices.** *Neuroimage* 2006, **29**:117-124.
31. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM: **White matter integrity and cognition in chronic traumatic brain injury: A diffusion tensor imaging study.** *Brain* 2007, **130**:2508-2519.
32. Niogi SN, Mukherjee P, Ghajar J, Johnson CE, Kolster R, Lee H, Suh M, Zimmerma RD, Manley GT, McCandliss BD: **Structural dissociation of**

attentional control and memory in adults with and without mild traumatic brain injury. *Brain* 2008, **131**:3209-3221.
33. Lipton ML, Gellella E, Lo C, Gold T, Ardekani BA, Shifteh K, Bello JA, Branch CA: **Multifocal white matter ultrastructural abnormalities in mild traumatic brain injury with cognitive disability: A voxel-wise analysis of diffusion tensor imaging.** *J Neurotrauma* 2008, **25**:1335-1342.
34. Miles L, Grossman RI, Johnson G, Babb JS, Diller L, Inglese M: **Short-term DTI predictors of cognitive dysfunction in mild traumatic brain injury.** *Brain Inj* 2008, **22**:115-22.
35. Levin HS, Goldstein FC, High WM Jr, Eisenberg HM: **Disproportionately severe memory deficit in relation to normal intellectual functioning after closed head injury.** *J Neurol Neurosurg Psychiatry* 1988, **51**:1294-1301.
36. Bigler ED: **Lesions in traumatic brain injury: implications for clinical neuropsychology.** *Arch Clin Neuropsychol* 2001, **16**:95-131.
37. Vakil E: **The effect of moderate to severe traumatic brain injury (TBI) on different aspects of memory: A selective review.** *J Clin Exp Neuropsychol* 2005, **27**:977-1021.
38. Squire LR: **Memory systems of the brain: A brief history and current perspective.** *Neurobiol Learn Mem* 2004, **82**:171-177.
39. Squire LR, Stark CE, Clark RE: **The medial temporal lobe.** *Annu Rev Neurosci* 2004, **27**:279-306.
40. Levin HS: **Memory deficit after closed-head injury.** *J Clin Exp Neuropsychol* 1990, **12**:129-153.
41. Wilson B: **Recovery and compensatory strategies in head injured memory impaired people several years after insult.** *J Neurol Neurosurg Psychiatry* 1992, **55**:177-180.
42. Zec RF, Zellers D, Belman J, Miller J, Matthews J, Ferneau-Belman D, Robbs R: **Long-term consequences of severe closed head injury on episodic memory.** *J Clin Exp Neuropsychol* 2001, **23**:671-691.
43. Squire LR, Zola-Morgan S: **The medial temporal lobe memory system.** *Science* 1991, **253**:1380-1386.
44. Eichenbaum H: **Hippocampus: Cognitive processes and neural representations that underlie declarative memory.** *Neuron* 2004, **44**:109-120.
45. Serra-Grabulosa JM, Junque C, Verger K, Salgado-Pineda P, Maneru C, Mercader JM: **Cerebral correlates of declarative memory dysfunctions in early traumatic brain injury.** *J Neurol Neurosurg Psychiatry* 2005, **76**:129-131.
46. Ariza M, Pueyo R, Junque C, Mataro M, Poca MA, Mena MP, Sahuquillo J: **Differences in visual vs. verbal memory impairments as a result of focal temporal lobe damage in patients with traumatic brain injury.** *Brain Inj* 2006, **20**:1053-1059.
47. Gale SD, Burr RB, Bigler ED, Blatter D: **Fornix degeneration and memory in traumatic brain injury.** *Brain Res Bull* 1993, **32**:345-349.
48. Baddeley A: **Working memory.** *Science* 1992, **255**:556-559.
49. Owen AM, McMillan KM, Laird AR, Bullmore E: **N-back working memory paradigm: A meta-analysis of normative functional neuroimaging studies.** *HumBrain Mapp* 2005, **25**:46-59.
50. Bonzano L, Pardini M, Mancardi GL, Pizzorno M, Roccatagliata L: **Structural connectivity influences brain activation during PVSAT in multiple sclerosis.** *Neuroimage* 2009, **44**:9-15.
51. Gong G, He Y, Concha L, Lebel C, Gross DW, Evans AC, Beaulieu C: **Mapping anatomical connectivity patterns of human cerebral cortex using in vivo diffusion tensor imaging tractography.** *Cereb Cortex* 2009, **19**:524-39.
52. Levin HS, O'Donnell VM, Grossman RG: **The Galveston Orientation and Amnesia Test. A practical scale to assess cognition after head injury.** *J Nerv Ment Dis* 1979, **167**:675-684.
53. Wechsler D: **Escala de intenteligencia de Wechsler para adultos (WAIS-III).** Madrid: TEA Ediciones; 1999.
54. Lezak MD, Howieson DB, Loring DD, Hannay HJ, Fisher JS: **Neuropsychological Assessment.** New York: Oxford University Press; 2004.
55. Cohen JD, Perlstein WM, Braver TS, Nystrom LE, Noll DC, Jonides J, Smith EE: **Temporal dynamics of brain activation during a working memory task.** *Nature* 1997, **386**:604-608.
56. Wilson B, Cockburn J, Baddeley AD: **The Rivermead Behavioural Memory Test.** Bury St. Edmunds, UK: Thames Valley Test; 1991.
57. Wills P, Clare L, Shiel A, Wilson BA: **Assessing subtle memory impairments in the everyday memory performance of brain injured people: exploring the potential of the extended Rivermead Behavioural Memory Test.** *Brain Inj* 2000, **14**:693-704.
58. Smith SM: **Fast robust automated brain extraction.** *Hum Brain Mapp* 2002, **17**:143-155.
59. Behrens TE, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, Matthews PM, Brady JM, Smith SM: **Characterization and propagation of uncertainty in diffusion-weighted MR imaging.** *Magn Reson Med* 2003, **50**:1077-1088.
60. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TEJ: **Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data.** *Neuroimage* 2006, **31**:1487-1505.
61. Jones DK, Symms MR, Cercignani M, Howard RJ: **The effect of filter size on VBM analyses of DT-MRI data.** *Neuroimage* 2005, **26**:546-554.
62. Nichols TE, Holmes AP: **Nonparametric Permutation Tests for Functional Neuroimaging: A Primer with Examples.** *Hum Brain Mapp* 2002, **15**:1-25.
63. Hayasaka S, Nichols TE: **Validating cluster size inference: random field and permutation methods.** *Neuroimage* 2003, **20**:2343-2356.
64. Smith SM, Nichols TE: **Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localization in cluster inference.** *Neuroimage* 2009, **44**:83-98.
65. Toga AW, Thompson PM, Mori S, Amunts K, Zilles K: **Towards multimodal atlases of the human brain.** *Nat Rev Neurosci* 2006, **7**:952-966.
66. Eickhoff SB, Paus T, Caspers S, Grosbras MH, Evans AC, Zilles K, Amunts K: **Assignment of functional activations to probabilistic cytoarchitectonic areas revisited.** *Neuroimage* 2007, **36**:511-521.
67. Mori S, Wakana S, Peter CM, Van Zijl PCM: **MRI Atlas of Human White Matter.** Amsterdam, The Netherlands: Elsevier; 2005.
68. Wakana S, Caprihan A, Panzenboeck MM, Fallon JH, Perry M, Gollub RL, Hua K, Zhang J, Jiang H, Dubey P, Blitz A, van Zijl P, Mori S: **Reproducibility of quantitative tractography methods applied to cerebral white matter.** *Neuroimage* 2007, **36**:630-644.
69. Hua K, Zhang J, Wakana S, Jiang H, Li X, Reich DS, Calabresi PA, Pekar JJ, van Zijl PC, Mori S: **Tract probability maps in stereotaxic spaces: analysis of white matter anatomy and tract-specific quantification.** *Neuroimage* 2008, **39**:336-47.
70. Klingberg T, O'Sullivan BT, Roland PE: **Bilateral activation of fronto-parietal networks by incrementing demand in a working memory task.** *Cereb Cortex* 1997, **7**:465-471.
71. Jonides J, Schumacher EH, Smith EE, Koeppe RA, Awh E, Reuter-Lorenz PA, Marshuetz C, Willis CR: **The role of parietal cortex in verbal working memory.** *J Neurosci* 1998, **18**:5026-5034.
72. Olesen PJ, Westerberg H, Klingberg T: **Increased prefrontal and parietal activity after training of working memory.** *Nat Neurosci* 2004, **7**:75-79.
73. Chumbley JR, Friston KJ: **False discovery rate revisited: FDR and topological inference using gaussian random fields.** *Neuroimage* 2009, **44**:62-70.
74. Dineen RA, Vilisaar J, Hlinka J, Bradshaw CM, Morgan PS, Constantinescu CS, Auer DP: **Disconnection as mechanism for cognitive dysfunction in multiple sclerosis.** *Brain* 2009, **132**:239-249.
75. Ranganath C, D'Esposito M: **Medial temporal lobe activity associated with active maintenance of novel information.** *Neuron* 2001, **31**:865-873.
76. Cabeza R, Dolcos F, Graham R, Nyberg L: **Similarities and differences in the neural correlates of episodic memory retrieval and working memory.** *Neuroimage* 2002, **16**:317-330.
77. Davachi L, Wagner AD: **Hippocampal contributions to episodic encoding: Insights from relational and item-based learning.** *J Neurophysiol* 2002, **88**:982-990.
78. Rissman J, Gazzaley A, D'Esposito M: **Dynamic adjustments in prefrontal, hippocampal and inferior temporal interactions with increasing visual working memory load.** *Cereb Cortex* 2008, **18**:1618-1629.
79. Lee I, Kesner RP: **Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory.** *J Neurosci* 2003, **23**:1517-1523.
80. Izaki Y, Takita M, Akema T: **Specific role of the posterior dorsal hippocampus-prefrontal cortex in short-term working memory.** *Eur J Neurosci* 2008, **27**:3029-3034.
81. Yoon T, Okada J, Jung MW, Kim JJ: **Prefrontal cortex and hippocampus subserve different components of working memory in rats.** *Learn Mem* 2008, **15**:97-105.
82. Rosene DL, Van Hoesen GW: **Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey.** *Science* 1977, **198**:315-317.

83. Barbas H, Blatt GJ: **Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey.** *Hippocampus* 1995, **5**:511-533.
84. Ferino F, Thierry AM, Glowinski J: **Anatomical and electrophysiological evidence for a direct projection from ammon's horn to the medial prefrontal cortex in the rat.** *Brain Res* 1987, **65**:421-426.
85. Laroche S, Davis S, Jay TM: **Plasticity at hippocampal to prefrontal cortex synapses: Dual roles in working memory and consolidation.** *Hippocampus* 2000, **10**:438-446.
86. Thierry AM, Gioanni Y, Degenetais E, Glowinski J: **Hippocampo-prefrontal cortex pathway: Anatomical and elecctrophysiological characteristics.** *Hippocampus* 2000, **10**:411-419.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2377/11/24/prepub

doi:10.1186/1471-2377-11-24
Cite this article as: Palacios *et al.*: Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury. *BMC Neurology* 2011 **11**:24.

ARTICLE IN PRESS

CORTEX XXX (2012) 1–12

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal homepage: www.elsevier.com/locate/cortex

Research report

Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume

Eva M. Palacios [a,b], *Roser Sala-Llonch* [a,b], *Carme Junque* [a,b,*], *Davinia Fernandez-Espejo* [c], *Teresa Roig* [d], *Jose M. Tormos* [d], *Nuria Bargallo* [e] *and Pere Vendrell* [a,b]

[a] *Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain*
[b] *August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain*
[c] *Centre for Brain and Mind, University of Western Ontario, London, Canada*
[d] *Institut Universitari de Neurorehabilitacio Guttmann, Badalona, Spain*
[e] *Centre de Diagnostic per la Imatge (CDIC), Hospital Clinic de Barcelona, Spain*

ARTICLE INFO

Article history:
Received 11 October 2011
Reviewed 15 December 2011
Revised 17 January 2012
Accepted 24 February 2012
Action editor Jordan Grafman
Published online xxx

Keywords:
Traumatic brain injury
Declarative memory
Cortical thickness
Diffusion tensor imaging
Hippocampus

ABSTRACT

We investigated structural brain damage in subjects who had suffered severe and diffuse traumatic brain injury (TBI), and examined its relationship with declarative memory impairment. Cortical thickness, diffusion tensor imaging (DTI), and volumetric and shape data of the hippocampus were assessed in a group of 26 adults with severe TBI in the chronic stage and 22 healthy matched controls. Declarative memory was evaluated by Rey's Auditory Verbal Learning Test (RAVLT). TBI patients performed significantly worse than controls on all RAVLT measures. The group comparison for cortical thickness and DTI revealed a pattern of widespread atrophy in TBI patients. In the TBI group DTI measures correlated with cortical thickness in the prefrontal and parietal regions, including the precuneus. Declarative memory correlated with both cortical thickness and DTI measures. However, although hippocampal volume was significantly decreased in TBI patients, no correlations were found. Multiple regression analysis of all the structural measures revealed that decreases in Fractional anisotropy (FA) and thinning of the left parietal region were the best predictors of memory impairment. In conclusion, cortical thickness reductions in the left hemisphere and a lack of white matter integrity are the main contributors to long-term impairment in declarative memory among patients suffering from severe and diffuse TBI. In this study the hippocampus did not make a significant contribution to memory dysfunctions, suggesting that damage to this structure is compensated for by other regions, with the definitive sequelae being mainly explained by alterations in cortico-subcortical connectivity.

* *Corresponding author*. Department of Psychiatry and Clinical Psychobiology, University of Barcelona, C/Casanova 143, 08036 Barcelona, Spain.
E-mail address: cjunque@ub.edu (C. Junque).
0010-9452/$ – see front matter © 2012 Elsevier Srl. All rights reserved.
doi:10.1016/j.cortex.2012.02.011

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

1. Introduction

Traumatic axonal injury (TAI) is considered a progressive event which involves widespread damage to axons throughout the white matter, and it is strongly associated with worse outcomes in severe cases (Adams et al., 2011). Axons first undergo primary injury caused by intense shear and strain forces resulting from rapid acceleration, deceleration and rotation mechanisms. This is followed by secondary injury, including cytoskeletal disorganization and protein accumulation, leading to delayed axonal disconnection (Povlishock and Katz, 2005). However, not only white matter is affected after TAI. Magnetic resonance imaging (MRI) studies have also shown decreased grey matter volumes (Ding et al., 2008), while a neuropathological study conducted by Maxwell et al. (2010) demonstrated that after TAI there was a greater loss of pyramidal neurons in several cortical regions, producing changes in cortical thickness.

Cortical thickness is the distance from the outer cortical surface to the inner cortical white matter/grey matter boundary, and it can provide an indirect measure of the changes undergone by cortical architecture after cellular events (Fischl and Dale, 2000). In MRI studies of traumatic brain injury (TBI), grey matter atrophy has mainly been investigated by means of voxel-based morphometry (VBM). However, grey matter atrophy measured by VBM includes the local cortical surface and cortical folding, and depends on the overall brain size (Hutton et al., 2009). A better alternative for measuring grey matter atrophy is to use the Freesurfer® software (http://surfer.nmr.mgh.harvard.edu), which provides a more accurate estimation of cortical thickness. Cortical thinning has been reported in paediatric samples after TBI. Merkley et al. (2008) described a diffuse pattern of cortical atrophy involving cortical regions in all lobes, while the pattern found by McCauley et al. (2010) showed frontal predominance. These studies do not excluded focal lesions, thus the effects of TAI cannot be isolated from those of regional grey matter loss due to contusions. Furthermore, studies based on children are influenced by neurodevelopmental brain changes that cannot be extrapolated to the TBI adult population (Shaw et al., 2008).

White matter integrity can be assessed using diffusion tensor imaging (DTI). Fractional anisotropy (FA) is a DTI measure of the degree of directionality of water diffusion and reflects white matter integrity (Mori and Zhang, 2006). In subacute and chronic TBI, FA is decreased (Kraus et al., 2007) and is related with functional outcome (Sidaros et al., 2008; Newcombe et al., 2011). Using a region-of-interest approach, Kraus et al. (2007) found that declarative memory correlated with FA reductions in several fasciculi, while a previous study by our group obtained a significant correlation for the corpus callosum and fornix (Palacios et al., 2011). With a more precise technique of analysis [Tract-Based Spatial Statistics (TBSS)], Kinnunen et al. (2011) identified the fornix as the region most strongly related with memory deficits. A longitudinal study by Wang et al. (2011) found that damage in networks involving several fasciculi in the acute stage could predict memory and learning deficits in the chronic stage. It has also been reported that inter-hemispheric functional connectivity correlates with delayed recall (DR) (Marquez de la Plata et al., 2011).

The aim of the present study was to assess structural brain damage in subjects with diffuse and chronic severe TBI, and to examine its relationship with declarative memory impairment. To this end we used a multimodal approach including measures of cortical thickness, DTI and volumetric and shape data for the hippocampus. To date, no studies have focused on the assessment of declarative memory when taking into account these three measures of structural damage after TAI as the main mechanism of injury in a group of patients without significant focal lesions. We hypothesized that (1) patients will show cortical thickness atrophy, altered white matter integrity and hippocampal reductions compared to controls; (2) cortical thinning will depend in part on white matter alterations; and (3) memory impairment will be explained by damage to the hippocampus and cortical grey matter regions, as well as to their structural connections.

2. Methods

2.1. Subjects

We applied the following inclusion criteria to a database ($n = 170$) of chronic stage TBI patients from the Head Injury Unit of the Institut de Neurorehabilitació Guttmann: severe closed-head injury and severe TBI defined as Glasgow Coma Scale (GCS) score ≤ 8; adults aged ≤ 40 years; chronic stage of recovery ≥ 2 years since the TBI; possible diffuse pathology reported in the MRI scans in the sub-acute stage without macroscopic lesions. The exclusion criteria were as follows: injury requiring craniectomy or craniotomy; previous history of TBI, drug intake, and neurological or psychiatric disorders. This left us with 48 candidates, who were scanned. Since we were interested in studying the consequences of TAI after TBI, the neuroradiologist (NB) described the chronic brain lesions seen in the MRI. Patients with large lesions were excluded, together with those whose images presented motion artifacts. The final sample included 26 patients with a mean of age of 27.40 ± 5.15 (see Table 1). They were investigated at a mean time of evolution of 4.20 ± 1.14 years and with a mean degree of severity measured by the GCS of 5.19 ± 1.70. All the patients showed alterations such as microbleeds as a sign diffuse pathology in the T2* and flair sequences. Supplementary Table 1 shows clinical and neuroradiology characteristics for each patient. The etiology of TBI was traffic accident in all cases.

The control group comprised 22 healthy volunteers matched by age, sex, handedness, and education (Table 1). None had a previous history of neurological or psychiatric diseases.

The study was approved by the research ethics committees of the Guttmann Institute for Neurorehabilitation and the University of Barcelona. All participants gave written informed consent.

2.2. Neuropsychological assessment

Declarative memory was assessed using a version of the Rey Auditory Verbal Learning Test (RAVLT). In this word

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

Table 1 – Demographic characteristics and memory scores of patients and controls.

	TBI group (mean/SD)	Control group (mean/SD)	Statistic (*p*)
Age	27.40 (±5.15)	28.57 (±6.74)	.60
Education (years)	13.69 (±2.70)	14.54 (±3.15)	.50
Sex (M/F)	16/10	12/10	.24
GCS	5.19 (±1.70)	–	–
Time since injury (years)	4.20 (±1.14)	–	–
Memory performance			
RAVLT (learning)	45.42 (±10.26)	60.10 (±5.86)	.013
RAVLT(DR)	7.81 (±3.14)	13.50 (±1.56)	.002
RAVLT recognition	12.89 (±2.3)	14.95 (±.21)	.001

M/F: male/female.

learning test, subjects are required to reproduce 15 words presented over five trials, including free recall of the list after each trial (A1-5). The ∑A1-5 provides a measure of the total number of words learned. The test also measures DR of the list (at 20 min), and includes a recognition list in which the target words are presented with distractors, thereby providing a measure of how much was learned regardless of spontaneous retrieval (Lezak et al., 2004). Since in this study we were interested in long-term memory we specifically selected the raw scores of the DR variable. Table 2 shows the detailed descriptive values for RAVLT performance.

2.3. *MRI acquisition*

The MRI data were acquired on a SIEMENS Magnetom TrioTim syngo 3-T at the Diagnostic Imaging Centre of the Hospital Clinic (CDIC) in Barcelona. For each subject a high resolution T1-weighted structural image scan was obtained using an MPRAGE 3D protocol (TR = 2300 msec; TE = 3 msec; TI = 900 msec; FOV = 244 mm; 1 mm isotropic voxel). Diffusion weighted images were sensitized in 30 non-collinear directions with a b-value = 1000 sec/mm^2, using an echo-planar (EPI) sequence (TR = 9300 msec, TE = 94 msec, slice thickness = 2.0 mm, voxel size = 2.0 × 2.0 × 2.0 mm, FOV = 240 mm, no gap). For the lesion description, the neuroradiologist (NB) took into account T1-weighted, FLAIR (TR = 9000 msec, TE = 85 msec, voxel size = 3.0 mm, voxel size = 1.3 × .9 × 3.0 mm, FOV = 240 mm), and T2* GE (TR = 518 msec, TE = 20 msec, voxel size = 3.0 mm, voxel size = .9 × .8 × 3.0 mm, FOV = 240 mm) sequences. All the images were visually inspected prior to analysis.

Table 2 – Brain structural characteristics of patients and controls.

	TBI group mean (SD)	Control group mean (SD)	Statistic t (*p*)
Cortical thickness (mm)	2.52 (.10)	2.63 (.06)	4.33 (<.001)
FA	0.42 (.02)	0.47 (.01)	7.56 (<.001)
Hippocampal volume (mm^3)	42.88 (7.05)	46.50 (4.91)	2.08 (.047)
BPV (mm^3)	1.41 (.94) × 10^6	1.58 (.58) × 10^6	8.10 (<.001)

2.4. *Imaging analysis*

2.4.1. *Cortical thickness analysis*

The reconstruction of cortical surfaces and estimation of cortical thickness were performed using the automated FreeSurfer stream, version 4.5.0 (Athinoula A. Martinos Center for Biomedical Imaging. The procedures carried out by FreeSurfer software include removal of non-brain data, intensity normalization (Sled et al., 1998), tessellation of the grey matter/white matter boundary, automated topology correction (Fischl et al., 2001; Segonne et al., 2007) and accurate surface deformation to identify tissue borders (Dale et al., 1999; Fischl and Dale, 2000). Cortical thickness is then calculated as the distance between the white and grey matter surfaces at each vertex of the reconstructed cortical mantle (Fischl and Dale, 2000). Results for each subject were visually inspected to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. The cortical parcellation used to describe the results was also provided by the FreeSurfer software (Desikan et al., 2006).

2.4.2. *DTI pre-processing and analysis*

Data pre-processing and analysis were performed using the FMRIB Software Library (FSL) Diffusion Toolbox (FDT, http://www.fmrib.ox.ac.uk/fsl). After calculation of the FA and MD maps for each subject we implemented a voxel-wise statistical analysis of the FA data using TBSS v.1.2 (Smith et al., 2006). In summary, within the TBSS stream, FA maps were aligned into the common FMRIB58 FA template, which is in Montreal Neurological Institute (MNI 152) standard space. A non-linear registration algorithm (FNIRT) was used, and aligned FA maps were visually inspected after registration. A mean FA image was then created from the images of all the subjects in this common space and narrowed to generate a mean FA skeleton that represented the centre of all tracts common to the entire group. The skeleton was thresholded to FA greater than .2 so as to include the major white matter pathways but to exclude peripheral tracts where there was significant inter-subject variability and partial volume effects with grey matter. The aligned FA image for each subject was then projected onto the skeleton by filling the skeleton with FA values from the nearest relevant tract centre. The resulting skeletonized data were then fed into voxel-wise cross-subject statistics. Global FA values were

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

obtained for each subject as the average of FA within all the voxels in the skeleton.

2.4.3. *Hippocampal volume and segmentation*

Automated segmentation of the hippocampus was performed using FMRIB's Integrated Registration and Segmentation Tool (FIRST) from FSL, which uses a Bayesian probabilistic approach based on multivariate Gaussian assumptions. The shape and appearance models used were constructed from segmented images provided by the Center for Morphometric Analysis, Massachusetts General Hospital, Boston. The manual labels are parameterized as surface meshes and then modelled as a point distribution. Using these models, FIRST searches through linear combinations of shape modes of variation for the most probable shape instance given the observed intensities from the input image. The hippocampus segmentation was performed by running a two-stage affine registration to standard space. Each hippocampus was then automatically segmented, producing both mesh and volumetric outputs from this structure in native space.

2.4.4. *Brain parenchymal volume (BPV)*

The SIENAX tool from FSL (FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl) was used to estimate BPV (Smith et al., 2002). In SIENAX, brain and skull images are extracted, and the brain image is affine-registered to an MNI standard template using the skull image to determine registration scaling, which is used as normalization for head size. Finally, segmentation with partial volume estimation is carried out to obtain the normalized white matter and BPV.

2.5. *Statistics*

2.5.1. *Cortical thickness*

Group comparisons of the cortical thickness maps were carried out using general linear modelling implemented in FreeSurfer (QDEC application), which performs inter-subject/group averaging and inference on the cortical surface. For group analyses, individual surfaces were registered to standard space and smoothed using a Gaussian kernel of full width at half maximum (FWHM) of 15 mm as suggested in the cortical thickness literature (Lerch and Evans, 2005). The different general linear model (GLM) matrices evaluated were: (1) group comparisons (t-test) to obtain maps of thickness differences between patients and controls; (2) correlations with global FA measures; and (3) correlations with declarative memory recall scores. The resulting maps were corrected for multiple comparisons using a Monte Carlo Null-Z simulation, performing 10,000 iterations (family-wise error [FWE]). Only corrected clusters with a final cluster-wise $p < .05$ were considered. For each individual, the mean cortical thickness within the areas that resulted significant was extracted to obtain summary statistics. Mean thickness values were extracted for both hemispheres.

2.5.2. *DTI*

Correlations between whole brain FA maps and RAVLT values were performed using Randomise v.2.1 from FSL. The statistical threshold was set at $p < .05$, FWE corrected, which is a conservative procedure that enables high control of Type I error; the probability of one or more false positives is the same as the significance level. The Threshold-Free Cluster Enhancement (TFCE) method was used to define the clusters.

2.5.3. *Hippocampal volumes*

Hippocampal volumes were obtained from outputs of the FIRST segmentation analysis. The ratio between hippocampal volume and the BPV was also calculated.

2.5.4. *Shape and vertex analysis*

Shape differences between groups were examined on a per-vertex basis. Vertex correspondence investigates focal shape differences by examining group differences in the spatial location of each vertex. The number of vertices and their labelling is fixed so that corresponding vertices can be compared across individuals and between groups. Group comparison of vertices was performed with F-statistics, using the total intracranial volume as a covariate. The result of this analysis was rendered on the shape surface, providing a map of those regions where the structure differed significantly between groups.

2.5.5. *Multiple regression analysis*

Individual values of averaged cortical thickness and mean FA were extracted for those areas which were shown to be significantly correlated with memory performance. For cortical thickness, values were extracted separately for each cluster within the significant map. These values, together with the ratio of hippocampal volume for each subject, were then introduced into a multiple regression analysis using SPSS (Statistical Package for the Social Sciences) v.17 (SPSS Inc., ChicagoIllinois). The dependent variable was the memory score, while the structural brain measures (FA, cortical thickness and hippocampal ratio) were considered as independent variables.

2.5.6. *Brain structural values and behavioural data*

Statistical tests on whole-brain measures and non-imaging data were performed using SPSS v.17.0. As the data were normally distributed, group differences were examined using the Student's *t*-test. The level of significance was set at $p < .05$.

3. Results

3.1. *Group comparison*

Table 2 shows group comparisons for the global measures of whole BPV, mean cortical thickness, whole brain mean FA values and hippocampal volumes. There were significant differences ($p < .05$) between patients and controls on all these measures of brain atrophy.

3.1.1. *Cortical thickness*

The cortical thickness analysis revealed a significant pattern of cortical atrophy in both hemispheres in the patient group. Regions with significant thinning were: rostral and middle frontal cortex, superior and middle temporal cortex (including the anterior parts of the temporal lobes), superior and inferior parietal cortex, precentral and postcentral

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

cortex, precuneus, parahippocampal cortex, lingual cortex, pericalcarine cortex, isthmus–cingulate, and anterior and posterior cingulate cortex (Fig. 1). The medial prefrontal cortex and the occipital and temporal lobes were widely preserved.

3.1.2. DTI

Group comparison showed a pattern of widespread white matter damage in TBI patients. TBSS analysis revealed several fasciculi whose FA values were significantly decreased: intra-hemispheric association fibres of the inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculi and the cingulum bundle; inter-hemispheric fibres of the corpus callosum (genu and splenium); projection fibres of the corti-cospinal tracts; and in the anterior thalamic radiations, anterior limb of the internal capsule, and anterior corona radiate. FWE-corrected maps ($p < .05$; TFCE) are shown in Fig. 2.

3.1.3. *Hippocampal volume and shape analysis*

There were significant differences between patients and controls in the ratio of global hippocampal volumes (Table 2). The shape analysis showed specific significant reductions in the left hippocampal head and tail corrected at FDR. However, at an uncorrected statistical level ($p < .001$), we observed a more global rather than a selective pattern of atrophy (Fig. 3).

3.2. *Correlation analyses*

3.2.1. *Correlations between cortical thickness and DTI measures*

In the TBI group cortical thickness correlated with global FA in several regions. The map of the regions that achieved statistical significance is shown in Fig. 4.

3.2.2. *Correlations between memory and structural measures*

In the TBI group cortical thickness was positively correlated with memory scores in several regions of the left hemisphere: superior frontal cortex and the inferior and superior parietal cortex (Fig. 5a). Individual values were computed as the average cortical thickness from these regions of interest (ROIs) and used to calculate the Pearson's correlation ($r = .70$; $p < .001$). No significant results were found for the right hemisphere. In the control group, by contrast, negative correlations were found between cortical thickness and memory scores in the rostral middle frontal and superior frontal cortex ($r = -.84$; $p < .001$; Fig. 5b).

The TBSS analysis showed that the FA maps of TBI patients correlated with memory scores in several fasciculi. MNI coordinates of the local maxima located the largest clusters in the left hippocampus and cingulum, followed by the left parietal part of the superior longitudinal fasciculi (Fig. 6). Pearson's r score was computed with the averaged FA within significant areas ($r = .59$; $p = .001$).

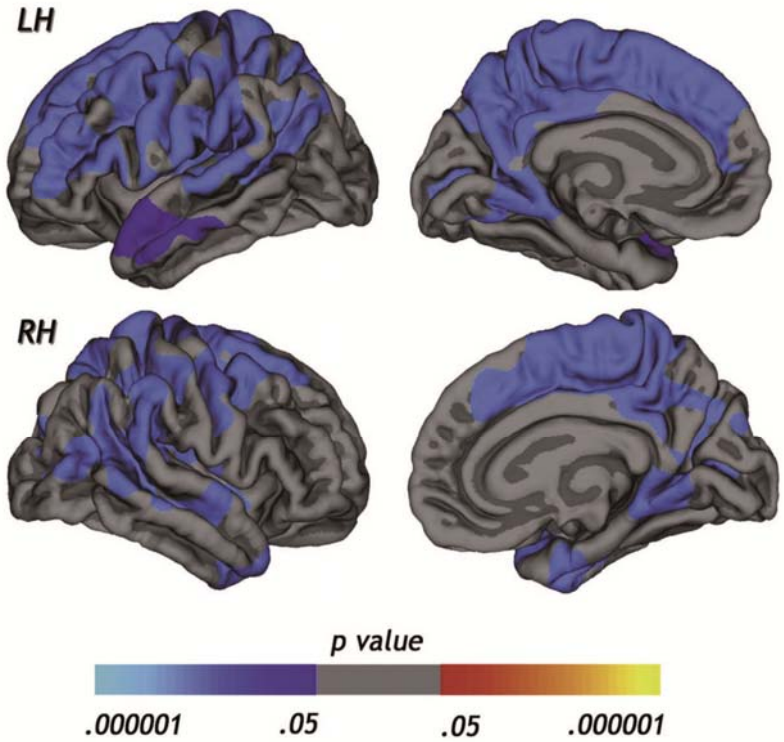


Fig. 1 – Regions of significant cortical thinning in TBI patients compared with controls. Only clusters surviving FWE multiple comparison correction with a final cluster-wise $p < .05$ are considered.

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

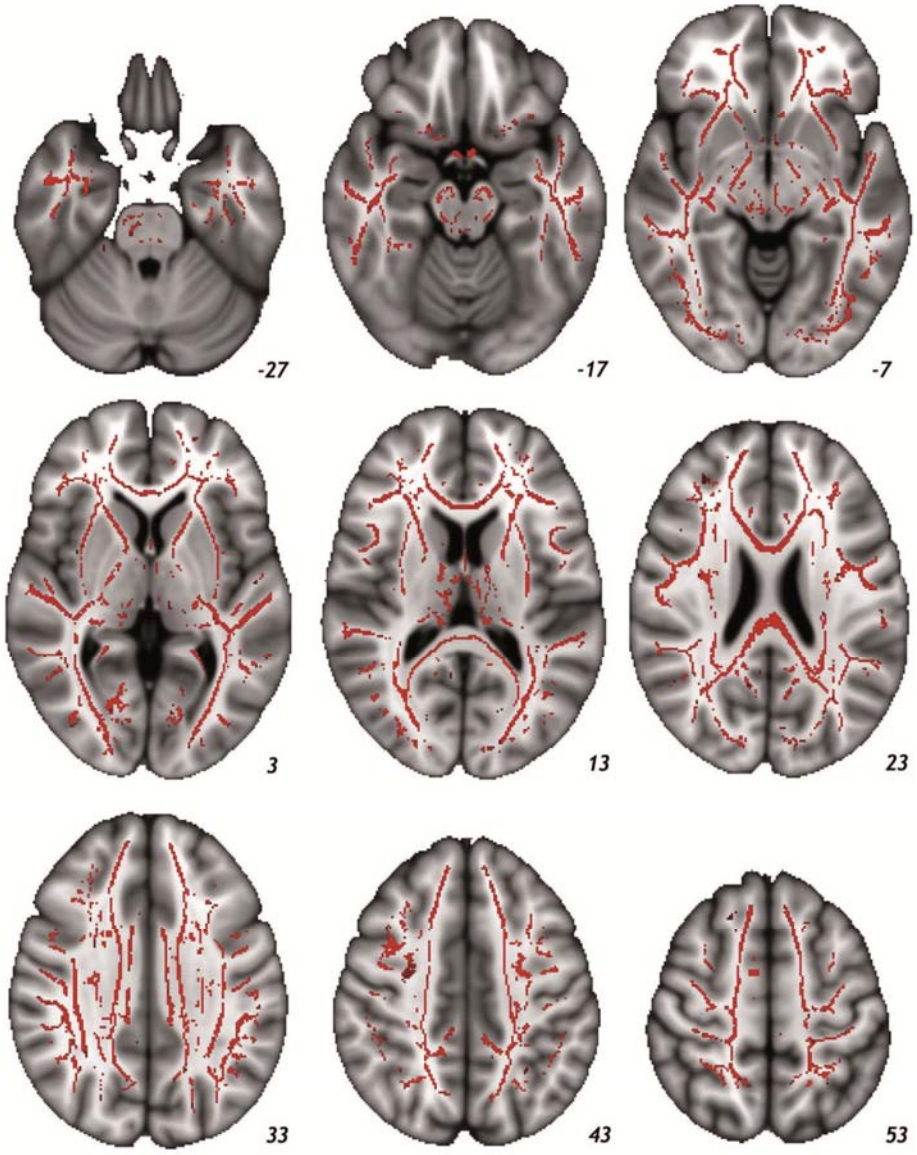


Fig. 2 – Results from TBSS analysis of FA maps showing (in red) the clusters of significantly reduced FA in TBI patients compared to controls. Widespread impairment of white matter integrity can be observed. Images are displayed in radiological convention (TFCE, $p < .05$ FWE-corrected).

The correlation analysis between the hippocampal ratio and declarative memory scores failed to reach statistical significance ($r = .27$; $p = .17$).

3.2.3. *Regression analysis*

The regression analysis was performed in order to investigate the best predictors of the memory deficits. The independent variables included in the regression analysis were: the volume hippocampal ratio, mean cortical thickness, and mean FA from the areas that correlated with memory.

This analysis revealed that the measures of mean FA, followed by cortical thickness of the parietal-precuneus ROI, were the best predictors of memory performance in TBI patients. Thickness of the frontal and superior parietal lobes, as well as hippocampal volumes, did not enter into the model.

4. Discussion

The aim of this study was to assess structural brain damage in subjects with diffuse and chronic TBI, and its relationship with declarative memory impairment. The main conclusion is that although patients had widespread grey and white matter atrophy, decreased cortical thickness in the parietal left

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

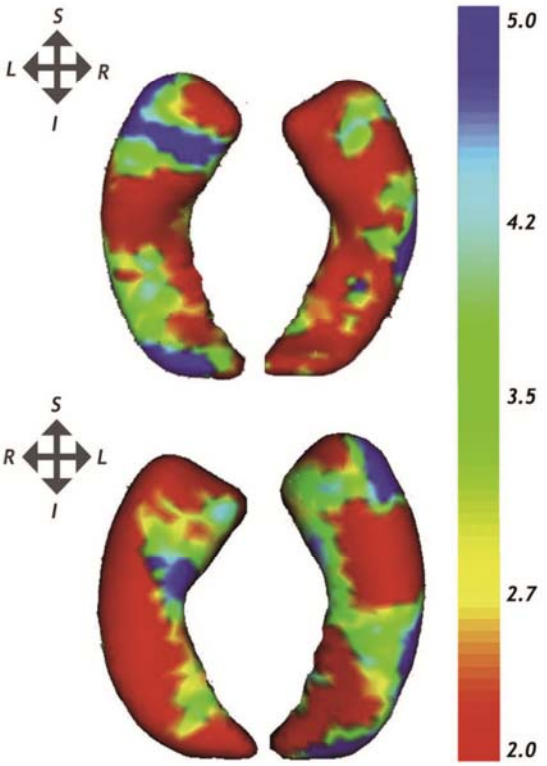


Fig. 3 – Hippocampal shape abnormalities in the TBI group compared to controls. The colour bar indicates F-statistics at an uncorrected level of $p < .001$. Widespread atrophy of both hippocampi can be observed, with a predominance of the hippocampal head.

hemisphere and white matter alterations were the main contributors to long-term memory deficits. By contrast, hippocampal atrophy did not play a primary role in explaining memory impairment, suggesting that damage to this structure may be compensated for by other regions, with the definitive sequelae being mainly explained by alterations in cortico-subcortical connectivity.

In group comparisons, the cortical thickness reductions observed in TBI patients reflect a pattern of widespread cortical atrophy, mainly involving the frontal and parietal lobes. The cortex of the occipital and temporal lobes was almost preserved. This pattern of cortical atrophy is similar to that described by Warner et al. (2010a) in a longitudinal study of patients with TAI. By using surface-based cortical volumetric analyses they found progressive cortical atrophy when comparing the acute stage (day 1) and follow-up (8 months) results. In addition to fronto-parietal atrophy we also found cortical thinning in the cingulate cortex and medial temporal regions. These differences in the degree of cortical atrophy can be explained in terms of the different time of evolution from injury between studies. The present sample involved patients in the chronic stage, with a minimum of 2 years post injury. There is clear evidence of progressive atrophy beyond 1 year after injury (Bigler, 2001; Sidaros et al., 2009; Bendlin et al., 2008). It should also be noted that cortical thickness comparisons between patients and controls have been reported in two paediatric samples. Merkley et al., 2008 described a more extensive pattern of cortical thinning than the one that we report, but may be due to the statistical threshold selected, as they presented the map of results at an uncorrected level. McCauley et al. (2010) obtained a pattern of prefrontal predominance. The differences between the studies are probably due to the clinical characteristics of the samples: McCauley et al.'s patients were in the subacute stage (3 months of evolution) while Merkeley et al.'s patients had a mean evolution of 3 years. Other factors that may explain the differences between the studies were the size and location of the focal lesions. However, those studies are not fully comparable to ours, because in children and adolescents the cortex is involved in a progressive process of physiological thinning (Shaw et al., 2008), and cortical thickness is thought to be dependent on the processes of grey matter dendritic arborization, pruning, and degree of myelinization at the gray/white matter interface (Huttenlocher, 1990; Sowell et al., 2004).

Although we found extensive cortical atrophy and widespread damage to white matter, FA was only correlated with the thickness of specific regions of the frontal and parietal lobes and the cingulate cortex. Interestingly, the cortical regions showing greater cerebral atrophy associated with decreased white matter integrity were those that are connected by the long associative fasciculi, such as the superior longitudinal fasciculi, the fronto-occipital and the cingulum. Given that the diffuse characteristics of our sample these results may suggest that the process of progressive cortical atrophy in these areas is a consequence of retrograde degeneration of these fasciculi. A recent histopathological study by Maxwell et al. (2010) demonstrated decreased cortical thickness in several frontal regions. This thinning is due to the loss and size reductions of pyramidal and non-pyramidal neurons, and it is seen in both TAI and non-TAI patients, although greater loss of pyramidal neurons occurs in the prefrontal cortex of patients with TAI.

The group comparison and correlational results obtained here may reflect different mechanisms of cerebral atrophy after severe TBI. Reduction of cortical thickness may be the result of neuronal loss due to several mechanisms, such as neuroexcitation, ischemic brain damage or microvascular injury directly affecting the grey matter as a consequence of primary and secondary damage; it could also be due to the progressive retrograde degeneration caused by TAI. It should also be noted that the general white matter damage found in the DTI analysis probably reflects primary and secondary axotomy, anterograde or Wallerian degeneration, demyelinization and other events that produce diffuse and extensive white matter alterations (Buki and Povlishock, 2006). Finally, the specific fronto-parietal cortical areas that were found to be related with white matter damage might be a direct result of a neurodegenerative processes caused by TAI and mainly involving retrograde degeneration.

Declarative memory correlated with cortical thickness in the left prefrontal cortex in both healthy controls and TBI patients. In the TBI group, patients who had decreased thickness in the frontal lobes were those who performed worse on the memory test. It has been demonstrated that

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

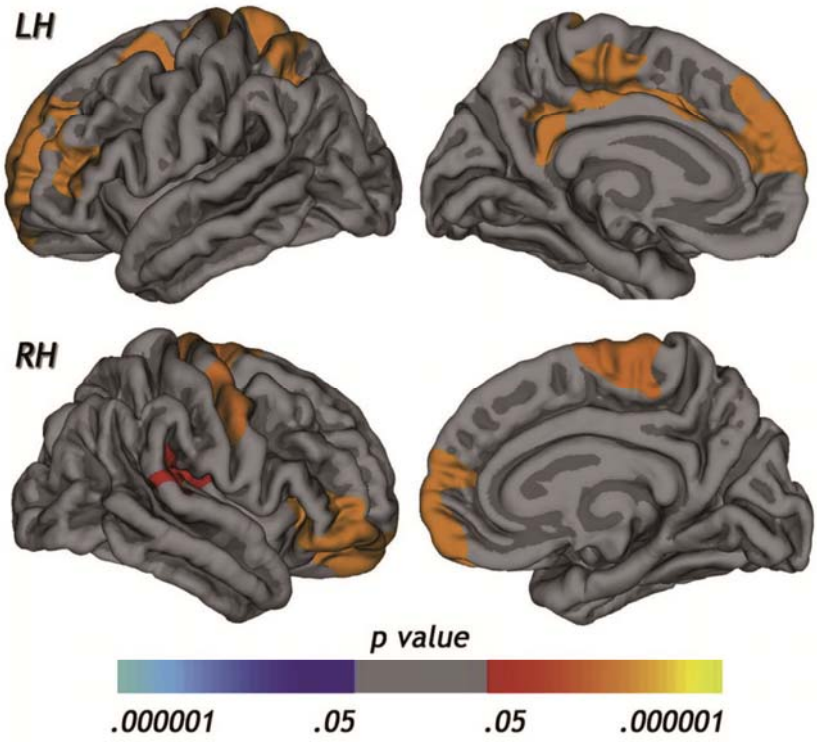


Fig. 4 – Maps of the correlation between cortical thickness and whole FA measures in the TBI group. The significant correlations are seen mainly in bilateral fronto-parietal regions, as well as in the left cingulum and right superior temporal cortex. Only clusters surviving FWE multiple comparison correction with a final cluster-wise $p < .05$ are considered.

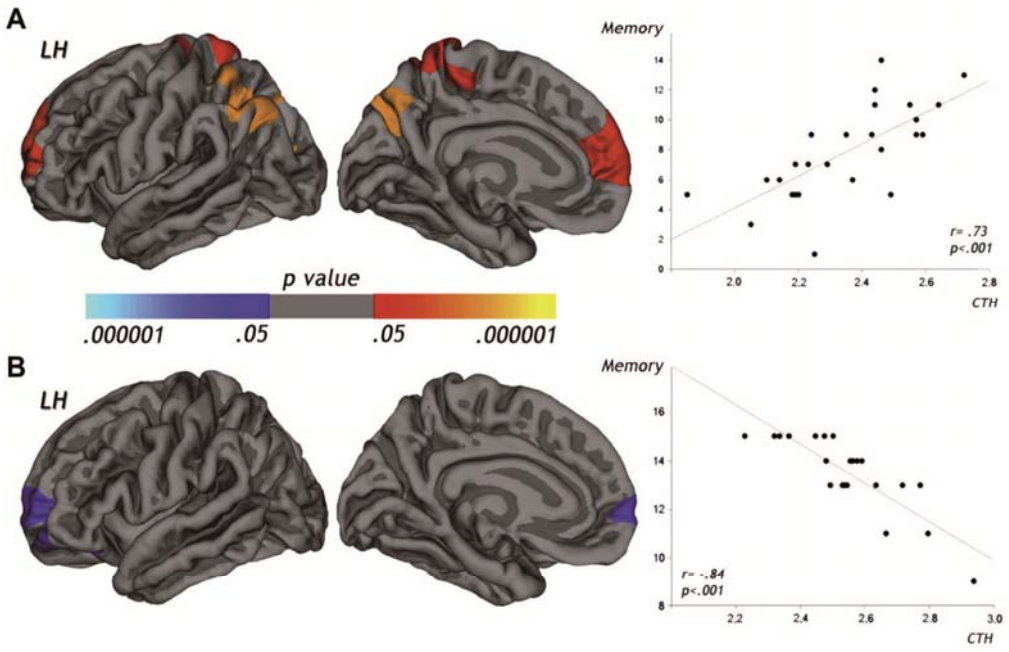


Fig. 5 – Correlation maps of cortical thickness and memory scores for patients and controls separately (FWE-corrected $p < .05$). Hot and cold colours represent positive and negative correlations, respectively. Scatter plots show the averaged cortical thickness within the significant clusters and the memory scores for each group of subjects.

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

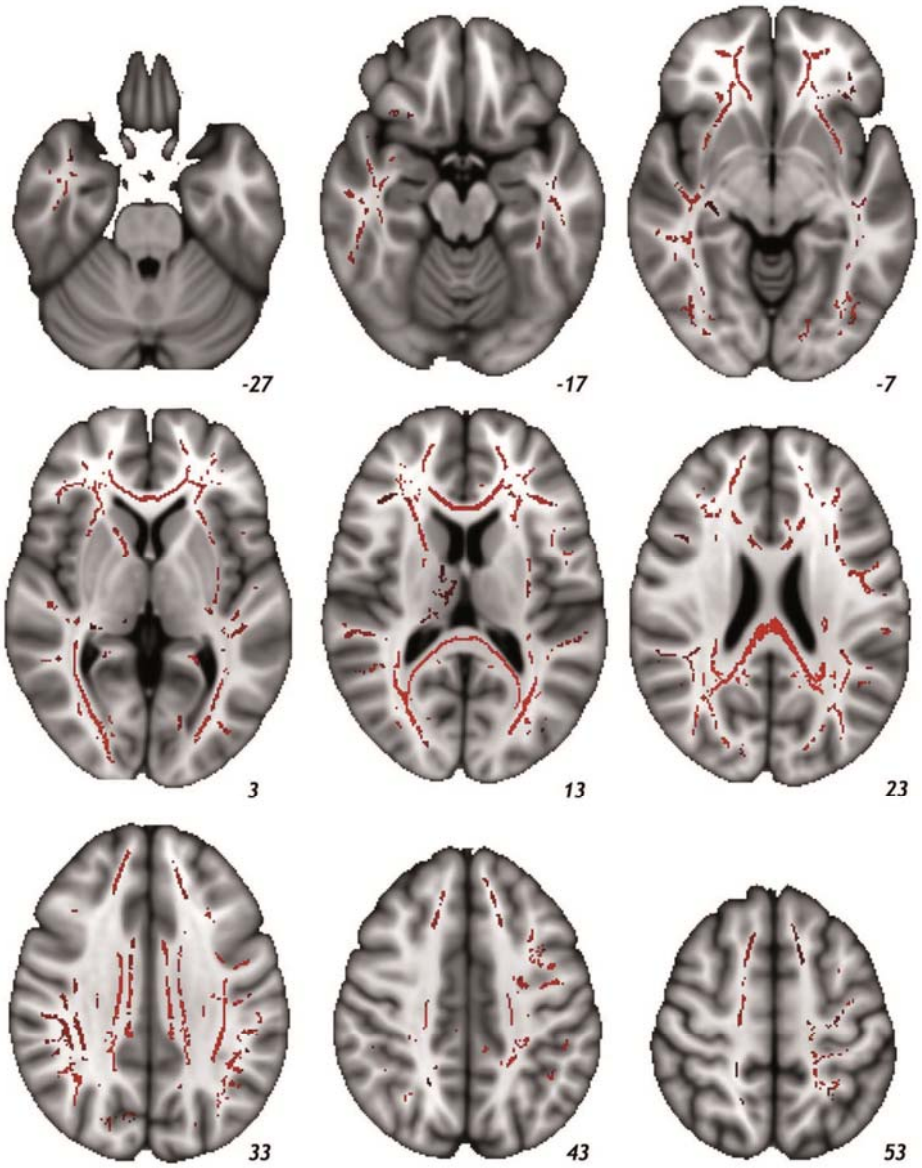


Fig. 6 – Maps of correlations between FA values and memory performance in patients (thresholded at $p < .05$, FWE-corrected).

after TBI there is a loss of neurons from the overlying grey/white matter boundary in the frontotemporal lobe, with axons being lost from frontotemporal white matter (Maxwell et al., 2010). Here, thinning in the left parietal lobe was only found to be related with worse declarative memory performance in the TBI group. The posterior parietal cortex is often activated during memory retrieval and these areas are connected directly or indirectly to the medial temporal lobe by the middle longitudinal fasciculi and inferior longitudinal fasciculi (Wagner et al., 2005; Cabeza et al., 2008). Furthermore, alterations in the parietal cortex per se cause attentional dysfunctions during the process that supports memory retrieval, searching, monitoring and verification (Cabeza et al., 2008). Here, we observed a relationship between memory performance and decreases in the thickness of the precuneus, a region that subserves successful episodic retrieval (Cavanna and Trimble, 2006 for review). Our results are in agreement with previous TAI studies involving similar samples, and which also reported that fronto-parietal regions and the precuneus are related with learning and memory scores (Warner et al., 2010b). Notably, the fronto-parietal cortical regions that we found to be correlated with memory deficits correspond to those that are correlated with white matter impairment, suggesting a role for disrupted connectivity between complex

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

nodes of memory network. Moreover, some of the areas that correlated with memory deficits in our sample, such as the precuneus and the middle frontal cortex, have a high correspondence with the spatial pattern of the default-mode network (DMN), (Buckner et al., 2008). Recent studies have found disruptions in the functional connectivity of the DMN in TBI patients which are related to white matter structural damage (Sharp et al., 2011). The alterations in the DMN have been associated with cognitive deficits involving attention and working memory (Sharp et al., 2011; Bonnelle et al., 2011; Palacios et al., 2012). In addition, the dysfunction in the connectivity of DMN nodes with other brain networks has been proposed as a biomarker of cognitive impairment in TBI (Mayer et al., 2011). We think that the structural damage in the connectivity between nodes of the complex networks may cause alterations in the functional connectivity of the DMN and may contribute to the residual cognitive deficits in TBI. Future studies on resting state fMRI networks and on cognitive functions will be required in order to further elucidate this hypothesis.

By contrast, in the control group, subjects with reduced thickness in the frontal region performed better on the memory test. This result is consistent with the literature on brain maturation, where it has been reported that the development of cortical thickness follows non-linear trajectories during childhood and early adulthood. A predominantly negative correlation between IQ and cortical thickness has also been demonstrated in adulthood, most notably in the prefrontal cortex (Shaw et al., 2008). Furthermore, Giedd et al. (1999) found decreases in frontal grey matter during post-adolescence, and suggested a relationship with the development of cognitive functions. As our control group included young adults this result might be evidence of the brain maturation that is characterized by cortical thinning in these stages of development.

Although we found a widespread correlation between the loss of white matter integrity and declarative memory impairment, the largest cluster included the left hippocampus and cingulum, followed by the left parietal part of the superior longitudinal fasciculi. These results are in agreement with the verbal component of the task. Using tractography Wang et al., 2011 showed that acute FA values are predictive of long-term impairment in learning, although they did not report specificity for the 28 tracks quantified. Unlike Kinnunen et al., 2011 who used the same approach to analyse DTI and found evidence that the fornices predicted associative memory performance in patients and controls, we failed to find specific fasciculi related to memory scores. The absence of a correlation between specific fasciculi and memory impairment in our sample may be due to the severity and chronicity of TBI.

Regarding the hippocampal volume and shape analysis, TBI patients presented significant bilateral hippocampal atrophy in comparison to controls. The shape group comparison showed that the pattern of atrophy was global in both hippocampi. Hippocampal atrophy and verbal memory impairment have been reported in several studies of TBI (Bigler et al., 1996; Tate and Bigler, 2000; Ariza et al., 2006). However, we found no significant correlations between memory scores and either total hippocampal volume or specific areas of the hippocampus. The absence of any correlation may be due to the chronic stage of our patients. At all events, the results suggest that hippocampal dysfunctions may be compensated for by other regions, with the definitive sequelae being mainly explained by alterations in cerebral connectivity.

Finally, multiple regression analysis, taking into account all the structural measures that were individually correlated with memory performance, showed that alterations in white matter connectivity are the best predictor of declarative memory sequelae, although there is also an independent contribution of cortical atrophy in the left precuneus.

One limitation of our study is that the results may not be generalizable across TBI with different profiles. Our sample was carefully selected to include patients with TAI without large focal lesions, like other studies in the TAI literature (e.g., Warner et al., 2010a, 2010b). However, despite the modest sample size, we believe that the homogeneity in the sample recruitment allows a better understanding of the structural damage after diffuse pathology and its relationship with memory deficits. Although all the patients were in the chronic stage, the time from injury ranged widely, some patients may still have been in an active state of functional reorganization. Longitudinal studies including acute and sub-acute stages would also be of interest to study structural brain connectivity reorganization after TBI with diffuse pathology and its relationship with cognitive improvements.

Acknowledgements

The authors thank MAPFRE Foundation for the financial support. We also thank Cesar Garrido from the Centre de Diagnostic per la Imatge of the Hospital Clinic (CDIC), for his assistance with data recollection.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cortex.2012.02.011.

REFERENCES

Adams JH, Jennett B, Murray LS, Teasdale GM, Gennarelli TA, and Graham DI. Neuropathological findings in disabled survivors of a head injury. *Journal of Neurotrauma*, 28(5): 701–709, 2011.

Ariza M, Serra-Grabulosa JM, Junque C, Ramirez B, Mataro M, Poca A, et al. Hippocampal head atrophy after traumatic brain injury. *Neuropsychologia*, 44(10): 1956–1961, 2006.

Bendlin BB, Ries ML, Lazar M, Alexander AL, Dempsey RJ, Rowley HA, et al. Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. *NeuroImage*, 42(2): 503–514, 2008.

Bigler ED. Quantitative magnetic resonance imaging in traumatic brain injury. *The Journal of Head Trauma Rehabilitation*, 16(2): 117–134, 2001.

Bigler ED, Johnson SC, Anderson CV, Blatter DD, Gale SD, Russo AA, et al. Traumatic brain injury and memory: The role

of hippocampal atrophy. *Neuropsychology*, 10(3): 333–342, 1996.

Bonnelle V, Leech R, Kinnunen KM, Ham TE, Beckmann CF, De Boissezon X, et al. Default mode network connectivity predicts sustained attention deficits after traumatic brain injury. *Journal of Neuroscience*, 31(38): 13442–13451, 2011.

Buckner RL, Andrews-Hanna JR, and Schacter DL. The brain's default network: anatomy, function, and relevance to disease. *Annals of the New York Acad Science*, 1124: 1–38, 2008.

Buki A and Povlishock JT. All roads lead to disconnection? Traumatic axonal injury revisited. *Acta Neurochirurgica*, 148(2): 181–193, 2006.

Cabeza R, Ciaramelli E, Olson IR, and Moscovitch M. The parietal cortex and episodic memory: An attentional account. *Nature Reviews. Neuroscience*, 9(8): 613–625, 2008.

Cavanna AE and Trimble MR. The precuneus: A review of its functional anatomy and behavioural correlates. *Brain*, 129(3): 564–583, 2006.

Dale AM, Fischl B, and Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage*, 9(2): 179–194, 1999.

Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage*, 31(3): 968–980, 2006.

Ding K, Marquez de la Plata CD, Wang JY, Mumphrey M, Moore C, Harper C, et al. Cerebral atrophy after traumatic white matter injury: Correlation with acute neuroimaging and outcome. *Journal of Neurotrauma*, 25(12): 1433–1440, 2008.

Fischl B and Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Sciences U.S.A.*, 97(20): 11050–11055, 2000.

Fischl B, Liu A, and Dale AM. Automated manifold surgery: Constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Transactions on Medical Imaging*, 20(1): 70–80, 2001.

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: A longitudinal MRI study. *Nature Neuroscience*, 2(10): 861–863, 1999.

Huttenlocher PR. Morphometric study of human cerebral cortex development. *Neuropsychologia*, 28(6): 517–527, 1990.

Hutton C, Draganski B, Ashburner J, and Weiskopf N. A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. *NeuroImage*, 48(2): 371–380, 2009.

Kinnunen KM, Greenwood R, Powell JH, Leech R, Hawkins PC, Bonnelle V, et al. White matter damage and cognitive impairment after traumatic brain injury. *Brain*, 134(2): 449–463, 2011.

Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, and Little DM. White matter integrity and cognition in chronic traumatic brain injury: A diffusion tensor imaging study. *Brain*, 130(10): 2508–2519, 2007.

Lerch JP and Evans AC. Cortical thickness analysis examined through power analysis and a population simulation. *NeuroImage*, 24(1): 163–173, 2005.

Lezak MD, Howieson DB, Loring DD, Hannay HJ, and Fisher JS. *Neuropsychological Assessment*. New York: Oxford University Press, 2004.

Marquez de la Plata CD, Garces J, Shokri Kojori E, Grinnan J, Krishnan K, Pidikiti R, et al. Deficits in functional connectivity of hippocampal and frontal lobe circuits after traumatic axonal injury. *Archives of Neurology*, 68(1): 74–84, 2011.

Mayer AR, Mannell MV, Ling J, Gasparovic C, and Yeo RA. Functional connectivity in mild traumatic brain injury. *Human Brain Mapping*, 32(11): 1825–1835, 2011.

Maxwell WL, MacKinnon MA, Stewart JE, and Graham DI. Stereology of cerebral cortex after traumatic brain injury matched to the glasgow outcome score. *Brain*, 133(1): 139–160, 2010.

McCauley SR, Wilde EA, Merkley TL, Schnelle KP, Bigler ED, Hunter JV, et al. Patterns of cortical thinning in relation to event-based prospective memory performance three months after moderate to severe traumatic brain injury in children. *Developmental Neuropsychology*, 35(3): 318–332, 2010.

Merkley TL, Bigler ED, Wilde EA, McCauley SR, Hunter JV, and Levin HS. Diffuse changes in cortical thickness in pediatric moderate-to-severe traumatic brain injury. *Journal of Neurotrauma*, 25(11): 1343–1345, 2008.

Mori S and Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron*, 51(5): 527–539, 2006.

Newcombe V, Chatfield D, Outtrim J, Vowler S, Manktelow A, Cross J, et al. Mapping traumatic axonal injury using diffusion tensor imaging: Correlations with functional outcome. *PLoS One*, 6(5): e19214, 2011.

Palacios EM, Fernandez-Espejo D, Junque C, Sanchez-Carrion R, Roig T, Tormos JM, et al. Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury. *BMC Neurology*, 11: 24, 2011.

Palacios EM, Sala-Llonch R, Junque C, Roig T, Tormos JM, Bargallo N, and Vendrell P. Altered structural connectivity is related to functional working memory networks in TBI. *Neurology*, 20(12): 852–860, 2012.

Povlishock JT and Katz DI. Update of neuropathology and neurological recovery after traumatic brain injury. *The Journal of Head Trauma Rehabilitation*, 20(1): 76–94, 2005.

Segonne F, Pacheco J, and Fischl B. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Transactions on Medical Imaging*, 26(4): 518–529, 2007.

Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, et al. Neurodevelopmental trajectories of the human cerebral cortex. *Journal of Neuroscience*, 28(14): 3586–3594, 2008.

Sharp DJ, Beckmann CF, Greenwood R, Kinnunen KM, Bonnelle V, De Boissezon X, et al. Default mode network functional and structural connectivity after traumatic brain injury. *Brain*, 134(8): 2233–2247, 2011.

Sidaros A, Engberg AW, Sidaros K, Liptrot MG, Herning M, Petersen P, et al. Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: A longitudinal study. *Brain*, 131(2): 559–572, 2008.

Sidaros A, Skimminge A, Liptrot MG, Sidaros K, Engberg AW, Herning M, et al. Long-term global and regional brain volume changes following severe traumatic brain injury: A longitudinal study with clinical correlates. *NeuroImage*, 44(1): 1–8, 2009.

Sled JG, Zijdenbos AP, and Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Transactions on Medical Imaging*, 17(1): 87–97, 1998.

Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage*, 31(4): 1487–1505, 2006.

Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, and De Stefano N. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *NeuroImage*, 17(1): 479–489, 2002.

Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, and Toga AW. Longitudinal mapping of cortical thickness and

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

brain growth in normal children. *Journal of Neuroscience*, 24(38): 8223–8231, 2004.
Tate DF and Bigler ED. Fornix and hippocampal atrophy in traumatic brain injury. *Learning and Memory*, 7(6): 442–446, 2000.
Wagner AD, Shannon BJ, Kahn I, and Buckner RL. Parietal lobe contributions to episodic memory retrieval. *Trends in Cognitive Sciences*, 9(9): 445–453, 2005.
Wang JY, Bakhadirov K, Abdi H, Devous MDS, Marquez de la Plata CD, Moore C, et al. Longitudinal changes of structural connectivity in traumatic axonal injury. *Neurology*, 77(9): 818–826, 2011.
Warner MA, Youn TS, Davis T, Chandra A, Marquez de la Plata CD, Moore C, et al. Regionally selective atrophy after traumatic axonal injury. *Archives of Neurology*, 67(11): 1336–1344, 2010a.
Warner MA, Marquez de la Plata CD, Spence J, Wang JY, Harper C, Moore C, et al. Assessing spatial relationships between axonal integrity, regional brain volumes, and neuropsychological outcomes after traumatic axonal injury. *Journal of Neurotrauma*, 27(12): 2121–2130, 2010b.

Please cite this article in press as: Palacios EM, et al., Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume, Cortex (2012), doi:10.1016/j.cortex.2012.02.011

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

E.M. Palacios, MSc
R. Sala-Llonch, MSc
C. Junque, PhD
T. Roig, PhD
J.M. Tormos, MD, PhD
N. Bargallo, MD, PhD
P. Vendrell, PhD

Correspondence & reprint requests to Dr. Junque: cjunque@ub.edu

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 connected[1] 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

Editorial, page XXX

Supplemental data at www.neurology.org

From the Department of Psychiatry and Clinical Psychobiology (E.M.P., R.S.-L., C.J., P.V.), University of Barcelona, Barcelona; Institute of Biomedical Research August Pi i Sunyer (IDIBAPS) (E.M.P., R.S.-L., C.J., P.V.), Barcelona; Institut Universitari de Neurorehabilitacio Guttmann (T.R., J.M.T.), Badalona; and Centre de Diagnostic per la Imatge Hospital Clinic de Barcelona (CDIC) (N.B.), Hospital Clinic de Barcelona, Spain.

Study funding: Sponsored by the MAPFRE Foundation.
Disclosure: Author disclosures are provided at the end of the article.

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 visuoperceptive 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.[12] 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.

Table 1 Clinical and neuroimaging characteristics of the TBI patients

Patient	Tev	GCS	MRI findings (T2*/FLAIR-hemosiderin deposits): MBs/contusions
1	6	3	MBs in R/L thalamus, internal capsule, midbrain, L hippocampus, L temporal, frontal lobe and genu CC. Parahippocampal contusion (0.9 mL).
2	5.9	6	MBs in R thalamus, L caudate, and frontotemporal lobes. Frontal and CC deep white matter hyperintensities due to demyelination. Frontal contusion (0.7 mL).
3	5.7	3	MBs in R caudate, R internal capsule, R temporal lobe, R lentiform nucleus, R/L frontoparietal lobes, R occipital lobe, and splenium CC. Frontoparietal deep white matter hyperintensities due to demyelination. No contusions evidenced.
4	3.4	7	MBs in R hippocampus, splenium CC, R cerebellum, R frontal hemorrhagic contusion (4.8 mL).
5	5.6	4	MBs in L thalamus, R insula, R/L hippocampus, R/L frontal lobes, and splenium CC. Frontal deep white matter hyperintensities due to demyelination. No contusions evidenced.
6	3.4	8	MBs in CC and frontoparietal lobes bilaterally. Periventricular and frontoparietal deep white matter affectation due to demyelinating or malacic myelopathy. No contusions evidenced.
7	2.6	3	MBs in R/L thalamus; R/L insula, R/L external capsule, L lentiform nucleus, L/R hippocampus, R/L cortico-subcortical frontoparietal junction, temporal and occipital lobes, splenium and body CC, and cerebellum. No contusions evidenced.
8	3.8	6	MBs in L frontal lobe, R temporal lobe, midbrain, and cerebellum. Small L parietal deep white matter focus of demyelination. No contusions evidenced.
9	7.4	6	MBs in L thalamus, midbrain, frontotemporal deep white matter affectation. Temporal contusions (L: 6.08 mL, 8.4 mL; R: 1.8 mL, 1.9 mL), frontal contusion (9.3 mL).
10	5	5	MBs in midbrain, cerebellum, splenium CC. Deep frontal white matter hyperintensities due to demyelination. No contusions evidenced.
11	3.4	3	MBs in L insula, basal ganglia, midbrain, L hippocampus, frontoparietal lobes. Deep R temporal and parietal white matter hyperintensities due to demyelination. Frontobasal (1.07 mL) and temporal (1.3 mL) contusions.
12	2.5	4	MBs in L thalamus, R hippocampus, and splenium CC. Deep frontal white matter hyperintensities. No contusions evidenced.
13	4.6	6	MBs R external capsule, pons, cerebellum, and splenium CC. Deep temporal white matter hyperintensities. Insular contusion (2.1 mL).
14	4.5	4	MBs in L caudate, R/L hippocampus, midbrain, frontoparietal lobes, and splenium and genu CC.
15	3.3	7	MBs in L parietal and temporal lobes, and R occipital lobe. Deep periventricular white matter hyperintensities.
16	2.3	8	MBs in L lentiform nucleus, midbrain, R frontoparietal lobes, R temporal lobe, and splenium CC. No contusions evidenced.
17	3	5	MBs in genu CC and parietotemporal lobes. Frontotemporal MBs related to contusions. Temporal lobe contusions (4.08 mL, 12.8 mL), R frontal contusion (3.9 mL), L frontal contusion (6.7 mL).
18	3.9	3	MBs R/L in frontal and temporal lobes. Deep temporal and frontal white matter hyperintensities due to demyelination. No contusions evidenced.
19	1.8	5	MBs in R/L frontal lobe. Deep frontal white matter hyperintensities. No contusions evidenced.

Abbreviations: CC = corpus callosum; FLAIR = fluid-attenuated inversion recovery; GCS = Glasgow Coma Scale; MBs = microbleeds; TBI = traumatic brain injury; Tev = time of evolution since the accident to the MRI evaluation (years).

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, visuoperception, 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 test,[13] Trail-Making Test A and B, Rey-Osterrieth complex figure, Rey Auditory Verbal Learning Test, Stroop reading and color-naming conditions, and verbal fluencies.[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 of the Hospital Clinic (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/mm^2, using an EPI sequence (TR = 9,300 msec; TE = 94 msec; slice thickness = 2.0 mm; voxel size = 2.0 × 2.0 × 2.0

Table 2 Demographic and neuropsychological characteristics of patients and controls

	TBI group, mean (SD)	Control group, mean (SD)	p Value
Age, y	26.78 (5.55)	27.47 (6.04)	0.72
Education			
Elementary school	3	4	
Secondary education	12	10	
College and university	4	4	0.86
Sex			
M	12	10	
F	7	9	0.52
Handedness			
R	15	15	
L	4	3	0.73
Neuropsychological performance			
TMT A	31.68 (10.9)	22.72 (6.23)	0.05
TMT B	79.16 (22.7)	57.28 (14.15)	0.001
TMT A & B	47.47 (21.43)	35.11 (16.1)	0.056
Digit forward	9.37 (1.9)	9.90 (2.32)	0.46
Digit backward	6.59 (1.12)	7.61 (1.6)	0.03
Symbol digit	69.74 (13.25)	86 (14.4)	0.001
Stroop reading	98 (14.67)	109.61 (13.28)	0.01
Stroop color naming	64.58 (8.46)	77.72 (9.12)	<0.001
Stroop interference	6.86 (6.18)	7.62 (4.36)	0.66
LNS	10.37 (2.06)	12.50 (1.6)	0.001
Fluencies			
Semantic	22.44 (11.68)	24 (4.85)	0.62
Phonemic	34.53 (12.4)	45.5 (9.4)	0.005
RAVLT (DR)	8.32 (3.44)	13.56 (1.25)	<0.001
ROCF (DR)	19.05 (5.9)	23.44 (4.44)	0.015
N-back task			
0-back (RT)	0.55 (0.08)	0.49 (0.07)	0.06
0-back d′	3.20 (1.24)	4.12 (0.18)	0.005
2-back (RT)	0.59 (0.1)	0.54 (0.08)	0.03
2-back d′	2.47 (0.85)	3.32 (0.66)	0.001

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 facial[15] n-back task. The n-back paradigm is widely used to investigate the neural basis of working memory.[16] 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.

fMRI preprocessing and analysis. For the fMRI data preprocessing we used the following tools of the FSL software (http://www.fmrib.ox.ac.uk/fsl): motion correction, removal of nonbrain structures, spatial smoothing, mean-based intensity normalization, and high-pass temporal filtering. The functional scans were then registered to the MNI152 standard space. We preprocessed the images using probabilistic ICA as implemented in MELODIC.[17] MELODIC decomposes the fMRI data into a set of ICs, where each IC is composed of a spatial map, a time course, and a vector of subject modes (Smodes) (appendix e-1 on the *Neurology*® Web site at www.neurology.org).

DTI preprocessing and analysis. We used FSL Diffusion Toolbox. After calculation of the FA map for each subject, we implemented a voxel-wise statistical analysis of the FA data using Tract-Based Spatial Statistics v1.2. We obtained global FA values from each subject's FA skeleton map (appendix e-1).

Gray matter volume analysis. We applied the SIENAX tool from FSL for estimation of brain gray matter volume (appendix e-1).

Statistics. For the fMRI data, we performed a post hoc regression analysis on the output estimated time series from the task-temporal series to identify the components related to each task condition. After identification of ICs of interest, data were regressed against 2 kinds of general linear model matrix. First we tested for differences between groups using a t test; we then assayed relationships between global FA values and patterns of functional activation, and then examined whether the activation correlated with task performance. We analyzed control and patient groups separately.

For the DTI data, we performed correlations between whole brain FA maps and mean blood oxygenation level–dependent (BOLD) response within the network for each subject and working memory performance (2-back accuracy measure d′) using Randomize v2.1 from FSL.[18,19] The statistical threshold was set at $p < 0.05$ family-wise error (FWE) corrected. The threshold-free cluster enhancement (TFCE) method was used to define the clusters.[20]

For statistical analyses of nonimaging data we used SPSS v17 (SPSS Inc., Chicago, IL). We examined group differences with the Student t test and used $p < 0.05$ as the threshold of significance.

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.

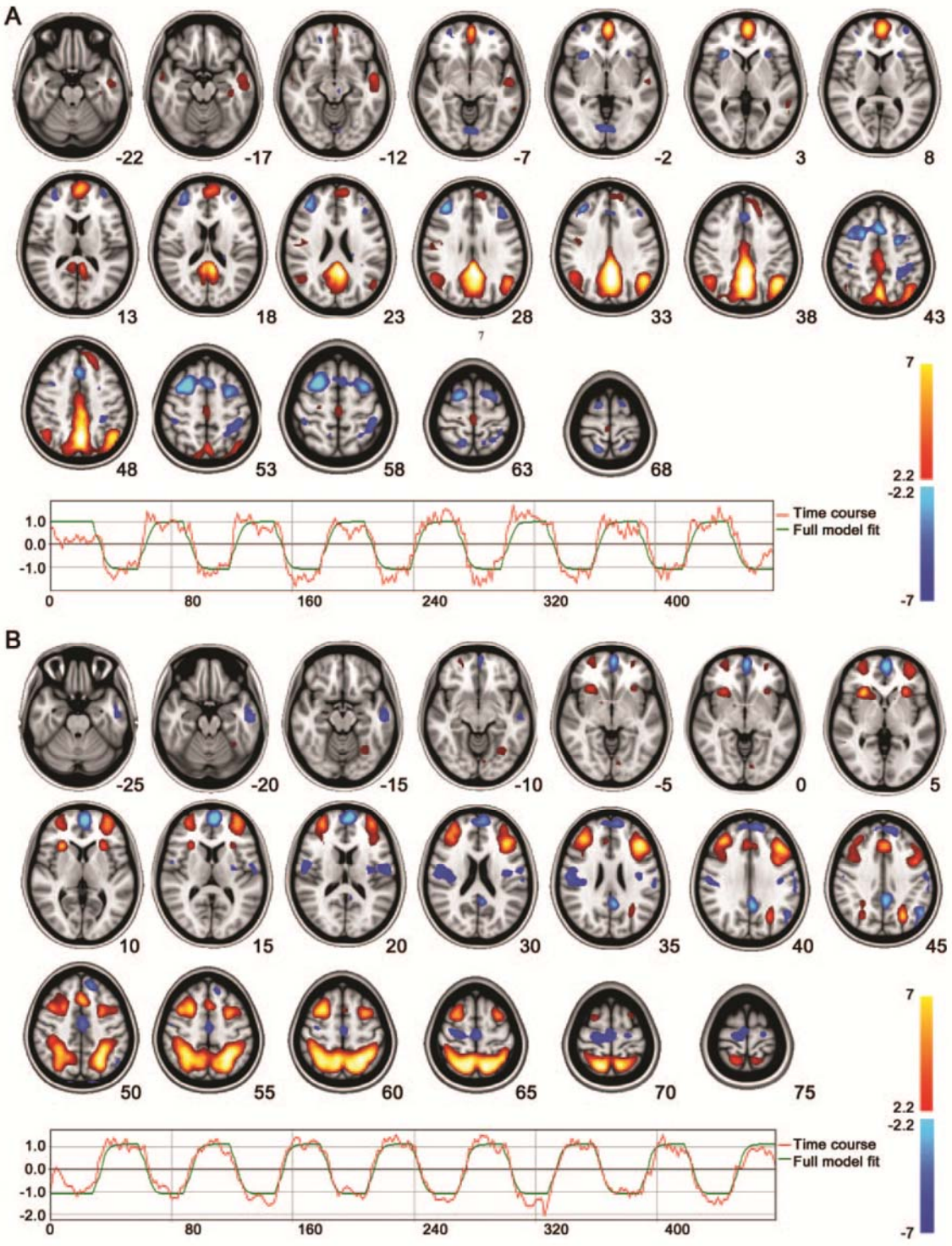
Working memory performance correlated with functional and structural measures. We found significant correlations for the TBI patients in the default mode and working memory networks with the accuracy measure d′ ($r = 0.53$, $p = 0.009$). These results indicate that patients with better performance had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

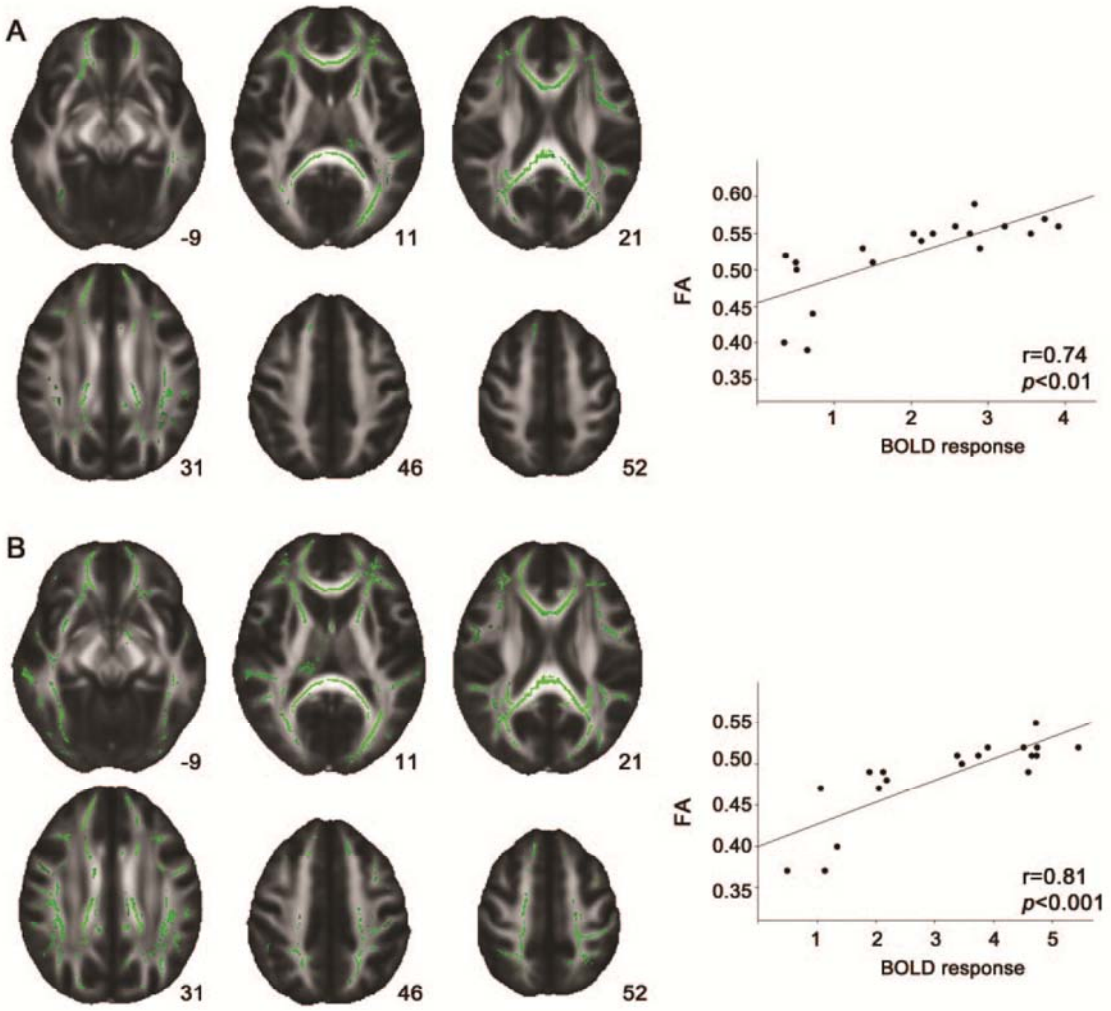
In our study, we found 2 different patterns of activation. One of these patterns corresponded to the

Figure 1 Default mode and working memory networks identified in the fMRI analysis of the groups



(A) Spatial map of IC1 (0-back >2-back) corresponding to the default mode network and its associated time series. (B) Spatial map of IC2 (2-back >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.

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



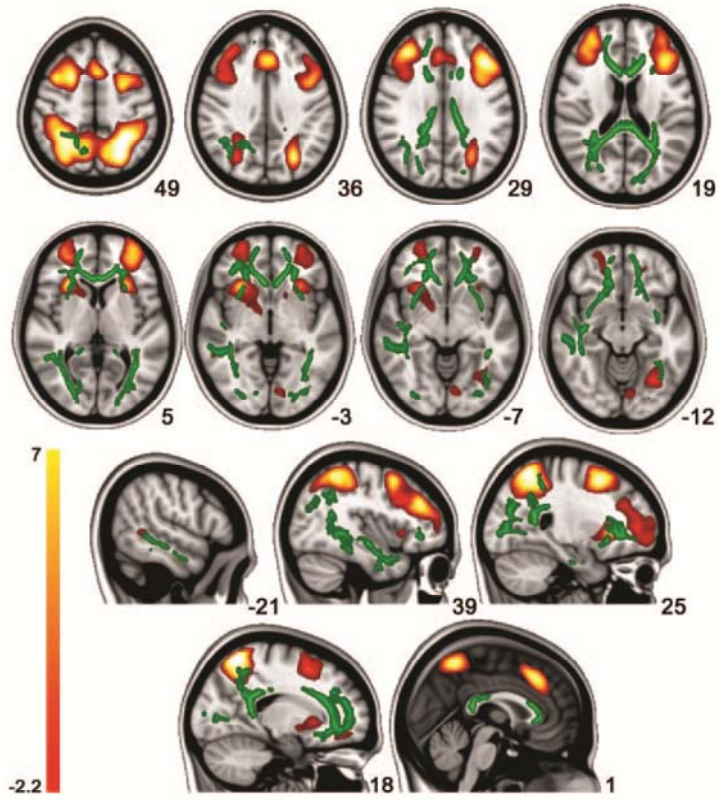
The relationship between fractional anisotropy (FA) values and functional activation scores is represented in green on the standard Montreal Neurological Institute brain (in A for default mode network, and in B for working memory network). Maps are thresholded at $p < 0.05$, family-wise error corrected. 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.

default mode network, which is known to be active during rest and deactivated during externally oriented tasks.[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.[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.[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.[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

Figure 3 White matter damage that correlates with altered functional working memory performance



In green, correlations of whole fractional anisotropy values and working memory performance. The fasciculi that achieved significance were posterior part of the superior longitudinal fasciculus (right), inferior longitudinal fasciculi, inferior fronto-occipital fasciculi, and corpus callosum (genu and splenium). Maps are thresholded at $p < 0.05$, family-wise error corrected. The activation maps for the working memory task are represented in hot colors. We can see that these significant fasciculi are linking cerebral regions that form part of the working memory network.

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[28] and in TBI.[9] 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.[29] 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.[30,31] As it connects both hemispheres, its damage interrupts the transfer of information that is crucial for efficiency in cognitive performance.[32,33] 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.[27,34] In aged subjects, global FA decreases correlated with speed of mental processing, probably indicating a widespread white matter integrity loss.[35]

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.[36,37] 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 study of the BOLD response in patients with vascular damage, such as severe TBI, may distort attempts to establish the relationship between cognitive impairment and cerebral activation. This is because diffuse vascular brain damage per se may reduce the BOLD response, and this reduction may be mistaken for lower cerebral activation in response to a cognitive task.[38] We used the activation in the visual network as a control for the working memory task, and we did not observe differences between patients and controls. Therefore our results for hypoactivation during working memory in patients do not seem to be due to a generalized effect of a decreased BOLD response secondary to vascular damage.

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. Neuroim-

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.

REFERENCES

1. Johansen-Berg H, Rushworth MF. Using diffusion imaging to study human connectional anatomy. Annu Rev Neurosci 2009;32:75–94.
2. Honey C, Sporns O, Cammoun L, et al. Predicting human resting- state connectivity from structural connectivity. Proc Natl Acad Sci USA 2009;106:2035–2040.
3. Povlishock JT, Katz DI. Update of neuropathology and neurological recovery after traumatic brain injury. J Head Trauma Rehabil 2005;20:76–94.
4. Scheid R, Walther K, Gurthke T, et al. Cognitive sequelae of diffuse axonal injury. Arch Neurol 2006;63:418–424.
5. Christodoulou C, DeLuca J, Ricker JH, et al. Functional magnetic resonance imaging of working memory impairment after traumatic brain injury. J Neurol Neurosurg Psychiatry 2001;71:161–168.
6. Newsome MR, Scheibel RS, Steinberg JL, et al. Working memory brain activation following severe traumatic brain injury. Cortex 2007;43:95–111.
7. Turner GR, Levine B. Augmented neural activity during executive control processing following diffuse axonal injury. Neurology 2008;71:812–818.
8. Sanchez-Carrion R, Gomez PV, Junque C, et al. Frontal hypoactivation on functional magnetic resonance imaging in working memory after severe diffuse traumatic brain injury. J Neurotrauma 2008;25:479–494.
9. Palacios EM, Fernandez-Espejo D, Junque C, et al. Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury. BMC Neurol 2011;11:24.
10. Biswal BB, Mennes M, Zuo XN, et al. Toward discovery science of human brain function. Proc Natl Acad Sci USA 2010;107:4734–4739.
11. Van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: A review on resting-state fMRI functional connectivity. Eur Neuropsychopharmacol 2010;20:519–534.
12. Warner MA, Youn TS, Davis T, et al. Regionally selective atrophy after traumatic axonal injury. Arch Neurol 2010;67:1336–1344.
13. Wechsler D. Escala de inteligencia de Wechsler para adultos (WAIS-III). Madrid: TEA Ediciones; 1999.
14. Lezak MD, Howieson DB, Loring DD, et al. Neuropsychological Assessment. New York: Oxford University Press; 2004.
15. Minear M, Park DC. A lifespan database of adult facial stimuli. Behav Res Methods Instruments Computers 2004;36:630–633.
16. Owen AM, McMillan KM, Laird AR, et al. N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. Hum Brain Mapp 2005;25:46–59.
17. Beckmann CF, Smith SM. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans Med Imaging 2004;23:137–152.
18. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 2002;15:1–25.
19. Hayasaka S, Nichols TE. Validating cluster size inference: random field and permutation methods. Neuroimage 2003;20:2343–2356.
20. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localization in cluster inference. Neuroimage 2009;44:83–98.
21. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci USA 2003;100:253–258.
22. Raichle ME, MacLeod AM, Snyder AZ, et al. A default mode of brain function. Proc Natl Acad Sci USA 2001;98:676–682.
23. Sumowski JF, Wylie GR, Deluca J, Chiaravalloti N. Intellectual enrichment is linked to cerebral efficiency in multiple sclerosis: functional magnetic resonance imaging evidence for cognitive reserve. Brain 2010;133:362–374.
24. Mayer AR, Mannell MV, Ling J, Gasparovic C, Yeo RA. Functional connectivity in mild traumatic brain injury. Hum Brain Mapp 2011;32:1825–1835.
25. Nakamura T, Hillary FG, Biswal BB. Resting network plasticity following brain injury. PLoS One 2009;4:e8220.
26. Vanhaudenhuyse A, Noirhomme Q, Tshibanda LJ, et al. Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. Brain 2010;133:161–171.
27. Kinnunen KM, Greenwood R, Powell JH, et al. White matter damage and cognitive impairment after traumatic brain injury. Brain 2011;134:449–463.
28. Bonzano L, Pardini M, Mancardi GL, Pizzorno M, Roccatagliata L. Structural connectivity influences brain activation during PVSAT in multiple sclerosis. Neuroimage 2009;44:9–15.
29. Kanwisher N. Functional specificity in the human brain: A window into the functional architecture of the mind. Proc Natl Acad Sci USA 2010;107:11163–11170.
30. Levin HS, Williams DH, Valastro M, Eisenberg HM, Crofford MJ, Handel SF. Corpus callosal atrophy following closed head injury: detection with magnetic resonance imaging. J Neurosurg 1990;73:77–78.
31. Kumar R, Saksena S, Husain M, et al. Serial changes in diffusion tensor imaging metrics of corpus callosum in moderate traumatic brain injury patients and their correlation with neuropsychometric tests: a 2-year follow-up study. J Head Trauma Rehabil 2010;25:31–42.
32. Benavidez DA, Fletcher JM, Hannay HJ, et al. Corpus callosum damage and interhemispheric transfer of infor-

Appendix e-1

METHODS

fMRI pre-processing and analysis:

The following pre-processing was applied to the fMRI data using tools from FSL software (http://www.fmrib.ox.ac.uk/fsl): motion correction, removal of non-brain structures, spatial smoothing, mean-based intensity normalization and high-pass temporal filtering. The functional scans were then registered to the MNI152 standard space. The pre-processed images were analyzed using probabilistic ICA as implemented in MELODIC as part of FSL[e1]. MELODIC decomposes the fMRI data into a set of Independent Components (ICs), where each IC is composed by a spatial map, a time-course and a vector of subject modes (Smodes), which quantifies the functional activation within each IC for each subject.

Data pre-processing was performed with FSL and included: motion correction using MCFLIRT, removal of non-brain structures with Brain Extraction Tool (BET), spatial smoothing by using a Gaussian kernel of 6 mm FWHM, mean-based intensity normalization of all volumes by the same factor (4D grand mean scaling), and high pass temporal filtering using a cut-off of 160 sec. Functional scans were first registered to the subject's high-resolution MPRAGE scan using affine linear registration (with FLIRT) with 6 degrees of freedom and further registered to the common MNI standard space also using linear affine registration with 12 degrees of freedom. Pre-processed functional images were introduced to an Independent Component Analysis (ICA), as implemented in MELODIC from FSL[e1]. ICA is a data-driven approach that decomposes the data into a set of Independent Components (ICs). Each IC consists of a triplet of a) time courses, b) spatial maps and c) subject modes, which characterize the signal variation across the temporal, spatial and subject domains. Subject modes are the quantification of the mean BOLD response for each subject within an IC. The number of ICs was estimated using the Laplace approximation to the Bayesian evidence for a probabilistic PCA model[e1]. In our study, data were decomposed into 36 ICs. Estimated Component maps were divided by the standard deviation of the residual noise and thresholded by fitting a mixture model to the histogram of intensity values. The threshold for the Z (Gaussianized T/F) statistic images was set so that the probability of being in the 'active' class (as modeled by the Gamma densities) exceeded the probability of being in the 'background' noise class.

DTI pre-processing and analysis:

DTI pre-processing and analysis was performed using tools from FSL. Image artifacts due to eddy current distortions were minimized by registering the diffusion images to the b0 images. The registered images were skull-stripped using BET. Fractional Anisotropy (FA) maps were calculated using the FDT tool[e2]. After calculation of the FA map for each subject, we implemented a voxel-wise statistical analysis of the FA

data using Tract-Based Spatial Statistics v1.2 (TBSS[e3,e4]). In summary, within the TBSS streamline, FA data were aligned into the common FMRIB58 FA template, which is in MNI152 standard space using a non-linear registration algorithm (FNIRT). Aligned FA maps were visually inspected after registration and we confirmed that the result of the previous step was correct. Next, a mean FA image was created from the images from all the subjects in this common space and narrowed to generate a mean FA skeleton that represented the center of all tracts common to the entire group. This was thresholded to FA greater than 0.2 to include the major white matter pathways but to exclude peripheral tracts where there was significant inter-subject variability and partial volume effects with gray matter. This ensured that each subject's skeleton was in the group space while also representing the center of the subject's unique white matter bundles. The aligned FA image for each subject was then projected onto the skeleton by filling the skeleton with FA values from the nearest relevant tract center. This is achieved for each skeleton voxel by searching perpendicular to the local skeleton structure for the maximum value in the FA image of the subject. The resulting skeletonized data were then fed into voxelwise cross-subject statistics. Mean FA values were obtained from each subject's FA skeleton map.

Gray matter volume analysis:

We applied SIENAX tool from FSL for estimation of brain gray matter volume. These methods are described in detail elsewhere[e5]. In SIENAX, brain and skull images are extracted, and the brain image is affine-registered to an MNI standard template using the skull image to determine registration scaling, to be used as a normalization for head size. Finally, segmentation into gray and white matter was carried out.

e-References

e1. Beckmann CF, Smith SM. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE trans Med Imaging 2004; 23(2):137-52.

e2. Behrens TEJ, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, Matthews PM, Brady JM, Smith SM. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. Magn Reson Med 2003; 50(5):1077-1088.

e3. Smith SM, JenkinsonM, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TEJ. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. Neuroimage 2006; 31:1487-1505.

e4. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localization in cluster inference. Neuroimage 2009; 44:83-98.

e5. Smith SM, Zhang Y, Jenkinson M, Chen JM, Mathews PM, Federico A, De Stefano N. Accurate, robust and automated longitudinal and cross-sectional brain change analysis. Neuroimage 2002; 17:479-489.

Table e-1. MNI maximum coordinates, cluster size, and statistical significance of the IC1 and IC2 spatial maps

IC1(0back>2back)	**Cluster size**	***x***	***y***	***z***	***Z***
Precuneus	82011	-2	-62	36	7.54
Frontal pole	19595	-2	58	0	5.43
Superior temporal gyrus	4811	-54	-6	-12	3.32
Postcentral gyrus	1401	42	-10	28	2.55
Middle temporal gyrus	631	58	-2	-16	2.56
Fusiform cortex	456	-38	-22	-20	2.76
IC2(2back>0back)	**Cluster size**	***x***	***y***	***z***	***Z***
Superior parietal lobe	76870	-26	-54	52	7.34
Middle frontal gyrus	54631	26	14	52	5.9
Middle frontal gyrus	38180	-38	30	24	6.33
Insular cortex	3204	-30	22	4	4.24
Fusiform gyrus	2614	-30	-66	12	3.21
Middle temporal gyrus	101	54	-46	0	2.47

The thresholds for the Z-maps established by MELODIC were 1.96 and 2.18 for IC1 and IC2 respectively.

[AME: Neuro PAGE: 1 SESS: 2 OUTPUT: Mon Apr 15 13:59:54 2013
ives/13jobs/neu/05_20_2013/noc130001

ORIGINAL CONTRIBUTION

ONLINE FIRST

Resting-State Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury

Eva M. Palacios; Roser Sala-Llonch; Carme Junque, PhD; Teresa Roig, PhD; Jose M. Tormos, MD, PhD; Nuria Bargallo, MD, PhD; Pere Vendrell

Importance: The study of brain activity and connectivity at rest provides a unique opportunity for the investigation of the brain substrates of cognitive outcome after traumatic axonal injury. This knowledge may contribute to improve clinical management and rehabilitation programs.

Objective: To study functional magnetic resonance imaging abnormalities in signal amplitude and brain connectivity at rest and their relationship to cognitive outcome in patients with chronic and severe traumatic axonal injury.

Design: Observational study.

Setting: University of Barcelona and Hospital Clinic de Barcelona, Barcelona, and Institut Guttmann–Neurorehabilitation Hospital, Badalona, Spain.

Participants: Twenty patients with traumatic brain injury (TBI) were studied, along with 17 matched healthy volunteers.

Interventions: Resting-state functional magnetic resonance imaging and diffusion tensor imaging data were acquired. After exploring group differences in amplitude of low-frequency fluctuations (ALFF), we studied functional connectivity within the default mode network (DMN) by means of independent component analysis, followed by a dual regression approach and seed-based connectivity analyses. Finally, we performed probabilistic tractography between the frontal and posterior nodes of the DMN.

Main Outcomes and Measures: Signal amplitude and functional connectivity during the resting state, tractography related to DMN, and the association between signal amplitudes and cognitive outcome.

Results: Patients had greater ALFF in frontal regions, which was correlated with cognitive performance. Within the DMN, patients showed increased connectivity in the frontal lobes. Seed-based connectivity analyses revealed augmented connectivity within surrounding areas of the frontal and left parietal nodes of the DMN. Fractional anisotropy of the cingulate tract was correlated with increased connectivity of the frontal node of the DMN in patients with TBI.

Conclusions and Relevance: Increased ALFF is related to better cognitive performance in chronic TBI. The loss of structural connectivity produced by damage to the cingulum tract explained the compensatory increases in functional connectivity within the frontal node of the DMN.

JAMA Neurol. Published online May 20, 2013.
doi:10.1001/jamaneurol.2013.38

Author Affiliations: August Pi i Sunyer Biomedical Research Institute (Mss Palacios and Sala-Llonch, Dr Junque, and Mr Vendrell) and Department of Psychiatry and Clinical Psychobiology, University of Barcelona (Ms Sala-Llonch, Dr Junque, and Mr Vendrell), Barcelona, Spain; Institut Guttmann–Neurorehabilitation Hospital, Badalona, Spain (Drs Roig and Tormos); and Centre de Diagnostic per la Imatge, Hospital Clinic, Barcelona (Dr Bargallo).

COGNITIVE DEFICITS AFTER traumatic brain injury (TBI) are a major cause of daily life disability.[1] Persistent disabling sequelae are caused by the structural brain damage that occurs not only in the early stage but also after a period of apparent recovery.[2] Traumatic axonal injury is associated with the most severely impaired outcomes after TBI. Neuropathological studies have shown that TBI affects structural brain networks progressively, from focal axon alteration to delayed axonal disconnection.[3] Functional magnetic resonance imaging (fMRI) studies suggest that white matter damage alters structural connectivity, which in turn can affect functional connectivity, and both contribute to cognitive dysfunctions in TBI.[4-7]

Resting-state fMRI studies have recently emerged as a useful tool for investigating brain functional connectivity after severe TBI[4,8-11] and in subjects with mild TBI examined during the early stages.[12,13] These studies provide information about brain activity and connectivity in the absence of task performance, a condition that allows researchers to investigate patient populations with broader ranges of in-

jury severity, because no specific cognitive ability is required. Advanced neuroimaging processing tools provide information about brain activity and functional connectivity during resting-state fMRI. Low signal fluctuations that occur during rest have been shown to reflect strong connectivity between functionally related brain regions.[14,15]

Although different functional connectivity networks can be identified during the resting state,[16] the most widely studied is the default mode network (DMN), which is reported to be affected in a broad range of brain disorders and is commonly related to cognitive processes.[17] The spatial pattern of the DMN includes the ventromedial prefrontal cortex, the posterior cingulate cortex, the lateral parietal cortex, and the precuneus. For the main nodes of the DMN, their functional connectivity is supported by an underlying structure of white matter pathways,[18] with the cingulum the key tract that interconnects the anterior and posterior core regions of the DMN.[19]

In addition to disrupted functional connectivity, studies of white matter integrity using diffusion tensor imaging[20-23] have shown that structural connectivity is also greatly affected after TBI. In a previous study,[3] we found that disrupted structural connectivity after traumatic axonal injury was responsible for altered functional connectivity during working memory performance. In the current research, we used new advances in resting state–related fMRI methods to determine whether the amplitude of spontaneous low-frequency fluctuations in brain activity and DMN connectivity may be sensitive biomarkers for cognitive dysfunction after TBI. To this end, we measured the amplitude of resting-state blood oxygen level-dependent signal fluctuations in the whole brain and the DMN, studying global and network-based functional connectivity, as well as structural connectivity of the main fasciculi within the DMN.

METHODS

STUDY PARTICIPANTS

Twenty patients with chronic and diffuse TBI were recruited from the Head Injury Unit of the Guttmann Institute–Neurorehabilitation Hospital. The criteria followed for sample selection have been described elsewhere.[3] This study is part of a project on long-term impairment of connectivity in diffuse TBI, and some results already have been published.[3,24] Patients' demographic and clinical characteristics are summarized in **Table 1**. Patients underwent magnetic resonance imaging (MRI) a mean (SD) of 4.1 (1.2) years after injury, and all showed microbleeds as a sign of diffuse disease in the T2* and fluid-attenuated inversion recovery sequences. Table 1 provides detailed clinical and neuroradiological characteristics for each patient in the study. The cause of TBI was motor vehicle crashes in all cases.

The control group comprised 17 healthy volunteers matched by age, sex, and educational level. None had a history of neurological or psychiatric diseases. Their demographic characteristics are provided in Table 1.

The study was approved by the research ethics committees of the Guttmann Institute–Neurorehabilitation Hospital and the University of Barcelona. All participants gave written informed consent.

Table 1. Demographic and Clinical Characteristics of Patients and Control Subjects

Characteristic	Patients With TBI (n = 20)	Control Subjects (n = 17)
Age, mean (SD), y[a]	27.50 (5.28)	26.29 (4.95)
Educational level, mean (SD), y[b]	15.20 (2.96)	14.64 (2.85)
Sex, No.[c]		
Male	11	10
Female	9	7
GCS score, mean (SD)	5 (1.74)	. . .
Time since injury, mean (SD), y	4.10 (1.18)	. . .
Microbleeds (TAI)		. . .
Frontal lobes	17	
Temporal lobes	12	
Corpus callosum	14	
Basal ganglia	10	
Parietal lobes	9	
Cerebellum	6	
Thalamus	5	
Midbrain	5	
Contusions (<10-mL volume)		. . .
Frontal lobes	4	
Temporal lobes	2	

Abbreviations: GCS, Glasgow Coma Scale; MRI, magnetic resonance imaging; TAI, traumatic axonal injury; TBI, traumatic brain injury.
[a] $t = 0.71$; $P = .48$.
[b] $t = 0.57$; $P = .57$.
[c] $t = 2.29$; $P = .82$.

IMAGE ACQUISITION

Data were acquired with a Siemens Magnetom Trio Tim syngo 3-T system at the Centre de Diagnostic per la Imatge of the Hospital Clinic, Barcelona. A high-resolution T1-weighted structural image was obtained for each subject with an MPRAGE (magnetization-prepared rapid acquisition gradient-echo) 3-dimensional protocol (repetition time [TR], 2300 milliseconds; echo time [TE], 3 milliseconds; inversion time [TI], 900 milliseconds; field of view [FOV], 244 mm; and 1-mm isotropic voxel) and a 5-minute fMRI resting-state single-shot gradient-echo echo-planar imaging sequence (TR, 2000 milliseconds; TE, 16 milliseconds; flip angle, 90°; FOV, 220 mm; and voxel size, 1.7 × 1.7 × 3.0 mm). Diffusion-weighted images were sensitized in 30 noncollinear directions with a b value of 1000 s/mm², in an echo-planar imaging sequence (TR, 9300 milliseconds; TE, 94 milliseconds; section thickness, 2.0 mm; voxel size, 2.0 × 2.0 × 2.0 mm; FOV, 240 mm; and no gap). For the lesion description, the neuroradiologist (N.B.) considered T1-weighted, fluid-attenuated inversion recovery (TR, 9000 milliseconds; TE, 85 milliseconds; voxel size, 3.0 mm; voxel size, 1.3 × 0.9 × 3.0 mm; and FOV, 240 mm), and T2* GE (TR, 518 milliseconds; TE, 20 milliseconds; voxel size, 3.0 mm; voxel size, 0.9 × 0.8 × 3.0 mm; and FOV, 240 mm) sequences. All the images were visually inspected to ensure that they did not contain MRI artifacts or excessive movement before analysis.

NEUROPSYCHOLOGICAL ASSESSMENT

A trained neuropsychologist masked to the clinical data administered tests to assess the main cognitive functions impaired after TBI. The assessment included the following: letter-number sequencing; digit span test (forward and backward measures); the Trail Making Test (parts A and B); the Rey Auditory Verbal Learning Test; the Rey-Osterrieth complex fig-

JOBNAME: Neuro PAGE: 3 SESS: 2 OUTPUT: Mon Apr 15 13:59:54 2013
/archives/13jobs/neu/05_20_2013/noc130001

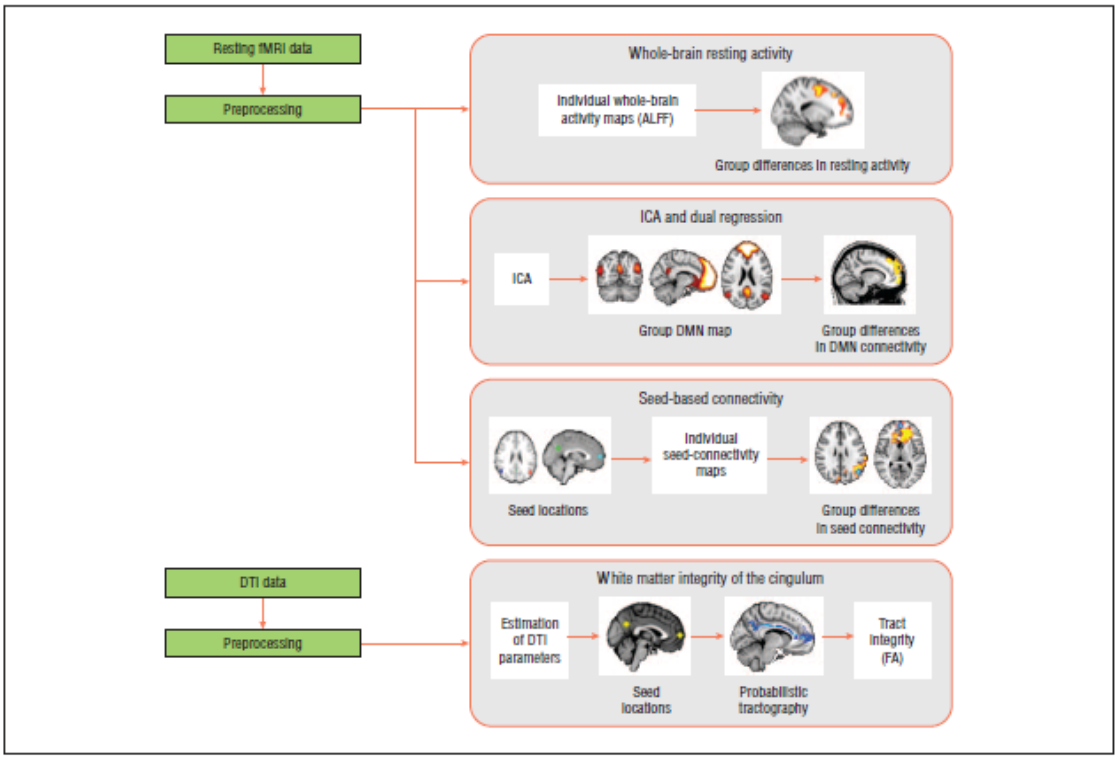


Figure 1. Image processing and analysis methods. (1) whole-brain analysis of the amplitude of low-frequency fluctuations (ALFF); (2) independent component analysis (ICA) and dual-regression analysis of the default mode network (DMN); (3) connectivity analysis using the predefined DMN regions of interest (ROIs); and (4) diffusion tensor imaging (DTI) tractography analysis of the cingulum tract from the DMN ROIs. (See the Methods section and eMethods for details.)

ure; reading, color naming, and reading word-color conditions from the Stroop test; and measures of verbal semantic and phonemic fluency.[25] Factor analysis was used to obtain a single measure that was representative of overall cognitive outcome, based on tests in which patients were significantly impaired compared with controls (eMethods and eTable; http://www.jamaneuro.com).

MRI: IMAGE PROCESSING AND ANALYSIS

A general overview of the image processing flow is shown in **Figure 1**.

Amplitude Measures of Resting-State Data

The amplitude of low-frequency fluctuations (ALFF) was measured using a method based on the fast Fourier transform of the resting-state time series for each voxel.[26] After individual ALFF maps were obtained, they were registered to the Montreal Neurological Institute standard space by means of linear registration (FLIRT [FMRIB's Linear Image Registration Tool] from FSL [http://www.fmrib.ox.ac.uk/fsl]).[27] Voxel-wise group comparisons were performed on these maps by using permutation-based comparisons with general linear modeling. Differences were considered significant at $P < .05$ (family-wise error corrected; see eMethods for a full description of the procedures used).

Independent Component Analysis of Resting-State Data

We entered preprocessed resting fMRI data into an independent component analysis (ICA) using MELODIC[28] software from FSL. This enabled us to obtain a set of independent components and identify the common resting-state functional networks.[14,16,19,29] Before group ICA decomposition, all individual fMRI data sets were linearly registered to the MNI standard space.[27] Finally, we selected the independent component map of the DMN. The procedure for selecting the DMN within the whole set of components was based on the computation of spatial cross-correlation between each independent component and a previously published template corresponding to the DMN.[16]

We then used a dual regression approach[4,30] to investigate between-group differences in the DMN maps. The significance threshold of the voxel-wise differences was set at $P < .05$ (family-wise error corrected) (eMethods).

Seed-Based Analysis of the DMN

The peak coordinates of the DMN identified with ICA were used to create 4 spherical regions of interest (ROIs) representing the main nodes of this network: medial prefrontal cortex (MPFC), precuneus/posterior cingulate (PPC), and left and right parietal cortices (eMethods).

For each seed (or DMN node), we created whole-brain func-

tional connectivity maps and tested group differences of these maps. All seed-based connectivity analyses were performed using the functional data sets that had been previously preprocessed and registered to the MNI standard space.

Analysis of MRI Diffusion Data

Diffusion MRI images were analyzed with FDT (FMRIB's Diffusion Toolbox) software from FSL. After first extracting individual fractional anisotropy (FA) maps, we then used diffusion tensor imaging data in a probabilistic tracking algorithm to estimate white matter pathways connecting the 2 DMN ROIs (ie, MPFC ROI and PPC ROI) extracted from the analysis of the resting-state fMRI data. These ROIs (from fMRI analysis) were originally in MNI standard space. They were then moved to each subject's diffusion space before we performed tractography (using linear registration implemented with FSL software).[27] Individual tracts were registered again to MNI to compute the group-average maps. White matter pathways were averaged across controls and patients separately. Finally, the average connectivity map of the controls was used, together with the registered FA maps, to estimate fiber integrity of this connection in the whole sample, as the mean FA within the pathway.

Cognitive Outcome and Structural and Functional Connectivity Data

Mean signal amplitude (ALFF scores) and connectivity (functional connectivity and structural connectivity) scores were extracted within the areas that resulted significantly from the whole-brain ALFF analysis, the ICA, and the seed-based functional connectivity and tractography analyses. We used Pearson correlations in SPSS software (IBM) (eMethods) to study these measures together with the measure of cognitive outcome.

RESULTS

AMPLITUDE OF RESTING-STATE FLUCTUATIONS

Compared with controls, patients had greater ALFF in several brain areas (corrected $P < .05$), including the frontal pole, superior frontal gyrus, middle frontal gyrus, paracingulate gyrus, and superior parietal lobe (see **Figure 2** and **Table 2** for MNI coordinates and cluster size). Because the map of increased ALFF in the middle frontal areas showed a large overlap with the frontal node of the DMN, we analyzed the brain connectivity of this network.

INDEPENDENT COMPONENT ANALYSIS

Using ICA with temporal concatenation, we obtained a set of 37 independent components and identified the main resting-state networks (eResults and eFigure 1). Within this set of networks, we selected the DMN for further analysis.

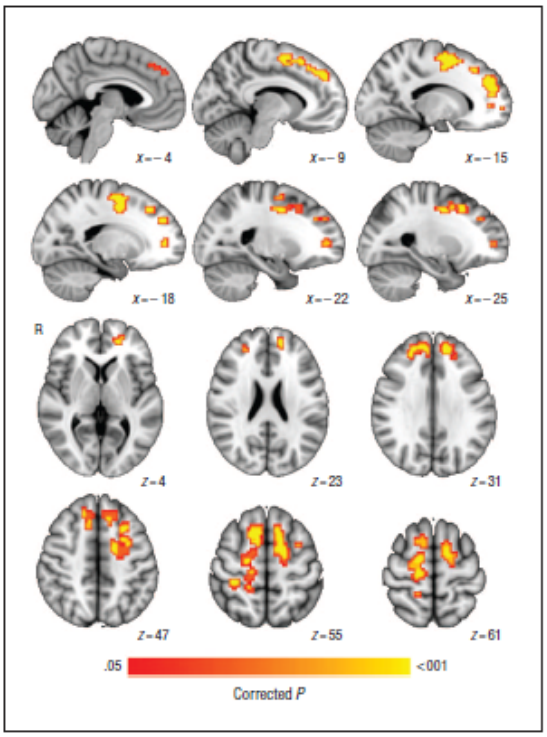


Figure 2. Increased amplitude of low-frequency fluctuations during the resting-state in patients with traumatic brain injury. Red-yellow regions represent areas with statistically significant differences between patients and controls (corrected $P < .05$).

DMN CONNECTIVITY

Group Comparisons of the ICA-Based DMN

Using the dual regression approach, patients with TBI had greater functional connectivity than controls within the DMN in certain areas of the frontal lobe (**Figure 3**). These regions included the frontal pole, the anterior cingulate and paracingulate gyrus, the superior frontal gyrus, and a small part of the precentral gyrus.

Seed-Based Connectivity Analysis

Using the ICA-based spatial map of the DMN, we created 4 spherical ROIs with which to compute seed-based connectivity maps in the MPFC, PPC, and left and right parietal cortex ROIs (see eResults and eFigure 2 for exact ROI locations).

With the MPFC ROI as a seed, we found increased functional connectivity of this region with other areas of the MPFC in patients compared with controls (corrected $P < .05$) (**Figure 4**). Moreover, with the left parietal cortex ROI, we also found a set of brain areas with increased functional connectivity in patients with TBI (corrected $P < .05$) (Figure 4). These areas were located in the left lateral occipital cortex, angular gyrus, supramarginal gyrus, temporo-occipital areas, middle and inferior temporal gyrus, occipital fusiform and lingual gyri, and precuneus.

Table 2. MNI Maximum Coordinates, Cluster Size, and Statistical Significance of the Increased Activity on Resting-State Spatial Maps

Brain Areas	Cluster Size (Voxels)	Coordinate			*P* Value
		x	*y*	*z*	
Frontal pole	274	−21	45	36	.02
Frontal pole	192	15	45	33	.02
Gyrus					
Superior frontal	75	15	−6	60	.02
Middle frontal	21	−27	18	45	.03
Paracingulate	14	−18	48	3	.04

Abbreviation: MNI, Montreal Neurological Institute.

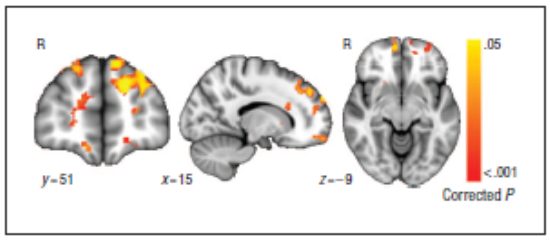


Figure 3. Increased connectivity within the default mode network in patients with traumatic brain injury to controls. Red-yellow regions represent areas where the connectivity differed significantly between patients and controls (corrected $P < .05$).

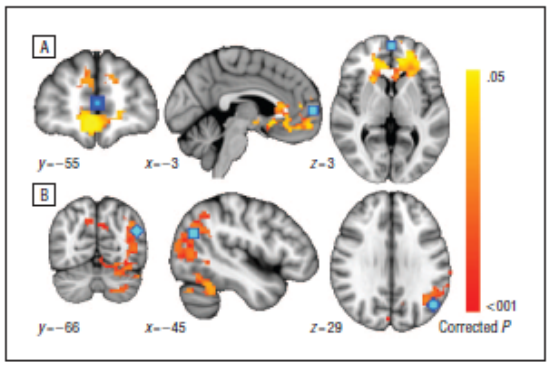


Figure 4. Areas showing increased functional connectivity in traumatic brain injury (red and yellow) and locations of the seeds (blue). A, Increased connectivity with the medial prefrontal cortex region of interest used as a seed. B, Increased connectivity with the left parietal cortex region of interest used as a seed. Significant at $P < .05$ (family-wise error corrected).

We found no differences in functional connectivity when using the remaining nodes as seeds.

STRUCTURAL CONNECTIVITY MEASURED WITH DIFFUSION TENSOR IMAGING

The MPFC and the PPC ROIs were used to reconstruct the white matter pathway connecting the 2 regions.

The probabilistic tractography map of each subject was used to create separate average maps for patients and controls. These maps indicate the probability of each voxel being part of a white matter pathway connecting the 2 regions. The main tracts identified were the left and right bundles of the cingulum. Visual inspection of the average maps for each group showed that the size of the cingulum was reduced in patients.

The average map for the control group was used as a mask to extract mean FA values within the cingulum for each subject. These values were significantly decreased in patients with TBI compared with controls (mean, 0.36 for patients vs 0.42 for controls; $t = 5.9$; $P < .001$) (**Figure 5**).

AMPLITUDE OF FLUCTUATIONS AND COGNITIVE OUTCOME

In patients, the amplitude of resting-state fluctuations was positively correlated with cognitive performance ($r = 0.48$; $P = .03$); the greater the activation, the better the cognitive outcome. There was no significant correlation between these measures in controls ($r = 0.35$; $P = .16$).

Within the patient group, functional connectivity scores for the frontal ROI were negatively correlated with the FA values of the cingulum tract ($r = -0.45$; $P = .04$).

DISCUSSION

The purpose of this study was to provide further insight into the ALFF and their connectivity in the resting state and the possible relationship of these findings with cognitive outcome after traumatic axonal injury. Our main finding was that higher ALFF at rest is associated with better cognitive outcome in patients with diffuse TBI. More specifically, these patients also had increased resting-state functional connectivity in regions surrounding the frontal node of the DMN. Moreover, the increased frontal connectivity could be explained by damage to the cingulum, the key tract connecting the anterior and posterior brain areas of the DMN. These findings suggest that the loss of structural connectivity is compensated for by an increase in the functional connectivity of local circuits.

Few studies to date have considered the ALFF at rest and its implications in terms of cognitive dysfunction. This measure has been found to be decreased in patients with mild cognitive impairment and Alzheimer disease.[31] In neurodegenerative diseases, these decreases in amplitude probably reflect a loss of neurons that consecutively provokes connectivity deficits and disorganization or breakdown of brain networks. In our study, when comparing whole-brain ALFF in the resting state

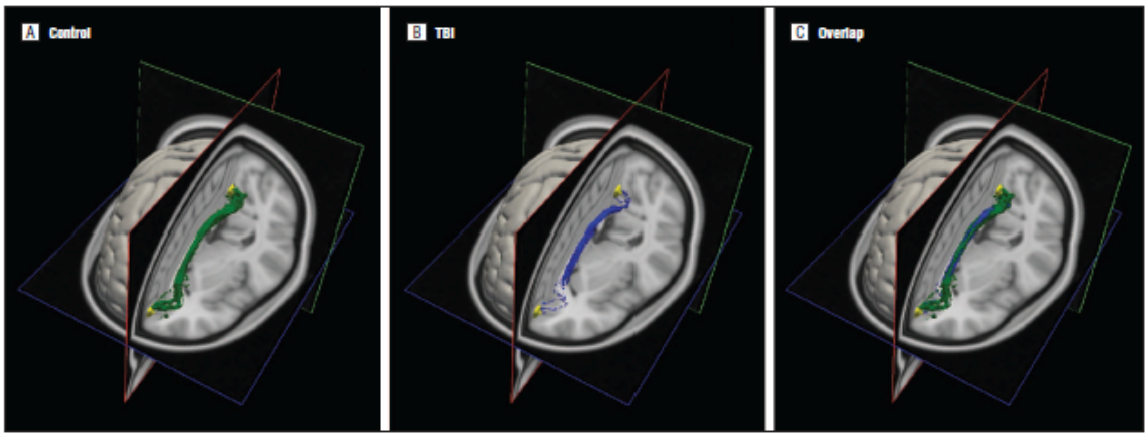


Figure 5. Results of tractography analysis shown as spatial maps of probabilistic tracking. Yellow represents the 2 regions of interest (medial prefrontal cortex and precuneus/posterior cingulate) used as seeds for the fiber-tracking algorithm. Average probabilistic connectivity maps are shown, with the standard Montreal Neurological Institute–fractional anisotropy template, for controls (in green) and patients with traumatic brain injury (TBI) (in blue).

between groups, the TBI group showed increased amplitudes that were predominantly focused within frontal lobe regions. Moreover, the measures of higher amplitudes in these areas predicted a better general cognitive outcome, suggesting that functional and efficient brain reorganization occurred to compensate for acute brain damage and improve cognitive performance. This finding, together with changes in structural connectivity, may constitute an objective measure of long-term cognitive outcome after TBI.

The specific analyses of the DMN in the resting state also showed increased connectivity in the frontal node of this network in patients with TBI compared with controls. Furthermore, connectivity from each core of DMN nodes to the whole brain revealed a widespread pattern of locally increased functional connectivity surrounding the medial frontal and left parietal nodes of the DMN. Increased functional connectivity has been found in other diseases involving white matter damage, such as multiple sclerosis; subjects with relapsing-remitting multiple sclerosis have compensatory increased connectivity in the posterior cingulate DMN node,[32] and in patients with early-stage disease, distinct networks exhibit increases in functional connectivity despite large reductions in white matter integrity.[33]

Previous studies investigating the DMN during the resting state in TBI have found alterations that reflect both decreases[11] and increases in functional connectivity.[12] The increases have been interpreted as compensatory or adaptive mechanisms because they were often positively correlated with cognitive outcome.[8,34] Another possible interpretation is that this increased connectivity, measured in voxels surrounding the DMN nodes, reflects a diffusion of the DMN nodes during recovery from TBI. This latter interpretation also may be supported by the fact that the white matter injury found in the cingulum in TBI precludes the functional inhibition of areas surrounding these nodes. We suggest that the increases found in frontal areas may reflect compensation because the amplitude of the fluctuations in these regions correlated with performance, but the increase in the spread of connectivity around the PPC may reflect a loss of directionality in the connectivity caused by the injury. On the other hand, Mayer et al[8] described a pattern of decreased long-distance functional connectivity between the nodes of the DMN (ie, between anterior and posterior nodes).

Although not directly examining the DMN, other authors have found alterations in resting-state connectivity in TBI, in the form of reduced interhemispheric connectivity of the hippocampus and increased ipsilateral connectivity, and these were associated with better cognitive outcome.[9] In addition, resting-state fMRI studies involving magnetoencephalographic recordings have also provided evidence of brain plasticity and network reorganization mechanisms, specifically increases or decreases in the extent of certain brain connections.[35]

To find a possible explanation for the observed pattern of augmented functional connectivity in the anterior and posterior areas of the DMN, we performed tractography of the fibers connecting the core of the anterior and posterior regions of this network. This showed reductions within the fibers corresponding to the cingulum, and the FA of this bundle was correlated with the increased connectivity of the frontal node of the DMN. The cingulum is a long, medial associative bundle that runs within the cingulate gyrus all around the corpus callosum, connecting the medial frontal and parietal lobes.[36] This finding suggests that damage to this fascicle leads to functional reorganization consisting of increased local connectivity surrounding the nodes of the network, probably resulting from decreased interconnectivity between the frontal and parietal nodes.

Altogether, our results suggest that increased frontal functional activity at rest, measured as the ALFF, is associated with better global cognitive performance and that the altered structural connectivity between related brain regions can be compensated for by increased functional connectivity. Two recent studies have accurately characterized the changes in the DMN connectivity at different time points. Hillary et al,[10] examining resting-state DMN connectivity in a sample of patients in the acute stage with predominant focal lesions, found increases in

the connectivity of this network during the first 6 months after injury.[10] On the other hand, Arenivas et al[11] examined DMN functional connectivity in a cohort of patients 6 to 11 months after TBI. Using 3 methodological approaches, they found decreased connectivity in the main nodes of the DMN. Although their sample characteristics were similar to our own (patients with white matter damage without significant contusions), our patients were studied a mean of 4 years after injury. Thus, our findings can be generalized only to patients in the late chronic stage and are not directly comparable to findings in the early chronic or postacute stages. However, whereas Arenivas et al[11] showed decreases in the core nodes of the DMN, we found diffuse increases in connectivity in areas surrounding DMN nodes. The apparent contradiction can be explained by the fact that Arenivas et al did not analyze the regions where we found increases because they studied connectivity within the nodes of the DMN. We suggest that the increased connectivity in areas surrounding DMN nodes, which we found in the late chronic stage, might represent the brain's attempt to compensate functionally for weaker connectivity within DMN nodes caused by structural damage after traumatic axonal injury. Nevertheless, the decreased connectivity of the DMN in this study did not predict the clinical or cognitive deficits. Future longitudinal studies with several follow-up points could clarify the dynamics of cerebral reorganization after TBI.

Accepted for Publication: January 4, 2013.
Published Online: May 20, 2013. doi:10.1001/jamaneurol.2013.38
Correspondence: Carme Junque, PhD, Department of Psychiatry and Clinical Psychobiology, University of Barcelona, C/Casanova 143, 08036 Barcelona, Spain (cjunque@ub.edu).
Author Contributions: Mss Palacios and Sala-Llonch contributed equally to the manuscript. *Study concept and design:* Palacios, Sala-Llonch, Junque, and Bargallo. *Acquisition of data:* Palacios, Sala-Llonch, Roig, Tormos, and Bargallo. *Analysis and interpretation of data:* Palacios, Sala-Llonch, and Vendrell. *Drafting of the manuscript:* Palacios, Sala-Llonch, and Junque. *Critical revision of the manuscript for important intellectual content:* All authors. *Statistical analysis:* Palacios, Sala-Llonch, and Vendrell. *Obtained funding:* Junque and Tormos. *Administrative, technical, and material support:* Roig, Tormos, and Bargallo. *Study supervision:* Junque and Tormos.
Conflict of Interest Disclosures: None reported.
Online-Only Material: The eMethods, eTable, and eReferences are available at http://www.jamaneuro.com.
Additional Contributions: Cesar Garrido, Rad Tech, from the Centre de Diagnostic per la Imatge, Hospital Clinic, Barcelona, assisted with data collection.

REFERENCES

1. Benedictus MR, Spikman JM, van der Naalt J. Cognitive and behavioral impairment in traumatic brain injury related to outcome and return to work. *Arch Phys Med Rehabil*. 2010;91(9):1436-1441.
2. Adams JH, Jennett B, Murray LS, Teasdale GM, Gennarelli TA, Graham DI. Neuropathological findings in disabled survivors of a head injury. *J Neurotrauma*. 2011;28(5):701-709.
3. Boki A, Povlishock JT. All roads lead to disconnection? traumatic axonal injury revisited. *Acta Neurochir (Wien)*. 2006;148(2):181-194.
4. Sharp DJ, Beckmann CF, Greenwood R, et al. Default mode network functional and structural connectivity after traumatic brain injury. *Brain*. 2011;134(pt 8):2233-2247.
5. Palacios EM, Sala-Llonch R, Junque C, et al. White matter integrity related to functional working memory networks in traumatic brain injury. *Neurology*. 2012;78(12):852-860.
6. Marquez de la Plata CD, Garces J, Shokri Kojori E, et al. Deficits in functional connectivity of hippocampal and frontal lobe circuits after traumatic axonal injury. *Arch Neurol*. 2011;68(1):74-84.
7. Kasahara M, Menon DK, Salmond CH, et al. Traumatic brain injury alters the functional brain network mediating working memory. *Brain Inj*. 2011;25(12):1170-1187.
8. Mayer AR, Mannell MV, Ling J, Gasparovic C, Yeo RA. Functional connectivity in mild traumatic brain injury. *Hum Brain Mapp*. 2011;32(11):1825-1835.
9. Bonnelle V, Ham TE, Leech R, et al. Salience network integrity predicts default mode network function after traumatic brain injury. *Proc Natl Acad Sci U S A*. 2012;109(12):4690-4695.
10. Hillary FG, Slocomb J, Hills EC, et al. Changes in resting connectivity during recovery from severe traumatic brain injury. *Int J Psychophysiol*. 2011;82(1):115-123.
11. Arenivas A, Diaz-Arrastia R, Spence J, et al. Three approaches to investigating functional compromise to the default mode network after traumatic axonal injury [published online July 31, 2012]. *Brain Imaging Behav*. .
12. Shumskaya E, Andriessen TM, Norris DG, Vos PE. Abnormal whole-brain functional networks in homogeneous acute mild traumatic brain injury. *Neurology*. 2012;79(2):175-182.
13. Slobounov SM, Gay M, Zhang K, et al. Alteration of brain functional network at rest and in response to YMCA physical stress test in concussed athletes: RsFMRI study. *NeuroImage*. 2011;55(4):1716-1727.
14. Biswal BB, Mennes M, Zuo XN, et al. Toward discovery science of human brain function. *Proc Natl Acad Sci U S A*. 2010;107(10):4734-4739.
15. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci*. 2007;8(9):700-711.
16. Smith SM, Fox PT, Miller KL, et al. Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A*. 2009;106(31):13040-13045.
17. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci*. 2008;1124:1-38.
18. Hagmann P, Cammoun L, Gigandet X, et al. Mapping the structural core of human cerebral cortex. *PLoS Biol*. 2008;6(7):e159 doi:10.1371/journal.pbio.0060159.
19. van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol*. 2010;20(8):519-534.
20. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. *Brain*. 2007;130(Pt 10):2508-2519.
21. Sidaros A, Engberg AW, Sidaros K, et al. Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. *Brain*. 2008;131(pt 2):559-572.
22. Kinnunen KM, Greenwood R, Powell JH, et al. White matter damage and cognitive impairment after traumatic brain injury. *Brain*. 2011;134(pt 2):449-463.
23. Newcombe V, Chatfield D, Outtrim J, et al. Mapping traumatic axonal injury using diffusion tensor imaging: correlations with functional outcome. *PLoS One*. 2011;6(5):e19214 doi:10.1371/journal.pone.0019214.
24. Palacios EM, Sala-Llonch R, Junque C, et al. Long-term declarative memory deficits in diffuse TBI: correlations with cortical thickness, white matter integrity and hippocampal volume. *Cortex*. 2013;49(3):646-657. Medline:22482692.
25. Lezak MD, Howieson DB, Loring DD, et al. *Neuropsychological Assessment*. New York, NY: Oxford University Press; 2004.
26. Zang YF, He Y, Zhu CZ, et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev*. 2007;29(2):83-91.
27. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*. 2002;17(2):825-841.
28. Beckmann CF, Smith SM. Tensorial extensions of independent component analysis for multisubject FMRI analysis. *NeuroImage*. 2005;25(1):294-311.
29. Damoiseaux JS, Rombouts SA, Barkhof F, et al. Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A*. 2006;103(37):13848-13853.
30. Filippini N, MacIntosh BJ, Hough MG, et al. Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A*. 2009;

JOBNAME: Neuro PAGE: 8 SESS: 2 OUTPUT: Mon Apr 15 13:59:54 2013
/archives/13jobs/neu/05_20_2013/noc130001

106(17):7209-7214.
31. Wang Z, Yan C, Zhao C, et al. Spatial patterns of intrinsic brain activity in mild cognitive impairment and Alzheimer's disease: a resting-state functional MRI study. *Hum Brain Mapp*. 2011;32(10):1720-1740.
32. Bonavita S, Gallo A, Sacco R, et al. Distributed changes in default-mode resting-state connectivity in multiple sclerosis. *Mult Scler*. 2011;17(4):411-422.
33. Hawellek DJ, Hipp JF, Lewis CM, Corbetta M, Engel AK. Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. *Proc Natl Acad Sci U S A*. 2011;108(47):19066-19071.
34. Bonnelle V, Leech R, Kinnunen KM, et al. Default mode network connectivity predicts sustained attention deficits after traumatic brain injury. *J Neurosci*. 2011; 31(38):13442-13451.
35. Castellanos NP, Paul N, Ordóñez VE, et al. Reorganization of functional connectivity as a correlate of cognitive recovery in acquired brain injury. *Brain*. 2010; 133(Pt 8):2365-2381.
36. Catani M, Thiebaut de Schotten M. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. *Cortex*. 2008;44(8):1105-1132.

SUPPLEMENTARY RESULTS

Supplementary Figure 1:

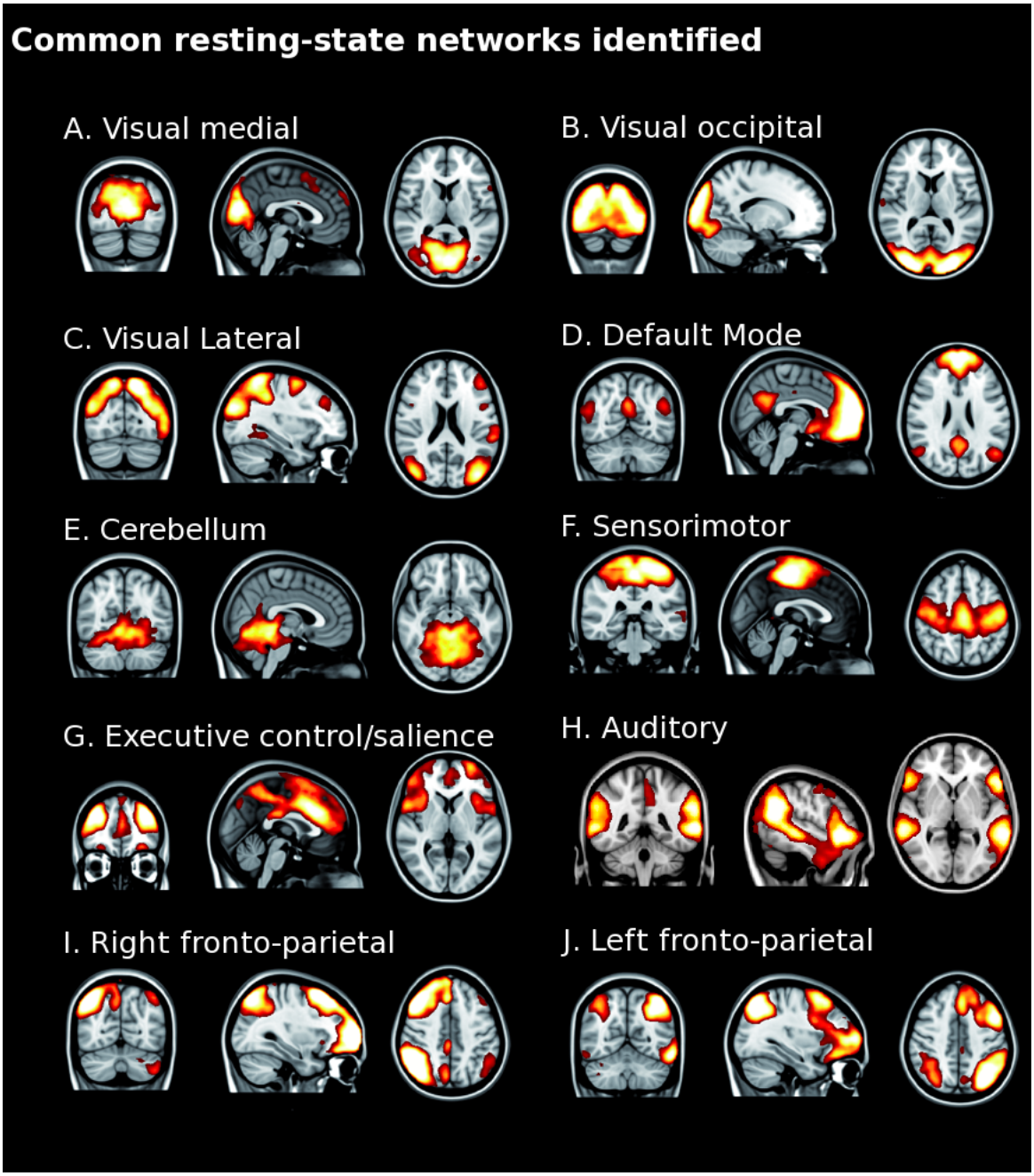


Figure 1. Ten common resting-state networks identified in the ICA analysis of the resting-state fMRI data of all the subjects.

Supplementary Table I.

Network	Areas
Visual medial	Medial visual areas
Visual occipital	Occipital pole visual areas
Visual lateral	Lateral visual areas
Default mode	Medial parietal areas (precuneus and posterior cingulate), bilateral inferior-lateral-parietal areas and medial/ventromedial prefrontal cortex.
Cerebellum	Cerebellum
Sensorimotor	Supplementary motor area, sensorimotor cortex and secondary somatosensory cortex
Auditory	Primary and association auditory cortices. Superior temporal gyrus, Heschl's gyrus and posterior insular cortex.
Executive control / salience	Medial-frontal areas, including anterior cingulate and paracingulate and insular cortex.
Right frontoparietal	Right dorsal-frontal and parietal areas. Anterior cingulate gyrus.
Left frontoparietal	Left dorsal-frontal and parietal areas. Anterior cingulate gyrus.

Table 1: Description of the main 10 resting-state networks that have been described in the literature and that can be found in our resting-state fMRI analysis.

SUPPLEMENTARY METHODS

Principal components analysis of cognitive data:

Principal component analysis (PCA) was implemented in PASW software. PCA is a dimensionality reduction method that enables a set of components to be extracted from a large amount of data, and where the first principal component accounts for as much of the variability in the data as possible (maximizing its variance).
PCA was used here to obtain a common factor of cognitive outcome. The variables introduced in the decomposition were the scores on the following neuropsychological tests: Letter-Number Sequencing; Digit Span Forward and Backwards; the Trail Making Test (TMT-A/B); the Rey Auditory Verbal Learning Test; the Rey-Osterrieth complex figure; Stroop reading, color-naming, and reading word-color conditions; verbal semantic and phonemic fluencies. The first principal component was used as the subject's cognitive outcome and it accounted for 50.3% of the total variance in the neuropsychological data.

Amplitude of low frequency fluctuations in fMRI data:

The amplitude of low frequency fluctuations (ALFF) was used to obtain a measure of the signal amplitude during resting fMRI in the frequency domain[1,2].
Prior to computing individual ALFF maps, resting fMRI data were preprocessed using tools implemented in AFNI (http://afni.nimh.nih.gov/afni) and FSL (http://www.fmrib.ox.ac.uk/fsl) software. These steps included motion correction, brain extraction, spatial smoothing with a Gaussian kernel of FWHM = 6 mm and grand mean scaling. A sample of the original fMRI data was also used to compute registration matrices between individual functional and MNI standard spaces, using a linear registration algorithm (FLIRT tool from FSL)[3].
Resting-state data were then transformed to frequency domain with a fast Fourier transform (FFT) algorithm, thereby enabling us to obtain a 4D image in which each voxel contained the amplitude of the signal across the whole spectrum. Finally, individual ALFF maps were computed as the square root of the sum of the squares of the amplitude at each frequency point for each voxel within the frequencies of interest (0.01 - 0.1 Hz).
Before conducting group statistical analyses, ALFF maps were transformed into Z scores and moved to standard MNI space using the registration matrices obtained previously.
Finally, ALFF maps of all subjects were concatenated and introduced into a permutation-based group analysis using Randomise[4] from FSL. In this analysis we tested for voxel-wise differences between groups using 5000 permutations. The size of significant clusters was selected using the threshold-free cluster enhancement (TFCE) method[5]. Difference maps were

finally thresholded using correction for family-wise error (FWE) with $p<0.05$.

Resting state pre-processing and ICA analysis:

Independent component analysis (ICA), as implemented in MELODIC[6] from FSL, was applied to the resting fMRI data in order to decompose the data into a set of independent components (ICs) that described common spatio-temporal and independent patterns of correlated brain activity across the whole group of subjects in the study. Before decomposition, fMRI data were preprocessed as follows: removal of the first five scans, motion correction, skull stripping, spatial smoothing with a Gaussian kernel of FWHM=6 mm, grand mean scaling, and temporal filtering (high-pass filter of FWHM=150 s and low-pass filter of FWHM=11.6 s). Functional scans were then registered to the MNI standard template using linear registration with 6 degrees of freedom[4]. Resampling resolution was set to 4 mm. The number of ICs was then estimated using the Laplace approximation to the Bayesian evidence of the model order[6]. Within all ICs obtained we identified the common resting-state functional networks[7,5,2], and specifically the default mode network (DMN). The selection procedure was performed by visual inspection together with template matching using data available online[5,2]. Template matching was performed by means of spatial cross-correlation between pairs of maps.

The spatial map of the DMN was then introduced into a dual regression analysis[8,9]. In this analysis the preprocessed functional data of each subject were first regressed against the spatial IC maps, yielding individual time series associated with the DMN. These time series were then used to regress again the individual preprocessed fMRI data and to obtain individual spatial maps. Spatial maps were finally tested for voxel-wise differences between groups using non-parametric testing with 5000 random permutations[4]. After family-wise error (FWE) correction, differences with $p<0.05$ were considered significant.

In all the regions that resulted significant in the comparison we extracted the DMN scores associated with each individual (patients and controls). These scores were further used to study the relationship between connectivity, structure and cognition.

Seed-based analysis of the DMN:

The peak coordinates of the DMN identified by the ICA were used to create four spherical ROIs representing the main nodes of this network: medial prefrontal cortex (MPFC), precuneus/posterior cingulate (PPC), and left and right parietal cortices (left-PAR and right-PAR). Connectivity maps were created using a procedure similar to that described in Biswal et al. (2010).

For each seed (or DMN node) we used the preprocessed and MNI-registered resting fMRI data to extract seed time series as the average time series within all the seed voxels. Then, for each 4D dataset we obtained a connectivity map representing the correlation between the seed and each voxel in the brain. These maps were transformed into Z

scores using Fisher's r-to-Z transformation.
Finally, Z-maps were tested for voxel-wise differences between groups using non-parametric testing with 5000 permutations[4]. Resulting maps were obtained directly in MNI standard space.
In all the regions of the connectivity maps where significant differences were found between groups, we extracted individual connectivity scores for further statistical analysis.

Analysis of diffusion MRI data:

Diffusion MRI images were analyzed using FDT (FMRIB's Diffusion Toolbox), a software tool for analysis of diffusion-weighted images included in FSL. Firstly, data were corrected for distortions caused by the eddy currents in the gradient coils and for simple head motion, using the B0 non-diffusion data as a reference volume. Fractional anisotropy (FA) maps from each subject were then obtained using a diffusion tensor model fit, and registered to MNI space.
A probabilistic tractography algorithm was also applied to the diffusion images. To this end, diffusion parameters were first estimated using the BEDPOSTX tool from FSL, which computes a Bayesian estimation of the parameters (i.e., diffusion parameters and local fiber directions) using sampling techniques and a model of crossing fibers[10]. The density functions obtained were subsequently used within PROBTRACKX from FSL to estimate connectivity between the two DMN ROIs (i.e., medial prefrontal ROI and posterior cingulate/precuneus ROI) extracted from the analysis of the resting fMRI data. Previous to tractography, these two ROIs were moved from standard MNI to individual diffusion space, using linear registration (FLIRT)[3]. Thus, the entire probabilistic tracking procedure was carried out in each subject's diffusion space and further registered to the standard MNI template. Using the probabilistic tractography algorithm we obtained individual normalized maps in which each voxel value indicated the probability of having fibers connecting the two regions.
Finally, we computed the average connectivity map for the group of controls and the group of patients separately. The average connectivity map of controls was used, together with the registered FA maps, to estimate fiber integrity of this connection in the whole sample, as the mean FA within the pathway.

Relationship between cognitive, structural and functional data:

The aim of this analysis was to study the relationship between the results obtained in all the above analyses and to determine how they are related to subjects' cognitive outcome.

SUPPLEMENTARY REFERENCES:

1. Zang, YF., He, Y., Zhu, CZ, Cao QJ, Sui MQ, Tian LX, Jiang TZ, and Wang YF. (2007) Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev., 29(2):83-91.

2. Biswal BB, Mennes M, Zuo XN, et al. (2010). Toward discovery science of human brain function. Proc Natl Acad Sci USA., 107(10):4734-9.

3. Jenkinson M, Bannister P, Brady M et al. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002;17(2):825-841.

4. Nichols T. E., and Holmes A. P., (2002) Nonparametric permutation tests for functional neuroimaging: a primer with examples. Human Brain Mapping, 15:1-25.

5. Smith SM and Nichols TE (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 44(1): 83-98.

6. Beckmann CF, Smith SM. Tensorial extensions of independent component analysis for multisubject FMRI analysis. Neuroimage 2005;25(1):294-311.

7. van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: A review on resting-state fMRI functional connectivity. Eur Neuropsychopharmacol 2010;20(8):519-534.

8. Filippini N, MacIntosh BJ, Hough MG et al. Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A 2009;106(17):7209-7214.

9. Sharp DJ, Beckmann CF, Greenwood R et al. Default mode network functional and structural connectivity after traumatic brain injury. Brain 2011;134(Pt 8):2233-2247.

10. Behrens, TEJ, Woolrich, MW, Jenkinson M., Johansen-Berg, H., Nunes, RG, Clare, S., Matthews, PM, Brady, JM, and Smith, SM. (2003). Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magn Reson Med*, 50(5):1077-1088.

11. T.E.J. Behrens, H. Johansen-Berg, S. Jbabdi, M.F.S. Rushworth, and M.W. Woolrich. (2007). Probabilistic diffusion tractography with multiple fibre orientations. What can we gain? *NeuroImage*, 23:144-155, 2007.

Supplementary Table1. Comparison of neuropsychological performance between patients and controls.

	TBI group (mean/SD)	Control group (mean/SD)	*Statistic t(p)*
RAVLT learning	44.77 (±10.20)	56.14 (±5.22)	4.15 (<0.001)
RAVLT (DR)	43.66 (±9.41)	57.45 (±3.24)	5.74 (<0.001)
ROCF (DR)	46.18 (±10.27)	54.48 (±7.75)	2.80 (0.008)
Symbol Digit	45.13 (±7.91)	55.72 (±9.28)	3.70 (0.001)
TMTA	54.40 (±10.55)	44.81 (±6.31)	3.28(0.002)
TMTB	54.89 (±10.55)	44.24 (±6.90)	3.88 (<0.001)
Digits forward	47.26 (±8.79)	53.21 (±10.62)	1.83 (0.076)
Digits backward	46.60 (±8.21)	53.98 (±10.65)	2.32 (0.027)
Semantic fluency	47.66 (±11.00)	52.74 (±8.14)	1.61 (0.116)
Phonetic fluency (PMR)	46.28 (±10.18)	54.36 (±8.02)	2.69 (0.011)
Letter-Number Sequencing	45.36 (±9.39)	55.45 (±7.88)	3.55 (0.001)
Stroop word reading	46.37 (±9.39)	54.26 (±9.18)	2.57 (0.014)
Stroop color naming	44.60 (±7.32)	56.34 (±9.06)	4.28 (<0.001)
Stroop color word naming	46.11 (±9.92)	54.56 (±8.19)	2.83 (0.07)

TBI: traumatic brain injury; RAVLT: Rey auditory verbal learning test; DR: delayed recall; ROCF: Rey-Osterrieth complex figure; CPT: Conners' Continuous Performance Test. Note: T- values mean scores.

5. GENERAL DISCUSSION

The neurobiological bases of cognitive impairment after traumatic brain injury are poorly understood due to both the heterogeneity and complexity of the pathology, as well as the limitations of clinical MRI. Traumatic axonal injury is associated with the widespread damage to axons throughout the white matter in the brain caused by sustained acceleration and deceleration forces at the moment of injury. Patterns of brain network connectivity are usually disrupted as a result of TBI, thus it is likely that TAI plays a major role in cognitive deficits after TBI. Advances in neuroimaging techniques have recently facilitated the study of the structural damage and brain dysfunction underlying the cognitive and behavioural sequelae MRI techniques such as diffusion tensor imaging have been demonstrated to be sensitive in detecting subtle white matter changes in different neurological pathologies (Ciccarelli et al., 2008). Moreover, studies of functional connectivity allow the investigation of the relationship between function and brain structure, showing that areas with strongly correlated activity are more likely to be anatomically connected (Johansen-Berg and Rushworth, 2009).

This thesis, which includes four research articles, has aimed to investigate the structural and functional alterations associated to TAI and related to the main cognition functions altered after TBI. Diffusion tensor imaging (DTI) is a good technique for the detection of subtle white matter changes given that it can reveal significant abnormalities in white matter in patients that are not observed in

conventional MRI (Rugg-Gunn et al., 2001). In our first study , we used DTI to investigate working and declarative memory deficits in a sample of diffuse TBI patients to determine the specific structural basis of memory alterations. Reduced FA values were found to be associated with executive functions, attention and memory domains as well as processing speed and visuoperceptual deficits (Kraus et al., 2007; Kumar et al., 2009b). However, previous studies include heterogeneous samples of patients with different degrees of severity and patients with focal lesions. All our studies, were performed with a sample of TBI patients with severe and diffuse pathology. Our results from the first study showed two different and restricted patterns of correlations with the FA and neuropsychological functions DTI analysis showed that decreased FA throughout the brain correlated with working memory measures but not with declarative memory measures. In a more regional analysis of the main association fasciculi, working memory specifically correlated with the SLF, corpus callosum, and, unexpectedly, with the fornix. Superior longitudinal fasciculi connect the associative frontal and parietal regions suggesting a relationship with working memory deficits and there is clear evidence of the involvement of parietal and frontal structures in working memory functions from functional and neuroanatomical studies (Jonides et al., 1998; Klingberg et al., 1997; Olesen et al., 2004; Owen et al., 2005). On the other hand, declarative memory deficits only correlated with the fornix, and the corpus callosum, although in this latter case the correlation was found in the ROI analysis of specific fasciculi but not with the global FA values. This suggests that declarative memory deficits are more directly dependent on damage to the hippocampus and its connections with the frontal lobe. We must consider the possibility that fornix damage in this study is a consequence not only of shearing forces but also of secondary excitotoxic injury induced by the trauma-related neuronal loss in the hippocampus and the resulting atrophy (Povlishock and Katz, 2005). However, a more atrophic hippocampus does not necessarily predict an atrophic fornix or vice versa (Tate and Bigler, 2000). The conclusion of this study was that working memory impairment reflects the diffuse white matter damage affecting large scale networks, whereas declarative memory deficits seem to be the result of more local disruption of the cerebral circuitry.

Given that memory impairment is one of the most common problems of patients suffering severe TBI and taking into account the white matter alterations resulting from

the first study, we performed a second study focusing on the structural brain damage and its relation with declarative memory considering not only the alterations of white matter but also other measures such as cortical thickness and hippocampal volume, which could also play a role in these deficits. To this end, we studied a sample of 26 patients with diffuse, severe and chronic TBI without major focal lesions. Earlier studies had shown some associations between white matter damage and memory deficits. Using a region-of-interest approach, Kraus et al., 2007, found that declarative memory correlated with FA reductions in several fasciculi, while in our own previous study we obtained significant correlations in the case of the corpus callosum and fornix (Palacios et al., 2011). With a more precise technique (tract-based spatial statistics), Kinnunen et al. (2011) identified the fornix as being the region that was most strongly related with memory deficits and, a longitudinal study by Wang et al. (2011) found that damage in the networks involving several fasciculi in the acute stage could predict memory and learning deficits in the chronic stage. Inter-hemispheric functional connectivity was also reported to correlate with delayed recall (Marquez de la Plata et al., 2011a). However, no previous study had combined the use of volumetric analyses and diffusion tensor imaging magnetic resonance to investigate the brain structural alterations sustaining declarative memory deficits and/or the relationship of cortical atrophy related to TAI. Our studies have resulted in several findings. Firstly, we found that the reductions in the cortical thickness observed in TBI patients reflected a pattern of widespread cortical atrophy. Similarly to a previous longitudinal study with diffuse TBI patients Warner et al., (2010b) we found that the cortex of the occipital and temporal lobes was almost preserved. This adds yet more weight to the already clear evidence of progressive atrophy even one year after TBI (Bigler et al., 2006; M. Farbota et al., 2012; Sidaros et al., 2009). Secondly, and as we found in the first study, DTI analyses revealed a widespread and diffuse pattern of white matter alteration but, interestingly, even in the case of the extensive cortical pattern of cortical atrophy and white matter damage, FA values, only correlated with the thickness of specific regions of the frontal, parietal and cingulate cortices. It is worth mentioning that the cortical regions showing greater cerebral atrophy associated with decreased white matter integrity were those connected by the long associative fasciculi, such as the superior longitudinal fasciculi, the fronto-occipital and the cingulum. Given the diffuse characteristics of our sample, these results may suggest that the process of progressive cortical atrophy in these areas is a consequence of retrograde degeneration of these

fasciculi. A recent histopathological study by (Maxwell et al., 2010) demonstrated decreased cortical thickness in several frontal regions due to the loss and size reductions of pyramidal and non-pyramidal neurons and that there was greater loss of pyramidal neurons in the prefrontal cortex of patients with TAI than in a non-TAI control group. Thirdly, declarative memory deficits were found to be related with reductions of cortical thickness in the frontal and parietal cortex involving the precuneus area in TBI patients who performed worse in the memory test. Notably, the fronto-parietal cortical regions found to correlate with memory deficits corresponded with regions that correlated with white matter impairment, suggesting a role for disrupted connectivity between complex nodes of the memory network. Fourthly, TBI patients presented significant bilateral hippocampal atrophy in comparison to controls. However, although hippocampal atrophy and verbal memory impairment have been reported in several studies of TBI (Ariza et al., 2006; Warner et al., 2010a), we found no significant correlations between memory scores and total hippocampal volume nor specific areas of the hippocampus, which suggests that damage to this structure may be compensated for by other regions that do not play a primary role in explaining memory impairment. Finally, a multiple regression analysis, revealed white matter connectivity to be the best predictor of declarative memory sequelae, although there was also an independent contribution of cortical atrophy in the left precuneus. We concluded that even if patients had widespread grey and white matter atrophy, alterations in cortico-subcortical connectivity explained long-term definitive memory sequelae together with the decreased thickness in the parietal left hemisphere in patients with diffuse chronic TBI.

Continuing our investigation of memory alterations, we performed a third study to better understand working memory deficits after TBI. Structural studies, could not tell us the extent to which structural connectivity alterations affect the brain while it is in action responding to external stimuli. We therefore conducted a study where we combined structural and functional MRI techniques. This was the first multimodal study combining fMRI and DTI techniques to obtain a better understanding of the connectivity alterations underlying working memory impairment after severe TAI. We studied the synchrony of brain activity among spatially distinct areas activated during the processing of a working memory task together with a baseline task (n-back task). These allowed us to identify two different patterns of activation, one of which

corresponded to the default mode network, which is known to be active during rest and deactivated during externally oriented tasks (Honey et al., 2009; Smith et al., 2012). The other pattern of activation corresponded to the task-related condition with activation of core areas of working memory circuitry. Group comparison showed that patients had decreased brain activity in the default mode network, a result which agrees with previous studies of TBI patients (Mayer et al., 2011; Nakamura et al., 2009; Vanhaudenhuyse et al., 2010). A DTI analysis, which was also performed, revealed a strong relationship between structural connectivity and fMRI activation maps. Patients with more white matter alterations showed lower brain connectivity for both default mode and working memory networks.

Working memory lower performance was related with white matter specific fasciculi such as the inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculi, cingulum, uncinate and corpus callosum. As we already demonstrated in the first study, and here reported again, 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. 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. Moreover, working memory performance also correlated with fMRI but not with grey matter volume. Our functional connectivity results indicated that working memory impairment may be a result of a deficit in activated task-related areas together with deficits in deactivating the DMN during task processing. Together with white matter alterations we demonstrated that structural connectivity impairment underlies alterations in the functional networks as well as working memory deficits.

In the same direction, with the fourth study we wanted to take advantage of the analysis of resting-state fMRI studies. These studies provide information about brain activity and connectivity in the absence of task performance, a condition that allows researchers to investigate patient populations with broader degrees of severities, since no specific cognitive ability is required. This study sought to determine whether the amplitude of spontaneous low-frequency fluctuations in brain activity and DMN connectivity may be sensitive biomarkers for cognitive dysfunction after traumatic brain injury. To this end, we measured the amplitude of resting-state BOLD signal

fluctuations of the whole brain and of the DMN, global and network-based functional connectivity, as well as the white matter structure within the cingulum tract. We then related these measures with a factorial measure of the global cognitive performance.

TBI group had an increased activity predominantly focused within the frontal lobe regions. Moreover, the resting activity of these areas predicted the better general cognitive outcome in TBI patients, suggesting that functional and efficient frontal brain reorganization occurred to compensate acute brain damage and improve cognitive performance. The specific analyses of the DMN during the resting state also showed increased connectivity in the frontal node of this network in TBI patients in comparison with controls. Furthermore, connectivity from each core of DMN nodes to the whole brain revealed a widespread pattern of locally increased functional connectivity surrounding the medial frontal and left parietal nodes of the DMN. Previous studies investigating the DMN during the resting state in TBI reported alterations that reflect both decreases and increases in functional connectivity. Increases in the functional connectivity of several DMN regions, as well as the recruitment of additional areas, have been interpreted as compensatory/adaptation mechanisms, since they positively correlated with cognitive outcome (Bonnelle et al., 2011; Mayer et al., 2011). Moreover, in our study, this frontal increase in the DMN connectivity was explained by the white matter fibres corresponding to the cingulum, a key tract connecting the core of the anterior and posterior regions of this network. The overall results of this study suggested that increased frontal functional activity at rest, measured as the amplitude of low-frequency fluctuations, is associated with better global cognitive performance, and that the altered structural connectivity between related brain regions can be compensated for by increased functional connectivity.

The most important contribution of these studies is to have reported a series of interesting findings that may contribute to our understanding of the neural substrates of cognitive deficits after severe, diffuse and chronic TBI. Amongst these that: (1) there is significant structural damage including global white matter alterations but that only specific alterations of certain tracts, such as the superior longitudinal fasciculi for working memory impairment and the fornix for declarative memory functions, are responsible for these deficits; (2) global grey matter atrophy could in part resulted from white matter degeneration; (3) the lack of white matter integrity explained declarative memory deficits among other measures such as the hippocampal volume or thickness;

(4) the decreased connectivity of synchronised areas involved in a working memory task together with the lack of deactivation of the DMN during a task, predicted working memory deficits. Moreover, these alterations in the connectivity during the performance of tasks, were explained by the specific white matter alterations showing evidence that white matter alterations of certain tracts were responsible for the functional alterations while performing a task (6) that the increased resting-state activity in the frontal lobe predicted better cognitive performance and that differences in the frontal connectivity of the DMN at rest in this case, were explained by the alteration of the cingulum tract which is the key tract connecting anterior and posterior areas of the DMN.

Taking all these results together, we can establish that, although after TAI there are clear widespread patterns of white matter damage and cortical atrophy, the evidence of these studies seems to suggest that cognitive sequelae after diffuse TBI follows a predominant frontal pattern of alteration. We suggest that frontal white matter connectivity and the basal activity of the frontal lobe are the main alterations sustaining cognitive outcome after diffuse and chronic TBI.

Frontal lobes are commonly damaged after as a result of TBI. This damage can produce changes in the neuroanatomy and neurophysiology of the brain disrupting cerebral networks that may ffect cognitive functions. Efficient cognitive performance depends on the widely distributed brain networks which are connected by association white matter tracts (Gong et al., 2009; Mesulam, 1998) being vulnerable after TBI. With the work presented in this thesis, we provide evidence that even in the absence of significant focal lesions and global white and grey matter alterations, white matter damage of the main fasciculi linking the frontal lobe with other brain areas, can affect functional connectivity, which in turn is an important factor for the prediction of long-term cognitive recovery. This frontal pattern of predominance as a predictor of cognitive functions may be important for neuropsychological rehabilitation programmes. Imaging measures of white matter potentially in combination with functional measures could be used to predict responses to an intervention in order to allow the tailoring of specific interventions and optimise outcomes (Dobkin, 2004; Johansen-Berg, 2010). Therefore, taking into account our results, cognitive programmes for patients suffering from TAI pathology, would benefit from the incorporation of

frontal function stimulation activities from an early stage as it is highly likely that this will benefit long-term global outcomes. Moreover, cognitive therapies combined with frontal drug enhancers or other non-invasive complementary therapies that would increase functional frontal baseline activity, would also improve reorganisation and neuroplasticity processes. Longitudinal neuroimaging of the patients from the acutest stages are also needed as those investigations could play an essential role in clinical and recovery management.

CONCLUSIONS

Taking together all the results from the present thesis, we can conclude that:

1. The structural MRI results demonstrated that patients with severe, diffuse and chronic TBI have a global reduction of cortical thickness, alongside a widespread pattern of white matter alteration involving intra-hemispheric association, inter-hemispheric, and projection fibres.

2. The loss of white matter integrity measured by FA is related to cortical thickness reductions of specific regions at frontal, parietal and cingulate cortices. This could indicate retrograde degeneration due to TAI.

3. In the fMRI working memory task, patients showed decreased functional connectivity within the working memory and default mode networks (DMN) as compared to controls.

4. In resting-state, DMN connectivity is increased in the frontal node of this network in TBI patients as compared to controls, probably reflecting compensatory mechanisms due to the underlying structural alteration of the cingulum tract.

5. The TBI group also showed increases in the resting state basal activity focused in frontal lobe regions, involving the anterior node of the DMN. This increase of resting activity is predictive of better cognitive outcome.

6. Alterations in the structural connectivity explained the abnormal patterns of functional connectivity within the DMN and working memory network.

7. The working memory impairment is explained by deficits in the functional connectivity of the task-related areas together with deficits in deactivation of the DMN during task processing. The white matter fasciculi more related to the functional connectivity alteration and lower performance are the inferior and superior longitudinal fasciculi, cingulum, uncinate and corpus callosum.

8. Deficits in declarative memory can be explained by the disruption of the cerebral cortico-subcortical circuitry, specifically with the fornix. In addition, the cortical thinning in the left precuneus also contributed to the declarative memory impairment.

In summary:

Although after TAI there are clear widespread patterns of white matter damage and cortical atrophy, the results of these studies seem to suggest that long-term cognitive sequelae after diffuse TBI follow a predominant frontal pattern of alteration. Disrupted frontal white matter structural connectivity and the basal activity of the frontal lobe at rest, are the main alterations explaining cognitive outcome after diffuse and chronic TBI.

ACKNOWLEDGEMENTS

I am enormously grateful to my supervisor, Dr. Junqué, for allowing me to collaborate in her team. The inspiring discussions we have had over these four years and the encouragement she has given me has been priceless. A special thanks also to the head of department, Dr. Vendrell, for his always critical point of view, his precise focus on detail, and his enthusiasm for science. Without their support this thesis would not have been possible.

I also wish to express my gratitude to the Rehabilitation Hospital Institut Guttmann, and very specially to Dr. Tormos, Dr. Roig, and Dr. Sánchez-Carrión, who are doing magnificent work in the rehabilitation institution. Thank you for facilitating my work and for putting their trust in me. I would also like to thank all the patients and relatives who agreed to participate in this research despite their difficult personal circumstances.

My special thanks to the University of Cambridge, Department of Medicine, Division of Anaesthesia. I am extremely grateful to Prof. David Menon and Dr. Emmanuel A. Stamatakis for making me welcome in their department and for sharing their professionalism and experience with me. The interaction I had with the members of their department proved to be an excellent learning experience.

To my dear friends and colleagues at the Department of Psychiatry and Clinical Psychobiology and at the Hospital Clinic of Barcelona. Thank you so much for creating such a friendly, enthusiastic, and inspiring environment. I will be taking many memorable moments away with me.

Finally, I want to thank my "families" for their support and for always being there for me.

SUPPLEMENTARY MATERIAL

EDITORIAL

Seeing into the traumatically injured brain

Diffuse tissue damage and cognition

Andrew M. Blamire, PhD

Correspondence & reprint requests to Dr. Blamire: andrew.blamire@newcastle.ac.uk

Neurology® 2012;78:1

Traumatic brain injury (TBI) is a major cause of long-term disability in the developed world.[1] TBI is associated with abnormalities in neuropsychological function, with particular deficits in working memory and attention. Perhaps surprisingly, the proportion of patients with long-term cognitive problems is largely independent of injury severity,[2] but there are opposing opinions on the extent to which these deficits recover over time, particularly in the more mildly injured patient. Some reports show ongoing problems at 12 months,[3] while others suggest that a complex interaction of neurobiological, psychosocial, and legal factors may obscure the true level of cognitive disability.[4]

One reason for the continuing discussion of how brain injury affects cognitive function has been the difficulty in visualizing the true extent of CNS damage. While CT and MRI can depict acute focal injury, conventional imaging is relatively insensitive to diffuse injury, resulting in only weak correlations between imaging findings and cognitive performance.[5] Recent developments in MRI have, however, provided tools to identify diffuse injury and to measure injury-related changes in brain activity. Diffusion tensor imaging (DTI) provides insight into tissue microstructure by measuring the interaction between water diffusion and the boundaries to diffusion presented by cell membranes, particularly in myelinated axons. DTI is sensitive to diffusion orientation, provides exquisite images of white matter structure, and hence has an obvious role in TBI, where the white matter tracts are known to undergo stretching and shearing forces during blunt traumatic injury. The cortical brain networks involved in performance of specific cognitive tasks can be identified using fMRI, but recently much interest has focused on the so-called resting state networks that reveal spatial patterns of synchronous activity throughout the brain.[6] One such network comprising the medial prefrontal, posterior cingulate, and inferior parietal cortices together with the hippocampal formation is active during rest or internally focused activity and deactivates when attending to externally derived tasks.[7] The activity in this so-called default mode network (DMN) therefore relates to attentional processing—a domain where TBI patients show consistent deficits. Together these MRI methods provide novel tools to measure the relationship among global tissue injury, damage to the white matter tracts, and alterations in both resting state and task-based brain network activity.

In this issue of *Neurology*®, Palacios et al.[8] report a combined DTI and fMRI study into the underlying neurobiological correlates of chronic cognitive dysfunction following severe TBI. By combining the 2 MRI techniques in the same subjects, this study shows how injury to the white matter tracts relates to cortical network activity during a working memory task. Poor performance is correlated with reduced activity in those cortical areas that form the memory network and also with less deactivation of the DMN. The ability to deactivate the DMN is considered essential for attentional processing and these findings suggest a network-related basis for the attentional deficits seen post-TBI. Further, by overlaying findings from the DTI and fMRI studies, Palacios et al. show that these patients have damage to the fasciculi (superior longitudinal, inferior longitudinal, inferior fronto-occipital fasciculi, and corpus callosum) that connect the distributed cortical areas of the memory network.[8] It is clear from these findings that long-term working memory problems following severe TBI are rooted in a true neurobiological basis that can be measured directly by MRI.

The field of TBI imaging research is often hampered by the assertion that every patient has a different injury, making the population highly heterogeneous and hence difficult to study. While heterogeneity is clearly a feature of these patients and does lead to wide variation in size and distribution of focal brain lesions, these, and other studies using DTI following trauma,[8–10] show strong consistency in diffuse white matter damage. It is clear that further development of these advanced imaging techniques holds tremendous promise for identification of patients

See page XXX

From the Institute of Cellular Medicine & Newcastle Magnetic Resonance Centre, Newcastle University, Newcastle, UK.

Disclosure: Author disclosures are provided at the end of the editorial.

with diffuse injury in regions subserving cognitive dysfunction. These current studies help to establish the base from which to improve our understanding of cognitive deficits in TBI. However, Palacios et al. (and others in the field) have generally focused on severely injured TBI patients with known cognitive deficits, which represent <5% of all TBI patients. The findings at this chronic postinjury time point are most likely indicative of permanent damage and deficits. To move forward we must now expand these investigations into the wider TBI population and into the acute postinjury phase, when findings in the individual could play an essential role in clinical management and provision of rehabilitation to those individuals who otherwise might become part of the expanding silent epidemic of TBI.

DISCLOSURE

Dr. Blamire receives research support from the Medical Research Council UK, EPSRC, Cancer Research UK, British Heart Foundation, and Sir Jules Thorn Charitable Trust.

REFERENCES

1. Zaloshnja E, Miller T, Langlois JA, Selassie AW. Prevalence of long-term disability from traumatic brain injury in the civilian population of the United States, 2005. J Head Trauma Rehab 2008;23:394–400.
2. Whitnall L, McMillan TM, Murray GD, Teasdale GM. Disability in young people and adults after head injury: 5–7 year follow up of a prospective cohort study. J Neurol Neurosurg Psychiatry 2006;77:640–645.
3. Vanderploeg RD, Curtiss G, Belanger HG. Long-term neuropsychological outcomes following mild traumatic brain injury. J Int Neuropsychol Soc 2005;11:228–236.
4. Kwok FY, Lee TMC, Leung CHS, Poon WS. Changes of cognitive functioning following mild traumatic brain injury over a 3-month period. Brain Inj 2008;22:740–751.
5. Wilson JTL, Wiedmann KD, Hadley DM, Condon B, Teasdale G, Brooks DN. Early and late magnetic resonance imaging and neuropsychological outcome after head injury. J Neurol Neurosurg Psychiatry 1988;51:391–396.
6. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 2007;8:700–711.
7. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann NY Acad Sci 2008;1124:1–38.
8. Palacios EM, Sala-Llonch R, Junque C, et al. White matter integrity related to functional working memory networks in traumatic brain injury. Neurology 2012;78:XXX–XXX.
9. Kinnunen KM, Greenwood R, Powell JH, et al. White matter damage and cognitive impairment after traumatic brain injury. Brain 2011;134:449–463.
10. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain 2007;130:2508–2519.

REFERENCES

▪ Adams, J.H., 1982. Diffuse axonal injury in non-missile head injury. Injury 13, 444–445.

▪ Adams, J.H., Doyle, D., Ford, I., Gennarelli, T.A., Graham, D I, McLellan, D.R., 1989a. Diffuse axonal injury in head injury: definition, diagnosis and grading. Histopathology 15, 49–59.

▪ Adams, J.H., Doyle, D., Ford, I., Graham, D I, McGee, M., McLellan, D.R., 1989b. Brain damage in fatal non-missile head injury in relation to age and type of injury. Scott. Med. J. 34, 399–401.

▪ Adams, J.H., Graham, D I, Murray, L.S., Scott, G., 1982. Diffuse axonal injury due to nonmissile head injury in humans: an analysis of 45 cases. Ann. Neurol. 12, 557–563.

▪ Adams, J.H., Scott, G., Parker, L.S., Graham, D I, Doyle, D., 1980. The contusion index: a quantitative approach to cerebral contusions in head injury. Neuropathol. Appl. Neurobiol. 6, 319–324.

▪ Arfanakis, K., Haughton, V.M., Carew, J.D., Rogers, B.P., Dempsey, R.J., Meyerand, M.E., 2002. Diffusion tensor MR imaging in diffuse axonal injury. Ajnr Am. J. Neuroradiol. 23, 794–802.

▪ Ariza, M., Serra-Grabulosa, J.M., Junqué, C., Ramírez, B., Mataró, M., Poca, A., Bargalló, N., Sahuquillo, J., 2006. Hippocampal head atrophy after traumatic brain injury. Neuropsychologia 44, 1956–1961.

▪ Baddeley, A., 2003. Working memory: looking back and looking forward. Nat. Rev. Neurosci. 4, 829–839.

▪ Bendlin, B.B., Ries, M.L., Lazar, M., Alexander, A.L., Dempsey, R.J., Rowley, H.A., Sherman, J.E., Johnson, S.C., 2008. Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. Neuroimage 42, 503–514.

▪ Bigler, Erin D, 2003. Neurobiology and neuropathology underlie the neuropsychological deficits associated with traumatic brain injury. Arch. Clin. Neuropsychol. Off. J. Natl. Acad. Neuropsychol. 18, 595–621; discussion 623–627.

▪ Bigler, Erin D, Maxwell, William L, 2011. Neuroimaging and neuropathology of TBI. NeuroRehabilitation 28, 63–74.

▪ Bigler, Erin D, Ryser, D.K., Gandhi, P., Kimball, J., Wilde, E.A., 2006. Day-of-injury computerized tomography, rehabilitation status, and development of cerebral atrophy in persons with traumatic brain injury. Am. J. Phys. Med. Rehabil. Assoc. Acad. Physiatr. 85, 793–806.

▪ Blumbergs, P., Reilly, P., and Vink, R. Trauma, in Greenfield's Neuropathology, 8th Edition, Eds., Arnold, London, 2008, p. 733

▪ Bonnelle, V., Ham, Timothy E, Leech, R., Kinnunen, Kirsi M, Mehta, M.A., Greenwood, R.J., Sharp, David J, 2012. Salience network integrity predicts default mode network function after traumatic brain injury. Proc. Natl. Acad. Sci. U. S. A. 109, 4690–4695.

▪ Bonnelle, V., Leech, R., Kinnunen Kirsi M, Ham, Tim E, Beckmann, Cristian F, De Boissezon, X., Greenwood, R.J., Sharp, David J, 2011. Default mode network connectivity predicts sustained attention deficits after traumatic brain injury. J. Neurosci. Off. J. Soc. Neurosci. 31, 13442–13451.

▪ Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L., 2008. The brain's default network: anatomy, function, and relevance to disease. Ann. N. Y. Acad. Sci. 1124, 1–38.

▪ Büki, A., Povlishock, J T, 2006. All roads lead to disconnection?--Traumatic axonal injury revisited. Acta Neurochir. (Wien) 148, 181–193; discussion 193–194.

▪ Ciccarelli, O., Catani, M., Johansen-Berg, H., Clark, C., Thompson, A., 2008. Diffusion-based tractography in neurological disorders: concepts, applications, and future developments. Lancet Neurol. 7, 715–727.

▪ D'Esposito, M., Postle, B.R., Ballard, D., Lease, J., 1999. Maintenance versus manipulation of information held in working memory: an event-related fMRI study. Brain Cogn. 41, 66–86.

▪ Dikmen, S.S., Corrigan, J.D., Levin, Harvey S, Machamer, J., Stiers, W., Weisskopf, M.G., 2009. Cognitive outcome following traumatic brain injury. J. Head Trauma Rehabil. 24, 430–438.

▪ Ding, K., Marquez de la Plata, C., Wang, Jun Yi, Mumphrey, M., Moore, Carol, Harper, C., Madden, Christopher J, McColl, Roderick, Whittemore, A., Devous, Michael D, Diaz-Arrastia, Ramon, 2008. Cerebral atrophy after traumatic white matter injury: correlation with acute neuroimaging and outcome. J. Neurotrauma 25, 1433–1440.

▪ Dobkin, B.H., 2004. Strategies for stroke rehabilitation. Lancet Neurol. 3, 528–536.

▪ Du Boisgueheneuc, F., Levy, R., Volle, E., Seassau, M., Duffau, H., Kinkingnehun, S., Samson, Y., Zhang, S., Dubois, B., 2006. Functions of the left superior frontal gyrus in humans: a lesion study. Brain J. Neurol. 129, 3315–3328.

▪ Eichenbaum, H., 2000. A cortical-hippocampal system for declarative memory. Nat. Rev. Neurosci. 1, 41–50.

▪ Farbota, K.D., Bendlin, B.B., Alexander, A.L., Rowley, H.A., Dempsey, R.J., Johnson, S.C., 2012. Longitudinal diffusion tensor imaging and neuropsychological correlates in traumatic brain injury patients. Front. Hum. Neurosci. 6, 160.

▪ Farbota, K.D.M., Sodhi, A., Bendlin, B.B., McLaren, D.G., Xu, G., Rowley, H.A., Johnson, S.C., 2012. Longitudinal volumetric changes following traumatic brain injury: a tensor-based morphometry study. J. Int. Neuropsychol. Soc. Jins 18, 1006–1018.

▪ Fischl, B., Dale, A.M., 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc. Natl. Acad. Sci. U. S. A. 97, 11050–11055.

▪ Gennarelli, Graham, 1998. Neuropathology of the Head Injuries. Semin. Clin. Neuropsychiatry 3, 160–175.

▪ Gennarelli, T.A., 1993. Mechanisms of brain injury. J. Emerg. Med. 11 Suppl 1, 5–11.

▪ Gennarelli, T.A., Thibault, L.E., Adams, J.H., Graham, D I, Thompson, C.J., Marcincin, R.P., 1982. Diffuse axonal injury and traumatic coma in the primate. Ann. Neurol. 12, 564–574.

▪ Gong, G., He, Y., Concha, L., Lebel, C., Gross, D.W., Evans, A.C., Beaulieu, C., 2009. Mapping Anatomical Connectivity Patterns of Human Cerebral Cortex Using In Vivo Diffusion Tensor Imaging Tractography. Cereb. Cortex New York Ny 19, 524–536.

▪ Graham, D I, Adams, J.H., Nicoll, J.A., Maxwell, W L, Gennarelli, T.A., 1995. The nature, distribution and causes of traumatic brain injury. Brain Pathol. Zurich Switz. 5, 397–406.

▪ Granacher, J.R., 2008. Traumatic brain injury: Methods for clinical and forensic neuropsychiatric assessment. 2nd edition. CRC Press.

▪ Greenfield, J.G., Love, S., Louis, D.N., Ellison, D.W., 2008. Greenfield's Neuropathology. CRC Press.

▪ Ham, Timothy E, Sharp, David J, 2012. How can investigation of network function inform rehabilitation after traumatic brain injury? Curr. Opin. Neurol. 25, 662–669.

▪ Hillary, F G, Slocomb, J, Hills, E.C., Fitzpatrick, N M, Medaglia, J D, Wang, J., Good, D C, Wylie, G.R., 2011. Changes in resting connectivity during recovery from severe traumatic brain injury. Int. J. Psychophysiol. Off. J. Int. Organ. Psychophysiol. 82, 115–123.

▪ Hillary, Frank G, Medaglia, John D, Gates, K., Molenaar, P.C., Slocomb, Julia, Peechatka, A., Good, David C, 2011. Examining working memory task acquisition in a disrupted neural network. Brain J. Neurol. 134, 1555–1570.

▪ Honey, C.J., Sporns, O., Cammoun, L., Gigandet, X., Thiran, J.P., Meuli, R., Hagmann, P., 2009. Predicting human resting-state functional connectivity from structural connectivity. Proc. Natl. Acad. Sci. U. S. A. 106, 2035–2040.

▪ Hutton, C., Draganski, B., Ashburner, J., Weiskopf, N., 2009. A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. Neuroimage 48, 371–380.

▪ Johansen-Berg, H., 2010. Behavioural relevance of variation in white matter microstructure. Curr. Opin. Neurol. 23, 351–358.

▪ Johansen-Berg, H., Rushworth, M.F.S., 2009. Using diffusion imaging to study human connectional anatomy. Annu. Rev. Neurosci. 32, 75–94.

▪ Jonides, J., Schumacher, E.H., Smith, E.E., Koeppe, R.A., Awh, E., Reuter-Lorenz, P.A., Marshuetz, C., Willis, C.R., 1998. The role of parietal cortex in verbal working memory. J. Neurosci. Off. J. Soc. Neurosci. 18, 5026–5034.

▪ Jr, R.P.G., 2007. Traumatic Brain Injury: Methods for Clinical and Forensic Neuropsychiatric Assessment, Second Edition. CRC Press.

▪ Kasahara, M., Menon, David K, Salmond, C.H., Outtrim, J.G., Tavares, J.V.T., Carpenter, T Adrian, Pickard, John D, Sahakian, B.J., Stamatakis, E.A., 2011. Traumatic brain injury alters the functional brain network mediating working memory. Brain Inj. Bi 25, 1170–1187.

▪ Katz, D I, Alexander, M P, 1994. Traumatic brain injury. Predicting course of recovery and outcome for patients admitted to rehabilitation. Arch. Neurol. 51, 661–670.

▪ Kinnunen, Kirsi Maria, Greenwood, Richard, Powell, Jane Hilary, Leech, R., Hawkins, P.C., Bonnelle, V., Patel, Maneesh Chandrakant, Counsell, Serena Jane, Sharp, David James, 2011. White matter damage and cognitive impairment after traumatic brain injury. Brain J. Neurol. 134, 449–463.

▪ Klingberg, T, O'Sullivan, B.T., Roland, P.E., 1997. Bilateral activation of fronto-parietal networks by incrementing demand in a working memory task. Cereb. Cortex New York N 1991 7, 465–471.

▪ Kraus, M.F., Susmaras, T., Caughlin, B.P., Walker, C.J., Sweeney, J.A., Little, D.M., 2007. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain J. Neurol. 130, 2508–2519.

▪ Kumar, R., Husain, M., Gupta, R.K., Hasan, K.M., Haris, M., Agarwal, A.K., Pandey, C.M., Narayana, P.A., 2009a. Serial changes in the white matter diffusion tensor imaging metrics in moderate traumatic brain injury and correlation with neuro-cognitive function. J. Neurotrauma 26, 481–495.

▪ Kumar, R., Husain, M., Gupta, R.K., Hasan, K.M., Haris, M., Agarwal, A.K., Pandey, C.M., Narayana, P.A., 2009b. Serial changes in the white matter diffusion tensor imaging metrics in moderate traumatic brain injury and correlation with neuro-cognitive function. J. Neurotrauma 26, 481–495.

▪ Langlois, J.A., Sattin, R.W., 2005. Traumatic brain injury in the United States: research and programs of the Centers for Disease Control and Prevention (CDC). J. Head Trauma Rehabil. 20, 187–188.

▪ Le Bihan, D., 2003. Looking into the functional architecture of the brain with diffusion MRI. Nat. Rev. Neurosci. 4, 469–480.

▪ Levin, H S, 1995. Prediction of recovery from traumatic brain injury. J. Neurotrauma 12, 913–922.

▪ Levin, H S, Goldstein, F.C., High, W.M., Jr, Eisenberg, H.M., 1988. Disproportionately severe memory deficit in relation to normal intellectual functioning after closed head injury. J. Neurol. Neurosurg. Psychiatry 51, 1294–1301.

▪ Lezak, M.D., 2004. Neuropsychological Assessment 4e. Oxford University Press.

▪ Maas, A.I.R., Stocchetti, N., Bullock, R., 2008. Moderate and severe traumatic brain injury in adults. Lancet Neurol. 7, 728–741.

▪ Marquez de la Plata, Carlos D, Garces, J., Shokri Kojori, E., Grinnan, J., Krishnan, K., Pidikiti, R., Spence, J., Devous, Michael D, Sr, Moore, Carol, McColl, Rodderick, Madden, C., Diaz-Arrastia, Ramon, 2011a. Deficits in functional connectivity of

hippocampal and frontal lobe circuits after traumatic axonal injury. Arch. Neurol. 68, 74–84.

▪ Marquez de la Plata, Carlos D, Yang, F.G., Wang, Jun Yi, Krishnan, K., Bakhadirov, Khamid, Paliotta, C., Aslan, S., Devous, Michael D, Moore, Carol, Harper, C., McColl, Roderick, Munro Cullum, C., Diaz-Arrastia, Ramon, 2011b. Diffusion tensor imaging biomarkers for traumatic axonal injury: analysis of three analytic methods. J. Int. Neuropsychol. Soc. Jins 17, 24–35.

▪ Maxwell, W L, Povlishock, J T, Graham, D.L., 1997. A mechanistic analysis of nondisruptive axonal injury: a review. J. Neurotrauma 14, 419–440.

▪ Maxwell, William L, MacKinnon, M.-A., Stewart, J.E., Graham, David I, 2010. Stereology of cerebral cortex after traumatic brain injury matched to the Glasgow outcome score. Brain J. Neurol. 133, 139–160.

▪ Mayer, A.R., Mannell, M.V., Ling, J., Gasparovic, C., Yeo, R.A., 2011. Functional connectivity in mild traumatic brain injury. Hum. Brain Mapp. 32, 1825–1835.

▪ McAllister, T.W., 2008. Neurobehavioral sequelae of traumatic brain injury: evaluation and management. World Psychiatry Off. J. World Psychiatr. Assoc. Wpa 7, 3–10.

▪ McCauley, S.R., Wilde, E.A., Merkley, T.L., Schnelle, K.P., Bigler, Erin D, Hunter, J.V., Chu, Z., Vásquez, A.C., Levin, Harvey S, 2010. Patterns of cortical thinning in relation to event-based prospective memory performance three months after moderate to severe traumatic brain injury in children. Dev. Neuropsychol. 35, 318–332.

▪ Medaglia, John D, Chiou, K.S., Slocomb, Julia, Fitzpatrick, Neal M, Wardecker, B.M., Ramanathan, D., Vesek, J., Good, David C, Hillary, Frank G, 2012. The less BOLD, the wiser: support for the latent resource hypothesis after traumatic brain injury. Hum. Brain Mapp. 33, 979–993.

▪ Menon, David K, Schwab, K., Wright, D.W., Maas, A.I., 2010. Position statement: definition of traumatic brain injury. Arch. Phys. Med. Rehabil. 91, 1637–1640.

▪ Merkley, T.L., Bigler, Erin D, Wilde, E.A., McCauley, S.R., Hunter, J.V., Levin, Harvey S, 2008. Diffuse changes in cortical thickness in pediatric moderate-to-severe traumatic brain injury. J. Neurotrauma 25, 1343–1345.

▪ Mesulam, M.M., 1998. From sensation to cognition. Brain J. Neurol. 121 (Pt 6), 1013–1052.

▪ Meythaler, J.M., Peduzzi, J.D., Eleftheriou, E., Novack, T.A., 2001. Current concepts: diffuse axonal injury-associated traumatic brain injury. Arch. Phys. Med. Rehabil. 82, 1461–1471.

▪ Minear M, Park DC. A lifespan database of adult facial stimuli. Behavior Research Methods, Instruments, & Computers 2004; 36: 630-633.

▪ Mohr, H.M., Goebel, R., Linden, D.E.J., 2006. Content- and task-specific dissociations of frontal activity during maintenance and manipulation in visual working memory. J. Neurosci. Off. J. Soc. Neurosci. 26, 4465–4471.

▪ Mori, S., Zhang, J., 2006. Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 51, 527–539.

▪ Nakamura, T., Hillary, Frank G, Biswal, B.B., 2009. Resting network plasticity following brain injury. Plos One 4, e8220.

▪ Nakayama, N., Okumura, A., Shinoda, J., Yasokawa, Y.-T., Miwa, K., Yoshimura, S.-I., Iwama, T., 2006. Evidence for white matter disruption in traumatic brain injury without macroscopic lesions. J. Neurol. Neurosurg. Psychiatry 77, 850–855.

▪ Newcombe, V F J, Williams, G B, Nortje, J., Bradley, P.G., Harding, S.G., Smielewski, P., Coles, J P, Maiya, B., Gillard, J.H., Hutchinson, P J, Pickard, J D, Carpenter, T A, Menon, D K, 2007. Analysis of acute traumatic axonal injury using diffusion tensor imaging. Br. J. Neurosurg. 21, 340–348.

▪ Newcombe, V., Chatfield, D., Outtrim, J., Vowler, S., Manktelow, A., Cross, J., Scoffings, D., Coleman, M., Hutchinson, P., Coles, J., Carpenter, T Adrian, Pickard, J., Williams, G., Menon, D., 2011. Mapping traumatic axonal injury using diffusion tensor imaging: correlations with functional outcome. Plos One 6, e19214.

▪ Newcombe, Virginia F J, Outtrim, J.G., Chatfield, D.A., Manktelow, A., Hutchinson, Peter J, Coles, Jonathan P, Williams, Guy B, Sahakian, B.J., Menon, David K, 2011. Parcellating the neuroanatomical basis of impaired decision-making in traumatic brain injury. Brain J. Neurol. 134, 759–768.

▪ Newsome, M.R., Scheibel, R.S., Steinberg, J.L., Troyanskaya, M., Sharma, R.G., Rauch, R.A., Li, X., Levin, Harvey S, 2007. Working memory brain activation following severe traumatic brain injury. Cortex J. Devoted Study Nerv. Syst. Behav. 43, 95–111.

▪ Olesen, P.J., Westerberg, H., Klingberg, Torkel, 2004. Increased prefrontal and parietal activity after training of working memory. Nat. Neurosci. 7, 75–79.

▪ Ommaya, A.K., Grubb, R.L., Jr, Naumann, R.A., 1971. Coup and contre-coup injury: observations on the mechanics of visible brain injuries in the rhesus monkey. J. Neurosurg. 35, 503–516.

▪ Owen, A.M., McMillan, K.M., Laird, A.R., Bullmore, E., 2005. N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. Hum. Brain Mapp. 25, 46–59.

▪ Palacios, E.M., Fernandez-Espejo, D., Junque, C., Sanchez-Carrion, R., Roig, T., Tormos, J.M., Bargallo, N., Vendrell, P., 2011. Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury. Bmc Neurol. 11, 24.

▪ Park, E., Bell, J.D., Baker, A.J., 2008. Traumatic brain injury: can the consequences be stopped? Cmaj Can. Med. Assoc. J. J. Assoc. Medicale Can. 178, 1163–1170.

▪ Povlishock, J T, Christman, C.W., 1995. The pathobiology of traumatically induced axonal injury in animals and humans: a review of current thoughts. J. Neurotrauma 12, 555–564.

▪ Povlishock, John T, Katz, Douglas I, 2005. Update of neuropathology and neurological recovery after traumatic brain injury. J. Head Trauma Rehabil. 20, 76–94.

▪ Raz, A., Buhle, J., 2006. Typologies of attentional networks. Nat. Rev. Neurosci. 7, 367–379.

▪ Roozenbeek, B., Maas, A.I.R., Menon, David K, 2013. Changing patterns in the epidemiology of traumatic brain injury. Nat. Rev. Neurol. 9, 231–236.

▪ Rosenfeld, J.V., Maas, A.I., Bragge, P., Morganti-Kossmann, M.C., Manley, G.T., Gruen, R.L., 2012. Early management of severe traumatic brain injury. Lancet 380, 1088–1098.

▪ Rugg-Gunn, F.J., Symms, M.R., Barker, G.J., Greenwood, R, Duncan, J.S., 2001. Diffusion imaging shows abnormalities after blunt head trauma when conventional magnetic resonance imaging is normal. J. Neurol. Neurosurg. Psychiatry 70, 530–533.

▪ Sánchez-Carrión, R., Gómez, P.V., Junqué, C., Fernández-Espejo, D., Falcon, C., Bargalló, N., Roig-Rovira, T., Enseñat-Cantallops, A., Bernabeu, M., 2008. Frontal hypoactivation on functional magnetic resonance imaging in working memory after severe diffuse traumatic brain injury. J. Neurotrauma 25, 479–494.

▪ Scheid, R., Walther, K., Guthke, T., Preul, C., von Cramon, D.Y., 2006. Cognitive sequelae of diffuse axonal injury. Arch. Neurol. 63, 418–424.

▪ Sharp, David J, Beckmann, Christian F, Greenwood, Richard, Kinnunen, Kirsi M, Bonnelle, V., De Boissezon, X., Powell, Jane H, Counsell, Serena J, Patel, Maneesh C,

Leech, R., 2011. Default mode network functional and structural connectivity after traumatic brain injury. Brain J. Neurol. 134, 2233–2247.

▪ Shaw, P., Kabani, N.J., Lerch, J.P., Eckstrand, K., Lenroot, R., Gogtay, N., Greenstein, D., Clasen, L., Evans, A., Rapoport, J.L., Giedd, J.N., Wise, S.P., 2008. Neurodevelopmental trajectories of the human cerebral cortex. J. Neurosci. Off. J. Soc. Neurosci. 28, 3586–3594.

▪ Sidaros, A., Engberg, A.W., Sidaros, K., Liptrot, M.G., Herning, M., Petersen, P., Paulson, O.B., Jernigan, T.L., Rostrup, E., 2008. Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. Brain J. Neurol. 131, 559–572.

▪ Sidaros, A., Skimminge, A., Liptrot, M.G., Sidaros, K., Engberg, A.W., Herning, M., Paulson, O.B., Jernigan, T.L., Rostrup, E., 2009. Long-term global and regional brain volume changes following severe traumatic brain injury: a longitudinal study with clinical correlates. Neuroimage 44, 1–8.

▪ Simons, J.S., Spiers, H.J., 2003. Prefrontal and medial temporal lobe interactions in long-term memory. Nat. Rev. Neurosci. 4, 637–648.

▪ Smith, D.H., Meaney, D.F., 2000. Axonal Damage in Traumatic Brain Injury. Neuroscientist 6, 483–495.

▪ Smith, S.M., Miller, K.L., Moeller, S., Xu, Junqian, Auerbach, E.J., Woolrich, M.W., Beckmann, Christian F, Jenkinson, M., Andersson, J., Glasser, M.F., Van Essen, D.C., Feinberg, D.A., Yacoub, E.S., Ugurbil, K., 2012. Temporally-independent functional modes of spontaneous brain activity. Proc. Natl. Acad. Sci. U. S. A. 109, 3131–3136.

▪ Squire, L.R., Stark, C.E.L., Clark, R.E., 2004. The medial temporal lobe. Annu. Rev. Neurosci. 27, 279–306.

▪ Stuss, D T, Stethem, L.L., Hugenholtz, H., Picton, T., Pivik, J., Richard, M.T., 1989. Reaction time after head injury: fatigue, divided and focused attention, and consistency of performance. J. Neurol. Neurosurg. Psychiatry 52, 742–748.

▪ Stuss, Donald T, Murphy, K.J., Binns, M.A., Alexander, Michael P, 2003. Staying on the job: the frontal lobes control individual performance variability. Brain J. Neurol. 126, 2363–2380.

▪ Tate, D.F., Bigler, E D, 2000. Fornix and hippocampal atrophy in traumatic brain injury. Learn. Mem. Cold Spring Harb. N 7, 442–446.

▪ Teasdale, G., Jennett, B., 1974. Assessment of coma and impaired consciousness. A practical scale. Lancet 2, 81–84.

▪ Traumatic brain injury: time to end the silence, 2010. . Lancet Neurol. 9, 331.

▪ Turner, G.R., McIntosh, A.R., Levine, B., 2011. Prefrontal Compensatory Engagement in TBI is due to Altered Functional Engagement Of Existing Networks and not Functional Reorganization. Front. Syst. Neurosci. 5, 9.

▪ Umarova, R.M., Saur, D., Schnell, S., Kaller, C.P., Vry, M.-S., Glauche, V., Rijntjes, M., Hennig, J., Kiselev, V., Weiller, C., 2010. Structural connectivity for visuospatial attention: significance of ventral pathways. Cereb. Cortex New York N 1991 20, 121–129.

▪ Van den Heuvel, M.P., Hulshoff Pol, H.E., 2010. Exploring the brain network: a review on resting-state fMRI functional connectivity. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 20, 519–534.

▪ Vanhaudenhuyse, A., Noirhomme, Q., Tshibanda, L.J.-F., Bruno, M.-A., Boveroux, P., Schnakers, C., Soddu, A., Perlbarg, V., Ledoux, D., Brichant, J.-F., Moonen, G., Maquet, P., Greicius, M.D., Laureys, S., Boly, M., 2010. Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. Brain J. Neurol. 133, 161–171.

▪ Velanova, K., Jacoby, L.L., Wheeler, M.E., McAvoy, M.P., Petersen, S.E., Buckner, R.L., 2003. Functional-anatomic correlates of sustained and transient processing components engaged during controlled retrieval. J. Neurosci. Off. J. Soc. Neurosci. 23, 8460–8470.

▪ Wang, J Y, Bakhadirov, K, Abdi, H, Devous, M D, Sr, Marquez de la Plata, C D, Moore, C, Madden, C J, Diaz-Arrastia, R, 2011. Longitudinal changes of structural connectivity in traumatic axonal injury. Neurology 77, 818–826.

▪ Wang, Jun Yi, Bakhadirov, Khamid, Devous, Michael D, Sr, Abdi, Hervé, McColl, Roddy, Moore, Carol, Marquez de la Plata, Carlos D, Ding, K., Whittemore, A., Babcock, E., Rickbeil, T., Dobervich, J., Kroll, D., Dao, B., Mohindra, N., Madden, Christopher J, Diaz-Arrastia, Ramon, 2008. Diffusion tensor tractography of traumatic diffuse axonal injury. Arch. Neurol. 65, 619–626.

▪ Warner, M.A., Marquez de la Plata, C., Spence, J., Wang, Jun Yi, Harper, C., Moore, Carol, Devous, M., Diaz-Arrastia, Ramon, 2010a. Assessing spatial relationships between axonal integrity, regional brain volumes, and neuropsychological outcomes after traumatic axonal injury. J. Neurotrauma 27, 2121–2130.

▪ Warner, M.A., Youn, T.S., Davis, T., Chandra, A., Marquez de la Plata, C., Moore, Carol, Harper, C., Madden, Christopher J, Spence, J., McColl, Roderick, Devous, M.,

King, R.D., Diaz-Arrastia, Ramon, 2010b. Regionally selective atrophy after traumatic axonal injury. Arch. Neurol. 67, 1336–1344.

▪ Whitnall, L., McMillan, T.M., Murray, G.D., Teasdale, G.M., 2006. Disability in young people and adults after head injury: 5-7 year follow up of a prospective cohort study. J. Neurol. Neurosurg. Psychiatry 77, 640–645.

▪ Xu, Jian, Rasmussen, I.-A., Lagopoulos, J., Håberg, A., 2007. Diffuse axonal injury in severe traumatic brain injury visualized using high-resolution diffusion tensor imaging. J. Neurotrauma 24, 753–765.

▪ Zang, Y.-F., He, Y., Zhu, C.-Z., Cao, Q.-J., Sui, M.-Q., Liang, M., Tian, L.-X., Jiang, T.-Z., Wang, Y.-F., 2007. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev. 29, 83–91.