Final Progress Report for an Individual Research Grant

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Name of Organization/Institution: Kessler Foundation, Inc.

Grant Title: Advanced Brain Imaging in Traumatic Brain Injury

Grant Number: 09-3214-BIR-N-0

Grant Period Covered by Report: 06/01/10 – 05/31/13

Date of Report Submission: 07/31/2013

Original Aims of project

In the clinical neurosciences, an understanding of the relationship between axonal integrity and information-processing efficiency has been sought in a range of studies including those examining normal aging [1], brain injury [2], and brain disease processes such as human immunodeficiency virus (HIV) [3] and multiple sclerosis (MS) [4]. Pathophysiological processes that disrupt axonal transmission have been theoretically linked to decrements in processing speed and efficiency, which are at the foundation of a number of cognitive functions, including learning, memory, and executive functions [5, 6]. Even given the implicit relationship between axonal injury and information-processing efficiency, the influence of local axonal injury on changes in brain function has rarely been directly examined. Advances in MRI techniques such as diffusion tensor imaging (DTI) now permit noninvasive examination of axonal integrity. Prior work using DTI to examine Traumatic Brain Injury (TBI) has demonstrated the relationship between fractional anisotropy (FA) and white matter degradation in mild and moderate and severe injuries [7-10], thus establishing the feasibility of using DTI to document axonal disruption TBI. This work has largely focused on brain sites where TBI has commonly occurred as opposed to examining specific white matter lesion sites [11]. In parallel with studies using diffusion imaging to examine TBI, investigators have documented changes in functional neural networks following TBI using simple fMRI paradigms that require working memory (WM) and speeded information processing [12-16]. While these studies have consistently documented recruitment of additional neural networks during speeded WM tasks, they also have been limited by between-subjects designs and have often not examined underlying pathophysiology which has lead to difficulty in interpreting the origin of these activation differences [17]. It is a second goal in this study to integrate information about local axonal disruption (measured via DTI) with functional brain changes (measured via fMRI) following TBI. The current proposal aims to foster collaborative, interdisciplinary efforts from investigators at three separate research institutions in order to facilitate the understanding of the TBI recovery process in humans via non-invasive MRI techniques. By examining sites of local axonal disruption as well as sites where white matter injury commonly occurs (e.g., splenium), the current proposal aims to examine the evolution axonal disruption and its influence on functional brain activation. Specifically, the goal is to compare changes in FA values during recovery with functional activation observed cognitive tasks requiring speeded information processing in order to obtain information about the inter-relationships between axonal integrity, functional brain activation and basic processing speed and efficiency. By directly examining these inter-relationships, the methods applied here may be used to better understand the nature of the recovery process following axonal and neural disruption in TBI, with the ultimate goal of standardizing these methods so that the efficacy of novel treatment interventions may be examined. This study thus represents an important opportunity to use non-invasive methods to model the recovery trajectory of axonal and neural networks simultaneously in order to determine their collective influence on cognitive outcome in humans.

Specific Aim 1: To use DTI to examine axonal changes during recovery from TBI both at focal lesion sites and at sites where axonal injury commonly occurs (e.g., splenium, internal capsule). Subjects will be examined at 3 and 6 months post PTA to determine the trajectory of white matter change measured by DTI.

Specific Aim 2: To examine changes in functional brain activation during recovery from TBI and the relationship between these functional activation changes and changes in axonal integrity (quantified in Specific Aim 1). It is anticipated that activation in PFC and ACC will diminish from the 3-month to 6-month time points and maintain a negative correlation with measures of axonal recovery (FA). This aim will also permit exploratory analyses using diffusion tensor tractography to examine the direct influence of local lesions on neural activity.

Specific Aim 3: To determine if early indicators of axonal and neural recovery (established in Aims 1 and 2, respectively) are predictive of cognitive functioning at 6 months post PTA. Several MRI variables including change in FA at local lesion sites, total FA in large white matter tracts (e.g., anterior corpus callosum), and change in PFC and ACC activation will be used to predict cognitive outcome. These indicators of axonal and neural recovery will be compared with traditional clinical descriptors of TBI severity (e.g., GCS, posttraumatic amnesia) to predict cognitive outcome.

Project successes

We have had great success with this project, despite having to overcome some challenges. Over the course of the three years of the grant (and one year of no-cost extension), we have tested 31 TBIs and 31 HCs. These numbers do not fully represent our productivity on the grant, since each participant came in multiple times. Thus, we conducted 75 total scans on TBIs and 55 total scans on HCs. We were able to achieve this

despite very demanding inclusion criteria and a need to follow each subject longitudinally.

The three Aims of this study were to better understand connectivity following a TBI, and to understand how that connectivity changes across recovery. When we conceptualized the grant, we envisioned that structural connectivity (DTI) would provide the clearest window into these issues, and we therefore emphasized DTI in the Specific Aims. However, in the intervening years, interest in functional connectivity has blossomed, and with good reason. Functional connectivity provides a more direct measure of cognition inasmuch as it is based on functional activation rather than on structural information. This is not to minimize what can be learned from the analysis of structural connectivity data—much can be learned, and we are currently using our DTI data to better understand the trajectory of recovery after a TBI (Aim 1)—however, functional connectivity data is both more direct and has also proved to be more sensitive to TBI than the structural connectivity data. These considerations have been fueled by recent advances in the tools available for the analysis of functional connectivity data (e.g., graph theoretical analyses and structural equation modeling of fMRI data), which we have applied to the data from this grant to motivate several grant applications (see below).

Because of the sensitivity of the functional connectivity data to TBI-related changes in brain function, we were able to use a subset of the entire dataset for a paper, which was published in 2012 (see below), and which supports Aim 2. In this paper, we reported a finding that—while counterintuitive—has proved to be highly replicable: individuals with TBI show increased functional connectivity relative to HCs. In our ongoing analyses of the full dataset, we are working to better understand this finding.

Although we initially focused on functional connectivity, we are currently working on the DTI data that was the original focus of the grant. We have analyzed these data not only in terms of fractional anisotropy (a measure of the integrity of the brain's white matter) and mean diffusivity (a different, but related, measure of white matter integrity), but also in terms of 'fractal dimension'. Fractal dimension is a technique that allows for the quantification of the overall shape of the brain (specifically, the brain's white matter). The shape of the white matter in a healthy brain is quite complex; the shape of the white matter in an atrophied or injured brain is less complex due to axonal loss. In a paper we are currently preparing for publication, we have shown that fractal dimension predicts cognition in TBI better than other measures of white matter integrity such as fractional anisotropy or mean diffusivity (this work addresses Aims 1 and 3).

In addition to the paper we have already published, we have presented data from this project at numerous International meetings/conferences (see below). Furthermore, now that the data has all been collected, we are working on several more manuscripts, which will be sent out for review and publication in the coming months.

Presentations:

Wylie, G.R., Hillary, F.G., Leavitt, V.M. & Chiaravalloti, N. Connectivity changes in Traumatic Brain Injury across recovery. The 39th Annual Meeting International Neuropsychological Society, February, 2011, Boston, MA.

Wylie, G.R. & Hillary, F.G. Change is good: brain activity in a working memory task is higher in TBI than in HC, but shows comparable changes across time. The 38th Annual Meeting International Neuropsychological Society, February, 2010, Acapulco, Mexico.

Chiou, K.S., Slocomb, J., Ramanathan, D., Medaglia, J.D., Wardecker B., Vesek, J., Wang, J., Hills, E., Good, D., Hillary, F.G. (2009, October). Longitudinal Investigation of White Matter Focal Lesions in Moderate to Severe TBI Using DTI. Poster presented at the annual meeting of the National Academy of Neuropsychology, New Orleans, LA.

Chiou, K.S., Wardecker, B.M., & Hillary, F.G. Effects of Task Structure on Metacognition in Traumatic Brain Injury. (February, 2010). Poster presented at the 38th annual meeting of the International Neuropsychological Society, Acapulco, Mexico. [Abstract published in the Journal of the International Neuropsychological Society, 2010, 16 (S1), pg 67].

Chiou, K.S., Cosentino, S., Carlson, R.A., Arnett, P.A., Wardecker, B.M., & Hillary, F.G. Relationship between Executive Functioning and Metacognitive Monitoring Following Traumatic Brain Injury. (February, 2010). Poster presented at the 38th annual meeting of the International Neuropsychological Society, Acapulco, Mexico. [Abstract published in the Journal of the International Neuropsychological Society, 2010, 16(S1), pg 118].

Wardecker, B.M., Medaglia, J.D., Ramanathan, D., Chiou, K.S., Slocomb, J., Hills, E., Good, B., & Hillary, F.G. (2010, March). Location of Functional Activation Changes During Recovery from Traumatic Brain Injury. Poster presented at the International Brain Injury Association's Eighth World Congress on Brain Injury, Washington, D.C.

Venkatesan, U.M., Medaglia, J.D., Slocomb, J., Hills, E.C., Fitzpatrick, N.M., Wang, J., Good, D.C., Wylie, G.R., & Hillary, F.G. (2011). Changes in Resting State Functional Connectivity during Recovery from Traumatic Brain Injury. Presented at the Brain 2012 Conference in Barcelona, Spain.

Peechatka, A., Medaglia, J.D., Chiou, K.S., Slocomb, J., Ramanathan, D.M. & Hillary, F.G. (February, 2012). A Longitudinal fMRI Study of Working Memory in TBI During Early Recovery. Poster presented at the 40th annual meeting of the International Neuropsychological Society, Montreal, Canada. [Abstract published in the Journal of the International Neuropsychological Society, 2012, 18 (S1), page 158].

Chiou, K.S., Bryer, E., Slocomb, J., & Hillary, F.G. Longitudinal Examination of Brain Volume Change and Cognitive Functioning in Moderate to Severe Traumatic Brain Injury. Poster presented at the 40th annual meeting of the International Neuropsychological Society, Montreal, Canada. [Abstract published in the Journal of the International Neuropsychological Society, 2012, 18 (S1), pg 222]

Venkatesan, U.M, Medaglia, J.D., Ram, N., Good, D.C., & Hillary, F.G. (2013, August) Dynamics in goal-directed and default mode networks during new learning after moderate or severe TBI. Paper to be presented at the annual meeting of Division 40 of the American Psychological Association. Recipient of the Blue Ribbon Award.

Publications:

Hillary, F.G., Medaglia, J.D., Venkatesan, U. & Wylie, G.R. (in preparation). Whole-brain connectivity changes during recovery from traumatic brain injury.

Medaglia, Wylie, G.R., J.D., Venkatesan, U. & Hillary, F.G. (in preparation). Network flexibility as an indicator of recovery after neurotrauma.

Wylie, G.R., Hillary, F.G., Venkatesan, U. & Medaglia, J.D. (in preparation). Local structural brain changes predict whole-brain connectivity after TBI: a graph theoretical analysis.

Rajagopalan, V, Das, A, Zhang, L, Wylie, GR, Yue, GH. (in preparation). Fractal dimension assessment of brain morphometry: a novel biomarker in Traumatic Brain Injury

Hillary, F.G., Slocomb, J., Hills, E.C., Fitzpatrick, N.M., Medaglia, J.D., Wang, J., Good, D.C. & Wylie, G.R. (2012). Changes in Resting Connectivity during Recovery from Severe Traumatic Brain Injury. *International Journal of Psychophysiology.*

Project challenges

We have encountered several challenges during the course of this study. The most challenging issue was recruitment: it was unremittingly difficult to recruit moderate/severe TBIs three months after they had emerged from post-traumatic amnesia. To overcome this, we set up an entirely new system that bridged the gap between The Kessler Foundation Research Center and the Kessler Institute for Rehabilitation (which are separate and independent institutions) that allowed us to contact patients at KIR but also protected the patients' private health information (PHI), in compliance with HIPPA regulations. Although it took an investment of time to get this system set up, that investment clearly 'paid off', inasmuch as it allowed us to meet our recruitment goals for the grant.

Another challenge had to do with the tasks used in the study. After collecting and analyzing the data from several subjects, we realized that the experimental paradigm we were using was not optimal. We therefore redesigned the paradigm for the remaining subjects. While this change necessarily meant that the data from the initial subjects was not fully compatible with the data from later subjects, it resulted in a stronger study because the bulk of the data was collected using the more robust experimental paradigm.

The last challenge we faced with this study had to do with using the relatively old 3T scanner at the University of Medicine and Dentistry of New Jersey (now Rutgers). Because this scanner is approaching 20 years old,

there were numerous times when it was down for repairs to its components (e.g., the gradient amplifiers) or to its peripherals (e.g., the HVAC system, or the projector for stimulus presentation). These issues delayed scans, but did not impact our ability to get the study done.

Implications for future research and/or clinical treatment

One implication of the research conducted in this grant is that TBI appears to be characterized by 'hyper-connectivity' in the brain (see our 2012 paper). That is, after sustaining a TBI, the brain seems to increase the extent to which disparate brain regions communicate with one another. This is a counterintuitive finding, and one that we are actively working to better understand. Without the data conducted in this grant, we might have continued to believe that the diffuse axonal damage that frequently attends a TBI would result in decreased (rather than increased) connectivity. One consequence of this increased connectivity appears to be a decrease in brain efficiency, which leads to slower processing speed and response time, and may also lead to increased cognitive fatigue.

Another implication from the data collected in this grant involves 'fractal dimension' (FD, see above). Because we have found that fractal dimension predicts cognition in TBI better than other measures of white matter integrity such as fractional anisotropy or mean diffusivity, this work promises to have important implications for future research and clinical treatment, because FD appears to be a sensitive marker for the overall health of the brain, and for the brain's ability to maintain cognition.

Plans to continue the research, including applications submitted to other sources for ongoing support
The data from this study have been used to motivate two R01 applications. The first application, entitled "Examining brain plasticity in neurotrauma using advanced connectivity modeling", was not funded. The second, entitled "Examining Neural Network Plasticity After Traumatic Brain Injury", is currently under review.

We have also recently begun to analyze the data from this grant from the standpoint of 'fractal dimension' (see above). As stated above, we have shown that fractal dimension predicts cognition in TBI better than other measures of white matter integrity such as fractional anisotropy or mean diffusivity. We anticipate submitting an R21 to investigate this issue further in the coming year.

Explain how you have leveraged NJCBIR funding to obtain additional federal or other support for brain injury research and list the appropriate funding organizations.
Our efforts to leverage NJCBIR funding to obtain additional federal support has yet to bear fruit. In the current funding climate, obtaining such funding requires patience and determination. The applications we have submitted have been strong and have received good scores. We are confident that we will soon have greater success.

List and include a copy of all publications emerging from this research, including those used in preparation.

Hillary, F.G., Medaglia, J.D., Venkatesan, U. & Wylie, G.R. (in preparation). Whole-brain connectivity changes during recovery from traumatic brain injury.

Medaglia, Wylie, G.R., J.D., Venkatesan, U. & Hillary, F.G. (in preparation). Network flexibility as an indicator of recovery after neurotrauma.

Wylie, G.R., Hillary, F.G., Venkatesan, U. & Medaglia, J.D. (in preparation). Local structural brain changes predict whole-brain connectivity after TBI: a graph theoretical analysis.

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Financial summary.

International Journal of Psychophysiology xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Psychophysiology

journal homepage: www.elsevier.com/locate/ijpsycho

Changes in resting connectivity during recovery from severe traumatic brain injury

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ARTICLE INFO

Article history:
Received 4 November 2010
Received in revised form 21 March 2011
Accepted 21 March 2011
Available online xxxx

Keywords:
Resting connectivity
Plasticity
Reorganization
Longitudinal

ABSTRACT

In the present study we investigate neural network changes after moderate and severe traumatic brain injury (TBI) through the use of resting state functional connectivity (RSFC) methods. Using blood oxygen level dependent functional MRI, we examined RSFC at 3 and 6 months following resolution of posttraumatic amnesia. The goal of this study was to examine how regional off-task connectivity changes during a critical period of recovery from significant neurological disruption. This was achieved by examining regional changes in the intrinsic, or "resting", BOLD fMRI signal in separate networks: 1) regions linked to goal-directed (or external-state) networks and 2) default mode (or internal-state) networks. Findings here demonstrate significantly increased resting connectivity internal-state networks in the TBI sample during the first 6 months following recovery. These findings were dissociable from repeat measurements in a matched healthy control sample.

1. Introduction

There is growing interest in the use of functional neuroimaging, and in particular blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI), to document brain changes associated with traumatic brain injury (TBI). This developing literature has focused primarily on the task-induced changes that differentiate clinical and healthy samples during cognitive, motor and sensory tasks. In one specific literature examining working memory (WM; or the ability to maintain a small amount of information "in mind" for online use) deficits after TBI, several consistent findings have emerged. Investigators have almost universally observed increased involvement of the regions critical for WM, including prefrontal cortex (PFC) and anterior cingulate cortex (ACC) and occasionally parietal regions in TBI (Christodoulou et al., 2001; Hillary et al., 2010; McAllister et al., 1999, 2001; Newsome et al., 2007; Perlstein et al., 2004; Scheibel et al., 2007). There is also rich literature examining the role of frontal systems disruption and the critical contribution of cognitive control to deficit after TBI (McDowell et al., 1997; Hillary et al., 2010; Perlstein et al., 2004; Larson et al., 2006, 2007; Scheibel et al., 2007, 2009). These studies offer heuristics as for how large-scale neuronal activity might adapt to TBI, including the potentially critical role of anterior networks and those involved in cognitive (or attentional) control.

While early studies of TBI have helped clarify some of the basic "activation" changes associated with injury and afford the opportunity to link specific cognitive deficits to brain activation changes, there are a number of important future directions for this literature. First, a majority of the studies to date have been cross-sectional observations which pose significant methodological challenges for investigators (e.g., differential task performance between groups) (Price et al., 2006; Price and Friston, 2002) and often do not permit the examination of critical within-subject dynamics. As a remedy to this, the current study makes use of a longitudinal design during a critical window of recovery (i.e., between 3 and 6 months post injury) in order to examine within-subject brain changes.

Second, most studies to date using functional imaging methods to examine the consequences associated with TBI have focused on task-related brain activation. The study of task "activation" offers the opportunity to examine task-specific alterations in neural networks after TBI, but such approaches limit the scope of study (focusing on task induced regions of interest (ROIs), instead of whole brain function) and are burdened by design challenges that are often difficult to resolve. For example, examining task-related activation requires the creation of appropriate control tasks and the need to guarantee equivalent task performance between groups [e.g., task

Abbreviations: TBI, traumatic brain injury; fMRI, functional magnetic resonance imaging; WM, working memory.

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0167-8760/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.ijpsycho.2011.03.011

accuracy in TBI vs. healthy control (HC)]. Moreover, the work to date has focused almost solely on the magnitude of the signal (i.e., topographical activation differences) as opposed to communication within the network or covariance in (and between) ROIs.

In pioneering work conducted over 15 years ago, Biswal et al. demonstrated that covariance in voxels comprising the primary motor cortex during rest showed spatial overlap with the observable change in the BOLD response during motor stimulation. These influential findings were the foundation to an intriguing method for investigating brain function through the use of "resting" BOLD data to understand underlying neural connectivity, or "resting state functional connectivity" (RSFC) throughout the brain (Biswal et al., 1995). In fMRI work, RSFC methods often focus on isolating the covariance in very low frequency (~0.1 Hz) fluctuations in the BOLD signal, thus permitting analysis of non-task related brain activity which likely plays a non-trivial role in both on-task and off-task functioning.

This early work was extended by Lowe et al. (1997) by demonstrating similar effects in larger regions of sensorimotor cortex (i.e., across multiple slices) and other examiners used these methods to examine relationships between motor and association cortex (Xiong et al., 1998, 1999). Of critical importance in this literature is the demonstration that task-induced activation maps underestimate the size and number of functionally connected regions and that functional networks are more fully revealed by RSC analysis (Biswal et al., 1995; Xiong et al., 1998, 1999). These studies established the foundation for "resting-state functional connectivity studies" using fMRI (Biswal et al., 1995; Greicius and Menon, 2004; Gusnard and Raichle, 2001; Hampson et al., 2002; Lowe et al., 1997) and a literature examining task negative or "default mode" networks (Fox et al., 2005; Raichle et al., 2001; Raichle and Snyder, 2007). In the case of the latter, examiners identified distinct "off-task" networks operating in concert as one transitions in and out of goal-directed behavior.

One general interpretation differentiating task-on and task-off networks is that they are reciprocal so that at moments where goal-directed behavior is necessary, the "inward" or self-reflective default mode network remits, giving way to neural activity relevant to task. However, interpreting this relationship as an opponent process may oversimplify this relationship; separate investigations have demonstrated that the default mode activity plays a role in task and the magnitude of deactivation in default mode regions contribute to task performance (Cole et al., 2010; Hampson et al., 2010). These findings offer guiding principles for understanding the role of resting states in healthy neural systems, but questions remain regarding how significant neural network disruption, such as that observed in TBI, might influence the interplay between task-related positive and negative brain activation.

The examination of both RSFC and default-mode networks in clinical samples remains novel, but there are already several findings that provide a framework for understanding how neurological disruption influences the resting signal and for developing expectations in TBI. To date, resting connectivity has been used to examine network changes in a number of clinical disorders including schizophrenia (Camchong et al., 2009; Rotarska-Jagiela et al., 2010; B. Zhou et al., 2010), normal aging (Koch et al., 2010), stroke (Carter et al., 2010), mood disorders (Chepenik et al., 2010; Hamilton et al., 2010; Sheline et al., 2010), multiple sclerosis (Rocca et al., 2010) and dementias (J. Zhou et al., 2010). There have also been whole brain analyses using resting data to examine alterations in cerebral blood flow (Kim et al., 2010) and we recently applied graph theory to examine "small-worldness" in networks after TBI (Nakamura et al., 2009). In one of the more intriguing applications of resting connectivity to date, Vanhaudenhuyse et al. (2010) used baseline BOLD measures to differentiate cognitively intact and comatose non-communicative brain injured patients. Not surprisingly, the outcome of these studies has varied and this is likely due as much to methodological differences as the effects of distinct pathophysiology in the clinical samples represented. Even so, two important findings emerge from this literature that may be relevant for TBI in the current study. The first is that neurological compromise has been demonstrated to influence resting connectivity (broadly defined). Second, one consequence for global brain connectivity is that connections between critical nodes may be greatly diminished or even unobservable after neurological disruption (see Ongur et al., 2010; Skudlarski et al., 2010; Vanhaudenhuyse et al., 2010).

We anticipate that network disruption results in less coherence in resting connectivity during periods of goal-directed behavior and, therefore, we should observe increased connectivity in internal-state networks during recovery. That is, significant neurological disruption of frontal systems (often observed in TBI), may result in a failure to effectively transition between self-reflective processing and outward goal-directed behavior.

1.1. Study purpose

The goal of this study is to examine resting state connectivity to determine if there are systematic changes in whole-brain connectivity during recovery from TBI. We aim to examine the changes in resting fMRI connectivity during the first 6 months following injury when behavioral recovery is known to occur (Millis et al., 2001; Pagulayan et al., 2006). The use of resting fMRI circumvents the methodological dilemmas that arise when using fMRI in clinical samples including difficulty guaranteeing task compliance and assumptions surrounding cognitive subtraction and pure insertion, where contributing components to a task are presumed to be linear and/or additive (Hillary, 2008; Price et al., 2006; Price and Friston, 2002). Moreover, the influences of diffuse neurological disruption (like that observed in TBI) on whole-brain neural networks remain largely unknown. Traditional fMRI studies in TBI have excluded much of the operating brain in order to isolate specific task-related networks. While this approach offers advantages for examining discrete cognitive deficits, it offers very little information about global brain changes secondary to injury. Consequently, there is very little work documenting how large-scale neural networks adapt to neural disruption and resting connectivity offers one approach to address this issue. For these reasons, resting connectivity is ideal for identifying alterations in the BOLD signal in the recovering brain. This approach may provide additional insight into how neural plasticity is expressed in the injured brain and offer context for findings in cross-sectional activation studies to date, including determining the meaning of increased neural involvement repeatedly observed in activation studies (for review see Hillary, 2008).

Of note, RSFC and the DMN and even the notion of the brain at "rest" have contextual meanings. For the purpose of this study, we will focus on the intrinsic, or resting, BOLD signal during off-task blocks that flank a visual working memory task. In this sense, we are not isolating the off-task "deactivations" that are often the focus in traditional studies of the DMN. Instead, we focus on covariance between four seeded ROIs and the intrinsic BOLD signal during these off-task blocks.

2. Materials and methods

2.1. Subjects

Ten participants with moderate and severe TBI between the ages of 19 and 56 years and ten healthy adults of comparable age underwent MRI scanning at separate time points for this study. Individuals with TBI underwent MRI data acquisition at 3 and 6 months after emerging from posttraumatic amnesia (PTA). These study participants were included from an original sample of 15 subjects. The data from five subjects were not included in the current study due to attrition (n = 1), an inability to adequately perform the cognitive task at

Please cite this article as: Hillary, F.G., et al., Changes in resting connectivity during recovery from severe traumatic brain injury, Int. J. Psychophysiol. (2011), doi:10.1016/j.ijpsycho.2011.03.011

3 months PTA (n = 3), and claustrophobia (n = 1). Because it was a primary goal in this study to examine alterations in PFC following disruption to frontal systems, subjects with small identified areas of contusion or hemorrhage in the frontal regions were included (7/10 subjects in this study sustained injuries to frontal regions). TBI severity was defined using the Glasgow Coma Scale (GCS) in the first 24 h after injury (Teasdale and Jennett, 1974) and GCS scores from 3 to 8 were considered "severe" and scores from 9 to 12 were considered moderate. One individual with a GCS score of 13 was included because acute neuroimaging findings were positive. During recruitment for either group, potential study participants were excluded if they had a history of previous neurological disorder such as seizure disorder, significant neurodevelopmental psychiatric history (such as schizophrenia or bipolar disorder), or had a history of substance abuse requiring inpatient treatment. To control for potential resting connectivity changes with repeated exposure to the MRI environment, a HC sample of comparable age was enrolled in the study and underwent two MRI scans separated by approximately 3 months (mean = 90.2 days, SD = 23.8).

2.2. TBI and focal lesions

There is accumulating evidence that, following moderate and severe TBI, even cases that appear to result in isolated injury (e.g., subdural hematoma), there are likely whole-brain consequences (Büki and Povlishock, 2006; Fujiwara et al., 2008). Findings from these and other studies indicate that diffuse axonal injury is a nearly universal finding (Wu et al., 2004). While there have been laudable efforts to examine diffuse injury in the absence of conspicuous focal lesions (Sanchez-Carrion et al., 2008a, 2008b), the samples examined in these instances may not often represent what is commonly observed in TBI which is most often represented by mixed pathophysiology. For this reason, focal injury was not an exclusionary criteria in the current study, unless the injury necessitated neurosurgical intervention and/or removal of tissue.

2.3. MRI procedure and data acquisition

Data were acquired using a Philips Achieva 3.0 T system (Philips Medical Systems, The Netherlands) with a 6-channel head coil and a Siemens Magnetom Trio 3.0 T system (Siemens Medical Solutions, Germany) with an 8-channel head coil both housed in the Department of Radiology, Hershey Medical Center, Hershey, PA. Subjects were made aware of the importance of minimizing head movement during all MRI scanning and any trials where motion was recognized were discontinued or repeated. For all subjects, high resolution brain anatomical images with isotropic spatial resolution of 1.2 mm × 1.2 mm × 1.2 mm were acquired using the MPRAGE sequence. Other imaging parameters for the MPRAGE sequence consisted of: 468.45 ms/16.1 ms/18°, repetition time (TR)/echo time (TE)/flip angle (FA), a 250 × 200 mm^2 field of view (FOV), and a 256 × 180 acquisition matrix. Echo planar imaging (EPI) was used for functional imaging. Imaging parameters for EPI were 2000 ms/30 ms/89°, TR/TE/FA, a 230 × 230 mm^2 FOV and a 128 × 128 acquisition matrix.

2.3.1. fMRI WM paradigm and off-task blocks

While the current study focuses on non-task related covariance in the BOLD fMRI signal, the resting connectivity observed here is influenced by task (we focus on the "resting" blocks flanked by a WM task), so a brief description of this visual working memory task is provided here. This study used a visual WM paradigm requiring rehearsal and memory of face stimuli. The task included exposure to two pictures of male and female Caucasian faces presented in black and white adapted from a standardized dataset (Beaupre and Hess, 2005). This paradigm requires the subject to match the identity and location of two face stimuli. For example, the subject views a box divided into quadrants and within two quadrants are two faces which appear for 3000 ms. After a delay of 3000 ms requiring focus on a fixation point, the subject is provided a target stimulus, or two face stimuli, presented in two of the four quadrants. At the time of presentation of the target, the subject is required to make a yes/no decision about the identity (match/no match) and the location of a single face presented in one of the four quadrants. This non-verbal working memory paradigm is initiated with a 20-second baseline followed by individual 42-second experimental trials (blocks) alternating with 20-second baseline measurements (i.e., fixation stimulus). The current study focuses on covariance in the BOLD fMRI signal during the 20-second baseline periods (between periods of task), including a mean of 165.3 (SD = 5.23) volumes per run (after eliminating volumes with movement, poor signal-to-noise ratio) across two runs (n = 19; data for 1 subject included 1 run at 1 time point).

2.3.2. fMRI resting connectivity: ROI determination and analytic procedure

2.3.2.1. Regions of interest. It was a focus here to examine RSFC in two separate classes of networks previously examined in this literature: 1) a network posited to have reciprocal interaction while engaging in goal-directed behavior or external stimulation and 2) a network believed to play a role in self-reflection or internal-states (Sheline et al., 2010). Almost universally, medial PFC (MedPFC) and posterior cingulate cortex (PCC) have been identified as central to the network organized around "internal-states" (Raichle and Snyder, 2007; Zou et al., 2009). In addition, because TBI most commonly disrupts frontal systems (and therefore, executive control processes) (Hillary et al., 2002; Whyte et al., 1998), we also aimed to examine fluctuation in the BOLD signal in regions believed to be directly involved in cognitive control and volitional behavior. Thus, we examined resting connectivity in dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) and related regions during rest that might be best conceptualized as a "goal directed" network. These two networks will henceforth be referred to as the "internal-state" and "goal-directed" networks. The internal-state network was examined by correlating activity in each voxel in the brain with two "seeds": one in PCC ([X Y Z] = [−6 −50 38]) and one in MedPFC ([X Y Z] = [0 48 −2]). In all cases, the seeds were the average time-series of a sphere with a radius of 3 mm. The PCC and MedPFC seeds were derived from a review of the literature on the "Default Mode Network" (e.g., Sumowski et al., 2010; Greicius et al., 2003). The goal-directed network was examined in the same way, but with seeds placed in the DLPFC (Brodmann's areas 9/46/10; [X Y Z] = [−36 31 13]) and ACC (Brodmann's areas 6/32; [X Y Z] = [−1 7 55]). The placement of these seeds was based on prior work summarizing attentional control networks (Wager and Smith, 2003). Overall, this approach permits the observation of networks reciprocal to those that are directly related to task. Fig. 1A and B illustrates the four distinct seeding locations.

2.3.2.2. Procedure for determining covariance in resting signal. For BOLD time series analyses, data were preprocessed using both Analysis of Functional NeuroImages (AFNI) software (Cox, 1996) and FSL (Smith et al., 2004). In data preprocessing, the first 5 images of each time-series were removed to ensure that magnetization had reached a steady state. The data from both runs were then realigned with the first image of the first run in the remaining time-series. The data were then smoothed, using a Gaussian smoothing kernel (FWHM = 6 × 6 × 6 mm), scaled to the mean intensity across the entire time-series, band-pass filtered (high-pass = 0.005 Hz; low-pass = 0.1 Hz), and detrended (to remove any linear drifts remaining in the data). The data were then deconvolved with a boxcar function representing the time spent on the working memory task (described above) and signal

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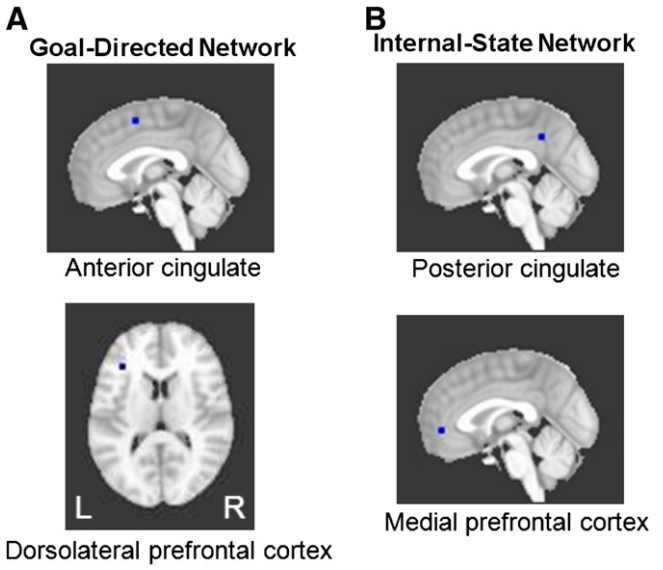

Fig. 1. A and B illustrate "seed" placement for determining the four networks examined in this study. A shows the seed for goal-directed networks (ACC and DLPFC) and B displays the seed placements for the internal-state networks (PCC and MedPFC).

attributable to physiological parameters, signals from cerebrospinal fluid and white matter, mean BOLD and signal six motion parameters were included as regressors of no interest. The residuals from the deconvolution were saved, and used as the resting-state data (see Biswal et al., 2010 for a similar approach). Regressing the global signal out of the data in this way has drawn some comment in relation to anticorrelations in the networks that show correlated activity (Murphy et al., 2009). However, because this is a commonly used preprocessing step (see Kelly et al., 2009; Di Martino et al., 2008), and because we do not specifically investigate anticorrelated activity in the present article, we elected to remove the global signal (particularly since the effect of gross differences in signal between groups on patterns of connectivity is unknown).

Resting data were then demeaned and resampled into standard (i.e., Montreal Neurologic Institute) space. Finally, correlations were calculated between each seed time-series and each voxel in the brain, resulting in 4 volumes per subject (the correlations between each of our 4 seed regions and every voxel in the brain). To determine statistical significance and in order to correct for multiple comparisons, we used a cluster-level threshold of 598 contiguous voxels that was determined using Monte Carlo simulations (using the AlphaSim program, available at http://afni.nimh.nih.gov). Because these are the first serial data to examine significant neurological disruption, we also employed an inclusive threshold in order to permit exploration of more subtle findings or those from spatially smaller regions (cluster size = 550, or the equivalent of correcting to $p<0.10$). Two significant findings with this second threshold are indicated as such in Table 3. To ensure that the resulting r-values were normally distributed, Fisher's r-to-z transformation was applied to the data.

The resulting z′ scores were used for two types of analyses: 1) group-level ANOVAs for each of the four seeded regions with the factors Group (HC vs. TBI) and Time (Time 1 vs. Time 2) and 2) within-group influence of time on connectivity in the four ROIs (Time 2 − Time 1 connectivity). The purpose of the ANOVAs was to comprehensively investigate the patterns of connectivity change using a voxelwise approach (for each of the four seeds). Because we were primarily interested in those regions that changed across time differentially in the two groups, we will focus on the interaction between Group and Time in the Results section. Moreover, because we were primarily interested in the change across time in the TBI group, we further limited the regions to those in which there was no statistically significant change across time in the HC group. The purpose of this second analysis was to examine subthreshold effects not observed in the ANOVA and to capture within-group effects, with specific interest in change during this window of recovery in TBI.

3. Results

3.1. Demographic, clinical descriptors

The groups were well-matched for age and gender, but there were significant between-group differences in education (see Table 1).

3.2. fMRI results: behavioral data

The current study does not focus on task-related BOLD signal change, however, task performance is a reasonable indicator of cognitive improvement from 3 to 6 months and here we compared the second run of each time point (to permit task acclimation during the first run and minimize the influence of early task practice effects). Compared to the HC sample, accuracy was significantly reduced in the TBI sample at Time 1 [TBI mean (SD) = 0.81 (0.15), HC mean = 0.95 (0.05); $t(17) = 2.38$; $p = 0.039$] and this difference diminished at Time 2 [TBI mean = 0.89 (0.1), HC mean = 0.95(0.07); $t(17) = 1.50$; $p = 0.15$]. Similar change was evident in RT for the task at Time 1 [in ms: TBI mean (SD) = 1454 (282), HC mean = 1205 (214); $t(17) = -2.15$; $p = 0.048$] compared to Time 2 [in ms: TBI mean (SD) = 1288 (253), HC mean = 1106 (202); $t(17) = -1.71$; $p = 0.107$]. Thus, these findings indicate that the improvements in performance observed in the TBI sample are greater than what was observed in the HC sample and cannot be attributed to task practice alone. Note: behavioral data at both time points were not available for one subject with TBI.

3.3. fMRI results: BOLD signal change during WM task

In order to provide context for understanding the current resting connectivity results, it is noted that the "on-task" period of the current paradigm elicits BOLD signal change primarily in PFC, parietal areas, and the cerebellum. These findings are consistent with other studies of non-verbal WM (Glahn et al., 2002).

3.4. Examining resting connectivity

It was a goal in the current study to examine both internal-state and goal-directed networks in off-task resting BOLD fMRI data. The current findings are separated into results for within-group change for the TBI sample from the first to the second time point for both networks (e.g., internal-state and goal-directed networks).

Table 1
Demographic characteristics for both groups and injury information for the TBI sample.

Demographic variables	TBI mean (SD)	HC mean (SD)	Group comparison
Age	29.4 (11.0)	27.5 (12.1)	$p = 0.71$
Education	12.2 (0.63)	14.6 (1.6)	$p = 0.001^*$
Gender	7 m, 3 f	4 m, 6 f	$p > 0.05$
Clinical variable	**TBI sample**		
GCS score	Mean = 5.4; SD = 3.5; range = 3–14		
Acute hospital days	Mean = 18.3; SD = 8.1; range = 5–32		
Acute CT/MRI result (n)	F (7); T (7); DAI (4); IVH (3); shift (3); P (1); P/O (1); Thal (1); BG (1); cerebellum (1)		

F = frontal lobe, T = temporal lobe, IVH = intraventricular hemorrhage; shift = ventricular compression; P = parietal; P/O = parietal–occipital; Thal = thalamus; BG = basal ganglia.

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3.4.1. Resting connectivity results: Group × Time interaction

There were a number of regions demonstrating significant Group × Time interaction effects. One important distinction between groups was the separation between internal state and external state networks in this analysis. That is, Group × Time effects dissociated the groups along the "internal" and "external" network divisions. Individuals with TBI demonstrated decreased connectivity in external state networks while the HC sample showed increased connectivity in these areas. By contrast, individuals with TBI demonstrated increased internal state connectivity between time points and the HC sample showed the opposite effect. Table 2 and Fig. 2 summarize the findings that differentiated the groups when considering change over time.

3.4.2. Resting connectivity results — within-group

As noted, it was also a goal to examine the greatest connectivity changes within the TBI sample from Time 1 to Time 2. In order to examine the gross influence of time on connectivity results within TBI (irrespective of the effect in HCs), the residual BOLD time series was analyzed by placing a "seed" in each of the four ROIs (i.e., ACC, PCC, MedPFC, and DLPFC) and comparing results between time points. When considering only effects within the TBI sample we again observed primarily increases in internal state networks and decreases in goal-directed networks (see Figs. 3–5). The one exception to this finding was increased connectivity observed between the DLPFC seed and the insula. This increased connectivity is consistent with that observed within the MedPFC seed (see Table 3).

3.4.3. Connectivity and performance change

While the current study does not focus on task-related BOLD signal change, there are certainly findings in the literature demonstrating that the relationship between internal and external-state networks has implications for task performance. To examine those relationships here, we conducted a Pearson correlational analysis for RT change scores and 8 connectivity change scores for the primary regions showing differences in Table 1 (ACC to BA40; DLPFC to thalamus/parietal; PCC to BA6; PCC to BA37) and Table 2 (ACC to parietal/precuneus; DLPFC to BA13; MedPFC to BA13; PCC to BA37). No analyses here revealed statistically significant results, although small effects were noted between change in RT and change in DLPFC to insula connections ($r = -0.44$; $p = 23$) and MedPFC to insula connections ($r = 0.43$; $p = 0.25$).

Table 2
Sites of significant connectivity change during Group × Time interaction analysis (mixed effects ANOVA).

	BA	X	Y	Z	Cluster	Direction
ACC seed						
Postcentral gyrus	3	30	−30	48	1267	+HC/TBI−
Postcentral gyrus (into inferior parietal lobule)	40	−22	−36	60	999	+HC/TBI−
DLPFC seed						
Thalamus [extending to parietal (precuneus/cuneus) and occipital areas]	−	−24	−20	0	963	+HC/TBI−
Medial frontal seed						
Inferior occipital gyrus	18	40	−88	−16	789	−HC/TBI+
PCC seed						
Middle frontal gyrus	6	−24	4	60	720	−HC/TBI+
Middle temporal gyrus	37	−54	−60	0	686	−HC/TBI+
Precuneus	31	10	−66	22	826	−HC/TBI+

Key: BA refers to Brodmann's areas; XYZ refers to the location of the voxel with the strongest connection to the seed, across the group; cluster refers to the number of voxels in the cluster (voxel size = 2 × 2 × 2 mm); direction refers to the direction of the difference in each group: green text indicates that the relationship with the seed was stronger at Time 2 than Time 1, and red text indicates that the relationship with the seed was weaker at Time 2 than Time 1. Note: all findings here were statistically significant at a corrected cluster size of 598 voxels ($p < 0.05$).

4. Discussion

The current study aimed to examine intrinsic, or "resting" brain connectivity during a period known to be of critical importance for recovery following moderate and severe TBI. The approach used here in a group of individuals sustaining moderate and severe TBI used BOLD fMRI to examine fluctuations in the "off-task" BOLD signal. Primary findings reveal increased involvement of internal-state networks during recovery from TBI (elaborated below). We arrived at this finding by examining two theoretically distinct resting networks: an internal-state network thought to be involved in self-reflective processes and a goal-directed network, that is thought to be associated with engaging external stimuli.

The clinical context for these findings must be emphasized. This is a sample of individuals with predominantly severe TBI where there was known disruption in neural functioning corroborated by clinical MRI (see Table 1) and we anticipate that these injuries have a direct consequence on neural connectivity. It is essentially unknown how large-scale neural networks adapt to significant neural disruption, in particular during the first 6 months following moderate to severe injury. While the findings clearly indicate changes in both internal and goal-directed networks during this period of recovery, these data should be interpreted only for the period of recovery measured here. That is, the increases observed in connectivity are quite different compared to the response in HCs and may be indicative of short or long-term changes in connectivity and therefore the long-term trajectory of these network connections remains uncertain. Of note, individuals with TBI demonstrated significantly improved performance from Time 1 to Time 2 and this is unlikely an improvement that can be accounted for by practice alone. Inasmuch as improvements in working memory function represent one metric of improvements in brain function, the changes in connectivity reported here occur in the context of recovery from TBI.

The following discussion outlines two specific observations permitted by the findings presented here. First when examining the regions that dissociated groups over time (i.e., mixed-effects ANOVA), the two groups were separated along the lines of "internal" and "goal-directed" networks between time points. Second, there were consistent increases in connectivity in several regions including the middle temporal lobe and insula and these findings are largely consistent with a larger literature and may have implications for transition between internal and goal-directed states and learning and task proceduralization.

4.1. Group by Time interactions

A Group × Time ANOVA revealed that, in multiple network regions (see Table 2), there was a Group by Time interaction so that the two groups showed clear dissociations along the two networks (i.e., internal-state and goal-directed). For those findings that dissociate the groups over time, primary findings reveal down-regulation of goal-directed attentional networks (ACC and DLPFC to parietal regions) and increased connectivity in regions associated with internal-state responsivity (i.e., MedPFC and PCC).

This finding reveals that these networks are shifting during this three month period. The reason for this shift is not entirely clear, but increased connectivity in similar networks has also been observed in clinical samples, such as substance abuse (Ma et al., 2010) and may be indicative of a period of recalibration between internal and goal-directed inputs permitting sufficient "release" from volitional on-task processing. The importance of this release has been documented previously, as the interplay between internal and external-state networks has been determined to predict task performance (Fox et al., 2007; Kelly et al., 2008). Finally, we do not anticipate that what is observed here is directly attributable to distinct task load issues between the groups. While there is some evidence that task "load"

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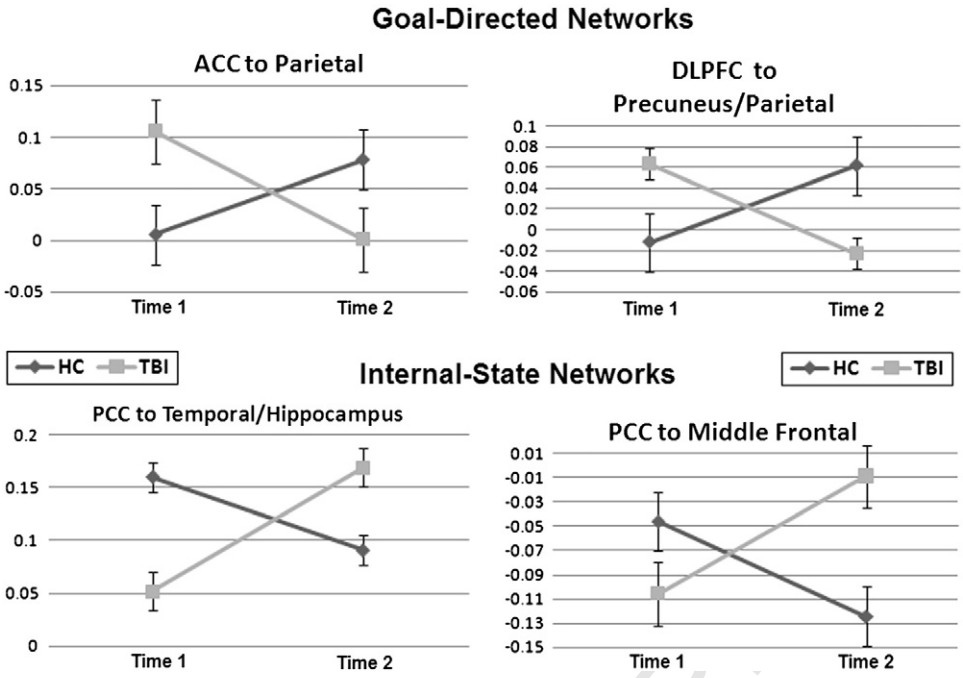

Fig. 2. TBI sample plotted for z′ values (y-axis) vs. Time 1 and Time 2 (x-axis) to demonstrate the findings differentiating groups over time (i.e., mixed effects ANOVA findings in Table 1). Error bars are averaged standard error for group for each seeded network. Note: mean standard z-scores summarize each set of scores (all interactions significant, $p<0.05$).

immediately during previous trials may influence internal-state connectivity (see Pyka et al., 2009; van Dijk et al., 2010), the PCC and MedPFC connections observed here are increasing over time and occur in the context of improving performance.

Unfortunately, there were no statistically significant relationships between RT change and resting connectivity change for either analyses (i.e., ANOVA and between-time comparison). Nevertheless, it does appear that DLPFC down-regulation may have at least some modest influence on RT change; analysis of connectivity change that dissociated these groups over time revealed a negative, but non-significant correlation between RT change and DLPFC change ($r=-0.44$). Moreover, increased connectivity in MedPFC was positively associated with RT albeit non-significantly ($r=0.43$). Thus, if permitted to interpret these data, the diminished DLPFC to parietal connections (referred to elsewhere as the "frontoparietal control network", see Spreng et al., 2010) coupled with increased MedPFC to insula connectivity may be an indicator of improving neural efficiency. That is, greater connectivity to internal-state connections, may operate to integrate demands from "internal" and "external" environments, providing greater continuity between these environments over time; the insula has been posited to play a critical role in developing and updating the "representations" of external demands (Mennes et al., 2011).

What is unclear is why change during a period of recovery would trend in the opposite direction with the network connections observed in the HC sample? While provisional, we might infer this trend to be a necessary, but temporary, release from control and "re-syncing" of external and internal states from 3 to 6 months PTA. A follow-up observation noting trends in these networks at 12–18 months post injury might provide information about the relative permanence of this down-regulation of DLPFC to parietal (i.e., "attentional control") connections.

In contrast with the diminished DLPFC connectivity observed over time, one of the most consistent findings dissociating these two groups was the increased involvement of PCC connectivity in the TBI sample. Certainly there is data demonstrating that these two networks (DLPFC and PCC) are dissociable and have often been observed to be negatively correlated (see Greicus et al., 2003), so the reciprocal findings here are consistent with a larger literature. However, analyses in separate seeding findings for PCC to medial temporal/hippocampus (i.e., BA37) and PCC to middle frontal areas (BA6) revealed no significant correlations with RT ($r=-0.01$, $r=-0.9$ and $r=-0.03$ respectively). Thus, connectivity change between PCC and medial temporal and anterior regions may have less direct consequence for behavior compared to the changes observed in DLPFC and MedPFC. In order to clarify the consequences large-scale connectivity changes have for behavior, future work should include larger samples observed at similar time points to isolate the clinical

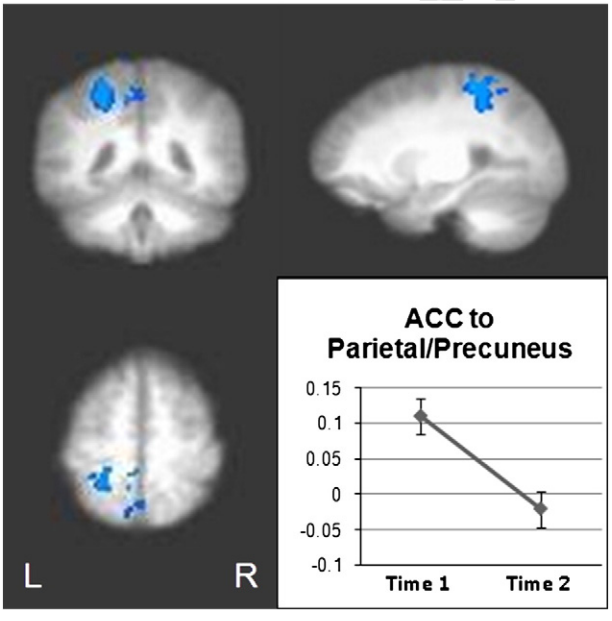

Fig. 3. Connectivity for the Time 2 — Time 1 findings in the TBI sample. Data here display the change in connectivity between the ACC seed and inferior parietal lobe and precuneus. Error bars are standard error averaged over time.

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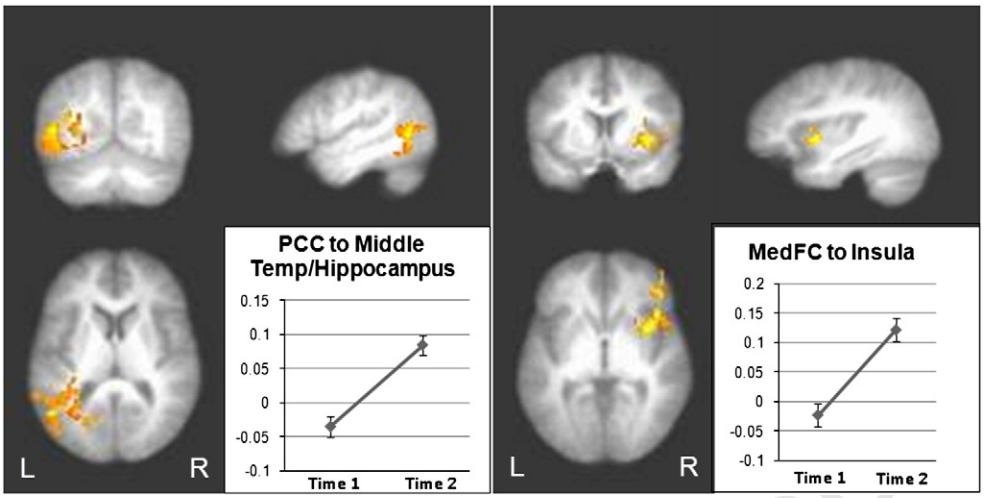

Fig. 4. Connectivity for the Time 2 – Time 1 findings in the TBI sample in "internal-state" networks. Data here display the change in connectivity between the PCC seed and middle temporal lobe and the MedPFC seed and the insula. For convenience, these views are presented together. Error bars are standard error averaged over time.

and performance factors that might influence the timing and magnitude of these network shifts.

4.2. Within-group change over time

While the interaction results reveal distinctions between groups, it was also a primary goal to observe those effects occurring over time within the TBI sample that may have been either consistent with or distinct from the HC sample, but did not rise to the level of statistical significance during ANOVA analysis. For this reason, we also conducted an analysis permitting observation of significant changes within the network observed within the TBI sample (see Figs. 3–5). This analysis of the direct effect of time (which we treat here as a surrogate for recovery) with performance reveals largely overlapping effects with the between-group comparison; internal-state networks demonstrated increased connectivity and external-state networks showed decreased connectivity. One important exception here was in a connection between DLPFC and the insula. This finding was unexpected, but converges with other connectivity data summarized above (i.e., between-group analysis) and is interpreted below.

Overall, Fig. 2 illustrates the change in connectivity over time for both groups and indicates that the internal state networks are more highly connected within the TBI sample over time and dissociable from the effects observed in the HC sample. The first 6 months represent a critical window of recovery following TBI, but certainly recovery can occur for several years following injury (Millis et al., 2001). Given this, what is unknown in TBI is if this result is maintained or if this is a nonlinear effect that later returns to approximate what is observed in HCs once greater recovery has occurred. We anticipate that the latter is an accurate depiction of this trajectory, but additional analyses with a more protracted time line would be necessary to confirm this.

4.3. Connectivity change and limbic system

The most consistent finding in these results is between seeded regions and changes in connectivity to the insula and middle temporal regions, including hippocampus. In all cases, connections to these regions are increasing from Time 1 to Time 2 and these findings are largely dissociable from the HC sample (see Table 1 and Fig. 2).

We offer here two interpretations of these primary shifts in the data. First, the movement in these data from anterior "attentional control" regions (e.g., DLPFC to parietal connections) to increased connectivity in posterior and medial temporal regions (e.g., PCC to

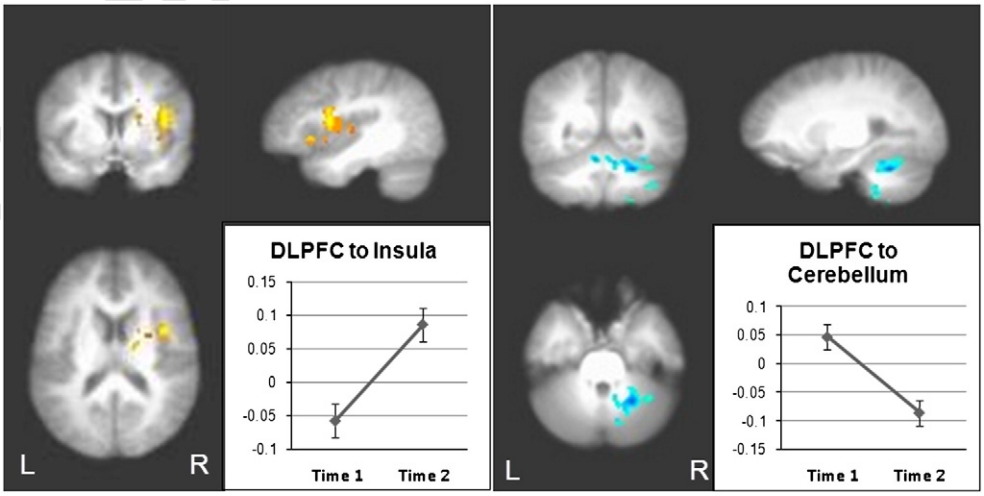

Fig. 5. Connectivity for the Time 2 – Time 1 findings in the TBI sample. Data here display the change in connectivity between the DLPFC seed and the insula and cerebellum. For convenience, these views are presented together. Error bars are standard error averaged over time.

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Table 3
Sites of significant connectivity change during Time 2 *minus* Time 1 analysis in the TBI sample.

	BA	X	Y	Z	Cluster	Direction
ACC seed						
Postcentral gyrus (extending back into parietal (precuneus) areas)	3/4	−4	−38	62	1380	TBI−
DLPFC seed						
Insula[a]	13	42	6	14	563	TBI+
Culmen (extending up into occipital areas (fusiform gyrus))	−	−24	−52	−26	767	TBI−
Tail of the caudate[a]	−	−32	−42	12	554	TBI−
Medial frontal seed						
Insula (extending forward into inferior frontal areas)	13	36	8	−2	1038	TBI+
PCC seed						
Middle temporal gyrus (extending into hippocampus)	37	−50	−60	8	1636	TBI+

Note: In no case was this contrast significant for the HC group.
[a] These areas were significant at a lower threshold, cluster size = 550, $p<0.10$; all other findings were statistically significant at a corrected cluster size of 598 ($p<0.05$).

hippocampus connections) likely reflects greater task proceduralization and formal integration of the task into memory. That is, at 6 months PTA, individuals with TBI may be better able to incorporate memorial systems and consolidate the constraints and demands of the task. This interpretation is consistent with the reductions in time-on-task (i.e., RT), as formal "representations" of the task permit more rapid processing and reduce demand on attentional control networks for effortful task processing.

Second, the increased connectivity from the insula to PCC and DLPFC is the only finding that does not dissociate the internal and goal-directed networks. In all statistically significant observations here, connectivity with the insula increased from 3 to 6 months PTA. The insula has been linked to a number of functions during task perturbation including emotion, task saliency, and monitoring internal states (for review see Kurth et al., 2010). In the examination of large-scale connections, there is recent evidence that the insula plays a critical role in "salience processing" and permits negotiation and shifting between internal-state and attentional control processes (Menon and Uddin, 2010). Other examiners have noted the unique topographical location of the insula placing it at the boundary between "cognitive, homeostatic, and affective systems of the human brain" serving as a conduit between external demands and the internal milieu (Craig, 2009). With this literature as context, the current data indicate that from 3 to 6 months PTA, increased connectivity to the insula may play a critical role in recovery by increasing interoception and permitting appropriate transitions between the sub-networks examined in this study.

5. Study limitations and future directions

The current study holds the advantage of examining TBI during a known window of recovery at separate time points and it is the first to do so using resting connectivity methods. Even so, there are several limitations to this study that require mention. The most significant shortcoming to this study is the small sample size for each of the groups; certainly to conduct sub-group analyses (e.g., injuries to right vs. left hemisphere), additional subjects would be required. In addition, the control group was significantly more educated than the TBI sample. However, it is unclear that a 2-year difference in education observed here could account for fundamental differences in internal and goal-directed networks examined. While there was almost no variance in the TBI sample with regard to education, a correlational analysis between education and connectivity change for each of the four change scores revealed no relationships (the highest correlation was $r = 0.19$ in ACC). Also, any between group differences in connectivity attributable to education should be at least partially ameliorated by the current emphasis on within-subject change over time. As noted, direct comparisons between connectivity change and task performance revealed only modest, non-significant relationships between connectivity change and RT change; a larger sample is required to determine if connectivity change observed here does indeed have direct implications for performance. Even given these concerns, the current data are unique and offer a preliminary look at how large-scale networks change during recovery from significant neurological disruption.

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Please cite this article as: Hillary, F.G., et al., Changes in resting connectivity during recovery from severe traumatic brain injury, Int. J. Psychophysiol. (2011), doi:10.1016/j.ijpsycho.2011.03.011

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Please cite this article as: Hillary, F.G., et al., Changes in resting connectivity during recovery from severe traumatic brain injury, Int. J. Psychophysiol. (2011), doi:10.1016/j.ijpsycho.2011.03.011

Fractal dimension assessment of brain morphometry: a novel biomarker in Traumatic Brain Injury

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Abbreviations

BET – Brain Extraction Tool

DTI – Diffusion Tensor Tmaging

FAST – FMRIB's Automated Segmentation Tool

FD – Fractal Dimension

FLIRT – FMRIB's Linear Image Registration Tool

FMRIB- Functional Magnetic Resonance Rmaging of Brain

FNIRT – FMRIB’s Nonlinear Image Registration Tool

FSL – Functional MRI of the brain Software Libraries

FWER – Family-Wise Error Rate

FWHM – Full-Width Half-Maximum

GLM – general linear model

GM – grey matter

MPRAGE – magnetization prepared rapid gradient echo

MRI – magnetic resonance imaging

SPM – statistical parametric mapping

TBI – Traumatic Brain Injury

VBM – voxel based morphometry

WM – white matter

Introduction

Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide with immense negative impact on society, economy and health-care systems. The Centers for Disease Control and Prevention estimates that every year 1.7 million people in USA sustain TBI and TBI contributes to 30% of all injury related deaths in the USA (Faul et al., 2010). An estimated 5.3 million people (close to 2% of the population) are currently living with TBI-related disabilities in USA with an estimated cost of more than $60 billion per year due to injury-related work loss and disability (Faul et al., 2010, Finkelstein et al., 2006). However, the overall impact may be much higher as recent studies suggest that published statistics underestimate the TBI burden (Powell et al., 2008, Bazarian et al., 2006). Despite these facts the approaches to the characterization of TBI severity and outcome have not changed in more than three decades (Maas et al., 2010). Currently, crude severity grades of mild, moderate, and severe TBI are defined by using clinical and radiographic indices. Glasgow Coma Scale (GCS), length of loss of consciousness, and post-traumatic amnesia form some of the clinical criteria; evidence of intracranial lesions on computed tomography (CT) scan or magnetic resonance imaging (MRI) are the basis of the radiographic criteria and outcome is measured using the Glasgow Outcome Scale Extended (GOS-E) (Teasdale and Jennett, 1976, Wilson et al., 1998). However, TBI is highly heterogeneous in cause, severity, pathology and clinical course and these measures often fail to capture this complexity (Yue et al., 2013). For example, current neuroimaging techniques have limited sensitivity to detect physiological alterations caused by TBI. The focal lesions detected by CT or MRI in TBI, such as contusions and axonal shearing injuries, are often not predictive of long-term functional disability after TBI, especially in mild cases. Overall, approximately 70% of patients with

TBI did not show any visible lesions using conventional MRI or CT techniques (Huang et al., 2009). In addition, the absence of abnormality on conventional neuroimaging techniques in the majority of mild TBI patients, even with post-concussive symptoms and cognitive and/or behavioral deficits, illustrates the limited prognostic value of conventional neuroimaging techniques (Johnston et al., 2001, Kirkwood et al., 2006). The heterogeneity of TBI, combined with the lack of accurate radiological indices for TBI, is thus hindering development of targeted treatment and neuroprotective strategy over the last four decades (Maas et al., 2010). A novel neuroimaging based biomarker, which can address this neurobiological heterogeneity, would enable the rapid classification, clinical trial stratification, and follow up rehabilitation care plans of patients with TBI.

Neuroimaging in TBI: the unsolved issues

The most consistent finding of brain tissue injury as a consequence of TBI is axonal injury (Gennarelli et al., 1982). TBI causes axon (nerve fiber) damage resulting from the stretching and shearing of white matter fibers due to rotational forces on the brain within the cranial cavity, a condition often referred to as diffuse axonal injury (DAI) (Adams et al., 1989). With newer MRI techniques to visualize the axonal damage, DAI has been almost universally demonstrated in fatal TBI (Gentleman et al., 1995). DAI is also considered as the common cause of poor outcome in TBI (Maas et al., 2008) and the mechanism most likely to be responsible for many of the cognitive deficits resulting from moderate to severe TBI (Scheid et al., 2006). However, current evaluation of DAI with MRI is often problematic as it depends on the sensitivity of the MR imaging sequences, selection of patients, and time between injury and scan (Skandsen et al., 2010). Due to these reasons the results of studies correlating MR evaluation of DAI and functional

outcome are often conflicting (Chelly et al., 2011). A better quantitative measure for detecting white-matter axonal injury will thus be a very suitable addition as a neuroimaging biomarker of TBI.

Fractal Dimension analysis: a novel way to measure WM changes

Because brain WM is consisted of axons, DAI would affect the WM network integrity at the system level, which can be detected by brain imaging techniques that can measure WM network structure's shape (morphology)complexity (Kinnunen et al., 2011, Kraus et al., 2007). Biomechanics models of TBI also indicated that shear forces causing DAI leads to elastic shape deformations in intracranial brain tissues(Sayed et al., 2008). Fractal dimension (FD) is a novel and quantitative technique to estimate WM shape complexity (WMc) in brain. "Fractal" is a term coined by Mandelbrot (Mandelbrot, 1982) to describe the irregular but self-similar shapes of natural objects. A fractal is defined as any rough and irregular object composed of smaller versions of itself. The cortical fractal structure, therefore, can be characterized by a single numerical value (the fractal dimension, FD) that summarizes the irregularity of the external cortical surface and the boundary between subcortical grey and white matter (Bullmore et al., 1994). FD measures have been successfully applied to reveal gender and age structural differences in the cerebral cortex in the absence of disease and to investigate various psychiatric and neurological disorders. (Esteban et al., 2007, Mustafa et al., 2012, Zhang et al., 2008, Zhang et al., 2007). However, to the best of our knowledge, FD is yet to be evaluated in the TBI population. There are several reasons why fractal analysis may be a useful paradigm for analyzing brain WM shape in TBI: (1) FD can capture very complicated morphology of structures in a simple and quantitative description and can characterize the

way in which the WM fills up the brain. (2) Given the high spatial resolution ($1X1X1mm^3$) of the 3D anatomic images based on which the analysis will be made, the WMc index can capture the WM changes which are beyond the resolution capability of other WM assessment techniques such as Diffusion Tensor Imaging (DTI). In this study, therefore, we examined whether white matter complexity (WMc) is reduced in chronic TBI subjects and whether the FD measures correlate with cognitive outcome in TBI patients.

Comparing FD with DTI and other volumetric markers

As FD offers additional benefits over other structural measurement of WM (e.g., DTI/WMVBM), we also examined how FD compares with these measures in predicting cognitive parameters in TBI. The comparison with DTI was of special interest. DTI provides quantitative information about integrity of WM microstructures by evaluating isotropic and anisotropic water diffusion within neuronal fiber tracts. DTI based measures are quite promising in evaluating DAI and emerging as important tool in TBI research (Kinnunen et al., 2011, Kraus et al., 2007). However, as noted above FD may provide additional benefits beyond the spatial resolution achieved by DTI. We also estimated whole brain intracranial WM volume using SPM. As FD will evaluate the WM complexity of the whole brain, it is important to know how this correlates with volumetric change of WM. In addition to WM, we also compared FD with GM volume and thickness changes using popular openware FreeSurfer. FreeSurfer is a set of automated tools for reconstruction of the brain's cortical surface from structural T1-weighted data. FreeSurfer can generate maps of whole brain GM atrophy. Generalized cerebral atrophy is a well-established consequence of moderate-to-severe TBI and the

degree of atrophy is related to injury severity (Ghosh et al., 2009). It is therefore imperative to evaluate how WM complexity change may relate to overlying GM changes.

Hence, given the potential importance of white matter pathology to outcome in TBI, and the sensitivity of FD in determining the integrity of white matter beyond currently available techniques, studies of FD are warranted in TBI population. In this study, a group of chronic TBI subjects of moderate-to-severe severity and a group of demographically matched healthy controls underwent MRI (anatomical and diffusion tensor imaging) and neuropsychological testing. The primary objective of the current investigation was to test the hypothesis that white matter complexity (WMc) is reduced in chronic TBI subjects. The secondary objective was to examine the relationship between WMc and cognition assessed with standard neuropsychological testing. Additionally we compared the WMc vs DTI/volumetric measures in predicting cognitive outcome.

Methods

Institutional review boards responsible for ethical standards at the Rutgers University - NJMS and the Kessler Foundation Research Center approved this study. Written informed consent was obtained from all subjects prior to participation.

Demographics

We recruited TBI patients of all severity (mild, moderate and severe) assessed using the Glasgow coma scale (n=17) and healthy controls (HC) (n=13) matched for age, gender and education. (Table 1)

Neuropsychological assessment

On the day of MRI, all participants completed a standardized neuropsychological test battery sensitive to cognitive impairment associated with traumatic brain injury. The following cognitive functions of specific interest were evaluated: (i) verbal short-term learning and memory performance via the Hopkins Verbal Learning Test – Revised (HVLT-R); (ii) the executive functions of set-shifting, inhibitory control and cognitive flexibility were measured using the Delis–Kaplan Executive Function System (Delis et al., 2001). We used the alternating-switch cost index (time to complete alternating letter and number Trails B—time to complete numbers-only Trail A) from the Trail Making subtest and the inhibition/switching minus baseline score from the Color–Word subtest (high scores indicating poor performance); (iii) information processing speed via the Symbol Digit Modalities Test (SDMT). (iv) Attention - via the Visual Search and Attention Test (VSAT).

Z-scores were calculated for all subjects, with the mean and SD of data from healthy subjects used to define z-scores for all subject groups. Negative scores indicate performance below the mean of healthy subjects. Domain scores for measures of executive function, attention, processing speed and memory were generated by averaging the standardized data from tests assessing these cognitive domains

Imaging Protocol

High resolution 3D T1-weighted axial magnetic resonance images (MRI) of the whole brain were obtained using magnetization prepared rapid gradient echo (MPRAGE) sequence on 3 T Siemens Allegra (Erlangen, Germany) scanner. TBI patients were scanned three months after their post traumatic amnesia. Imaging parameters were: TR (repetition time) = 2000 ms, TE (echo time) =4.9 ms, flip angle=8°, inversion time (TI) =900 ms, slice thickness=0.96 mm, in-plane resolution=0.96×0.96mm^2, and number of slices=172. DTI data were also acquired using single shot echo planar imaging (SS-EPI) sequence along 12 diffusion weighted (b = 1000 s/mm^2) directions and one b = 0 s/mm^2. Imaging parameters were: 26 slices, thickness 4 mm, with 2.0×2.0 mm in-plane resolution; pulse sequence parameters were: repetition time TR = 7300 ms, echo time TE = 88 ms, number of averages = 8.

Data processing

A comprehensive quantitative analysis was performed on brain grey and white matter structures of TBI and control subjects. The techniques used to quantitatively assess WM damage were; a) FD dimension analysis, b) DTI and c) WM whole brain intracranial volume changes. Grey matter changes were quantitatively assessed using cortical thickness analysis. The purpose of this comprehensive analysis was to evaluate the sensitivity of FD measure (in detecting structural change of the WM system in TBI patients) over currently available techniques such as DTI. More details on each of these methods are given below.

White matter analysis

Fractal Dimension (FD) analysis

FD analysis was carried out using our customized in-house routines (details described elsewhere) . Briefly the image processing included: skull stripping of T1-weighted images using FMRIB Software Library (FSL) Brain Extraction Tool (BET)(Smith et al., 2004) (http://www.fmrib.ox.ac.uk/fsl/ Center for Functional Magnetic Resonance Imaging of the Brain (FMRIB), Oxford, UK). Brain extraction was followed by segmentation into WM, grey matter (GM) and cerebrospinal fluid (CSF) probability maps using FSL's FAST tool(Zhang et al., 2001). WM probability maps were then binarized using a threshold value of 0.5. A 3D thinning method was then applied to the WM binary image in order to obtain the 3D WM skeleton. The 3D thinning algorithm removed as many boundary voxels as possible without changing the general shape of the WM, until a center line of one voxel width (skeleton) remained. Left and right hemispheres were then separated from the whole brain using FSL tools. Masks of left and right hemispheres separated in the previous step were applied to WM skeleton and WM general structure images to get the WM skeleton and WM general structure of left and right hemispheres. FD values were estimated using a 3D box-counting method (details described elsewhere) (Zhang et al., 2006). The box-counting method was preferred since it can be applied to structures without self-similarity, such as the human brain. (The box-counting method works by repeatedly applying different-sized meshes (r) to the fractal image and counting the number of boxes (N) needed to completely cover the fractal.) Finally, a linear regression fit after log transformation was used to estimate FD values using equation 1 given below

$$\ln N = \mathrm{FD} \ln(1/r) + \ln k, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

where *k* is a nuisance parameter, in self-similar scale (linear portion in the logarithmic function).

In this study we estimated FD values of the three WM features (shape representations): skeleton, surface and general structure. Skeleton FD was calculated by counting the boxes needed to cover the WM skeleton; surface FD was evaluated by counting the boxes needed to cover the boundary of WM/GM interface; general structure FD was estimated by counting the boxes needed to cover all the WM voxels (which included skeleton and surface). The skeleton (consists of central line of each WM tract/bundle), also known as WM interior structure that preserves the topological and geometric information of the WM. The skeleton configuration represents the interior structure complexity of the brain WM. The surface structure consists of voxels at the boundary i.e. GM/WM interface, reflecting the shape of the gyral and sulcal convolutions in the GM/WM interface. General structure comprises of all WM voxels (including voxels in the GM/WM boundary and skeleton in WM segmented images, representing the volume changes. Because the WM skeleton, general structure and surface represent three different aspects of brain WM structure, it was expected that they may serve as more comprehensive and distinct shape complexity measures to evaluate the WM structure shape/structure changes brought out by pathophysiological mechanisms of TBI.

DTI Analysis

DTI images were processed using FSL openware (http://fsl.fmrib.ox.ac.uk/fsl). The processing steps include correction for eddy current distortion effects. The b-matrix was rotated after eddy current distortion effects in order to preserve correct orientation

information. Corrected images were then fitted to the diffusion tensor model using "*dtifit"* routine and maps of diffusion tensor metrics namely fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) radial diffusivity (RD) were obtained. Whole brain, right and left hemisphere WM masks obtained by segmenting T1-weighted images were then multiplied (after registering with the DTI images) with FA,MD,AD and RD maps.Mode values of whole brain, right and left hemisphere FA, MD, AD and RD were measured from the histogram plots of each subject. Statistical analysis was performed using either T-test or Mann-Whitney U test depending on data meeting the assumptions of normality.

White matter intracranial volume analysis

Whole brain (intra-cranial) white matter, grey matter and cerebrospinal fluid volumes were obtained for both control and TBI patients using SPM software. A T-test was performed to compare the difference in whole brain WM and cerebrospinal fluid (CSF) intracranial volume between TBI and controls.

FreeSurfer based automated image analysis

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis version 4.0.4 (Athinoula A. Martinos Center for Biomedical Imaging, c2005; http://surfer.nmr.mgh.harvard.edu.libproxy2.umdnj.edu/). Details described elsewhere (Fischl et al., 2004a and Jovicich et al., 2009). Briefly, this processing includes the removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical WM and deep GM volumetric structures (Fischl et al., 2002 and Fischl et al.,

2004a), intensity normalization (Sled et al., 1998) tessellation of the GM–WM boundary, automated topology correction (Fischl et al., 2001 and Segonne et al., 2007), and surface deformation following intensity gradients to optimally place the GM/WM and GM/CSF borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale et al., 1999, Dale and Sereno, 1993 and Fischl and Dale, 2000). The resulting cortical models were registered to a spherical atlas, utilizing individual cortical folding patterns to match cortical geometry across subjects (Fischl et al., 1999) The cerebral cortex was parcellated into regions based on gyral and sulcal structure (Desikan et al., 2006 and Fischl et al., 2004b). Results for each subject were visually inspected to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction.

Both intensity and continuity information from the entire 3D MR volume were used to produce representations of cortical thickness, where thickness was measured as the closest distance from GM-WM to GM-CSF boundary at each vertex on the tessellated surface (Fischl and Dale, 2000). A p value of <0.05 corrected for multiple comparisons using a false discovery rate (FDR) was considered the level of significance for measures cortical thickness, cortical area and cortical volume.

Statistical Analysis

IBM SPSS for Windows version 21 (Armonk, New York) was used for database and statistical analysis. A two-tailed unpaired Student's t-tests or Fisher's exact test was used to compare demographic, neuropsychological and FD findings between healthy controls and patients with TBI. To investigate the correlation between FD and cognitive variables, univariate correlations between continuous variables were assessed using the Pearson

correlation coefficient and those between discrete variables were assessed with the Spearman rank correlation coefficient. A stepwise linear regression analysis was performed to assess the relative contributions of the main demographic (age, education and sex) and WM parameters (FD, DTI parameters) in predicting the cognitive domain index scores. Forward and backward stepwise analyses were conducted using the Wald statistic as a criterion, with P = .05 for entry and P = .10 for removal.

Results

Standard magnetic resonance imaging

Among the patients with TBI all had moderate to severe TBI according to GCS. T1 imaging was normal in 2 patients (11.7%) and T2* normal in 3 patients (17.6%). Definite and possible intraparenchymal microbleeds indicative of diffuse axonal injury were found in 35% of the patients (Microbleed group: 6 patients, 5 males, mean age 28.2 ± 6.9 years, average time since injury 26 months; Non-microbleed group: 11 patients, 8 males, mean age 30.2 ± 14.5 years, average time since injury 25 months). There was no group difference for age, gender and duration of TBI. Microbleeds were mainly found in frontal and temporal white matter bilaterally.

Neuropsychological testing

Group means are presented for the each neuropsychological test in Table 2. Mean cognitive domain scores are also presented in Fig. 2. Patients with TBI differed from the controls on all cognitive domains - memory/learning (p=0.02), executive function (p = 0.001), attention (p = 0.001) and processing speed (p=0.002).

Fractal dimension and demographic parameters

To understand the relation between FD parameters with age and education we ran a series of correlation analysis. There was no relation between age and any of the FD parameters (surface, general structure or skeleton). However, education in years was positively correlated with whole brain skeleton FD values ($r=0.42$, $p=0.03$), which meant higher education increased white matter complexity. There was no difference in any of the FD parameters (surface, general structure or skeleton) between males and females (t tests, $p>0.05$).

Fractal dimension changes in TBI

Significant ($p < 0.05$) reduction were observed in the skeleton FD values of right (TBI - 2.24 ± 0.06; HC - 2.30 ± 0.06 (Mean±SD)) and left hemispheres (TBI - 2.25 ± 0.04; HC - 2.30 ± 0.05 (Mean±SD)) with TBI group showing lower structural complexity compared to controls. This is shown in Figure 1. However; we failed to observe any significant difference in surface or general structure FD values between TBI and controls.

Relation between intraparenchymal microbleed and fractal dimension

Significant ($p < 0.05$) reduction were observed in the skeleton FD values of right (Nonmicrobleed - 2.28 ± 0.05; Microbleed - 2.19 ± 0.04 (Mean±SD)) and left hemispheres (Nonmicrobleed - 2.27 ± 0.03; Microbleed - 2.21 ± 0.05 (Mean±SD)) with patients with microbleeds showing lower structural complexity compared to TBI patients without microbleeds. However, we failed to observe any significant difference in surface or general structure FD values between microbleed and nonmicrobleed group.

DTI

No significant differences in FA, MD or RD values were observed between TBI and controls either in the whole brain or on the right or left hemisphere WM tissue. The only significant difference was observed in AD values in left hemisphere ($p=0.03$) and right hemisphere ($p<0.05$).

White matter intracranial volume changes

Whole brain WM intracranial volume (in units of ml) was slightly reduced in TBI patients (mean =512.36 ml) when compared to controls (mean =514.43 ml) but did not reach statistical significance. On the other hand CSF volume was found to be significantly ($p<0.001$) increased in TBI (mean = 271.43 ml) when compared to controls (mean = 218.57 ml).

Grey matter volume and cortical thickness changes

No significant difference in either grey matter volume or cortical thickness or cortical area was observed in any of the brain regions between TBI and healthy controls. Among the deep grey matter structures, however, right thalamus ($p=0.01$) was significantly atrophied in the TBI group.

Relationship between white matter shape change and neuropsychological function in TBI

To examine the contribution of white matter shape change (skeleton FD values) in neuropsychological outcome in TBI, hierarchical linear regressions were performed, separately for each of four cognitive domains: executive function, memory, attention and

processing speed. The education was not included in the first step as it was highly correlated with FD-skeleton.

For executive function domain, the model explained 63% of variance ($F_{6,18} = 5.12$, $p = 0.003$). The FD-skeleton accounted for additional 19.5% of variance beyond demographic and DTI variables ($p = 0.02$).

For processing speed, the model explained 47% of variance ($F_{5,19} = 3.32$, $p = 0.02$). The FD-skeleton accounted for additional 22% of variance beyond demographic and DTI variables ($p = 0.03$).

For memory domain, the model explained 57% of variance ($F_{6,18} = 3.99$, $p = 0.01$). The FD-skeleton accounted for additional 13.1% of variance beyond demographic and DTI variables ($p < 0.1$), which was borderline significant.

For attention, , the model explained 43% of variance ($F_{6,18} = 2.33$, $p = 0.07$). The FD-skeleton accounted for additional 12.1% of variance beyond demographic and DTI variables ($p < 0.1$), which was not significant. For this domain the DTI contributed significantly ($\Delta R^2 = 0.24$, $p = 0.04$)

Discussion

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Spare the rich: the most highly connected network nodes predict recovery of function after neurotrauma

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Keywords: traumatic brain injury, functional imaging, graph theory, connectivity, plasticity, network, flexibility

Introduction

Recent developments in network connectivity have broadened the scope of investigation in the neurosciences, providing unparalleled opportunity to examine whole-brain communication. One applied mathematical approach, graph theory, has received significant recent attention in literatures using functional brain imaging methods (e.g., functional MRI) to examine the flow of information in dynamical networks. While graph theory has a much longer history in the areas of topology (refs) and social networks (refs), in its relatively brief application to the neurosciences, this approach has already influenced how we conceptualize network communication. For example, in non-human primates (refs) and humans (refs), graph theory approaches have demonstrated that neural systems hold "small-world" properties characterized by high "clustering", or extensive connectivity within groups of neural nodes while also retaining short net communication paths between nodes. These network characteristics afford specialized processing of information locally (refs) while permitting large scale information transfer throughout the network (refs). It is a goal in the current study to apply these methods in order to better understand the systems-level network changes occurring after severe traumatic brain injury (TBI), where significant neurological disruption is evident. We anticipate that whole-brain connectivity patterns will provide previously unavailable information about how neural systems adapt to catastrophic disruption.

Clinical network neuroscience

In the clinical neurosciences it remains an important goal to understand the basic brain changes associated with neurological disruption and the implications these changes have for behavioral deficit and recovery trajectory. In the systems neurosciences, there has been widespread use of functional imaging methods to examine task-related brain changes (e.g., mean signal differences) in localized regions of the brain. In several literatures examining brain injury and disease (e.g., TBI, multiple sclerosis), several consistent findings have emerged including increased involvement of neural resources near sites of injury (refs) and in regions that may share functional responsibilities (e.g., homologous regions in the contralateral hemisphere, refs).

With the more recent emphasis of connectivity modeling in the systems neurosciences, the scope of investigations in neurological disorders has mirrored this change; there is an expanding literature documenting the network alterations associated with brain injury and disease (see xx for review). Several studies to date have demonstrated that neurological disruption results in altered connectivity in large scale neural networks (refs) including evidence that even focal injury has widespread consequences for broader network functioning (ref-sharp? Or D'Esposito?) . For example, both focal and diffuse injuries observed in TBI may disrupt distal connectivity which is a distinct and crucial feature to the small-world topology observed in efficient neural systems (Nakamura et al., 2009; Pandit et al., 2013). As a paradoxical consequence to this network disruption, we have proposed that a primary response in dynamical systems is hyperconnectivity within parts of the network (see Hillary et al., 2009; Hillary et al., in review).

In TBI, much remains to be determined with respect to network dynamics early after the injury, including the timeline for connectivity alterations and the regions within the network most sensitive to disruption. We anticipate that hyperconnectivity observable within the typical regions serving as “hubs” (or “rich club”, the most highly connected brain regions in neural systems; see van den Heuvel et al., 2012) have important consequences for network functioning and recovery. Work outside the neurosciences has demonstrated that small-world topology is particularly resilient to non-selective or “random” connectivity loss (see Albert & Barabasi, 200x), but that targeted “attack” on critical network hubs can lead to catastrophic consequences for network communication. For example, the focused loss of anterior-posterior connectivity (e.g., frontal to PCC to hippocampal connections) in Alzheimer’s has devastating consequences for functioning in the areas of memory, spatial navigation, and maintaining semantic associations (see xxxx). The network disruption occurring in TBI is, nearly by definition, idiosyncratic, but we anticipate that injury resulting in connectivity loss to network hubs should have more widespread consequences for functioning. Thus, we anticipate that selective connectivity loss in the rich club and failure to bring specific hubs online from 3-6 months will show the greatest behavioral consequence after TBI. In order to examine the influence of TBI on network hubs, we will make use of functional MRI and graph theory to examine whole-brain connectivity in severe TBI during a critical window of recovery.

Study Hypotheses:

Hypothesis 1: We propose that the most common response to moderate and severe TBI is hyperconnectivity. We anticipate this results in increased number and strength of connections during this window of recovery when compared to a healthy control sample.

Hypothesis 2: There is evidence that disruption of network hubs can have catastrophic results for network functioning (see Albert & Barabasi, 2000). For this reason, we anticipate that individuals showing the least connectivity in the "rich club" or regions commonly considered to be indispensable network hubs (i.e., PCC, insula, and DLPFC) will show the greatest impairment of functioning.

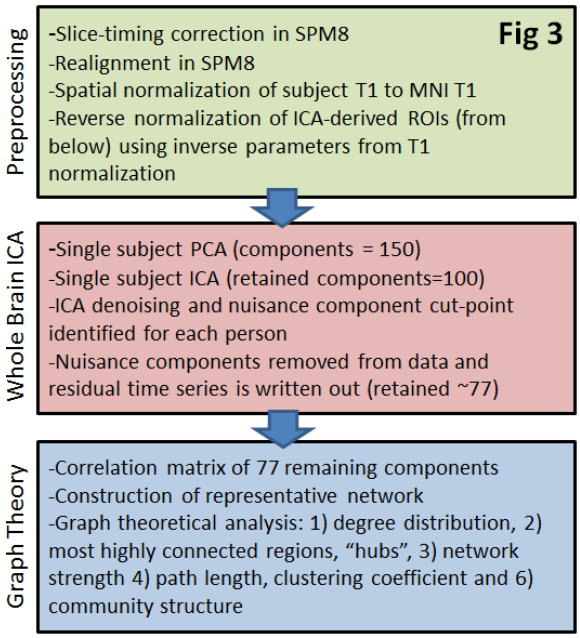

Fig 3

Method

Materials and Methods

Subjects

The participants included 20 participants with moderate and severe TBI between the ages of 19 and 56 years and 15 healthy adults of comparable age and education. All study participants underwent two MRI scanning sessions separated by three months. For the TBI sample, data were acquired at 3 and 6 months after emerging from posttraumatic amnesia (PTA). TBI severity was defined using the Glasgow Coma Scale (GCS) in the first 24 hours after injury (Teasdale and Jennett 1974) and GCS scores from 3-8 were considered "severe" and scores from 9-12 were considered moderate. One individual

with a GCS score of 13 was included because acute neuroimaging findings were positive. Participants were excluded if they remain in treatment for concomitant spinal cord injuries, orthopedic injury, or other injury making it difficult to remain still in the MRI environment. Patients with focal contusions and hemorrhagic injuries were included unless injuries required neurosurgical intervention and removal of tissue resulting in gross derangement of neuroanatomy. The HC sample of comparable age was enrolled in the study and underwent two MRI scans separated by approximately three months (mean = 90.2 days, SD =23.8).

Focal lesions: There are often whole-brain structural and functional brain changes evne in cases of TBI where the primary injury is isolated (e.g., subdural hematoma), (Büki and Povlishock 2006; Fujiwara et al. 2008) and diffuse axonal injury (DAI) is a nearly universal finding (Wu et al. 2004). Moreover, focal injuries can have widespread consequences for brain function (…desposito, 2012). For these reasons, focal injury was not an exclusionary criteria in the current study, unless the injury was so severe so as to require neurosurgical intervention (i.e., (i.e., craniotomy) and/or gross derangement of neuroanatomy. Inclusion of cases where identifiable injruy was evident permitted direct examination of TBI as it naturally occurs even in brain regions directly influenced by injury.

MRI procedure and Data acquisition

Data were acquired using a Philips Achieva 3.0 T system (Philips Medical Systems, The Netherlands) with a 6-channel head coil or a Siemens Magnetom Trio 3.0 T system

(Siemens Medical Solutions, Germany) with a 8-channel head coil both housed in the Department of Radiology, Hershey Medical Center, Hershey, PA. Between time point subject data were always collected on the same scanner to maximize reliability. Subjects were made aware of the importance of minimizing head movement during MRI scanning and trials containing significant motion were discontinued or repeated. High resolution brain anatomical images with isotropic spatial resolution of 1.2 mm × 1.2 mm × 1.2 mm were acquired using an MPRAGE sequence: 468.45 ms/ 16.1 ms / 18º, repetition time (TR)/echo time (TE)/flip angle (FA), 250 × 200 mm^2 field of view (FOV), and a 256 × 180 acquisition matrix. Echo planar imaging (EPI) was used for functional imaging. Imaging parameters for EPI were 2000 ms/30 ms/89º, TR/TE/FA and a 230 × 230 mm^2 FOV, 128 × 128 acquisition matrix.

Data processing and region parcellation:

Figure 1 presents the processing stream for fMRI time series analysis. Initial steps of the processing stream involve pre-processing including realignment of the functional time series to gather movement parameters for correction, coregistration of the EPI data with a high resolution T1 image, and spatial normalization and smoothing (see Figure 1; consistent with Hillary et al., 2011; Medaglia et al., 2012).

Nuisance signal removal and connectivity: To obtain the "rest" fMRI data, we used a single 142 volume run of a simple working memory task (1-back; refence). In preparation for graph theory analyses, "nuisance signal" was removed from each time series using the Conn toolbox (Whitfield-Gabrieli &Nieto-Castonon , 2012). To address signal from the CSF

and white matter, masks in these regions were created and statistically removed from the time series using univariate regression. There were three distinct task-onset timing parameters for the n-back, so as a first step, subject-specific task-onset vectors were entered into the regression using the Conn Toolbox (see Gabrielie-Whitfield &) and task related signal was removed at the individual level first. In addition, six subject movement parameters were input as a temporal confound and regressed at each voxel across the time series (see Behzadi et al., 2007) and a band-pass filter was applied to the resulting time series. After all regressors of no interest were removed, the residuals from the deconvolution were saved and used as the "resting-state" data.

Region of interest determination: In neural network modeling, possibly the most important early decision is determining the nodes, or brain regions, that will contribute to the model. In fact, recent efforts to examine "small-world" properties in TBI have used 20-30 anatomically-determined ROIs [29, 30] within unweighted (i.e., binary) networks and in large-scale network analyses, the characterization of the network ndoes has a direct influence on the graph properties observed (see 32, 41). Separately, anatomical ROIs are often used to avoid biased data selection and circularity in data interpretation (see 42); yet these approaches aggregate a number of functionally distinct oscillatory signals within each ROI. For example, Brodmann's area 46 in PFC is one of the largest ROIs in anatomical atlases and maintains critical roles in a number of functions, yet in the absence of additional parcellation, the ~600 2mm isotropic voxels from this region are averaged and treated as a single homogenous signal. To address these concerns, we use a data-driven approach for ROI parcellation through the use of spatial independent component analysis (ICA; 43, 44). In this way, each ROI is represented as

a functional signature as opposed to an anatomically bound average of many functional signals (see 45). Moreover, in studies using fMRI to examine neurotrauma there is concern regarding the influence of brain lesions on the BOLD signal (see 46) and this is particularly problematic in local areas of hemorrhage where blood products cause susceptibility artifact and local signal attenuation (see 47, 48). However, the ICA procedure implemented here can isolate the effects of local signal drop-out as a "component" and model these data or remove the signal during "denoising and nuisance" identification.

This approach addresses basic differences in brain morphology and local signal drop-out due to the effects of TBI early after injury. Finally, we will use a spatially constrained ICA (scICA) which provides a hybrid approach enabling us to focus on specific subnetworks of interest in this paper (i.e., the rich club) by providing a set of masks or images to the algorithm while also allowing the data to refine the resulting component (see 49). Overall, we anticipate that the approaches used here provide safeguards for conservative data analysis and interpretation while retaining optimal sensitivity to dynamic network effects over time in TBI.

Graph theory analysis:

Procedure for Correlation Matrix

A representative network graph was created using the data parcellation approach described above, such that each "node" in the graph represented a resultant component from whole-brain ICA (see 75, 94). The pair wise correlations amongst all component time series was determined and, after thresholding with false discovery rate,

components with statistically significant correlations were joined by an edge in the graph, weighted by the value of the corresponding correlation (see 38 for similar method). Graph indices were calculated for the weighted network.

Graph theory was applied to investigate two levels of analysis. For the first level of analysis we test Hypothesis 1 using whole brain analyses documenting graph properties. Basic graph metrics of interest include : 1) the degree distribution, i.e. the histogram of the number of connections per node, 2) total number and strength of network connections, 3) clustering coefficient (i.e. the density of triangles in the network graph) for a) frontal systems and b) whole brain, and 4) average global path length as well as local path length for specified regions of interest. For the second level of analysis we test Hypothesis 2 by examining changes in regional connectivity in the most highly connected network nodes from 3 to 6 months post injury. The ROIs included are based upon prior work establishing the most critical and highly connected regions in the brain (the Rich Club): precuneus, superior frontal cortex, superior parietal cortex, anterior and posterior cingulate cortex, and the insula (van den Heuvel et la., 2012); (Fig. 1).

Structural MRI analysis: In order to examine the morphometric changes associated with the Early and Late TBI subgroups, voxel-based morphometry (VBM) analysis was conducted using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/). VBM was used to quantify the white, gray and CSF compartments for all subjects. Gray matter volumes where then used as covariates to determine if connectivity differences between groups were influenced by volume change. First, T1 images were segmented into gray

matter, white matter, and CSF volumes. These segmented images from each individual were then aligned to a template brain (Montreal Neurological Institute (MNI) space) during a normalization step.

Cognitive Assessment:

The most common cognitive deficits following TBI are in the areas of working memory and processing speed (see Demaree et al., 2000; Hillary et al., 2009) and common deficits in abnormal aging are episodic memory deficits (see xxx). All participants completed a brief battery of tests examining these areas of functioning to examine: 1) areas of cognitive deficit compared to a HC sample, 2) change from Time 1 to Time 2 in TBI, and 3) relationship between connectivity changes and cognitive deficit. To assess working memory and processing speed we used the Digit Symbol Modalities Test[78] and the visual search and attention task, the Stroop task[79, 80], and select tests from the Wechsler Adult Intelligence Scale –Fourth Edition (Digit Span and Letter Number Sequencing for WM; Coding and Cancellation for speed)[81]. In addition, verbal episodic memory is the most common deficit observed in DAT (see Summers&Saunders) and this was assessed using the Hopkins Verbal Learning Test (HVLT). Consistent with Steffener & Stern (2012)[85], cognitive reserve will be determined using the Vocabulary subtest from the WAIS-IV, education, socioeconomic status, employment.

Results

Demographic and Neuropsychological Data

Tables 1 and 2 provide the demographic information and neuropsychological information for the two samples.

Graph Theory Results: global graph metrics

The data in Table 3 support a hyperconnectivity hypothesis during this critical recovery window characterized by increased number and strength of connections globally (all significantly greater in TBI compared to HCs, $p<.05$).

Graph theory Results: connectivity within the “Rich Club”

Discussion

We used functional MRI and graph theory methods to examine whole brain connectivity changes during a critical recovery window after moderate and severe TBI. There was support for Hypothesis 1 that the brain hyperconnects during early recovery.

There were also significant changes in clustering coefficient between 3 and 6 months post injury and we anticipate that data collection at 1-year post injury will permit observation of these networks after greater recovery and network stabilization.

Tables

Table 1: Demographic information for both samples

	TBI Mean (sd)	Healthy Controls Mean (sd)	
Age (years)	29.1 (10.4)	28.8 (11.9)	
Education (years)	12.5 (1.5)	13.4 (1.7)	
Gender	19 M, 3 F	9 F, 6 M	
Time-post injury	100.4 (26.6)	-	
GCS	5.7 (4.3)	-	

Table 2: Neuropsychological performance

	TBI mean (sd) 3 months	TBI mean (sd) 6 months	Healthy Controls Mean (sd)
Digit span			
Stroop			
VSAT			
HVLT			

Table 2: Graph properties in TBI and HC groups

Table 2: Hyperconnectivity after TBI indexed as increased number and strength of connections. *= statistically significant difference	**3 months after TBI Mean (sd)**	**6 months after TBI Mean (sd)**	**Healthy Controls Mean (sd)**
Total Number of Connections	341.93 (70.38)*	333.93 (47.96)	282.19 (55.89) *
Total Strength of Connections	104.69 (23.97)*	101.54 (15.79)	84.72 (18.13)*
Average (Weighted) Node Degree	2.72 (0.62) *	2.64 (0.41)	2.20 (0.47) *
Average (Unweighted) Node Degree	9.88 (1.83)*	9.67 (1.35)	8.33 (1.45)*
Average (Weighted) Clustering Coefficient	0.082 (0.032)*	0.069 (0.008)*	0.065 (0.011)
Average (Unweighted) Clustering Coefficient	0.24 (0.04)	0.22 (0.03)	0.21 (0.03)