GREENE BROILLET & WHEELER, LLP
LAWYERS
100 WILSHIRE BOULEVARD, SUITE 2100
P.O. BOX 2131
SANTA MONICA, CALIFORNIA 90407-2131
TEL. (310) 576-1200
FAX. (310) 576-1220

BRUCE A. BROILLET, State Bar No. 63910
ALAN VAN GELDER, State Bar No. 221820
TAYLOR RAYFIELD, State Bar No. 272300

Attorneys for Plaintiffs

(SPACE BELOW FOR FILING STAMP ONLY)

SUPERIOR COURT OF THE STATE OF CALIFORNIA

FOR THE COUNTY OF LOS ANGELES – CENTRAL DISTRICT

WILLIAM ANDROLIA and LINDA ANDROLIA,

Plaintiffs,

vs.

ENTERTAINMENT CENTER, LLC; CBRE, INC.; and DOES 1-100, inclusive,

Defendants.

AND RELATED CROSS-ACTIONS.

CASE NO. BC534479
[Re-Assigned for All Purposes to the Honorable Yvette M. Palazuelos, Dept. 28]

PLAINTIFFS' OPPOSITION TO DEFENDANTS' MOTION *IN LIMINE* NO. 3; MEMORANDUM OF POINTS AND AUTHORITIES; DECLARATION OF TAYLOR RAYFIELD, ESQ.; EXHIBITS

Complaint Filed: January 29, 2014
FSC Date: May 11, 2017
Trial Date: May 22, 2017

///

///

///

///

///

///

///

///

MEMORANDUM OF POINTS AND AUTHORITIES

I. INTRODUCTION.

In July of 2012 Plaintiff walked into a glass door and hit his head on the door. He then started having symptoms of concussion. Plaintiff had a MRI scan which did not show any evidence of a brain bleed or brain damage. Plaintiff then went to a neurologist, Dr. Chang. Dr. Chang began treating him for his concussion symptoms with the hopes that the symptoms would resolve in a few weeks like most concussions. However, Plaintiff's symptoms have continued to last, so Dr. Chang diagnosed Plaintiff with post-concussive syndrome. Dr. Chang tried various treatments and medications and was not having any luck treating Plaintiff's symptoms. Then Dr. Chang had advanced neuroimaging studies performed on Plaintiff with the hopes that it would provide some ideas of some different treatments that she could try. At the time the advanced imaging studies were performed Dr. Chang had already diagnosed Plaintiff with post concussive syndrome. She was not using the advanced imaging studies to diagnose brain injury. The advanced imaging studies merely confirmed what Dr. Chang already diagnosed.

Defendant disputes that Plaintiff has a mild traumatic brain injury and is blaming his underlying medical history and depression for the symptoms that Plaintiff is experiencing. Defendant is seeking to exclude any and evidence relating to the advanced neuro imaging studies that were performed. Defendant cites to one article amidst the hundreds of articles on this issue to support its' contention that advanced neuroimaging is unreliable across the board. This court should not be persuaded.

II. ADVANCED NEUROIMAGING TECHNIQUES ARE RELIABLE AND ACCEPTED IN THE SCIENTIFIC COMMUNITY TO DETERMINE THE EXTENT OF PLAINTIFF'S TRAUMATIC BRAIN INJURY.

The federal courts have already determined admissibility of advanced neuroimaging. In *White v. Deere & Company*, Slip Copy, 2016 WL46290, Defendant moved to exclude the opinions of Plaintiff's expert Dr. Benson. Dr. Benson opined Plaintiff suffered a traumatic brain injury and based that opinion in part on DTI (advanced neuro imaging). The Court denied Defendant's motion to exclude Dr. Benson's opinions based on DTI because it found DTI to be

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

reliable and relevant. The Court noted DTI finds have been admitted by multiple courts. *Id.* at 10-12. "In df, the evidence submitted shows DTI has been tested and has a low error rate; DTI has been subject to peer review and publications; and DTI is generally accepted method for detecting TBI." *Id.* The Court also noted Dr. Benson's conclusion of TBI was not based solely on the DTI findings but on other sources of data. *Id.* at 5. See Exhibit 1 to Taylor Rayfield's Declaration, Slip Copy of Opinion.

Other federal courts have similarly concluded that DTI is a reliable tool, and that the findings of DTI may be testified to by appropriate experts. See *Andrew v. Patterson Motor Freight, Inc.* (W.D. La. Oct. 23, 2014) 2014 WL 5449732; *Chiulli v. Newberry Fine Dining Inc.*, No. 10-10488-JLT (D. Mass. Sept. 30, 2013) 2013 WL 5494723; *Ruppel v. Kucanin* (N.D. Ind. 2011) 2011 WL 2470621 ("the evidence shows that DTI and analysis of white matter in DTI images are generally accepted methods for determining mild TBI"); *Booth v. Kit* (D.N.M. Mar. 23, 2009) 2009 WL 4544743.

Here, Dr. Chang is not using the advanced neuro imaging solely to form her opinions about Plaintiff's brain injury. Dr. Chang already formed the opinion that Plaintiff was suffering from post concussive syndrome before she even did the advanced neuro imaging. This imaging simply confirmed what Dr. Chang had already diagnosed, that Plaintiff has a brain injury.

Advanced neuro imaging has been admitted in this very Courthouse. In fact Judge Feffer allowed evidence of a functional MRI into evidence in 2014. In an unpublished California case, *Diao v. Southern California Gas Company*, the Second District, Division 1, Court of Appeals affirmed a lower court judgment which considered evidence of an abnormal fMRI for purposes of proving mTBI caused by the blasting force of ignited natural gas. (*Diao v. Southern California Gas Company* (Jan. 8, 2016) 2016 WL 110000 [not officially published].) While not specifically deciding on the admissibility of the fMRI, the Court of Appeals concluded that the fMRI, along with a SPECT scan, neuropsychological testing, constituted substantial evidence of TBI. (*Id.* at p. 8.) Judge Feffer allowed the fMRI into evidence in 2014 as evidence that the Plaintiff suffered a TBI.

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

Other State court too have determined that DTI is reliable and generally accepted in the medical community. For example, New York has led the way in helping the legal community recognize the reliability and accuracy of DTI that the medical community accepted years ago. In *Lamasa v. Bachman,* a published opinion in which the New York appellate court held that DTI evidence was properly admitted as it could not be characterized as novel science, but rather, "objective medical tests." *Lamasa v. Bachman,* 869 N.Y.S. 2d 17, 18. As the trial court stated, "DTI provides anatomical information about tissue structure and composition," the properties of which can often by correlated with disease and traumatic brain injury. *Lamasa v. Bachman,* 2005 N.Y. Slip Op. 50882(U) (N.Y. Sup. Ct. April 13, 2005) 2005 WL 1364515, at fn. 3.

In an article entitled, A Decade of DTI I Traumatic Brain Injury: 10 years and 100 Articles Later, the *American Journal of NeuroRadiology* (2013) reported the findings of *Hulkower, et al.* who concluded that "A unifying theme can be deduced from this large body of research: DTI is an extremely useful and robust tool for the detection of TBI-related brain abnormalities." *See* Exhibit 3 to Rayfield Dec, "Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI patients."

Further studies support use of fMRI for traumatic brain injury patients. In the Journal of Neurotrauma (July 15, 2016), "Is Resting State Functional Connectivity in Mild Traumatic Brain Injury at the Acute Stage: Independent Component and Seed Based Analysis," fMRI is a viable alternative in detecting injury related abnormalities that structural MRI's do not. Rayfield Dec. Exhibit 4.

The Radiological Society of North America (October 2012), "Default Mode Network Disruption in Mild Traumatic Brain Injury" utilized fMRI to measure reduced connectivity in the PCC and parietal regions and increased frontal connectivity around the MPFC in patients with mild traumatic brain injury. *See* Rayfield Dec., Exhibit 5.

The Journal of the American Medical Association, "Resting State Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury," discussed fMRI abnormalities in signal amplitude and brain connectivity at rest and their

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

relationship to cognitive outcome in patients with chronic and severe axonal injury. Rayfield Dec. Exhibit 6.

The Journal of Neurotrauma (June 1, 2016), "Is Traumatic Brain Injury Associated with Reduced Inter-Hemispheric Functional Connectivity? A Study of Large Scale Resting State Networks following Traumatic Brain Injury" discussed resting state fMRI and how it was utilized to display a specific pattern of aberrant FC following a traumatic brain injury. Rayfield Dec. Exhibit 7.

Thus, as Plaintiff's experts will state, the advanced neuroimaging is like other imaging modality where it does not reveal the etiology of a particular pathology all by itself. In the case of traumatic brain injury the advanced neuroimaging studies is used in conjunction with clinical data, such as historical data, physical examination, and neurological examination. It is disingenuous to assert that advanced neuroimaging should be, in and of itself, diagnostic of TBI or any other pathology as no radiologic technique is used in isolation.

Functional MRI's are not a novel area of scientific inquiry as Defendants imply in their moving papers. DTI/fMRI is widely and routinely used with their very own CPT insurance codes. A set of CPT codes was assigned to fMRI by the AMA in 2007 (70554, 70555, 96020) allowing radiology facilities to bill insurance companies. The following is from the Practice Guidelines published by the American College of Radiology concerning fMRI:

"The guidelines are an educational tool designed to assist practitioners in providing appropriate radiologic care for patients. The ultimate judgment regarding the propriety of any specific procedure or course of action must be made by the physician or medical physicist in light of all the circumstances presented. Thus, an approach that differs from the guidelines, standing alone, does not necessarily imply that the approach was below the standard of care. To the contrary, a conscientious practitioner may responsibly adopt a course of action different from that set forth in the guidelines when, in the reasonable judgment of the practitioner, such course of action is indicated by the condition of the patient, limitations on available resources, or advances in knowledge or technology subsequent to publication of the guidelines. The practice of medicine involves not only the science but also the art of dealing with the prevention, diagnosis, alleviation,

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

and treatment of disease...all that should be expected is that the practitioner will follow a reasonable course of action based on current knowledge, available resources, and the needs of the patient to deliver effective and safe medical care."

Regarding qualifications of responsible personnel:

"The physician supervising and interpreting fMRI must be clinically informed and understand the specific questions to be answered prior to the procedure in order to plan and perform it safely and effectively. Additionally, physicians performing this procedure should have experience or formal training in performing fMRI."

Dr. Randall Benson who presented at the U.S. Army Telemedicine and Advanced Technology Research Command sponsored the "Diffusion MRI TBI Roadmap Development Workshop", wherein the acknowledged purpose was, *"DTI has detected abnormalities associated with brain trauma at several single centers and the workshop seeks to identify and remove barriers to rapid translation of advanced diffusion MRI technology for TBI...in order to expedite getting the benefits of diffusion MRI to reach those who need it most, especially injured soldiers and veterans."* As one of 50 or so "experts from academia, industry, government agencies and several European nations," He also presented in a session entitled, "Experience in Neuroimaging Translation to Clinical Use." His talk entitled, "Global and Voxel-based approaches to DTI in TBI" included a comprehensive approach to imaging mild TBI, which was the culmination of over six years of peer-reviewed published research on DTI and TBI. He used both group and single cases to demonstrate the clinical validity and reliability of DTI in TBI. In addition to demonstrating the excellent correlation between DTI and injury severity, he showed repeatability of DTI for a single mTBI case scanned in two different cities, and for a different mTBI case scanned twice 6 weeks between scanning sessions. He also used a third mtbi case to demonstrate the excellent correspondence between hemorrhage location (using susceptibility imaging) and abnormally low FA on DTI in these cases. Other speakers presented data showing the correlations of DTI with neurocognitive outcome and experience using DTI on Iraq war veterans.

Finally, like Dr. Benson's DTI analysis of his database and known rate of error, Dr. Chang also has a database for analysis with a known rate of error and standard deviation.

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

In conclusion it is generally accepted in the scientific community throughout the peer review literature that advanced neuro imaging is a reliable and accurate tool to detect microscopic damage done to the brain.

Defendant relies on an article authored by M. Wintemark, P.C. Sanelli, Y. Anzai, A.J. Tsiouris, and C.T. Whitlow. These authors have been critiqued in their positions in an article published in the American Journal of Bioethics Neuroscience April-June, Volume5, Number 2, 2014, the authors, Michael Lipton and Eric Bigler state, "The misleading and often entirely unsubstantiated opinions and positions of Wortzel, Tsiouris, and Filippi (2014), in opposition to diffusion tensor imaging (DTI) as a useful measure in mTBI, are at odds with the clear consensus of the scientific literature regarding mild traumatic brain injury (mTBI), its clinical assessment, and its natural history. The authors' critique contains numerous errors." Rayfield Dec, Exhibit 8.

Dr. Chang is a neurologist and expert in this matter. She is intimately familiar with the field of advanced neuroimaging. She uses advanced neuroimaging in her medical practice as one more piece of data to attempt to synthesize several independent lines of evidence. Dr. Chang has been Plaintiff's treating physician since July of 2012. Based on the symptoms that Plaintiff was having and his medical history Dr. Chang diagnosed Plaintiff with post concussive syndrome. After this diagnosis was made, Dr. Chang used advanced neuroimaging which gave further support to her opinions.

III. CONCLUSION.

For the reasons stated above, Defendants' motion should be denied in its entirety. However, if the Court is not willing to deny this motion then Plaintiff requests a 402 hearing.

DATED: May 8, 2017

GREENE BROILLET & WHEELER, LLP

Taylor Rayfield

Bruce A. Broillet, Esq.
Alan Van Gelder, Esq.
Taylor Rayfield, Esq.
Attorneys for Plaintiffs

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

DECLARATION OF TAYLOR RAYFIELD

I, TAYLOR RAYFIELD, declare and say that:

I am an attorney at law licensed to practice before all of the courts of the State of California, and am a member of the law firm of Greene Broillet & Wheeler, LLP, attorneys of record for plaintiff. As such, I have personal knowledge of the facts surrounding the present action and all facts herein stated. If called as a witness, I could testify competently to the following:

1. Attached as Exhibit 1 is a true and correct copy of the slip copy of *White v. Deere & Company,* Slip Copy, 2016 WL 46290.

2. Attached as Exhibit 2 is a true and correct copy of the Declaration of Dr. Benson, the expert in the *White v. Deere & Company* matter. Dr. Benson's declaration is attached for the purposes of providing the court with the data Plaintiff's expert, Dr. Chang will explain.

3. Attached as Exhibit 3 is a true and correct copy of the article "Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI patients."

4. Attached as Exhibit 4 is a true and correct copy of an article published in the Journal of Neurotrauma (July 15, 2016), "Is Resting State Functional Connectivity in Mild Traumatic Brain Injury at the Acute State: Independent Component and Seed-Based Analysis."

5. Attached as Exhibit 5 is a true and correct copy of an article published by the Radiological Society of North American (October 2012), "Default-Mode Network Disruption in Mild Traumatic Brain Injury."

6. Attached as Exhibit 6 is a true and correct copy of an article published in the Journal of the American Medical Associated, "Resting-Stage Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury.

7. Attached as Exhibit 7 is a true and correct copy of an article published in the Journal of American Medical Association, "The Journal of Neurotrauma (June 1, 2016), "Is Traumatic Brain Injury Associated with Reduced Inter-Hemispheric Functional Connectivity? A Study of Large Scale Resting Networks following Traumatic Brain Injury" discussed resting state fMRI and how it was utilized to display a specific pattern of aberrant FC following a traumatic brain injury.

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

8. Attached as Exhibit 8 is a true and correct copy of an article published by American Journal of Bioethics Neuroscience April-June, Volume5, Number 2, 2014 titled "Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI Patients."

9. Attached as Exhibit 9 is a true and correct copy of the unpublished opinion *Diao v. Southern California Gas Company.*

10. Attached as Exhibit 10 is a true and correct copy of *Lamasa v. Bachman,* (N.Y. Sup. Ct. April 13, 2005) 869 N.Y.S. 2d 17.

I declare under penalty of perjury under the laws of the State of California that the foregoing is true and correct.

Executed this 8th day of May, 2017, at Santa Monica, California.

Taylor Rayfield

TAYLOR RAYFIELD, ESQ.
Declarant

GREENE BROILLET & WHEELER, LLP
P.O. BOX 2131
SANTA MONICA, CA 90407-2131

2016 WL 462960
Only the Westlaw citation is currently available.
United States District Court,
D. Colorado.

Miriam White, Plaintiff,
v.
Deere & Company, John Deere Limited, and John Does 1-5, Defendants.

Civil Action No. 13-cv-02173-PAB-NYW
|
Signed February 8, 2016

Attorneys and Law Firms

Alejandro Daniel Blanco, Blanco Law Firm, PC, Glendale, CA, Richard P. Poormon, Riggs, Abney, Neal, Turpen, Orbison & Lewis, PC, Denver, CO, Stephen Reed Morgan, S. Reed Morgan, P.C., Comfort, TX, for Plaintiff.

Jacqueline Ventre Roeder, Charles L. Casteel, Jordan Lee Lipp, Davis Graham & Stubbs, LLP, Denver, CO, for Defendants.

ORDER

PHILIP A. BRIMMER, United States District Judge

*1 This matter is before the Court on defendants' Motion to Exclude Randall Benson's Opinions Derived from Neuroimaging [Docket No. 103].

I. BACKGROUND

This is a products liability action that arises out of an accident that occurred on August 17, 2011 while plaintiff Miriam White was operating her Deere Model 4600 compact utility tractor and Model 460 loader. Ms. White claims that she suffered facial injuries and traumatic brain injury ("TBI") as a result of a hay bale falling onto her head while she was operating the tractor. Docket No. 103 at 1. Ms. White alleges that her tractor had design defects that created an unreasonable risk of injury from falling hay bales and that her injuries resulted from these defects. Docket No. 150 at 2-3.

Ms. White has designated Randall Benson, a board-certified neurologist, as a medical expert. Docket No. 103 at 1. Dr. Benson opines that Ms. White suffered a traumatic brain injury as a result of the August 17, 2011 incident. Docket No. 116-3 at 18. He bases his opinion, in part, on results derived from a Magnetic Resonance Imaging ("MRI") sequence called diffusion tensor imaging ("DTI"). *Id* at 20-21. Defendants move to exclude Dr. Benson's DTI findings on two grounds. First, defendants argue that Dr. Benson's DTI findings are unreliable. Docket No. 103 at 3. Second, defendants argue that Dr. Benson's DTI findings will not assist the trier of fact to determine whether Ms. White's alleged brain injuries were caused by the August 17, 2011 accident. *Id.* at 4.

II. FEDERAL RULE OF EVIDENCE 702

Rule 702 of the Federal Rules of Evidence provides that:

> A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if: (a) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (b) the testimony is based on sufficient facts or data; (c) the testimony is the product of reliable principles and methods; and (d) the expert has reliably applied the principles and methods to the facts of the case.

Fed. R. Evid. 702. As the rule makes clear, while required, it is not sufficient that an expert be qualified based upon knowledge, skill, experience, training, or education to give opinions in a particular subject area. Rather, the Court must "perform[ ] a two-step analysis." *103 Investors I, L.P. v. Square D Co.*, 470 F.3d 985, 990 (10th Cir. 2006). After determining whether the expert is qualified, the specific proffered opinions must be assessed for reliability. *See id.*; Fed. R. Evid. 702 (requiring that the testimony be "based on sufficient facts or data," be the "product of reliable principles and methods," and reflect a reliable application of "the principles and methods to the facts of the case").

EXHIBIT 1

Rule 702 imposes on the district court a "gatekeeper function to 'ensure that any and all scientific testimony or evidence admitted is not only relevant, but reliable.' " *United States v. Gabaldon*, 389 F.3d 1090, 1098 (10th Cir. 2004) (quoting *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 589 (1993)). To perform that function, the Court must "assess the reasoning and methodology underlying the expert's opinion, and determine whether it is both scientifically valid and applicable to a particular set of facts." *Dodge v. Cotter Corp.*, 328 F.3d 1212, 1221 (10th Cir. 2003) (citing *Daubert*, 509 U.S. at 592-93). Where an expert relies on experience, the expert " 'must explain how that experience leads to the conclusion reached, why that experience is a sufficient basis for the opinion, and how that experience is reliably applied to the facts.' " *United States v. Medina-Copete*, 757 F.3d 1092, 1104 (10th Cir. 2014) (quoting Fed. R. Evid. 702, advisory committee notes).

***2** Although it is not always a straightforward exercise to disaggregate an expert's method and conclusion, when the conclusion simply does not follow from the data, a district court is free to determine that an impermissible analytical gap exists between premises and conclusion. *Gen. Elec. Co. v. Joiner*, 522 U.S. 136, 146 (1997). In examining an expert's method, however, the inquiry should not be aimed at the "exhaustive search for cosmic understanding but for the particularized resolution of legal disputes." *Daubert*, 509 U.S. at 597. It is the specific relationship between an expert's method, the proffered conclusions, and the particular factual circumstances of the dispute that renders testimony both reliable and relevant.

In addition to the expert having appropriate qualifications and methods, the proponent of the expert's opinions must demonstrate that the process by which the expert derived his or her opinions is reliable. *United States v. Crabbe*, 556 F. Supp. 2d 1217, 1220 (D. Colo. 2008). When assessing reliability, "the court may consider several nondispositive factors: (1) whether the proffered theory can and has been tested; (2) whether the theory has been subject to peer review; (3) the known or potential rate of error; and (4) the general acceptance of a methodology in the relevant scientific community." *103 Investors I*, 470 F.3d at 990 (citing *Daubert*, 509 U.S. at 593-94). These considerations are not exhaustive. Rather, "the trial judge must have considerable leeway in deciding in a particular case how to go about determining whether particular expert testimony is reliable." *Kumho Tire Co. v. Carmichael*, 526 U.S. 137, 152 (1999). Ultimately, the test requires that the expert "employs in the courtroom the same level of intellectual rigor that characterizes the practice of an expert in the relevant field." *Id.*

While plaintiff, as the proponent of the challenged testimony, has the burden of establishing admissibility, the proffer is tested against the standard of reliability, not correctness; she need only prove that "the witness has sufficient expertise to choose and apply a methodology, that the methodology applied was reliable, that sufficient facts and data as required by the methodology were used and that the methodology was otherwise reliably applied." *Crabbe*, 556 F. Supp. 2d at 1221.

Once the standard of reliability "is met, the court will still consider other non-exclusive factors to determine whether the testimony will assist the trier of fact: (1) whether the testimony is relevant; (2) whether it is within the juror's common knowledge and experience; and (3) whether it will usurp the juror's role of evaluating a witness'[ ] credibility." *United States v. Rodriguez-Felix*, 450 F.3d 1117, 1123 (10th Cir. 2006).

In sum, assuming an objection is properly made, expert testimony must be excluded if the expert is unqualified to render an opinion of the type proffered, if the opinion is unreliable, if the opinion will not assist the trier of fact, or if the opinion is irrelevant to a material issue in the case.

III. ANALYSIS

Defendants do not challenge Dr. Benson's qualifications, the application of MRI techniques other than DTI, [1] or the four sources of data other than DTI on which Dr. Benson bases his conclusions. defendants' challenge focuses squarely on Dr. Benson's use of DTI and his opinions based on DTI. The Court's Practice Standards regarding Rule 702 objections require that the party seeking to exclude an opinion of an opposing expert identify the opinion sought to be excluded. *See* Practice Standards (Civil Cases), Judge Philip A. Brimmer, § III.G. The only specific opinion that defendants identify in their motion is Dr. Benson's fifth piece of evidence regarding brain imaging, including DTI. Docket No. 103 at 2. The Court therefore assumes that the opinion defendants seek to exclude is that finding in Dr. Benson's report that states as follows: "DTI voxel-wise analysis revealed a large number of white matter tracts with abnormally

reduced FA." Docket No. 116-3 at 20. Dr. Benson also refers to these findings later in his report in support of his conclusion that the DTI "reveals axonal injury predominantly in bilateral frontal lobes." *Id.* at 21-22.

1 In their reply, defendants appear to broaden their argument to include Dr. Benson's conclusions drawn from Susceptibility Weighted Imaging (SWI) and Fluid Attenuated Inversion Recovery (FLAIR) imaging. Docket No. 130 at 3. Defendants admit that SWI and FLAIR are "methodologically sound." *Id.* A party generally may not raise an issue for the first time in a reply brief. *See Ulibarri v. City & Cty. of Denver*, No. 07-cv-01814-WDM-MJW, 2011 WL 1336388, at *2 (D. Colo. April 6, 2011) (citing *Hill v. Kemp*, 478 F.3d 1236, 1250 (10th Cir. 2007)); *LNV Corporation v. Hook*, No. 14-cv-00955-RM-CBS, 2015 WL 5679723, at *3 (D. Colo. Sept. 25, 2015) (citing *Conroy v. Vilsack*, 707 F.3d 1163, 1179 n.6 (10th Cir. 2013)). Accordingly, the Court will not consider defendants' arguments related to SWI and FLAIR imaging.

A. Reliability of DTI for Identifying a TBI

*3 Defendants argue Dr. Benson should be precluded from presenting his opinions based on DTI because DTI is unreliable as a means for diagnosing individual patient injuries. Docket No. 103 at 3. Defendants cite a November 2014 research paper by Wintermark et al. that finds DTI to be suitable only for research and concludes that there is insufficient evidence to support its routine clinical use at the individual patient level. Docket No. 103 at 3-4; Docket No. 103-1 at 76.

Plaintiff responds that the non-exclusive *Daubert* reliability factors establish that Dr. Benson's opinions based on DTI are admissible. Docket No. 116 at 11-14. While the Wintermark article may undermine the weight of Dr. Benson's DTI findings, plaintiff cites articles that support DTI's reliability. *See, e.g.*, Docket No. 116-1 at 7, ¶ 10; Docket No. 116-6. The articles cited by plaintiff appear to support the conclusion that DTI is a generally accepted diagnostic measure for TBI. One peer-reviewed article cited by plaintiff reviews the last decade of research conducted on DTI and finds that "[a] unifying theme can be deduced from this large body of research: DTI is an extremely useful and robust tool for the detection of TBI-related brain abnormalities. The overwhelming consensus of these studies is that low white matter FA [fractional anisotrophy] is characteristic of TBI." M.B. Hulkower et al., *A Decade of DTI in Traumatic Brain Injury: 10 Years and 100 Articles Later*, 34 AM J NEURORADIOL 2064, 2071 (2013). This article also found "an overwhelming consensus that imaging abnormalities detected with DTI are associated with important clinical outcomes. This further validates DTI as a meaningful measure of clinically important brain injury." *Id.* Another peer-reviewed article cited by plaintiff states that the "overwhelming consensus of a substantial body of scientific inquiry supports DTI for detecting pathology in [mild TBI ("mTBI")] patients," Docket No. 116-6 at 4, and directly challenges the criticisms of DTI proffered by defendants' expert, Dr. Hal Wortzel. *Id.* at 2 ("The misleading and often entirely unsubstantiated opinions and positions of Wortzel, Tsiouris, and Filippi (2014), in opposition to diffusion tensor imaging (DTI) as a useful measure in mTBI, are at odds with the clear consensus of the scientific literature regarding [mTBI], its clinical assessment, and its natural history."). The Court notes that the November 2014 research paper cited by defendants acknowledges that "there is evidence from group analyses that DTI can identify TBI-associated changes in the brain across a range of injury severity, from mild to severe TBI. Evidence also suggests that DTI has the sensitivity necessary to detect acute and chronic TBI-associated changes in the brain, some of which correlate with injury outcomes." Docket No. 103-1 at 78. Thus, the Court finds that defendants have not shown that the November 2014 research paper, or other evidence, establishes that DTI is an unreliable technology to detect mild TBI-associated changes in the brain.

In his affidavit, Dr. Benson discusses some of the testing that he has conducted "to demonstrate the clinical validity and reliability of DTI in TBI" as part of his work with the U.S. Army Telemedicine and Advanced Technology Research Command at a "Diffusion MRI TBI Roadmap Development Workshop." Docket No. 116-1 at 11-12, ¶ 18. As part of his research for his presentation at that workshop, Dr. Benson found "excellent correlation between DTI and injury severity" and "repeatability of DTI for a single mTBI case scanned in two different cities." *Id.* Dr. Benson also notes that "[o]ther speakers presented data showing the correlations of DTI with neurocognitive outcome and experience using DTI on Iraq war veterans." *Id.* Dr. Benson states the known rate of error for DTI analysis is .4%, Docket No. 116-1 at 14, ¶ 28; however, he provides no support for this rate.

*4 Application of the four non-dispositive *103 Investors* factors supports plaintiff's argument that DTI is a reliable methodology. *See 103 Investors I*, 470 F.3d at 990 (citing *Daubert*, 509 U.S. at 593-94). Regarding whether DTI can be and has been tested, Dr. Benson's affidavit discusses the testing he has conducted to confirm DTI results. Docket No. 116-1 at 11-12, ¶ 18. The publications and workshops cited by Dr. Benson support the conclusion that DTI has been subjected to peer review and is generally accepted in the medical community as a tool for detecting TBI. *Id.* at 10-12, ¶¶ 16, 18. While plaintiff has not supported her argument that DTI has a known error rate, no single *103 Investors* factor is dispositive. *See 103 Investors I*, 470 F.3d at 990 (citing *Daubert*, 509 U.S. at 593-94). The Court notes that DTI findings have been admitted by multiple courts. *Andrew v. Patterson Motor Freight, Inc.*, 2014 WL 5449732, at *8 (W.D. La. Oct. 23, 2014) ("In sum, the evidence submitted shows DTI has been tested and has a low error rate; DTI has been subject to peer review and publication; and DTI is a generally accepted method for detecting TBI.") (citation omitted); *Ruppel v. Kucanin*, 2011 WL 2470621, at *6 (N.D. Ind. June 20, 2011) (finding DTI to be a reliable method); *Booth v. KIT, Inc.*, 2009 WL 4544743, at *3 (D.N.M. Mar. 23, 2009) (denying motion to exclude expert testimony regarding findings from DTI). Accordingly, the Court finds that plaintiff has carried its burden of showing that DTI is a reliable technology and that Dr. Benson applied a reliable methodology in arriving at his challenged opinion.

B. "Fit" of Dr. Benson's DTI Findings

Defendants argue that Dr. Benson's opinions derived from DTI do not "fit" this case. Docket No. 103 at 4; *see Bitler v. A.O. Smith Corp.*, 400 F.3d 1227, 1234 (10th Cir. 2004) ("A trial court must look at the logical relationship between the evidence proffered and the material issue that the evidence is supposed to support to determine if it advances the purpose of aiding the trier of fact. Even if an expert's proffered evidence is scientifically valid and follows appropriately reliable methodologies, it might not have sufficient bearing on the issue at hand to warrant a determination that it has relevant 'fit.' ") (citing *Daubert*, 509 U.S. at 591). Defendants assert that Dr. Benson's DTI findings show that plaintiff has only one or two white matter lesions and that Dr. Benson has not adequately addressed other possible causes for such findings in light of Ms. White's medical history, specifically, her injuries after being kneed in the head by a horse. Docket No. 103 at 5-6. On June 10, 2012, Ms. White was hit on the left side of her face by a horse's knee. Docket No. 81-3 at 6. After emergency medical services arrived and evaluated Ms. White, they determined that she should be transferred to the Medical Center of the Rockies. *Id.* There, Chris Cribari, M.D., noted that Ms. White was admitted with a diagnosis of a concussion and that the EMTs said she was repeating herself, had retrograde amnesia, and was slow to respond. *Id.* Defendants claim that these are signs of brain trauma that Dr. Benson ignores. Docket No. 103 at 5. Defendants also argue that Dr. Benson does not "adequately consider or explain why the white matter lesions are so definitively attributable to the 2011 incident and not to [p]laintiff's psychiatric issues." *Id.* at 6. The Court notes that both the June 10, 2012 incident and plaintiff's psychiatric history are mentioned in Dr. Benson's report. *See* Docket No. 81-3 at 6, 8. Defendants also argue that "a fact-finder needs to determine ...whether [p]laintiff's alleged brain injury was caused by the 2011 incident at issue in this case" and claim that Dr. Benson's DTI findings are not relevant to the issue of causation. Docket No. 103 at 5.

In support of his conclusion that "[i]t is probable that [Ms. White's] permanent cognitive, emotional, and physical symptoms...are the direct result of the 8/17/11 injury and not the subsequent injury of 6/10/12," Dr. Benson relied on five sources of data: (1) the available biomechanical information regarding the August 17, 2011 injury event; (2) Ms. White's symptoms following the August 17, 2011 injury event; (3) findings from a neurobehavioral examination; (4) findings from a neuropsychological assessment; and (5) Ms. White's neuroimaging. Docket No. 81-3 at 18-20. Thus, DTI is not the only source of information Dr. Benson uses to diagnose TBI. The neuroimaging he relies upon consists of FLAIR, SWI, and Gradient Echo imaging in addition to DTI. *Id.* at 20. Dr. Benson pairs the neuroimaging results with the neuropsychological assessment, which notes impaired processing speed and working memory and delayed verbal memory, coding, and symbol search, to determine the presence of brain damage. *Id.* at 21. The reasons Dr. Benson articulates for identifying the August 17, 2011 incident as the source of plaintiff's traumatic brain injury are not based on DTI, and Dr. Benson readily admits that "[n]o standalone imaging technique allows for unequivocal determination of etiology absent clinical information." Docket No. 116-1 at 6. Dr. Benson compares the imaging findings to the other data sources and states that the "imaging findings match the

biomechanics, chronic symptoms, neurobehavioral and neuropsychological findings." Docket No. 116-1 at 9. Applying the differential diagnosis procedure, Dr. Benson asserts that Ms. White's "injury/accident of 8/17/11 was the much more significant injury and rendered her vulnerable to the more mild[ ] concussion of 6/10/12." Docket No. 116-4 at 6. He also states that the "injury of 6/10/12, while inducing a mild concussion, does not explain her clinical deficits that began when her head was crushed under the weight of a heavy hay bale on 8/7/11." *Id.*

***5** The Court finds that defendants present no basis to exclude Dr. Benson's causation opinions on the grounds of the alleged unreliability or irrelevance of DTI for identifying a TBI suffered by Ms. White.

IV. CONCLUSION

For the foregoing reasons it is

ORDERED that defendants' Motion to Exclude Randall Benson's Opinions Derived from Neuroimaging [Docket No. 103] is **DENIED**.

All Citations

Slip Copy, 2016 WL 462960

End of Document

2015 WL 10439167 (D.Colo.) (Expert Report and Affidavit)
United States District Court, D. Colorado.

Miriam WHITE, Plaintiff,

v.

DEERE & COMPANY, John Deere Limited, and John Does 1-5, Defendants.

No. 13-cv-02173.
July 13, 2015.

Affidavit of Randall Benson, MD in Support of Plaintiff White Opposition to Defendant Deere's Motion to Exclude Dr. Benson's Opinions Re Neuroimaging

Case Type: Products Liability >> Tools/Equipment/Machinery
Jurisdiction: D.Colo.
Name of Expert: Randall R. Benson, M.D.
Area of Expertise: Health Care-Physicians & Health Professionals >> Neurologist

Representing: Plaintiff

I, Randall Benson, MD, declare and state as follows:

1. I have personal knowledge of the facts set forth in this affidavit and, if called upon to testify as a witness, I could and would competently testify to them.

2. 1 am a board-certified neurologist with fellowship training in Neuroimaging. I completed my Residency Training in Neurology at Boston University School of Medicine in 1991. Thereafter, I completed Fellowship Training in both Functional Neuroimaging and Behavioral Neurology in 1993. From 2001-20111 I served as an Assistant Professor of Neurology at Wayne State University School of Medicine in Detroit, Michigan. I also served as voluntary faculty in the Departments of Radiology and Neurology in 2011 and continue to collaborate with faculty in research at the Medical School. I also served as the Co-Director for Traumatic Brain Injury Research at Wayne State University. A copy of my *Curriculum Vitae* is attached as Exhibit 1. I conducted a full neuro-behavioral evaluation of Ms. White, which consisted in a medical (neurological and neurobehavioral) exam, review of medical records, review of neuropsychological report and assessment by Dr. James Berry, and review of MRI scan of the brain.

Attached as Exhibit 2 is a true and correct copy of my Comprehensive Medical Report, dated January 18, 2015. All of these materials and medical examination pertain to Plaintiff Miriam White in the matter of *White v. Deere & Co., et al.,* No. 13-cv-02173-PAB-NYW. I hereby incorporate by this reference and adopt all statements in the Comprehensive Medical Report in this declaration.

Attached as Exhibit 3 is a true and correct copy of a Rebuttal report to Dr. Wortzel, dated May 20, 2015, 1 authored in response to the report of Dr. Wortzel, dated May 20, 2015, after a careful review of Dr. Wortzel's report. All of these materials pertain to the medical and scientific evaluation of Dr. Wortzel's opinions and basis in the matter of *White v. Deere & Co., et al.,* No. 13-cv-02173-PAB-NYW. I hereby incorporate by this reference and adopt all statements in the Rebuttal Report to Dr. Wortzel in this declaration.

EXHIBIT 2

Attached as Exhibit 4 is a Region of Interest Analysis of Fractional Anisotropy showing the areas of interest in rebuttal to Dr. Wortzel's report. All of these materials pertain to the medical evaluation of MRI evidence of the Plaintiff, Miriam White in the matter of *White v. Deere & Co., et al.,* No. 13-cv-02173-PAB-NYW. I hereby incorporate by this reference and adopt all statements in the Region of Interest Analysis of Fractional Anisotropy in this declaration.

3. I have reviewed Deere's motion to exclude Dr. Benson's opinions derived from neuroimaging as well as the report of defense expert, Dr. Wortzel. The motion references an article by a psychiatrist who does not perform DTI and has no training or firsthand experience in imaging.

4. DTI is simply another MRI sequence type and has been used since the early 1990's. There are a growing number of clinical facilities throughout the U.S., Asia, and Europe that are routinely relying on DTI to diagnose axonal injury from TBI, multiple sclerosis, and hypoxic-ischemic encephalopathy. There are good scientific reasons for this. Since 1994, there have been 10,282 peer-reviewed articles published on DTI; 8,481 articles concerning DTI and the brain and another 1,194 articles concerning DTI and the spinal cord; and 982 articles concerning DTI and head trauma. There were 124 peer reviewed articles published concerning DTI and the brain in 2014 alone, compared with 58 in 2007 (113% increase). These numbers reflect the exponential increase in scientific and clinical interest in an imaging method that has repeatedly demonstrated the unique and clinically relevant ability to detect microscopic alterations in white matter fibers (axons), reflecting injury to these same structures. In 2001, DTI was FDA approved for use on a clinical magnet (Philips) and then, in 2003, General Electric followed suit. DTI was initially used clinically to locate white matter tracts involved in motor function to help neurosurgeons avoid inducing paralysis during tumor and epilepsy surgery. This began shortly after FDA approval throughout the country and I performed this, in addition to fMRI of language areas in patients, which I had been performing since 1994. DTI is performed throughout the western world in all continents. It is no different from any other imaging sequence in terms of its accessibility. A full explanation of the science behind DTI is given below starting at paragraph 19.

5. The motion also suggests (pages 3 and 4) that DTI is inappropriate for use on single subjects and is not standardized. This is misleading.

An analogous situation is functional MRI (fMRI) for presurgical mapping of brain tumors and epilepsy in patients. A set of CPT codes was assigned to fMRI by the AMA in 2007 (70554, 70555, 96020) allowing radiology facilities to bill insurance companies. The following is from the Practice Guidelines published by the American College of Radiology concerning fMRI (*http://www.asfnr.org/docs/IMRI_Clinical_Guidelines.pdf): "These guidelines are an educational tool designed to assist practitioners in providing appropriate radiologic care for patients... The ultimate judgment regarding the propriety of any specific procedure or course of action must be made by the physician or medical physicist in light of all the circumstances presented. Thus, an approach that differs from the guidelines, standing alone, does not necessarily imply that the approach was below the standard of care. To the contrary, a conscientious practitioner may responsibly adopt a course of action different from that set forth in the guidelines when, in the reasonable judgment of the practitioner, such course of action is indicated by the condition of the patient, limitations on available resources, or advances in knowledge or technology subsequent to publication of the guidelines... The practice of medicine involves not only the science but also the art of dealing with the prevention, diagnosis, alleviation, and treatment of disease... all that should be expected is that the practitioner will follow a reasonable course of action based on current knowledge, available resources, and the needs of the patient to deliver effective and safe medical care."*

Regarding qualifications of responsible personnel: *"The physician supervising and interpreting fMRI must be clinically informed and understand the specific questions to be answered prior to the procedure in order to plan and perform it safely and effectively. Additionally, physicians performing this procedure should have experience or formal training in performing fMRI."*

A similar set of Guidelines is posted by the ASFNR regarding DTI (*http://www.asfnr.org/wp-content/uploads/ASFNR-Guidelines-for-DTI.pdf: "As for DTI acquisition, the specifics of DTI post-processing largely depend on the hardware/ software configuration available for image processing and the preferences and experience of the user."* It is evident that the clinical governing bodies, the ACR, and ASFNR do not require strict standardization of methods. Regarding Findings and Impressions, the ASFNR Guidelines continues: *"Scalar parametric maps: Although these maps are inherently quantitative, they should be described qualitatively. Quantitative reporting (e.g. FA values in a region of interest) is discouraged unless also provided is an estimate of the normal range to be expected for the reported parameter. "* Note that I follow this guideline and report both qualitative and quantitative results with normal ranges provided.

They include the disclaimer: *"It is critical that physicians basing clinical decisions on DTI be familiar with the limitations and potential pitfalls inherent to the technique... their results should be interpreted in conjunction with conventional anatomical imaging as well as other clinical data including physical examination..."* I have been doing clinical research using DTI for eleven years as well as publishing peer-reviewed articles on clinical methods regarding the use of advanced neuroimaging for TBI. Furthermore, I routinely integrate "other clinical data" such as history, neurobehavioral examination, and all other relevant medical records.

6. The motion states in page 4, *"Dr. Benson seeks to use DTI to diagnose an organic brain injury tied to a specific incident. "* This is not true. Imaging findings are used in conjunction with other sources of evidence. The diagnosis of a traumatic brain injury is never made using imaging as definitive proof. This is not different from the use of a spinal MRI revealing a disc herniation. The imaging finding is used in the context of other relevant facts. I used all of the relevant medical facts, including other sequences of MRI, i.e. SWI, FLAIR and GE, as well as all medical facts obtained through my medical evaluation of the patient in person and review of medical records in arriving at my conclusions to reasonable degree of medical probability. *No standalone imaging technique* allows for unequivocal determination of etiology absent clinical information. This is why radiologists require historical information and usually conclude their reports with "clinical correlation is advised." Again, all radiologic scans often result in differential diagnoses. DTI images reveal brain regions showing significantly reduced directional diffusion. This is critically important information since it is quantitative and objective. When white matter fibers (axons) are injured, DTI detects this change. We certainly feel that the probative value of DTI for mTBI is high. There are no absolutes in medicine or science.

7. Fluid Attenuated Inversion Recover, FLAIR, is a MRI sequence, different than DTI, which is utilized to look for macroscopic damage in the brain. These findings must be clinically correlated to the behavioral and neurological changes shown by the patient. There are regions of typical distribution of lesions in Traumatic Brain Injury shown by FLAIR. FLAIR is scientifically valid and reliable as an MRI sequence. In Ms. White's case, FLAIR/T2 imaging revealed two areas of hyperintensity, which I concluded, to a reasonable degree of medical probability, to be brain abnormalities resulting from the 8/17/11's farming incident.

8. Susceptibility Weighted Imaging, SWI, is an MRI sequence, different than DTI, utilized to detect bleeding residue, hernosiderin, in the brain. These findings must be clinically correlated to the behavioral and neurological changes shown by the patient. SWI is scientifically valid and reliable as an MRI sequence. In Ms. White's case, SWI imaging revealed contusion (bleeding) on the left frontal lobe, which I concluded, to a reasonable degree of medical probability, to be a brain abnormality resulting from the 8/17/1 I's farming incident.

9. Diffusion Tensor Imaging, DTI, is an MRI sequence used to examine the microstructure of the white matter of the brain, allowing for the detection of microscopic pathology or abnormality of the white matter (axons) in the brain. DTI is an FDA approved, peer reviewed and approved, commercially marketed, and widely available MRI method, which has been in clinical use for many years. DTI is scientifically valid and reliable as an MRI sequence.

10. The motion suggests that DTI is *unreliable.* DTI is not appropriately termed 'unreliable.' It has been used since 1994 and is FDA-approved with clinical guidelines issued by the clinical radiologists governing body. It is already being

done clinically and is an add-on sequence for preoperative protocols. In a recent article entitled, A Decade of DTI in Traumatic Brain Injury: 10 years and 100 Articles Later, the *American Journal of NeuroRadiology* (2013) reported the findings of Hulkower, et al. who concluded that "A unifying theme can be deduced from this large body of research: DTI is an *extremely useful and robust tool for the detection of TBI-related brain abnormalities."*

11. The motion states in page 5, *"Dr. Benson's findings derived from DTI would not assist a trier of fact, because they only show, if anything, that Plaintiff has one or two small white matter lesions. That information is not relevant to a fact finder determination of whether Plaintiff's incident with the compact tractor caused an organic brain injury"*. This is misleading. The FLAIR scan reveals two white matter lesions while the DTI reveals more. DTI is like any other imaging technique. Imaging does not reveal the etiology of a particular pathology all by itself DTI reveals damage to axons. DTI is used as other imaging modalities are used-to add important information regarding a differential diagnosis. In the case of TBI, DTI is used in conjunction with clinical history, physical examination, and other MRI sequences/scans. TBI abnormalities have a predilection for particular locations on DTI and on FLAIR/T2 and SWI. We apply the same principles that are used routinely in the diagnosis of stroke, MS, degenerative disease, and other neurologic diagnoses. Other pathologies have different patterns of abnormality on imaging, which are distinct from TBI. It is disingenuous to assert that DTI should be, in and of itself, diagnostic of TBI or any other pathology. No radiologic technique is used in isolation. Radiologic scans reveal intensity differences based on tissue contrast. Conventional MRI scans, CT, and x-ray reveal *imaging patterns* only. DTI should not be held to a different standard than other imaging techniques.

12. The motion further states in page 5, that "Dr. Benson's report fails to adequately address the possibility that the white matter lesion visible on the DTI scan (although the lesions are seen on FLAIR) could have resulted from a horse knee to the head of plaintiff suffered in June of 2012". Again, this is misleading since DTI cannot address the etiology as explained above. However, I addressed this possibility in my Comprehensive Medical Report (exhibit 2), ruling out to a reasonable degree of medical probability that the medical condition and findings on MRI were due to the horse incident. I then more specifically addressed it in the rebuttal to Dr. Wortzel's report, Exhibit 3, pages one through three, inclusive.

13. The motion goes on to state on page 6 "Dr. Benson fails to explain other possible alternatives for the small white lesions detected on DTI (again, FLAIR). For example, Dr. Benson admits that "a few white matter hyper-intensities on FLAIR/T2 in a 60 year old is not unusual." (see Benson Rebuttal, at page 3) "But then, with no scientific backing, Dr. Benson disregards the possibility that the white lesions are simply the result of age." This is untrue. On Exhibit 3, page 3, the Rebuttal report goes on to mention, right after the Motion chooses to end the quote, as follows: "However, there are a number of reasons to consider these lesions as traumatic." I fully discuss the medical and scientific reasons why the two hyperintensity lesions are more likely to be traumatically in nature than "other possible alternatives" which I ruled out to a reasonable degree of medical probability (bottom of page 3 and top of page 4 of the Rebuttal report, Exhibit 3).

14. The motion also suggests that I failed *"to adequately consider or explain why the white matter lesions are so definitely attributable to the 2011 incident and not to Plaintiff's psychiatric issues, for which she was diagnosed and treated in the late 1980s"*. Again, this is simply untrue. I fully considered her history and upon review of the data and conclusions of Dr. James Berry, neuropsychologist at Craig's Hospital. As reported in pages 5 through 6, Dr. Berry's conclusion was that her psychiatric diagnosis was correctly not carried forward and was a misdiagnosis. Dr. Berry did not validate the diagnosis of the late 1980s in his formulation but emphasized that her frontal-executive dysfunction and her altered emotional processing were consequences of her traumatic brain injury o 201 1. Furthermore, the medical literature does not conclusively suggest DTI abnormalities are associated with pure psychiatric diagnoses. Therefore, based upon neuropsychological data and testimony, along with my training, education and experience, I concluded that the lesions observed on MRI are attributable to the 2011 farming incident, again to a reasonable degree of medical probability.

15. My summary in rebuttal is that "Miriam White sustained a significant traumatic injury to her frontal lobes bilaterally, which has left her cognitively and emotionally permanently impaired. She was high functioning and active prior to the injury. Her injury/accident of 8/17/1 1 was the much more significant injury and rendered her vulnerable to the

mild concussion of 6/10/12. Her imaging findings match the biomechanics, chronic symptoms, neurobehavioral and neuropsychological findings. The right parietal injury of 6/10/12, while inducing a mild concussion, does not explain her clinical deficits that began when her head was crushed under the weight of a heavy hay bale on 8/17/11." I expressed these opinions to a reasonable degree of medical probability under the pains and penalties of perjury, using my best judgment, based upon my training, education, research, and clinical experience.

16. The motion indicates, on page 7, *"As Dr. Wortzel notes, when used in the litigation context, DTI Imaging is subject to interpretations that are subjective rather than evidence-based and thus unreliable in this context."* Dr. Wortzel has been *critiqued* in the literature for the position and opinions he has taken. Recently, in an article published in the American Journal of Bioethics Neuroscience April-June, Volume 5, Number 2, 2014, the authors, Michael Lipton and Eric Bigler state, "The misleading and often entirely unsubstantiated opinions and positions of Wortzel, Tsiouris, and Filippi (2014), in opposition to diffusion tensor imaging (DTI) as a useful measure in mTBI, are at odds with the clear consensus of the scientific literature regarding mild traumatic brain injury (mTBI), its clinical assessment, and its natural history. The authors' critique contains numerous errors." (Please see Hulkower et al., A Decade of DTI in Traumatic Brain Injury: 10 years and 100 articles later, paragraph 10 for the majority view) Attached as Exhibit 6 is a true and correct copy of Lipton & Bigler, *"Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI Patients"* in AJOB Neuroscience, 2014.

17. I have been actively involved in MR imaging research since 1992 and in Diffusion Tensor Imaging (DTI) since 2004. I am a fellowship-trained behavioral neurologist who has evaluated and treated hundreds of patients with head trauma and have been engaged in brain imaging research using advanced MRI methods for 18 years. My focus has been TBI imaging for 7 years at the MR Research Program at Detroit Medical Center together with an MR scientist, E. Mark Haacke, Ph.D. My clinical and research activities are even more focused on TBI imaging since co-founding the Center for Neurological Studies in 2011. I published a seminal paper in 2007 delineating the alterations in DTI parameters in TBI and the correlation of DTI (FA) with injury severity, including mild TBI. Along with the members of my research group, I have presented or published extensively on the use of DTI for TBI emphasizing proper methodology and precautions to avoid misinterpretation. A true and correct list of my research in DTI and lecturing is included in my Curriculum Vitae, Exhibit 1 to this declaration.

18. On June 2-3, 2010, in recognition of the already demonstrated importance of diffusion tensor imaging to diagnose TBI, the U.S. Army Telemedicine and Advanced Technology Research Command (TATRC) sponsored the "Diffusion MRI TBI Roadmap Development Workshop", wherein the acknowledged purpose was, *"DTI has detected abnormalities associated with brain trauma at several single centers and the workshop seeks to identify and remove barriers to rapid translation of advanced diffusion MRI technology for TBI... in order to expedite getting the benefits of diffusion MRI to reach those who need it most, especially injured soldiers and veterans."* As one of 50 or so "experts from academia, industry, government agencies and several European nations," I presented in a session entitled, "Experience in Neuroimaging Translation to Clinical Use." My talk entitled, "Global and Voxel-based approaches to DTI in TBI" included a comprehensive approach to imaging mild TBI, which was the culmination of over six years of peer-reviewed published research on DTI and TBI. I used both group and single cases to demonstrate the clinical validity and reliability of DTI in TBI. In addition to demonstrating the excellent correlation between DTI and injury severity, I showed the repeatability of DTI for a single mTBI case scanned in two different cities, and for a different mTBI case scanned twice 6 weeks between scanning sessions. I also used a third mTBI case to demonstrate the excellent correspondence between hemorrhage location (using susceptibility imaging) and abnormally low FA on DTI in these cases. Other speakers presented data showing the correlations of DTI with neurocognitive outcome and experience using DTI on Iraq war veterans.

19. A traditional MRI shows the structure of the brain. The majority of people who have sustained mild traumatic brain injury ("mTBI") have normal MRI findings, even when they have significant impairment. An overwhelming majority of people with mTBI have normal CT scans, even with significant impairments. In fact, I have personally been involved

with patients in coma who have normal CT Scans. And so it appeared in the case of Ms. White after the initial recognized bleed.

20. DTI examines the microstructure of the white matter of the brain, allowing for the detection of microscopic pathology or abnormality of the white matter. DTI is a more sensitive technique that can reveal abnormalities that are not visible on standard MRIs. In fact, a major drive in the research and development of DTI has been its ability to detect that which is largely invisible to MRI and CT.

21. DTI measures the direction of movement or flow (known as diffusion) of water molecules through tissue. DTI is based upon the basic biophysics of the flow of water. With *no barriers* to flow, water will move in an isotropic distribution, which means it will move equally in all directions. If there *are barriers* to flow, it will move anisotropically or unequally in all directions.

22. DTI has the ability to measure the distribution of water throughout the brain by specifically measuring the flow of water in the many voxels of the brain. Voxels are like the pixels in a digital camera. Unlike an image from a digital camera, however, each of the MRI pixels has *three* dimensions, the left-right and up-down dimensions of the slice as well as the thickness of the slice. When multiple slices are stacked atop one another, the result is the fl.111 volumetric representation of the brain.

23. DTI measures the distribution of water in each voxel with the degree of anisotropy (non-sphericity) expressed as a fraction of the total diffusion, i.e. fractional anisotropy (FA), which can range between zero (completely isotropic diffusion) and one (completely anisotropic diffusion).

24. White matter of the brain is comprised of axons, which are long processes extending from the nerve cells that constitute the gray matter. Axons are organized into thick, tubular tracts that extend from one brain region to another similar to electrical cables. Water diffusion is much greater along the long axon than across it and, therefore, has a relatively anisotropic distribution (higher FA). Closed head injury (or non-penetrating TBI), induced by sudden acceleration or deceleration of the head, results in widely scattered damage to white matter fibers known as "diffuse axonal injury." This damage includes segmental breakdown in the outer membrane of the axon, increasing diffusion in the short axis dimension, leading to more isotropic distribution (decreased FA). In other words, in the white matter of a normal/healthy brain, the direction of water diffusion is very uniform. Injury disrupts the normal structure of white matter (axons) leading to a less uniform direction of diffusion.

25. Since microscopic traumatic injuries, which are not visualized on standard clinical MRI scans, cause a relatively modest reduction in FA that cannot be seen by visual inspection, quantitative analysis of images is performed, whereby a TBI patient's FA images are statistically analyzed using a set of non-TBI controls' brain images as the reference standard. This method is performed in an automated fashion on the white matter *globally, regionally* and *voxel-by-voxel* after co-registration of brain images or tracts into a standard space. Comparing TBI images against a set of non-TBI brain images has been demonstrated by my group and other groups to be a sensitive, reliable, and objective means of distinguishing TBI from non-injured brains.

26. We have described our methods along with making our methods of analysis available by publishing them in peer reviewed journals (*See Curriculum Vitae).* We have also published the scientific rationale and the basis for the choice of using fractional anisotropy (FA) as the parameter to compare a patient's brain image to controls. This methodology has been utilized by multiple imaging specialists and is well-described in the literature.

27. We use DTI in our medical practice as one more piece of data and attempt to synthesize several independent lines of evidence including acute injury characteristics, chronic symptoms, neurobehavioral findings, neuropsychological findings and imaging, including other scans. This is the age-old Differential Diagnosis procedure used in Medicine.

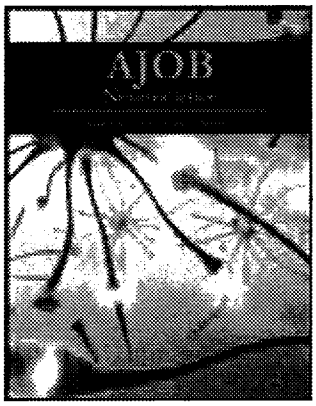
The clinical history and examination helps by limiting the differential diagnosis. Imaging then functions to further limit the possibilities. As most diseases and disorders do, TBI has a particular "profile" on imaging that emerges from the biomechanics, pathology, and pathophysiology that are different from other disorders.

28. DTI analysis has a known rate of error. In fact it is 0.4%. We use 2 standard deviations, which represents 95% of the normal values. We use a 1-tailed test since we are explicitly looking only for reduced FA in the patients. That means our false positive rate would be 0.025. We additionally impose a post hoc correction (cluster analysis) to further reduce the false positive rate by keeping only regions with reduced FA, which are significantly larger than observed in the controls. The false positive (error) is 0.025 x 0.16 (one standard deviation) = 0.004 (0.4%). Finally, we do a second statistical analysis using tract-based statistical analysis (TBSS) and only keep voxels that are significant on TBSS (t > 2). The methods employed are conservative by design.

29. General acceptance-DTI is acknowledged to be the best method for imaging white matter injury (see Defense Centers of Excellence Clinical Recommendation, July 2013, *https://dvbic.dcoe.mil/sites/default/files/2013_NeuroimangingR#ecs_CR_070#81#3_13* 50.pdf) It is generally accepted in the scientific community throughout the peer reviewed literature that DTI is a reliable and accurate tool to detect microscopic damage done to the white matter of the brain.

I declare under the penalty of perjury under the laws of the United States of America that the foregoing (including the statements in the attached reports) is true and correct.

End of Document



AJOB Neuroscience

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uabn20

Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI Patients

Michael L. Lipton [a] & Erin D. Bigler [b]

[a] Albert Einstein College of Medicine and Montefiore Medical Center

[b] Brigham Young University

Published online: 18 Mar 2014.

To cite this article: Michael L. Lipton & Erin D. Bigler (2014) Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI Patients, AJOB Neuroscience, 5:2, 41-43, DOI: 10.1080/21507740.2014.884191

To link to this article: http://dx.doi.org/10.1080/21507740.2014.884191

EXHIBIT 3

issues that concerned Wortzel and colleagues, such research can be a valuable aid in dealing with the basic inverse inference problem pertaining to DTI interpretation in medicolegal settings.

REFERENCES

Fisher, R. A. 1973. *Statistical methods and scientific inference, 3rd ed.* London, UK: Collier Macmillan.

Krueger, J. 2001. Null hypothesis significance testing: On the survival of a flawed method. *American Psychologist* 56(2): 16–26.

Nickerson, R. S. 2000. Null hypothesis significance testing: A review of an old and continuing controversy. *Psychological Methods* 5(2): 241–301.

Trafimow, D. 2003. Hypothesis testing and theory evaluation at the boundaries: Surprising insights from Bayes's theorem. *Psychological Review* 110(3): 526–535.

Trafimow, D. 2005. The ubiquitous Laplacian assumption: Reply to Lee and Wagenmakers. *Psychological Review* 112(3): 669–674.

Trafimow, D. 2006. Using epistemic ratios to evaluate hypotheses: An imprecision penalty for imprecise hypotheses. *Genetic, Social, and General Psychology Monographs* 132(4): 431–462.

Trafimow, D., and S. Rice. 2009. A test of the null hypothesis significance testing procedure correlation argument. *Journal of General Psychology* 136(3): 261–269.

Wortzel, H. S., A. J. Tsiouris, and C. G. Filippi. 2014. The potential for medicolegal abuse: Diffusion tensor imaging in traumatic brain injury. *AJOB Neuroscience* 5(2): 9–15.

Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI Patients

Michael L. Lipton, Albert Einstein College of Medicine and Montefiore Medical Center
Erin D. Bigler, Brigham Young University

As clinicians and scientists, we believe scientific evidence and prudent clinical practice form the proper basis for determining the utility of diagnostic measures, which should subsequently inform forensic use. The misleading and often entirely unsubstantiated opinions and positions of Wortzel, Tsiouris, and Filippi (2014), in opposition to diffusion tensor imaging (DTI) as a useful measure in mTBI, are at odds with the clear consensus of the scientific literature regarding mild traumatic brain injury (mTBI), its clinical assessment, and its natural history. The authors' critique contains numerous errors. We focus on four areas: (1) the clinical reality of mTBI, (2) the true substance of the scientific evidence supporting use of DTI in mTBI, (3) the authors' erroneous and off-target opinions regarding DTI analysis, and (4) critical appraisal and integration of clinical information for diagnosis of mTBI.

First, an underlying theme of the authors' arguments claims that lasting sequelae from mTBI is not a clinical reality. For example, "best available evidence does not support notions that mTBI results in long-term cognitive impairments" (12). mTBI is a reality that results in lasting sequelae in a substantial minority (Bigler et al. 2013; McMahon et al. 2014). mTBI is modeled in animals, yielding reproducible microstructural neuropathological and behavioral findings, as well as with finite biomechanical models of human mTBI. The impact of mTBI cannot be argued away by focusing on the majority who recover. Clinical, scientific, and even forensic focus must be on the affected minority. Moreover, traditional neuropsychological approaches are problematic in assessing the cognitive effects of mTBI; they were never designed to assess subtle but important deficits.

Second, the authors' critique of DTI challenges the "believability" of quantitative DTI findings, juxtaposing visual detection of spinal disk herniation and detection of microscopic mTBI pathology. They imply that because the microstructural abnormality cannot be "seen" without quantification its existence is in question. This "straw man" argument would also imply that other neuroimaging findings that cannot be seen without quantification, such as spectroscopic and perfusion-based detection of tumor infiltration into normal-appearing white matter, are not real or reliable. The substance and implications of the American Society for Functional Neuroradiology (ASFNR) guideline are mischaracterized to support the authors' position: "the guidelines . . . detailing the limitations in using DTI clinically, especially at the individual level and when analyzed by voxel-based techniques" (11). The guideline deals exclusively with clinical use in patients and does not isolate single patient

Address correspondence to Michael L. Lipton, MD, PhD, FACR, The Gruss Magnetic Resonance Research Center, Departments of Radiology, Psychiatry and Behavioral Sciences and The Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA. E-mail: michael.lipton@einstein.yu.edu

assessment for scrutiny. Important cautions are listed, but the message is: When DTI is used in accordance with the guideline, reliable clinical use can be achieved. Assessment of DTI parameters is not singled out as most of concern; greater attention is paid to limitations of tractography and its misuse.

Third, the authors argue that method variance renders DTI research studies and clinical assessments inconclusive. Statements similar to "Numerous factors can influence results without current consensus as to the best parameters" (10) recur throughout the article, often without supporting citations, and imply that method variance across published studies undermines reliability and leads to (even willful) type 1 errors (i.e., false positives). The authors claim differences in acquisition, analysis, and so on preclude salient conclusions. This approach completely misses the point of a very large literature, which speaks with essentially one voice: Low fractional anisotropy (FA) is characteristic of TBI patients, *despite significant variability across studies* (e.g., Aoki et al. 2012; Hulkower et al. 2013; Niogi and Mukherjee 2010; Shenton et al. 2012). Even if we assume that DTI metrics, such as FA, vary across scanners and institutions, we will not encounter bias in the identification of abnormalities in any individual; this issue is simply not germane to a properly conducted analysis. What does matter is that patient and control data are acquired, processed, and analyzed in the same manner and that temporal variation be substantially less than the magnitude of the effect sought. This latter requirement, of course, is out of concern for type 2 errors (i.e., false negatives). It is even more illogical to expect that method variance would yield regionally localized "abnormalities" that in fact represent type 1 errors. Bias due to acquisition and processing variation across subjects, if it were in fact a problem, would lead to a uniform bias at all brain locations. This would be the result, not the manufacture of lesions, if it were true that "technological parameters can be manipulated in ways that impact results" (10). Digging deeper into the authors' case for fatal variability of DTI metrics, we again note a void of supporting evidence. The authors conclude that "unlike traditional MR sequences ... the very existence of a lesion ... in any given single patient identified via DTI is fundamentally questionable" (10). The only relevant citation (Vollmar et al. 2010), however, is completely misconstrued by the authors; it in fact documents the high degree of intra- and interinstitutional fidelity of FA measurements, also reported by others (Fox et al. 2012). Such misunderstanding of the science suffuses the discussion of technical issues. Glib citations such as "Not too surprisingly, when the same DTI data set was provided for analysis to nine different research groups ..., nine different results were obtained" (11) entirely misrepresent the substance of an unpublished abstract to suit the authors' bias. The authors of the cited abstract actually conclude: "This serves as a reminder of what is being tested under the null hypothesis, i.e. just because one method finds a particular difference, it does NOT mean that there were NO other differences—a fact that can be easily overlooked" (Jones et al. 2007, 74). The concern is not that any of the findings are not "real", but that additional real findings may be missed in any analysis.

Another methods-specific argument is that abnormalities could occur simply by chance: "Statistical science also portends problems for the analysis of DTI" (11). After exaggerating the typical number of simultaneous comparisons by at least 50% and invoking a "typical 5% chance of error," the authors conclude that "statistical realities represent yet another potential avenue for abuse" (11). This rudimentary analysis does not acknowledge that 5% is not a typical threshold and that corrections should be and are made for multiple testing (not just that they "fortunately exist"). Most glaring is the authors' omission of spatial clustering, which dramatically reduces type 1 errors. DTI analyses do not seek individual voxel abnormalities, but ask, "What is the likelihood that hundreds of voxels comprising a contiguous tissue volume several milliliters in size will all appear abnormal by mere chance alone?" Along these lines, the authors state, "Given that even carefully selected healthy controls will feature areas of 'abnormality' ..., it should be anticipated that most unselected patients/litigants will feature areas of abnormality when compared to such normative databases" (11). This is a gross misrepresentation of Kraus and colleagues (2007), in the same way that Wortzel misused it previously (Wortzel et al. 2011). The criterion for "abnormality" in the Kraus article (1SD) is well within all concepts of normal. That some controls had some regions of interest outside of 1SD is expected and does not bear on the finding that patients had significantly more regions of interest outside of 1SD (Kraus et al. 2007, Figure 5). This citation provides no basis whatsoever for inferring that normals will have "abnormalities" when reasonable thresholds for abnormality are employed. This sentence and especially its italicized emphasis have no basis in the cited paper or any scientific communication.

Fourth, diagnosis of mTBI, or any other disorder, is based on integration of clinical information, not the result of one diagnostic test. The authors offer another "straw man" argument that insinuates DTI should not be used as a standalone definitive diagnostic test, a use for which it has not been proposed. The realities of DTI use in the clinic entail weighing the strength of all clinical evidence. The authors argue that "neuropsychiatric conditions are common in the general population, and are often present in individual litigants. The potential impact of common psychiatric conditions on DTI findings is well illustrated" (11). Placing these two sentences back to back is blatantly misleading. The authors cite White and colleagues (White et al. 2008), who reviewed studies of psychiatric patients, not of healthy people who unknowingly harbor as-yet-undiagnosed psychopathology. No literature exists to support that such individuals can be identified with DTI. Moreover, the authors do not consider that the literature on psychiatric diagnosis is comprised of studies detecting modest group differences, whereas TBI studies have specifically shown that individuals, though not all individuals, can differ from population norms to a degree that normals do not (see Hulkower et al. 2013). It is not at all clear that DTI abnormalities in

individuals with as-yet-undiagnosed psychiatric disease can be detected in the way that such abnormalities may be detected in TBI patients. Moreover, statements, such as "early life stress and/or parental verbal abuse may result in differences in white matter integrity as measured by DTI" (11) give equal weight to single reports of small samples and fail to assess subject/control overlap and whether any inference at the individual level might even be supported. In stark contrast, the overwhelming consensus of a substantial body of scientific inquiry supports DTI for detecting pathology in mTBI patients.

ACKNOWLEDGMENTS

The authors acknowledge the critical review and helpful suggestions of Jacqueline A. Bello, MD, FACR.

REFERENCES

Aoki, Y., R. Inokuchi, M. Gunshin, N. Yahagi, and H. Suwa. 2012. Diffusion tensor imaging studies of mild traumatic brain injury: A meta-analysis. *Journal of Neurology, Neurosurgery & Psychiatry* 83(9): 870–876.

Bigler, E. D., T. J. Farrer, J. L. Pertab, K. James, J. A. Petrie, and D. W. Hedges. 2013. Reaffirmed limitations of meta-analytic methods in the study of mild traumatic brain injury: A response to Rohling et al. *Clinical Neuropsychology* 27(2): 176–214.

Fox, R. J., K. Sakaie, J. C. Lee, et al. 2012. A validation study of multicenter diffusion tensor imaging: reliability of fractional anisotropy and diffusivity values. *American Journal of Neuroradiology* 33(4): 695–700.

Hulkower, M. B., D. B. Poliak, S. B. Rosenbaum, M. E. Zimmerman, and M. L. Lipton. 2013. A decade of DTI in traumatic brain injury: 10 Years and 100 articles later. *American Journal of Neuroradiology* 34(11): 2064–2074.

Jones D. K., X. A. Chitis, D. Job D., P. L. Khong, L. T. Leung, S. Marenco, S. M. Smith, and M. R. Symms. 2007. What happens when nine different groups analyze the same DT-MRI data set using voxel-based methods? *Proceedings of the International Society for Magnetic Resonance in Medicine* 15: 74.

Kraus, M. F., T. Susmaras, B. P. Caughlin, C. J. Walker, J. A. Sweeney, and D. M. Little. 2007. White matter integrity and cognition in chronic traumatic brain injury: A diffusion tensor imaging study. *Brain* 130(Pt 10): 2508–2519.

McMahon, P., A. Hricik, J. K. Yue, et al. 2014. Symptomatology and functional outcome in mild traumatic brain injury: Results from the prospective TRACK-TBI study. *Journal of Neurotrauma* 31(1): 26–33.

Niogi, S. N., and P. Mukherjee. 2010. Diffusion tensor imaging of mild traumatic brain injury. *Journal of Head Trauma Rehabilitation* 25(4): 241–255.

Shenton, M., H. Hamoda, J. Schneiderman, et al. 2012. A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain Imaging and Behavior* 6(2): 137–192.

Vollmar, C., J. O'Muircheartaigh, G. J. Barker, et al. 2010. Identical, but not the same: intra-site and inter-site reproducibility of fractional anisotropy measures on two 3.0T scanners. *Neuroimage* 51(4): 1384–1394.

White, T., M. Nelson, and K. O. Lim. 2008. Diffusion tensor imaging in psychiatric disorders. *Topics in Magnetic Resonance Imaging* 19(2): 97–109.

Wortzel, H. S., M. F. Kraus, C. M. Filley, C. A. Anderson, and D. B. Arciniegas. 2011. Diffusion tensor imaging in mild traumatic brain injury litigation. *Journal of the American Academy of Psychiatry and the Law* 39(4): 511–523.

Wortzel, H. S., A. J. Tsiouris, and C. G. Filippi. 2014. The potential for medicolegal abuse: Diffusion tensor imaging in traumatic brain injury. *AJOB Neuroscience* 5(2): 9–15.

Functional Magnetic Resonance Imaging in Court

Lyn M. Gaudet, The Mind Research Network
Julia R. Lushing, The Mind Research Network
Kent A. Kiehl, The Mind Research Network

It was more than 30 years ago that neuroimaging evidence was first presented in criminal court in the United States. The charges included two counts of murder and one count of attempted murder, and the defendant asserted the insanity defense. Defense experts testified that the defendant suffered from schizophrenia with prominent psychotic features and was thus legally insane at the time of the crimes. Prosecution experts testified that the defendant did not have psychosis and was legally sane at the time of the crimes. The defense presented a computer-assisted tomography (CAT) scan that revealed the defendant had widened sulci and enlarged ventricles. These structural characteristics are

Address correspondence to Lyn M. Gaudet, The Mind Research Network, 1101 Yale Blvd, Albuquerque, NM 87106, United States. E-mail: lkiehl@mrn.org

Original Investigation

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

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

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

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

DESIGN Observational study.

SETTING University of Barcelona and Hospital Clinic de Barcelona, Barcelona, and Institut Guttmann-Neurorehabilitation Hospital, Badalona, Spain.

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

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

MAIN OUTCOMES AND MEASURES Signal amplitude and functional connectivity during the resting state, tractography related to DMN, and the association between signal amplitudes and cognitive outcome.

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

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

JAMA Neurol. 2013;70(7):845-851. doi:10.1001/jamaneurol.2013.38
Published online May 20, 2013.

Supplemental content at jamaneurology.com

CME Quiz at jamanetworkcme.com and CME Questions

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

Corresponding Author: Carme Junque, PhD, Department of Psychiatry and Clinical Psychobiology, University of Barcelona, C/Casanova 143, 08036 Barcelona, Spain (cjunque@ub.edu).

EXHIBIT 4

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

Resting-state fMRI studies have recently emerged as a useful tool for investigating brain functional connectivity after severe TBI[4,8-11] and in subjects with mild TBI examined during the early stages.[12,13] These studies provide information about brain activity and connectivity in the absence of task performance, a condition that allows researchers to investigate patient populations with broader ranges of injury severity, because no specific cognitive ability is required. Advanced neuroimaging processing tools provide information about brain activity and functional connectivity during resting-state fMRI. Low signal fluctuations that occur during rest have been shown to reflect strong connectivity between functionally related brain regions.[14,15]

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

In addition to disrupted functional connectivity, studies of white matter integrity using diffusion tensor imaging[20-23] have shown that structural connectivity is also greatly affected after TBI. In a previous study,[5] we found that disrupted structural connectivity after traumatic axonal injury was responsible for altered functional connectivity during working memory performance. In the current research, we used new advances in resting state-related fMRI methods to determine whether the amplitude of spontaneous low-frequency fluctuations in brain activity and DMN connectivity may be sensitive biomarkers for cognitive dysfunction after TBI. To this end, we measured the amplitude of resting-state blood oxygen level-dependent signal fluctuations in the whole brain and the DMN, studying global and network-based functional connectivity, as well as structural connectivity of the main fasciculi within the DMN.

Methods

Study Participants

Twenty patients with chronic and diffuse TBI were recruited from the Head Injury Unit of the Guttmann Institute-Neurorehabilitation Hospital. The criteria followed for sample selection have been described elsewhere.[5] This study is part of a project on long-term impairment of connectivity in diffuse TBI, and some results already have been published.[5,24] Patients' demographic and clinical characteristics are summarized in **Table 1**. Patients underwent magnetic resonance imaging (MRI) a mean (SD) of 4.1 (1.2) years after injury, and all showed microbleeds as a sign of diffuse disease in the T2* and fluid-attenuated inversion recovery sequences. Table 1 provides detailed clinical and neuroradiological characteristics for each patient in the study. The cause of TBI was motor vehicle crashes in all cases.

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

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

Abbreviations: GCS, Glasgow Coma Scale; MRI, magnetic resonance imaging; TAI, traumatic axonal injury; TBI, traumatic brain injury.

[a] $t = 0.71$; $P = .48$.

[b] $t = 0.57$; $P = .57$.

[c] $t = 2.29$; $P = .82$.

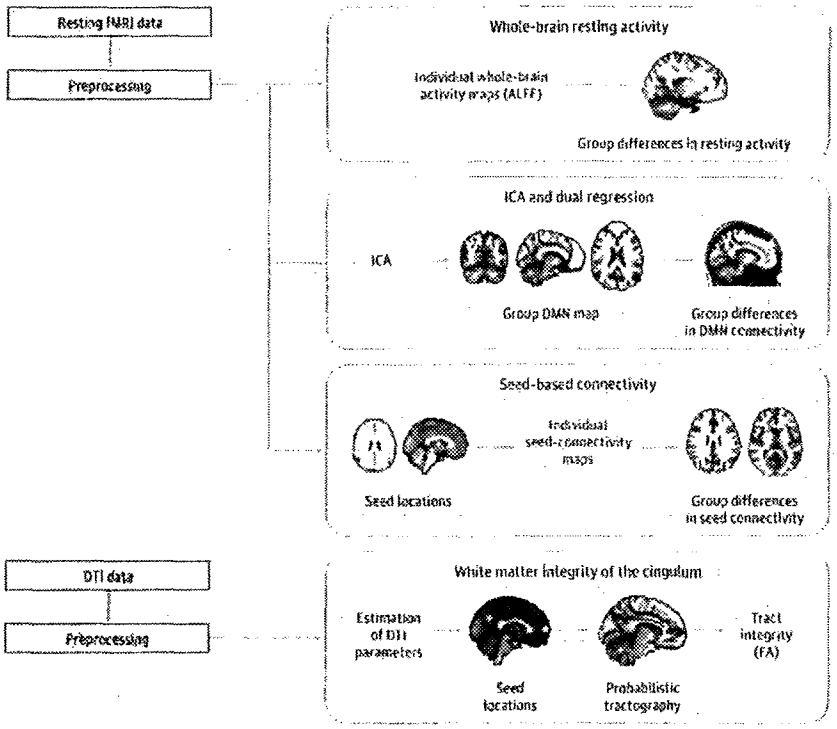
The control group comprised 17 healthy volunteers matched by age, sex, and educational level. None had a history of neurological or psychiatric diseases. Their demographic characteristics are provided in Table 1.

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

Image Acquisition

Data were acquired with a Siemens Magnetom Trio Tim syngo 3-T system at the Centre de Diagnostic per la Imatge of the Hospital Clinic, Barcelona. A high-resolution T1-weighted structural image was obtained for each subject with an MPRAGE (magnetization-prepared rapid acquisition gradient-echo) 3-dimensional protocol (repetition time [TR], 2300 milliseconds; echo time [TE], 3 milliseconds; inversion time, 900 milliseconds;

Figure 1. Image processing and analysis methods



(1) Whole-brain analysis of the amplitude of low-frequency fluctuations (ALFF); (2) independent component analysis (ICA) and dual-regression analysis of the default mode network (DMN); (3) connectivity analysis using the predefined DMN regions of interest (ROIs); and (4) diffusion tensor imaging (DTI) tractography analysis of the cingulum tract from the DMN ROIs. (See the Methods section and the eMethods in Supplement for details.) FA indicates fractional anisotropy; fMRI, functional magnetic resonance imaging.

field of view [FOV], 244 mm; and 1-mm isotropic voxel) and a 5-minute fMRI resting-state, single-shot, gradient-echo, echo-planar imaging sequence (TR, 2000 milliseconds; TE, 16 milliseconds; flip angle, 90°; FOV, 220 mm; and voxel size, 1.7 × 1.7 × 3.0 mm). Diffusion-weighted images were sensitized in 30 noncollinear directions with a b value of 1000 s/mm^2 in an echo-planar imaging sequence (TR, 9300 milliseconds; TE, 94 milliseconds; section thickness, 2.0 mm; voxel size, 2.0 × 2.0 × 2.0 mm; FOV, 240 mm; and no gap). For the lesion description, the neuroradiologist (N.B.) considered T1-weighted, fluid-attenuated inversion recovery (TR, 9000 milliseconds; TE, 85 milliseconds; section thickness, 3.0 mm; voxel size, 1.3 × 0.9 × 3.0 mm; and FOV, 240 mm), and T2* gradient-echo sequence (TR, 518 milliseconds; TE, 20 milliseconds; section thickness, 3.0 mm; voxel size, 0.9 × 0.8 × 3.0 mm; and FOV, 240 mm) sequences. All the images were visually inspected to ensure that they did not contain MRI artifacts or excessive movement before analysis.

Neuropsychological Assessment

A trained neuropsychologist masked to the clinical data administered tests to assess the main cognitive functions impaired after TBI. The assessment included the following: letter-number sequencing; digit span test (forward and backward measures); the Trail Making Test (parts A and B); the Rey Auditory Verbal Learning Test; the Rey-Osterrieth complex figure; reading, color naming, and reading word-color conditions from the Stroop test; and measures of verbal semantic and phonemic fluency.[25] Factor analysis was used to obtain a single measure that was representative of overall cognitive outcome, based on tests in which patients were significantly impaired compared with controls (eMethods and eTable in Supplement).

MRI: Image Processing and Analysis

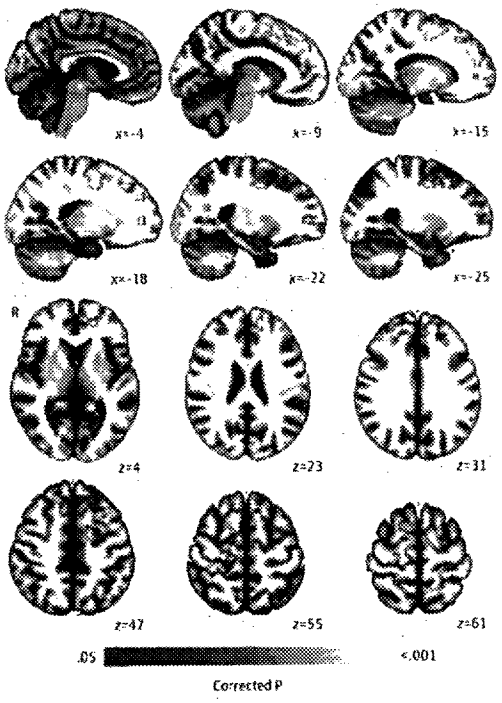
Amplitude Measures of Resting-State Data

The amplitude of low-frequency fluctuations (ALFF) was measured using a method based on the fast Fourier transform of the resting-state time series for each voxel.[26] After individual ALFF maps were obtained, they were registered to the Montreal Neurological Institute (MNI) standard space by means of linear registration (FLIRT [FMRIB's Linear Image Registration Tool] from FSL [http://fmrib.ox.ac.uk/fsl]).[27] Voxel-wise group comparisons were performed on these maps by using permutation-based comparisons with general linear modeling. Differences were considered significant at $P < .05$ (family-wise error corrected; see the eMethods in Supplement and Figure 1 for a full description of the procedures used).

Independent Component Analysis of Resting-State Data

We entered preprocessed resting fMRI data into an independent component analysis (ICA) using MELODIC[28] software from FSL. This enabled us to obtain a set of independent components and identify the common resting-state functional networks.[14,16,19,29] Before group ICA decomposition, all individual fMRI data sets were linearly registered to the MNI standard space.[27] Finally, we selected the independent component map of the DMN. The pro-

Figure 2. Increased amplitude of low-frequency fluctuations during the resting-state in patients with traumatic brain injury



Red-yellow regions represent areas with statistically significant differences between patients and controls (corrected $P < .05$).

cedure for selecting the DMN within the whole set of components was based on the computation of spatial cross-correlation between each independent component and a previously published template corresponding to the DMN.[16]

We then used a dual regression approach[4,30] to investigate between-group differences in the DMN maps. The significance threshold of the voxel-wise differences was set at $P < .05$ (family-wise error corrected) (eMethods in Supplement and Figure 1).

Seed-Based Analysis of the DMN

The peak coordinates of the DMN identified with ICA were used to create 4 spherical regions of interest (ROIs) representing the main nodes of this network: medial prefrontal cortex (MPFC), precuneus/posterior cingulate (PPC), and left and right parietal cortices (eMethods in Supplement and Figure 1).

For each seed (or DMN node), we created whole-brain functional connectivity maps and tested group differences of these maps. All seed-based connectivity analyses were performed using the functional data sets that had been previously preprocessed and registered to the MNI standard space.

Analysis of MRI Diffusion Data

Diffusion MRI images were analyzed with FDT (FMRIB's Diffusion Toolbox) software from FSL. After first extracting individual fractional anisotropy (FA) maps, we then used diffusion tensor imaging data in a probabilistic tracking algorithm to estimate white matter pathways connecting the 2 DMN ROIs (ie, MPFC ROI and PPC ROI) extracted from the analysis of the resting-state fMRI data. These ROIs (from fMRI analysis) were originally in MNI standard space. They were then moved to each subject's diffusion space before we performed tractography (using linear registration implemented with FSL software).[27] Individual tracts were registered again to MNI to compute the group-average maps. White matter pathways were averaged across controls and patients separately. Finally, the average connectivity map of the controls was used, together with the registered FA maps, to estimate fiber integrity of this connection in the whole sample as the mean FA within the pathway (Figure 1).

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

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

Abbreviation: MNI, Montreal Neurological Institute.

Cognitive Outcome and Structural and Functional Connectivity Data

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

Results

Amplitude of Resting-State Fluctuations

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

Independent Component Analysis

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

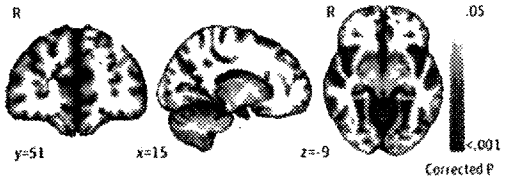
DMN Connectivity

Group Comparisons of the ICA-Based DMN

Using the dual regression approach, patients with TBI had greater functional connectivity than controls within the DMN

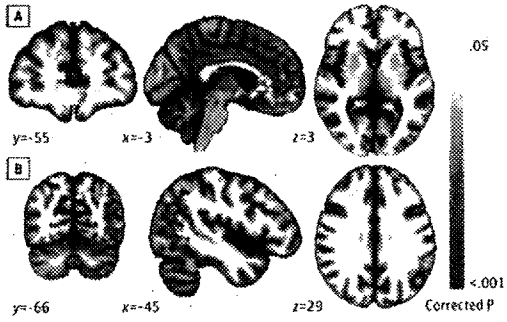
in certain areas of the frontal lobe (Figure 3). These regions included the frontal pole, the anterior cingulate and paracingulate gyrus, the superior frontal gyrus, and a small part of the precentral gyrus.

Figure 3. Increased connectivity within the default mode network in patients with traumatic brain injury to controls



Red-yellow regions represent areas where the connectivity differed significantly between patients and controls (corrected $P < .05$).

Figure 4. Areas showing increased functional connectivity in traumatic brain injury and locations of the seeds



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

Seed-Based Connectivity Analysis

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

With the MPFC ROI as a seed, we found increased functional connectivity of this region with other areas of the MPFC in patients compared with controls (corrected $P < .05$) (Figure 4). Moreover, with the left parietal cortex ROI, we also found a set of brain areas with increased functional connectivity in patients with TBI (corrected $P < .05$) (Figure 4). These areas were located in the left lateral occipital cortex, angular gyrus, supramarginal gyrus, temporo-occipital areas, middle and inferior temporal gyrus, occipital fusiform and lingual gyri, and precuneus. We found no differences in functional connectivity when using the remaining nodes as seeds.

Structural Connectivity Measured With Diffusion Tensor Imaging

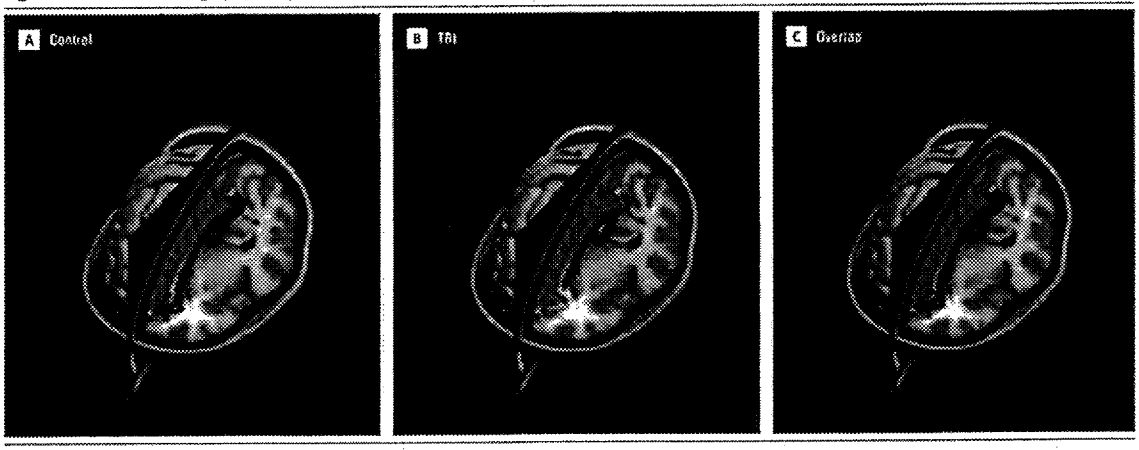
The MPFC and the PPC ROIs were used to reconstruct the white matter pathway connecting the 2 regions. The probabilistic tractography map of each subject was used to create separate average maps for patients and controls. These maps indicate the probability of each voxel being part of a white matter pathway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

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

Amplitude of Fluctuations and Cognitive Outcome

In patients, the amplitude of resting-state fluctuations was positively correlated with cognitive performance ($r = 0.48$; $P = .03$);

Figure 5. Results of tractography analysis shown as spatial maps of probabilistic tracking



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

the greater the activation, the better the cognitive outcome. There was no significant correlation between these measures in controls ($r = 0.35$; $P = .16$).

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

Discussion

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

Few studies to date have considered the ALFF at rest and its implications in terms of cognitive dysfunction. This measure has been found to be decreased in patients with mild cognitive impairment and Alzheimer disease.[31] In neurodegenerative diseases, these decreases in amplitude probably reflect a loss of neurons that consecutively provokes connectivity deficits and disorganization or breakdown of brain networks. In our study, when comparing whole-brain ALFF in the resting state between groups, the TBI group showed increased amplitudes that were predominantly focused within frontal lobe regions. Moreover, the measures of higher amplitudes in these areas predicted a better general cognitive outcome, suggesting that functional and efficient brain reorganization occurred to compensate for acute brain damage and improve cognitive performance. This finding, together with changes in structural connectivity, may constitute an objective measure of long-term cognitive outcome after TBI.

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

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

Although not directly examining the DMN, other authors have found alterations in resting-state connectivity in TBI in the form of reduced interhemispheric connectivity of the hippocampus and increased ipsilateral connectivity, and these were associated with better cognitive outcome.[6] In addition, resting-state fMRI studies involving magnetoencephalographic recordings have also provided evidence of brain plasticity and network reorganization mechanisms, specifically increases or decreases in the extent of certain brain connections.[35]

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

Altogether, our results suggest that increased frontal functional activity at rest, measured as the ALFF, is associated with better global cognitive performance and that the altered structural connectivity between related brain regions can be compensated for by increased functional connectivity. Two recent studies have accurately characterized the changes in the DMN connectivity at different time points. Hillary et al,[10] examining resting-state DMN connectivity in a sample of patients in the acute stage with predominant focal lesions, found increases in the connectivity of this network during the first 6 months after injury. On the other hand, Arenivas et al[11] examined DMN functional connectivity in a cohort of patients 6 to 11 months after TBI. Using 3 methodological approaches, they found decreased connectivity in the main nodes of the DMN. Although their sample characteristics were similar to our own (patients with white matter damage without significant contusions), our patients were studied a mean of 4 years after injury. Thus, our findings can be generalized only to patients in the late chronic stage and are not directly comparable to findings in the early chronic or postacute stages. However, whereas Arenivas et al[11] showed decreases in the core nodes of the DMN, we found diffuse increases in connectivity in areas surround-

ing DMN nodes. The apparent contradiction can be explained by the fact that Arenivas et al did not analyze the regions where we found increases because they studied connectivity within the nodes of the DMN. We suggest that the increased connectivity in areas surrounding DMN nodes, which we found in the late chronic stage, might represent the brain's attempt to compensate functionally for weaker connectivity within DMN nodes caused by structural damage after traumatic axonal injury. Nevertheless, the decreased connectivity of the DMN in this study did not predict the clinical or cognitive deficits. Future longitudinal studies with several follow-up points could clarify the dynamics of cerebral reorganization after TBI.

ARTICLE INFORMATION

Accepted for Publication: January 4, 2013.

Author Contributions: Mss Palacios and Sala-Llonch contributed equally to the manuscript. *Study concept and design:* Palacios, Sala-Llonch, Junque, Bargallo.
Acquisition of data: Palacios, Sala-Llonch, Roig, Tormos, Bargallo.
Analysis and interpretation of data: Palacios, Sala-Llonch, Vendrell.
Drafting of the manuscript: Palacios, Sala-Llonch, Junque.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Palacios, Sala-Llonch, Vendrell.
Obtained funding: Junque, Tormos.
Administrative, technical, and material support: Roig, Tormos, Bargallo.
Study supervision: Junque, Tormos.

Published Online: May 20, 2013. doi:10.1001/jamaneurol.2013.38.

Conflict of Interest Disclosures: None reported.

Additional Contributions: Cesar Garrido, Rad Tech, from the Centre de Diagnostic per la Imatge, Hospital Clinic, Barcelona, assisted with data collection.

REFERENCES

1. Benedictus MR, Spikman JM, van der Naalt J. Cognitive and behavioral impairment in traumatic brain injury related to outcome and return to work. *Arch Phys Med Rehabil*. 2010;91(9):1436-1441.

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

3. Büki A, Povlishock JT. All roads lead to disconnection? traumatic axonal injury revisited. *Acta Neurochir (Wien)*. 2006;148(2):181-194.

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

5. Palacios EM, Sala-Llonch R, Junque C, et al. White matter integrity related to functional working memory networks in traumatic brain injury. *Neurology*. 2012;78(12):852-860.

6. Marquez de la Plata CD, Garces J, Shokri Kojori E, et al. Deficits in functional connectivity of hippocampal and frontal lobe circuits after traumatic axonal injury. *Arch Neurol*. 2011;68(1):74-84.

7. Kasahara M, Menon DK, Salmond CH, et al. Traumatic brain injury alters the functional brain network mediating working memory. *Brain Inj*. 2011;25(12):1170-1187.

8. Mayer AR, Mannell MV, Ling J, Gasparovic C, Yeo RA. Functional connectivity in mild traumatic brain injury. *Hum Brain Mapp*. 2011;32(11):1825-1835.

9. Bonnelle V, Ham TE, Leech R, et al. Salience network integrity predicts default mode network function after traumatic brain injury. *Proc Natl Acad Sci U S A*. 2012;109(12):4690-4695.

10. Hillary FG, Slocomb J, Hills EC, et al. Changes in resting connectivity during recovery from severe traumatic brain injury. *Int J Psychophysiol*. 2011;82(1):115-123.

11. Arenivas A, Diaz-Arrastia R, Spence J, et al. Three approaches to investigating functional compromise to the default mode network after traumatic axonal injury [published online July 31, 2012]. *Brain Imaging Behav*. doi:10.1007/s11682-012-9191-2.

12. Shumskaya E, Andriessen TM, Norris DG, Vos PE. Abnormal whole-brain functional networks in homogeneous acute mild traumatic brain injury. *Neurology*. 2012;79(2):175-182.

13. Slobounov SM, Gay M, Zhang K, et al. Alteration of brain functional network at rest and in response to YMCA physical stress test in concussed athletes: RsFMRI study. *Neuroimage*. 2011;55(4):1716-1727.

14. Biswal BB, Mennes M, Zuo XN, et al. Toward discovery science of human brain function. *Proc Natl Acad Sci U S A*. 2010;107(10):4734-4739.

15. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci*. 2007;8(9):700-711.

16. Smith SM, Fox PT, Miller KL, et al. Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A*. 2009;106(31):13040-13045.

17. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci*. 2008;1124:1-38.

18. Hagmann P, Cammoun L, Gigandet X, et al. Mapping the structural core of human cerebral cortex. *PLoS Biol*. 2008;6(7):e159. doi:10.1371/journal.pbio.0060159.

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

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

21. Sidaros A, Engberg AW, Sidaros K, et al. Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. *Brain*. 2008;131(pt 2):559-572.

22. Kinnunen KM, Greenwood R, Powell JH, et al. White matter damage and cognitive impairment after traumatic brain injury. *Brain*. 2011;134(pt 2):449-463.

23. Newcombe V, Chatfield D, Outtrim J, et al. Mapping traumatic axonal injury using diffusion tensor imaging: correlations with functional outcome. *PLoS One*. 2011;6(5):e19214. doi:10.1371/journal.pone.0019214.

24. Palacios EM, Sala-Llonch R, Junque C, et al. Long-term declarative memory deficits in diffuse TBI: correlations with cortical thickness, white matter integrity and hippocampal volume. *Cortex*. 2013;49(3):646-657.

25. Lezak MD, Howieson DB, Loring DO, et al. *Neuropsychological Assessment*. New York, NY: Oxford University Press; 2004.

26. Zang YF, He Y, Zhu CZ, et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev*. 2007;29(2):83-91.

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

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

29. Damoiseaux JS, Rombouts SA, Barkhof F, et al. Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A*. 2006;103(37):13848-13853.

30. Filippini N, Macintosh BJ, Hough MG, et al. Distinct patterns of brain activity in young carriers of the APOE-ε4 allele. *Proc Natl Acad Sci U S A*. 2009;106(17):7209-7214.

31. Wang Z, Yan C, Zhao C, et al. Spatial patterns of intrinsic brain activity in mild cognitive impairment and Alzheimer's disease: a resting-state functional MRI study. *Hum Brain Mapp*. 2011;32(10):1720-1740.

32. Bonavita S, Gallo A, Sacco R, et al. Distributed changes in default-mode resting-state connectivity in multiple sclerosis. *Mult Scler*. 2011;17(4):411-422.

33. Hawellek DJ, Hipp JF, Lewis CM, Corbetta M, Engel AK. Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. *Proc Natl Acad Sci U S A*. 2011;108(47):19066-19071.

34. Bonnelle V, Leech R, Kinnunen KM, et al. Default mode network connectivity predicts sustained attention deficits after traumatic brain injury. *J Neurosci*. 2011;31(38):13442-13451.

35. Castellanos NP, Paúl N, Ordóñez VE, et al. Reorganization of functional connectivity as a correlate of cognitive recovery in acquired brain injury. *Brain*. 2010;133(Pt 8):2365-2381.

36. Catani M, Thiebaut de Schotten M. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. *Cortex*. 2008;44(8):1105-1132.

Default-Mode Network Disruption in Mild Traumatic Brain Injury[1]

Yongxia Zhou, PhD
Michael P. Milham, MD, PhD[2,3]
Yvonne W. Lui, MD
Laura Miles, PhD
Joseph Reaume, BSRT
Daniel K. Sodickson, MD, PhD
Robert I. Grossman, MD
Yulin Ge, MD

Purpose: To investigate the integrity of the default-mode network (DMN) by using independent component analysis (ICA) methods in patients shortly after mild traumatic brain injury (MTBI) and healthy control subjects, and to correlate DMN connectivity changes with neurocognitive tests and clinical symptoms.

Materials and Methods: This study was approved by the institutional review board and complied with HIPAA regulations. Twenty-three patients with MTBI who had posttraumatic symptoms shortly after injury (<2 months) and 18 age-matched healthy control subjects were included in this study. Resting-state functional magnetic resonance imaging was performed at 3 T to characterize the DMN by using ICA methods, including a single-participant ICA on the basis of a comprehensive template from core seeds in the posterior cingulate cortex (PCC) and medial prefrontal cortex (MPFC) nodes. ICA z images of DMN components were compared between the two groups and correlated with neurocognitive tests and clinical performance in patients by using Pearson and Spearman rank correlation.

Results: When compared with the control subjects, there was significantly reduced connectivity in the PCC and parietal regions and increased frontal connectivity around the MPFC in patients with MTBI ($P < .01$). These frontoposterior opposing changes within the DMN were significantly correlated ($r = -0.44$, $P = .03$). The reduced posterior connectivity correlated positively with neurocognitive dysfunction (eg, cognitive flexibility), while the increased frontal connectivity correlated negatively with posttraumatic symptoms (ie, depression, anxiety, fatigue, and postconcussion syndrome).

Conclusion: These results showed abnormal DMN connectivity patterns in patients with MTBI, which may provide insight into how neuronal communication and information integration are disrupted among DMN key structures after mild head injury.

[1] From the Center for Biomedical Imaging, Department of Radiology, New York University School of Medicine, 660 First Ave, 4th Floor, New York, NY, 10016 (Y.Z., Y.W.L., L.M., J.R., D.K.S., R.I.G., Y.G.); and Phyllis Green and Randolph Cowen Institute for Pediatric Neuroscience, New York University Child Study Center, New York, NY (M.P.M.). Received March 30, 2012; revision requested May 15; revision received June 4; accepted June 8; final version accepted June 29. **Address correspondence to** Y.G. (e-mail: *yulin.ge@nyumc.org*).

[2] **Current address:** Center for the Developing Brain, Child Mind Institute, New York, NY

[3] **Current address:** Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY

Mild traumatic brain injury (MTBI) is a substantial public health problem, representing 75% of traumatic brain injury cases (1). As defined by the World Health Organization (2,3), MTBI results from a traumatic event that causes a brief loss of consciousness, lasting less than 30 minutes, transient memory dysfunction or disorientation that lasts less than 24 hours, or both. Although conventional neuroimaging findings are often normal in patients with MTBI, 20%–30% of individuals develop long-term symptoms, including behavioral, cognitive, and neuropsychologic problems (4), ultimately preventing some from ever successfully reengaging in their normal lives (5). These posttraumatic symptoms, referred to as *postconcussion syndrome* (PCS), manifest as a history of traumatic brain injury along with the presence of three or more common symptoms (ie, headache, dizziness, fatigue, irritability, insomnia, poor concentration, memory difficulty, or an intolerance of stress, emotion, or alcohol) that have lasted at least 3 months (6), as well as evidence of deficits in attention and memory (7). PCS is frequently overlooked at the time of initial injury and can develop and persist for variable periods of time. However, the underlying pathophysiology of PCS is still poorly understood.

In recent years, resting-state functional magnetic resonance (MR) imaging has enabled evaluation of critical brain networks on the basis of baseline energy expenditure in awake and resting states of the brain. Resting-state networks are composed of brain regions with highly correlated time courses of robust low-frequency (<0.1 Hz) blood oxygen level–dependent signal fluctuations (8), which are believed to represent the maintenance of baseline human cognition and metabolic equilibrium (ie, oxygen balance) (9). These intrinsic, spontaneous neuronal activities have been considered to represent important functional architecture for understanding the cognitive functions and can be altered in various neurologic and psychiatric disorders (10). Among these networks, the default-mode network (DMN) is of particular interest. The DMN is a well-established network that is active at rest and suppressed during tasks that require attention and decision making (9,11). The DMN typically comprises the posterior cingulate cortex (PCC), precuneus, inferior parietal, and medial prefrontal cortex (MPFC) nodes (12). Anatomically, the cytoarchitectonic areas of the DMN are suggested to be strongly interconnected (13). Alterations in DMN functional connectivity have been found in several psychiatric disorders (14–17). It is also known that several key cognitive functions are supported by this network (18). For example, the main role of the PCC is memory encoding, consolidation, and environmental monitoring, while the MPFC participates in self-relevance, rapid error identification, and social functions (19). These higher-order cognitive functions are often disrupted in patients with MTBI (20).

Several studies of patients with severe forms of traumatic brain injury, including vegetative states, have shown disrupted DMN connectivity in terms of evaluating patients with consciousness impairment (21–24). Despite considerable interest in the DMN and its relationship with cognitive function, little is known about DMN connectivity changes in MTBI. A recent study by Mayer et al (25) showed decreased connectivity within DMN nodes and increased

Advances in Knowledge

- In patients with mild traumatic brain injury (MTBI), as compared with healthy control subjects, the integrity of default-mode network (DMN) functional connectivity was disrupted, as evidenced by significantly reduced connectivity in the posterior cingulate cortex and parietal regions and increased frontal connectivity around the medial prefrontal cortex ($P < .01$).
- These opposed changes were significantly correlated with each other ($r = -0.44$, $P = .03$), suggesting that frontoposterior DMN nodes are not only intrinsically interdependent but also highly complementary and dynamically equilibratory in their functions after mild injury.
- The reduced posterior connectivity correlated clinically with neurocognitive dysfunction (eg, cognitive flexibility), while the increased frontal connectivity correlated with posttraumatic symptoms (ie, depression, anxiety, fatigue, and postconcussion syndrome [PCS]).

Implication for Patient Care

- Our results of disrupted DMN synchrony and connectivity in MTBI suggest that resting-state functional MR imaging can be used as an additional clinical tool for detecting subtle brain injury that is not apparent with conventional MR imaging and for better understanding the underlying disease pathophysiology of PCS.

Published online
10.1148/radiol.12120748 **Content code:** NR

Radiology 2012; 265:882–892

Abbreviations:
DMN = default-mode network
FSL = Functional Magnetic Resonance Imaging of the Brain Software Library
ICA = independent component analysis
MELODIC = Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MNI = Montreal Neurologic Institute
MPFC = medial prefrontal cortex
MTBI = mild traumatic brain injury
PCC = posterior cingulate cortex
PCS = postconcussion syndrome

Author contributions:
Guarantors of integrity of entire study, Y.W.L., Y.G.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, Y.Z., M.P.M., Y.W.L., R.I.G., Y.G.; clinical studies, Y.Z., L.M., J.R., R.I.G., Y.G.; experimental studies, Y.Z., M.P.M.; statistical analysis, Y.Z., Y.G.; and manuscript editing, Y.Z., M.P.M., Y.W.L., Y.G.

Funding:
This research was supported by the National Institutes of Health (grants R01 NS039135 and NS039135-06S1).

Conflicts of interest are listed at the end of this article.

connectivity between DMN nodes and the lateral prefrontal cortex in MTBI by using seed-based analysis, which is a single time-course correlation approach based on predefined regions of interest. To the best of our knowledge, these findings have not been replicated by using multiple-regression methods, such as independent component analysis (ICA), which is a more robust technique involving the blind source separation method that captures the essential components of multivariate resting-state functional MR imaging data (26). The ICA model is a powerful tool for the extraction of imaging patterns of synchronized neural activity from resting-state functional MR imaging time series. ICA can help separate the whole-brain signal fluctuations from physiologic noise and automatically capture the entire DMN as a single major component (27). This decreases the heterogeneity of DMN patterns when using the seed-based method (28). We hypothesized that there are abnormal neuronal activations of DMN subregions in the resting brain in patients with MTBI, which can be detected with advanced ICA techniques.

To validate our findings, we used three different strategies of ICA, which included a template-based ICA method (29), a hybrid ICA seed-based method (30), and a group ICA method (31) in patients with MTBI compared with healthy individuals. The purpose of this study was to investigate the integrity of the DMN by using ICA methods in patients shortly after MTBI compared with control subjects and to correlate DMN connectivity changes with neurocognitive tests and clinical symptoms.

Materials and Methods

Participants

This institutional review board–approved study was performed between July 2008 and April 2011 and was in compliance with the Health Insurance Portability and Accountability Act. A total of 26 patients with MTBI (recruited from a university-affiliated level I trauma center and a university hospital) and 21 healthy control subjects were identified and recruited from a large database in our center of several ongoing neuroimaging projects. All individuals signed a consent form before undergoing MR imaging. All patients experienced a closed head injury with either posttraumatic amnesia of less than 24 hours duration or loss of consciousness of approximately 30 minutes or less, with Glasgow Coma Scale scores between 13 and 15 obtained by the emergency or ambulatory care staff after injury. In this study cohort, all patients experienced loss of consciousness (minimum time of 10 seconds), and all had various posttraumatic symptoms at the time of MR imaging, including headache, insomnia, fatigue, sensitivity to light, irritability, and deficits in attention, memory, and executive function. Exclusion criteria included history of alcohol or drug abuse, neuropsychologic disease before injury, and prior brain injury or other neurologic disease, including stroke, epilepsy, and somatic disorders. On the basis of these criteria, three patients were excluded owing to previous head injury, presence of other neurologic disease, or extensive motion artifacts. Thus, 23 patients with clinically defined MTBI (32) (17 men, six women; mean age, 37.8 years ± 12.9 [standard deviation]; educational attainment, 15 years ± 2.6) were included in this study, with a mean interval of 22 days (range, 3–53 days) between trauma and MR imaging. One control subject was excluded due to an incidental finding of brain lesions at MR imaging, and another two control subjects were excluded due to excessive movement. Eighteen healthy control subjects (mean age, 32.6 years ± 10.0; educational attainment, 16 years ± 2.1) were finally included for comparison, and they were confirmed to have no brain diseases according to patient history and imaging. The two groups had no significant demographic differences in age, sex, or education ($P > .2$).

MR Imaging

MR imaging data were obtained with a 3-T whole-body MR imager (Siemens Tim Trio; Siemens Medical Systems, Erlangen, Germany) by using a 12-channel head coil. Standard gradient-echo echo-planar resting-state functional MR imaging (repetition time msec/echo time msec, 2000/30; flip angle, 75°; field of view, 220 × 220 mm; matrix, 128 × 128; 153 volumes) was performed in the axial plane, parallel to a line through the anterior and posterior commissures (section thickness, 5 mm; section gap, 1 mm) and positioned to cover the entire cerebrum (spatial resolution, 1.72 × 1.72 × 6.00 mm) with an acquisition time of 5 minutes 6 seconds. Individuals were instructed to close their eyes for better relaxation but to stay awake during the imaging protocol. For coregistration and normalization of resting-state functional MR imaging data, three-dimensional T1-weighted magnetization-prepared rapid gradient-echo imaging (repetition time msec/echo time msec/inversion time msec, 2300/2.98/900; flip angle, 9°; resolution, 1 × 1 × 1 mm) was performed. In addition, T2-weighted fast spin-echo and high-spatial-resolution susceptibility-weighted imaging sequences (45/20/900; flip angle, 15°; resolution, 0.5 × 0.5 × 2.0 mm; 52 axial sections) were also implemented to help detect hemorrhagic or other lesions. The conventional T1- and T2-weighted images together with susceptibility-weighted images were reviewed carefully by two experienced radiologists (Y.W.L and Y.G.), and lesions, if present, were documented.

Neuropsychologic Assessment

Neuropsychologic tests were performed within 12 hours of MR imaging for patients with MTBI. The neuropsychologic tests were performed by a psychologist (L.M) who had more than 7 years of experience and was blinded to MR imaging results. Neuropsychologic measures to evaluate cognitive functioning included *(a)* the Symbol Digit Modalities Test (33) to measure information processing speed; *(b)* the Digit Span subtest of the Wechsler Adult Intelligence Scale III (34) to measure verbal attention and concentration, as well as working memory; *(c)* the Trail Making Test A to assess speed and visual attention; *(d)* the Trail Making Test B (35) to assess mental flexibility, specifically the

ability to shift rapidly between cognitive sets; *(e)* the California Verbal Learning Test II to assess verbal learning, as well as immediate and delayed verbal memory (36); and *(f)* the Rey-Osterreith Complex Figure Test to assess visuoperceptual ability and immediate and delayed visual memory (37). These test results were reported in *z* scores. Posttraumatic symptoms, including anxiety, depression, and fatigue, were assessed by using self-report questionnaires and scales, for which higher scores indicate heightened symptoms. The Beck Depression Inventory (38) was used to assess depressive symptoms, fatigue was measured with the Fatigue Severity Scale (39), and subjective symptoms were assessed by using the Postconcussion Symptoms Scale (40). This scale is used to assess the severity of 19 symptoms, such as dizziness, balance problems, headache, and sensitivity to light, which are associated with PCS, mild cognitive impairment, or both. Items are rated on a Likert scale, indicating severity of the symptom, from 0 (none) to 6 (severe). Past research has validated the use of such inventories in the use of assessing individuals after minor head injuries (41).

Image Processing and Data Analysis

All data pre- and postprocessing were performed by an imaging scientist (Y.Z.) with 11 years of experience in functional MR imaging data analysis and interpretation.

Preprocessing.—The three-dimensional magnetization-prepared rapid gradient-echo imaging data were first reoriented, skull stripped, and segmented into gray matter, white matter, and cerebrospinal fluid regions. Thereafter, they were coregistered and normalized to the Montreal Neurologic Institute (MNI) 152-brain template (2 × 2 × 2 mm). Preprocessing steps for resting-state functional MR imaging data included realignment, spatial Gaussian smoothing with full width at half maximum (6 mm), band-pass temporal filtering of 0.005–0.100 Hz, coregistration to magnetization-prepared rapid gradient-echo imaging and removal of nuisance signals (motion parameters, global signal, and signals derived from cerebrospinal fluid and white matter), and transformation to MNI standard space. The anatomic and functional data were preprocessed by using both the Functional Magnetic Resonance Imaging of the Brain, or FMRIB, Software Library (FSL) (eg, tissue segmentation, registration, smoothing, and regression) and Analysis of Functional NeuroImages, or AFNI, programs (ie, reorientation, skull stripping, motion correction, filtering, and region of interest time courses extraction) (adapted scripts from *http://www.nitrc.org/projects/fcon_1000*).

For correction of motion artifacts, head movement–constraint headphones and a cushion were used to prevent movement during imaging, and all individuals were instructed to keep still during the imaging protocol. AFNI motion-correction algorithms were used to perform more realignment steps with three angular rotation (roll, pitch, and yaw in units of degrees) and three directional displacement (in millimeters) adjustments to further minimize motion artifacts. After these correction steps, no statistical differences were observed in *(a)* the previous six motion parameters and *(b)* frame displacement (42) along all temporal frames between MTBI and control groups ($P > .05$). Additionally, with the ICA approach, movement artifacts can be identified easily and removed separately by applying a band-pass filter.

Seed-based analysis.—After preprocessing, the Pearson correlation coefficient was computed and *z* transformed between the averaged time series of either the PCC- or the MPFC-centered seed and the time course of each voxel in the brain. Two regions of interest, including the MPFC seed (MNI: 0, 48, −3 mm) and the PCC seed (MNI: −6, −48, 39 mm), both with 896-mm^3 volumes, were derived from the script seed library and were well evaluated previously by Fox and Raichle (18). Since the individual seeding approach with the seed placed within either the PCC or the MPFC generates different and incomplete DMN connectivity patterns (43), the combined template from these two DMNs was used and converted into a binary image after applying a threshold of correction of $P < .05$ for subsequent single-participant ICA. The overlap similarity index between PCC- and MPFC-seeding DMN group patterns was calculated as the Dice coefficient, defined as twice the intersection over the sum of cardinalities (44,45). The total number of voxels *(N)* and average correlational *z* value were computed from the obtained entire, anterior, or posterior DMN connectivity maps by using a threshold of correction of $P < .05$. The anterior and posterior portions of the DMN were defined according to MNI coordinates as $y \geq 0$ and $y < 0$, respectively.

Single-participant ICA.—The conventional single-participant ICA was implemented by using information maximization criteria (46) to decompose the data from each individual into 16 spatially statistically independent and intrinsically connected components (47) (*http://cnl.salk.edu/~tewon/ica_cnl.html*). Each component was shown by a *z* score map by subtracting the global mean from each voxel and dividing by the global standard deviation with a threshold of 1.96 (equivalent $P < .05$). After excluding very-low-frequency (<0.01 Hz) or high-frequency (>0.1 Hz) artifacts due to imager drift and cardiac and respiratory motion by applying a band-pass filter, the component that had the maximum difference was chosen to identify the DMN component (C_{DMN}) and generate a *z*-score map by using the following equation:

$$C_{DMN} = \max_i \{C_{i(T)} - C_{i(1-T)}\} \qquad (1)$$

where *i* represents the *i*th low-frequency component, *T* stands for the placement of voxels within the binarized DMN template image generated from the previous seed-based method; (1 − *T*) represents the region outside the DMN template; and C_i is the average *z* score of the *i*th component inside or outside the template region.

Hybrid ICA.—The DMN detected automatically by means of single-participant ICA was used as a new seed to iterate for the next-round ICA

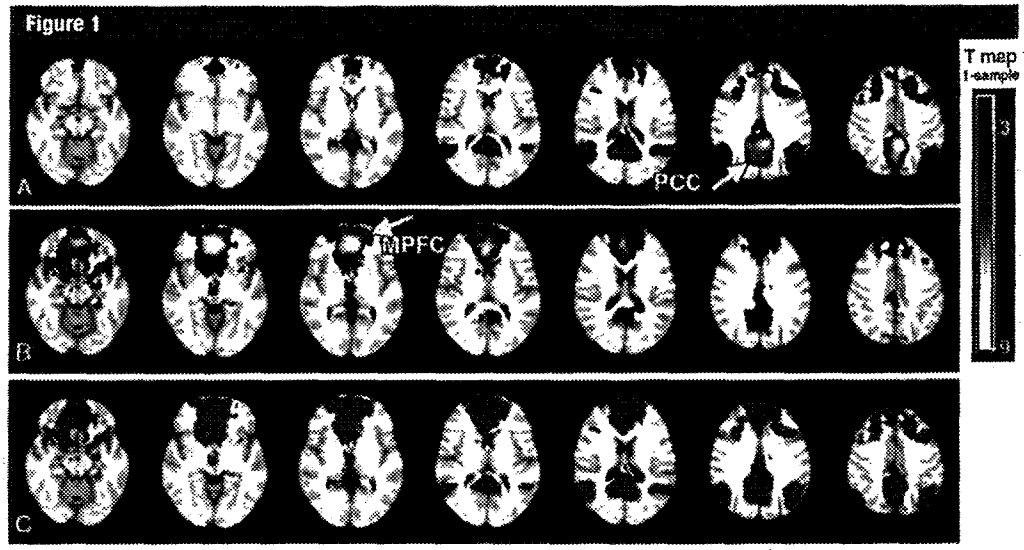


Figure 1: DMN templates created with the seed-based method in healthy control subjects. *A*, PCC-seed–based template, with predominant PCC, bilateral inferior parietal, and MPFC connectivity (corrected, $P < .05$; cluster size, $K \geq 20$). *B*, MPFC-seed–based template shows predominant MPFC connectivity with PCC nodes detected (corrected, $P < .05$; cluster size, $K \geq 20$). *C*, Binarized image of combined-network seeding from the MPFC and PCC show a more inclusive connectivity pattern of the DMN.

template, which is termed "hybrid ICA seed-based method," as proposed by Kelly et al (30). By deriving seed information from single-participant ICA, this hybrid algorithm uses the exploratory power of single-participant ICA to increase the confidence of automatic detection of spatial relationships and to reduce possible original simple seed-template–matching errors. The hybrid ICA is particularly useful in cases where seed-based functional connectivity MR imaging maps would otherwise be generated from seed voxels where multiple components overlap, which is often the case with DMN.

Group ICA.—To validate the single-participant ICA results, group ICA with the FSL Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) algorithm *(http://www.fmrib.ox.ac.uk/fsl/melodic/index.html)* (31) was also implemented. The final DMN component of each group was identified with visual inspection on the basis of periodic temporal fluctuation, spatial pattern, and distinct peak of power spectrum at low-frequency (<0.1 Hz) range.

Statistical Analysis

For seed-based analysis, a two-sample *t* test for comparing the voxel numbers or mean *z* values was performed between the patients and control subjects. The FSL FMRIB Local Analysis of Mixed Effects with Ordinary Least Square option (FLAME0) command was used to analyze the variance of DMN maps within each group and between two groups with corrected $P < .05$. For single-participant ICA, one- and two-sample *t* tests were implemented by applying SPM8 software (Statistical Parametric Mapping, *http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/*) to the *z* image of the DMN made up of ICA components from the two participant groups to generate statistical maps. An integrated threshold was used at a significance level of $P < .01$ and a cluster size of at least 20 voxels for small-volume correction to the whole brain to remove false-positive error and maintain true-positive sensitivity (48). Multiple-comparison corrections at the cluster level were performed on the whole brain on the basis of Gaussian random field theory by using FSL easythresh (minimum, $z > 2.3$; cluster significance, $P < .05$, corrected).

Neuropsychologic measurements were input as covariants for second-level covariance analysis, and Pearson or Spearman rank correlations were performed between either the neurocognitive tests or posttraumatic symptoms (ie, anxiety, depression, fatigue, and PCS) scales and the ICA *z* images of DMN. Bonferroni corrections were performed in patients with a total correction factor of 12 for multiple tests ($n = 6$), and two averaged z values ($n = 2$) were obtained from either decreased or increased regions (49).

Results

In the healthy control group, different spatial distribution patterns of DMN were shown when the seed was placed in the standard MPFC versus PCC region, which showed anterior-predominant connectivity with MPFC seeding and posterior-predominant connectivity with PCC seeding (Fig 1, *A* and *B*) (one-sample *t* test with corrected $P < .05$ and cluster size of $K \geq 20$), suggesting an intrinsic region-dependent blood oxygen level–dependent signal coherence of resting-state

networks. The total number of DMN voxels in the healthy control group with PCC seed (n = 16877) had only a 41.2% (DICE coefficient) overlap with MPFC seed (n = 20152). A combined DMN (n = 29397) from both PCC and MPFC seeds was shown to represent a more comprehensive network (Fig 1, *C*) for selecting ICA components. In the control group, this combined DMN showed a more inclusive network pattern (74.2% and 45.9% increase from a PCC- and MPFC-seed–only resting-state network, respectively) involving PCC, precuneus, MPFC, and bilateral parietal regions. By means of visual inspection, patients showed decreased connectivity in the parietal region (PCC seeding), whereas increased connectivity was observed in the frontal region (MPFC seeding) when compared with control subjects. However, such differences did not reach statistical significance (two-sample t test) regarding the voxel numbers of DMN or average z values.

No hemorrhagic brain lesions were detected with conventional imaging, including T2-weighted MR imaging and susceptibility-weighted MR imaging in patients with MTBI. By using a combined DMN template (a binarized image) from both core seeds, single-participant ICA (one-sample t test, $P <$ .01; cluster size, $K \geq 20$) showed a similar spatial connectivity pattern of DMN with seed-based analysis in healthy control subjects (Fig 2, *A*). In patients with MTBI, there was a significantly different distribution pattern of DMN connectivity (Fig 2, *B*) when compared with that of healthy control subjects. Figure 2, *C*, shows comparison results between the two groups (two-sample t test, $P < .01$), demonstrating significantly increased connectivity primarily in the anterior MPFC region (Table 1) and decreased connectivity primarily in the posterior medial PCC and parietal regions (Table 2) within the DMN in patients with MTBI. Specifically, the increased connectivity in the anterior DMN regions includes the left caudate nucleus, the bilateral medial prefrontal cortex, and the right superior temporal pole, whereas the decreased connectivity in the posterior DMN regions includes the left calcarine cortex (V1), the left posterior cingulate cortex, and the bilateral cuneus. In patients with MTBI, such increased connectivity in the frontal (anterior) regions significantly correlated with decreased connectivity in the posterior regions (r = −0.44, P = .03) (Fig 3), suggesting that these opposite within-network changes are essentially associated.

Figure 2

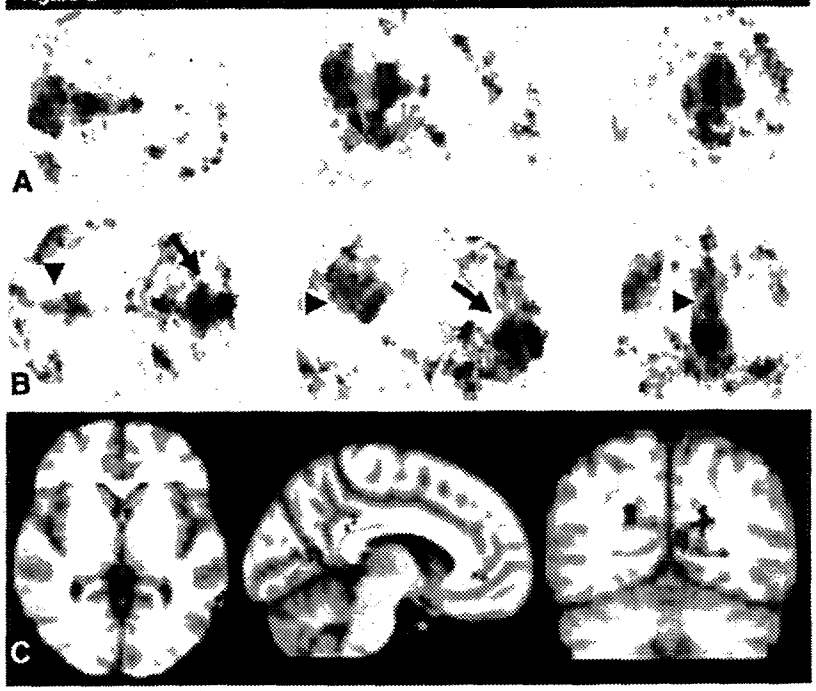


Figure 2: Group t test maps of single-subject ICA results and comparison between patients with MTBI and control subjects. When compared with DMN patterns in the control group *(A)*, the patient group *(B)* showed significantly decreased connectivity in the posterior portion (arrowheads) and increased connectivity in the anterior portion (arrows) of the DMN ($P < .01$; cluster size, $K \geq 20$). *C*, The group-level voxelwise image of single-participant DMN pattern comparing MTBI to control groups shows decreased connectivity regions in blue and increased connectivity regions in yellow.

Table 1

Group Comparison of Increased DMN with Single-Subject ICA in Patients with MTBI versus Healthy Control Subjects

Brain Region	Brodmann Area	MNI Coordinates (x, y, and z in mm)	Cluster Size	z Score	P Value
Left caudate nucleus	....	−2, 12, 2	45	3.19	.001
Left MPFC	10	−12, 44, 2	49	3.08	.001*
Right superior temporal pole	38	34, 10, −24	41	3.04	.001
Right medial frontal	10	8, 44, 2	32	2.78	.004*

Note.—Single-subject ICA was based on whole-brain voxelwise analysis, showing regions with increased DMN. $P < .01$; cluster size, $K \geq 20$.

* Clusters still survived after use of a Gaussian random-field algorithm (eg, FSL easythresh) for multiple-comparison correction at the cluster level (minimum z score > 2.3; cluster significance, $P < .05$).

Table 2

Group Comparison of Decreased DMN with Single-Subject ICA in Patients with MTBI versus Healthy Control Subjects

Brain Region	Brodmann Area	MNI *x*, *y*, *z* Coordinates (mm)	Cluster Size	*z* Score	*P* Value
Left calcarine cortex (V1)	17	−8, −62, 10	42	3.35	<.001*
Left PCC	23	−4, −38, 28	22	3.18	.001
Right cuneus	19	14, −80, 38	78	2.54	.002
Left cuneus/PCC	23	−16, −64, 26	25	2.75	.003*

Note.—Single-subject ICA was based on whole-brain voxelwise analysis, showing regions with decreased DMN. $P < .01$; cluster size, $K \geq 20$.

* Clusters still survived after use of a Gaussian random-field algorithm (eg, FSL easythresh) for multiple-comparison correction at the cluster level (minimum *z* score > 2.3; cluster significance, $P < .05$).

Figure 3

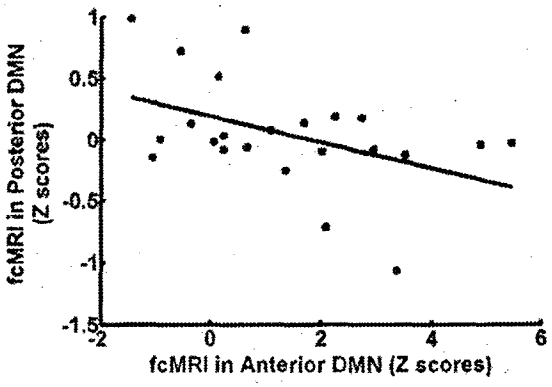


Figure 3: Plot of patients with MTBI shows that increased functional connectivity (*z* scores) in the MPFC region correlates negatively ($r = -0.44$, $P = .03$) with decreased functional connectivity (*z* scores) in the PCC and parietal regions, which may suggest a highly complementary and dynamically equilibratory relationship between the two key substructures of the DMN in terms of function after injury. *fcMRI* = functional connectivity MR imaging.

By using the hybrid ICA seed-based method, we found a similar but augmented pattern of increased frontal connectivity and decreased posterior connectivity of the DMN in patients compared with control subjects (Fig 4). We also performed MELODIC group ICA, the results of which are consistent with single-participant ICA, which showed 43.4% increases of the voxel numbers of the DMN in the anterior regions (MNI coordinate, $y \geq 0$), primarily in the MPFC region, and 24% decreases in the posterior regions (MNI coordinate, $y < 0$) in patients with MTBI compared with control subjects.

In patients with MTBI, there was a significant correlation after Bonferroni correction between decreased DMN in the posterior regions with the Trail Making Test B (Pearson correlation coefficient, $r = 0.60$; corrected, $P = .02$) (Fig 5, *A*), which is a measure of executive functioning to assess mental flexibility—specifically, the ability to shift rapidly between cognitive sets. There was no significant correlation between DMN changes and other neurocognitive tests after Bonferroni adjustment. By using covariance analysis, a significant correlation ($P < .01$) between functional connectivity MR imaging (*z* score) of anterior MPFC and clinical symptoms, including anxiety, depression, fatigue, and postconcussive symptoms, was also found. Figure 5, *B*, shows a representative example of a negative correlation between MPFC connectivity and depression score in patients with MTBI (Spearman correlation coefficient, $r = -0.56$; corrected, $P = .01$), indicating that patients with higher MPFC functional connectivity have a lower degree of depression.

Discussion

Our results show significantly decreased intrinsic functional connectivity in the PCC and parietal regions and increased functional connectivity in the MPFC regions at functional MR imaging in patients shortly after MTBI. We used three different algorithms of ICA to covalidate our findings, and our results are consistent and independent of data processing algorithms, which all suggested posterior hypoconnectivity and anterior hyperconnectivity during the resting state of the brain after injury in patients with MTBI. The observed relationships between DMN disruption and neurocognitive dysfunction, as well as clinical symptoms, provide an additional important clue to the pathophysiology underlying PCS after injury.

Regarding the normal DMN pattern, previous study findings (43,50), together with the current study results in which seed-based methods were used, demonstrated very different connectivity patterns of the DMN in healthy control subjects when the seed was placed separately in PCC versus MPFC regions. For example, Johnson et al reported a dissociation between MPFC and PCC activity by using functional MR imaging during cognitive tasks (51). They provided evidence with task-related functional MR imaging that anterior and posterior regions of the DMN may subserve different functions with respect to different self-reflection conditions. In the current study, we used a comprehensive core-seed–based template for single-participant ICA to select the best fit of coactivation of DMN substructures. The template-based single-participant ICA is a novel automated approach in sorting independent components without subjective identification of the DMN (52,53). By using template-based ICA analysis, Greicius et al also showed improved specificity

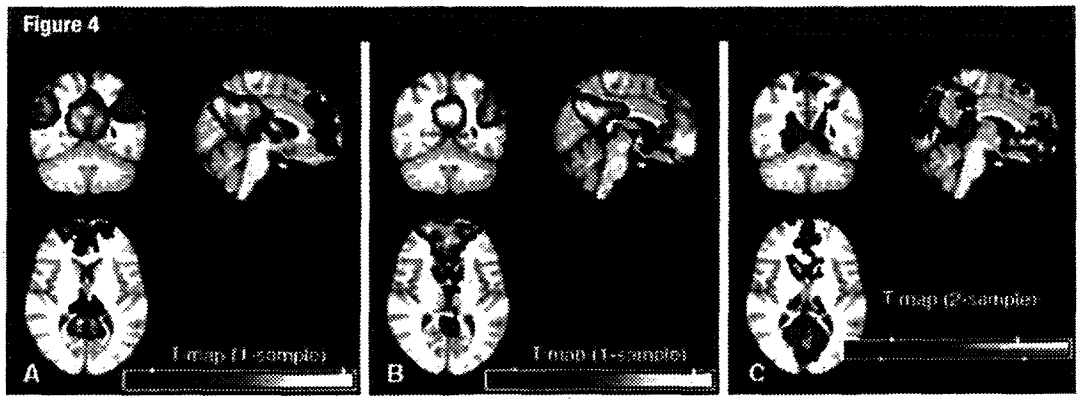


Figure 4: DMN templates obtained with the hybrid ICA seed method in patients and control subjects (corrected, $P < .05$; $K \geq 20$). *A*, Typical but enhanced connectivity pattern of the DMN was identified in the healthy control group. *B*, Disrupted DMN pattern is shown in the patient group. *C*, Group-level voxelwise image of hybrid DMN template differences comparing MTBI to control groups. Red = increased functional connectivity, blue = decreased functional connectivity in patients compared with control subjects. These changes in DMN pattern were consistent with the single-subject ICA results by means of visual inspection.

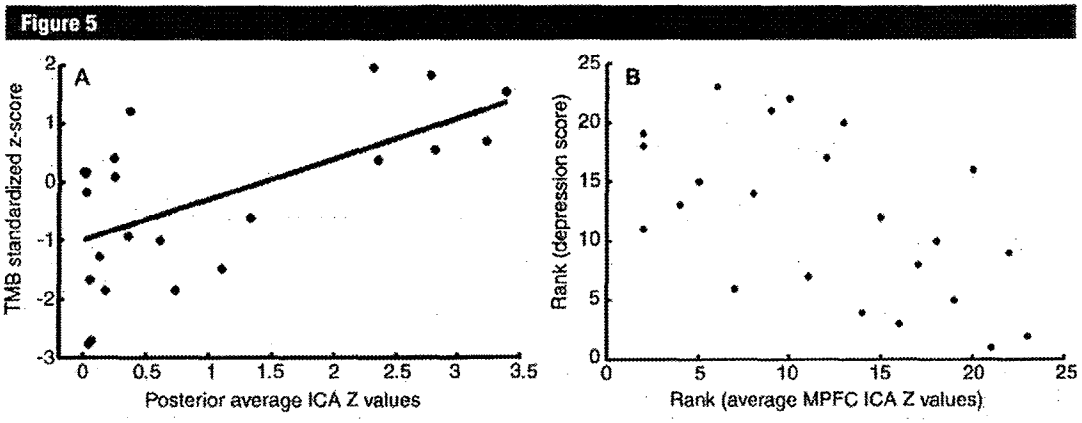


Figure 5: Plots show correlation analyses after Bonferroni correction between neuropsychologic tests and single-subject ICA *z* images in patients with MTBI. *A*, Pearson correlation analysis shows significant positive correlation ($r = 0.60$, $P = .02$) between functional connectivity in posterior regions and Trail Making Test B *z* score. *B*, Spearman rank correlation shows significant negative correlation ($r = -0.56$, $P = .01$) between functional connectivity of the anterior MPFC and depression scores in patients with MTBI.

of detecting DMN abnormalities in patients with Alzheimer disease when the entire template was used, as compared with a PCC-only template (29).

On the basis of single-participant analysis, the regions of decreased functional connectivity within the DMN at functional MR imaging in the present study were found to be predominantly located in the posterior part of the brain, including the cuneus, left calcarine cortex (V1), and PCC. These posterior components of the DMN (eg, the PCC) have been reported to be vulnerable to injury after traumatic brain injury; for example, Yount et al found decreased PCC volume (54) and Levine et al (55) reported that there was gray matter atrophy predominantly involved in the PCC and retrosplenial regions after traumatic brain injury. Relatively recent study findings (24) have implicated DMN connectivity as being reflective of level of consciousness in patients with brain damage. Although the precise role of the DMN in conscious processes remains to be elucidated, all patients with MTBI in the present study experienced a short period of loss of consciousness at the time of trauma. In addition, the reduced posterior functional connectivity of the DMN might indicate subtle axonal pathway injury (56,57). We also found a highly significant correlation between reduced posterior connectivity and decreased cognitive flexibility, as measured with a Trail Making Test B in patients with MTBI, due to the highly involved cognitive processes of this network (20,58,59). Our results of increased MPFC functional connectivity

at functional MR imaging in MTBI may, on the other hand, reflect a compensatory mechanism of increased frontal baseline activity. Increased MPFC activation has been reported previously in moderate and severe traumatic brain injury (60) and is hypothesized to represent brain neuroplasticity operating in recovery and neural repair after injury (61). Our finding of a negative correlation between increased MPFC connectivity and neuropsychologic symptoms also supports the notion of increased usage of MPFC neural resources as compensation in response to the impaired neurocognitive function. Previous studies, outside of traumatic brain injury, have also shown MPFC connectivity changes in patients with anxiety and depression, although the pattern of changes in connectivity appears to be different compared with MTBI (17,62,63). The abnormally increased MPFC usage over the long run, however, might lead to persistent psychologic symptoms, such as the depression, anxiety, and fatigue seen in these patients.

Our finding of a significant negative correlation between increased MPFC functional connectivity and decreased PCC functional connectivity at functional MR imaging in these patients suggests that frontoposterior DMN nodes are not only intrinsically interdependent but also highly complementary in their functions. The within-network discrepant changes have also been identified in other diseases; for example, Zhang et al (53) reported bilaterally decreased MPFC and medial temporal lobe connectivity and increased PCC connectivity in patients with epilepsy, suggesting that DMN components can have distinct alteration patterns with different pathologic circumstances. Jones et al also found increased anterior DMN connectivity and decreased posterior DMN connectivity in aging populations and those with Alzheimer disease (64). These studies support the notion that DMN cannot be considered a homogeneous entity but instead comprises several nodes that serve different, highly complex cognitive functions that are independent but are maintained in a dynamic equilibrium (65). Thus, the functional connectivity and interaction between the MPFC and PCC, which was considered a robust, converged ventral-dorsal compartment model (28), appears to be critical when these structures are injured.

When compared with single-participant ICA, the results of our hybrid ICA seed-based method by using an iterative ICA algorithm showed similar but enhanced patterns of DMN changes in MTBI, providing more converged information about spatial relationships with less heterogeneity and reproducibility problems. In line with the previous two methods, our group ICA, in which we used the FSL MELODIC algorithm on the basis of temporal concatenations of all individuals within each group, also showed increased frontal MPFC connectivity and decreased posterior connectivity around the PCC in patients with MTBI compared with healthy control subjects. Although group ICA is a robust measure (66), it is limited by its inapplicability in individual quantification and correlation with clinical measures. Although our seed-based method did not show statistical significance between patients with MTBI and control subjects when we used the significance level of corrected $P < .05$, the direct visual inspection of seed-based results indeed revealed a similar pattern of differences as when ICA approaches were used. Currently, the seed-based approach is known to have limitations of noise and high dependence on seed location (67,68). By using a seed-based method, Mayer et al (25) showed decreased anterior and posterior functional connectivity within the DMN at functional MR imaging. This discrepancy in findings is likely due to several factors, including the following: *(a)* Their seed-based method was based on interregional correlation between averaged time courses of two specific regions instead of global voxel-wise correlation between time courses of each voxel and template, as used in the current study. *(b)* The PCC seed in their study was chosen to be smaller (679 mm^3) and more anteriorly centered (at MNI coordinates 0, −47, 33) when compared with our seed (896 mm^3, centered at −6, −48, 39). *(c)* There were differences in the interval time between injury and MR imaging, with a mean of 11.5 days postinjury in their study versus 22 days in our study.

A few potential limitations of the current study should be considered. First, this study was limited in scope to the DMN, a most reliable and robust network that supports the "default mode of brain function" during rest. The functional connectivity MR imaging abnormalities of other networks have not been investigated in the current study, and only the thalamic network was reported on previously in MTBI (69). Second, the difference between patients and control subjects with a seed-based method did not reach statistical significance, which raises cautions for the interpretation of our ICA-based findings, despite the fact that the patterns based on the two methods are similar by means of visual inspection. One possibility is that the sample size is relatively small in our groups, which warrants further investigation in a larger population. Third, the results of disrupted DMN connectivity can be caused by structural connectivity abnormalities (eg, axonal injury), which were not investigated in the current study. Future studies designed to explore the relationship between structural and functional network deficits within DMN nodes will be valuable.

Given the important role of the DMN in brain cognitive function, our results of significant changes of DMN functional connectivity in patients with MTBI may provide insights into the underlying mechanisms of the reduced performance in neurocognitive testing. Longitudinal studies are warranted to further evaluate whether the DMN can serve as a biomarker to monitor disease progression and recovery in MTBI.

Acknowledgments: The authors thank Kelly-Anne McGorty, BS, HT, for technical support and Damon Kenul, BS, for manuscript preparation.

Disclosures of Conflicts of Interest: **Y.Z.** No relevant conflicts of interest to disclose. **M.P.M.** No relevant conflicts of interest to disclose. **Y.W.L.** No relevant conflicts of interest to disclose. **L.M.** No relevant conflicts of interest to disclose. **J.R.**

No relevant conflicts of interest to disclose. **D.K.S.** No relevant conflicts of interest to disclose. **R.I.G.** No relevant conflicts of interest to disclose. **Y.G.** No relevant conflicts of interest to disclose.

References

1. Sosin DM, Sniezek JE, Thurman DJ. Incidence of mild and moderate brain injury in the United States, 1991. Brain Inj 1996;10(1):47–54.
2. Cassidy JD, Carroll LJ, Peloso PM, et al. Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med 2004;(43 Suppl):28–60.
3. Carroll LJ, Cassidy JD, Peloso PM, et al. Prognosis for mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med 2004;(43 Suppl):84–105.
4. Rutland-Brown W, Langlois JA, Thomas KE, Xi YL. Incidence of traumatic brain injury in the United States, 2003. J Head Trauma Rehabil 2006;21(6):544–548.
5. Silver JM, McAllister TW, Yudofsky SC. Textbook of traumatic brain injury. 2nd ed. Washington, DC: American Psychiatric Publishing, 2011.
6. World Health Organization. International Classification of Diseases and Related Health Problems. 10th ed. Geneva, Switzerland: World Health Organization, 2007.
7. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington, DC: American Psychiatric Association, 2010.
8. Cordes D, Haughton VM, Arfanakis K, et al. Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. AJNR Am J Neuroradiol 2001;22(7):1326–1333.
9. Gusnard DA, Raichle ME, Raichle ME. Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2001;2(10):685–694.
10. Zhang D, Raichle ME. Disease and the brain's dark energy. Nat Rev Neurol 2010;6(1):15–28.
11. Shulman GL, Corbetta M, Fiez JA, et al. Searching for activations that generalize over tasks. Hum Brain Mapp 1997;5(4):317–322.
12. Raichle ME, Snyder AZ. A default mode of brain function: a brief history of an evolving idea. Neuroimage 2007;37(4):1083–1090; discussion 1097–1099.
13. Greicius MD, Supekar K, Menon V, Dougherty RF. Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb Cortex 2009;19(1):72–78.
14. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci 2008;1124:1–38.
15. Rocca MA, Valsasina P, Absinta M, et al. Default-mode network dysfunction and cognitive impairment in progressive MS. Neurology 2010;74(16):1252–1259.
16. Slobounov SM, Gay M, Zhang K, et al. Alteration of brain functional network at rest and in response to YMCA physical stress test in concussed athletes: RsFMRI study. Neuroimage 2011;55(4):1716–1727.
17. Zhao XH, Wang PJ, Li CB, et al. Altered default mode network activity in patient with anxiety disorders: an fMRI study. Eur J Radiol 2007;63(3):373–378.
18. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 2007;8(9):700–711.
19. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A 2001;98(2):676–682.
20. Lux WE. A neuropsychiatric perspective on traumatic brain injury. J Rehabil Res Dev 2007;44(7):951–962.
21. Boly M, Phillips C, Tshibanda L, et al. Intrinsic brain activity in altered states of consciousness: how conscious is the default mode of brain function? Ann N Y Acad Sci 2008;1129:119–129.
22. Hillary FG, Slocomb J, Hills EC, et al. Changes in resting connectivity during recovery from severe traumatic brain injury. Int J Psychophysiol 2011;82(1):115–123.
23. Sharp DJ, Beckmann CF, Greenwood R, et al. Default mode network functional and structural connectivity after traumatic brain injury. Brain 2011;134(Pt 8):2233–2247.
24. Vanhaudenhuyse A, Noirhomme Q, Tshibanda LJ, et al. Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. Brain 2010;133(Pt 1):161–171.
25. Mayer AR, Mannell MV, Ling J, Gasparovic C, Yeo RA. Functional connectivity in mild traumatic brain injury. Hum Brain Mapp 2011;32(11):1825–1835.
26. Lee TW, Girolami M, Sejnowski TJ. Independent component analysis using an extended infomax algorithm for mixed subgaussian and supergaussian sources. Neural Comput 1999;11(2):417–441.
27. De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. Neuroimage 2006;29(4):1359–1367.
28. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 2003;100(1):253–258.
29. Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 2004;101(13):4637–4642.
30. Kelly RE, Wang Z, Alexopoulos GS, et al. Hybrid ICA-seed-based methods for fMRI functional connectivity assessment: a feasibility study. Int J Biomed Imaging 2010;2010. pii: 868976.
31. Beckmann CF, Smith SM. Tensorial extensions of independent component analysis for multisubject FMRI analysis. Neuroimage 2005;25(1):294–311.
32. Petchprapai N, Winkelmann C. Mild traumatic brain injury: determinants and subsequent quality of life—a review of the literature. J Neurosci Nurs 2007;39(5):260–272.
33. Smith A. Symbol Digit Modalities Test Manual. Los Angeles, Calif: Western Psychological Services, 1973.
34. Wechsler D. Wechsler Adult Intelligence Scale-III. New York, NY: The Psychological Corporation, 1985.
35. Reitan R. Trail Making Test: Manual for Administration and Scoring. Tucson, Ariz: Reitan Neuropsychology Laboratory, 1992.
36. Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test. Adult Version. Manual. 2nd ed. San Antonio, Tex: The Psychological Corporation, 2000.
37. Meyers JE, Meyers KR. Rey Complex Figure Test and Recognition Trial: Professional manual. Odessa, Fla: Psychological Assessment Resources, 1995.
38. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561–571.
39. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale: application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46(10):1121–1123.
40. Aubry M, Cantu R, Dvorak J, et al. Summary and agreement statement of the First International Conference on Concussion in

Sport, Vienna 2001: recommendations for the improvement of safety and health of athletes who may suffer concussive injuries. Br J Sports Med 2002;36(1):6–10.

41. Chen JK, Johnston KM, Collie A, McCrory P, Ptito A. A validation of the post concussion symptom scale in the assessment of complex concussion using cognitive testing and functional MRI. J Neurol Neurosurg Psychiatry 2007;78(11):1231–1238.

42. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 2012;59(3):2142–2154.

43. Uddin LQ, Kelly AM, Biswal BB, Xavier Castellanos F, Milham MP. Functional connectivity of default mode network components: correlation, anticorrelation, and causality. Hum Brain Mapp 2009;30(2):625–637.

44. Dice LR. Measures of the amount of ecologic association between species. Ecology 1945;26(3):297–302.

45. Rombouts SA, Barkhof F, Hoogenraad FG, Sprenger M, Valk J, Scheltens P. Test-retest analysis with functional MR of the activated area in the human visual cortex. AJNR Am J Neuroradiol 1997;18(7):1317–1322.

46. Bell AJ, Sejnowski TJ. An information-maximization approach to blind separation and blind deconvolution. Neural Comput 1995;7(6):1129–1159.

47. Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci 2005;360(1457):1001–1013.

48. Bennett CM, Wolford GL, Miller MB. The principled control of false positives in neuroimaging. Soc Cogn Affect Neurosci 2009;4(4):417–422.

49. Vialatte FB, Cichocki A. Split-test Bonferroni correction for QEEG statistical maps. Biol Cybern 2008;98(4):295–303.

50. Daniels JK, McFarlane AC, Bluhm RL, et al. Switching between executive and default mode networks in posttraumatic stress disorder: alterations in functional connectivity. J Psychiatry Neurosci 2010;35(4):258–266.

51. Johnson MK, Raye CL, Mitchell KJ, Touryan SR, Greene EJ, Nolen-Hoeksema S. Dissociating medial frontal and posterior cingulate activity during self-reflection. Soc Cogn Affect Neurosci 2006;1(1):56–64.

52. Cauda F, Micon BM, Sacco K, et al. Disrupted intrinsic functional connectivity in the vegetative state. J Neurol Neurosurg Psychiatry 2009;80(4):429–431.

53. Zhang Z, Lu G, Zhong Y, et al. Altered spontaneous neuronal activity of the default-mode network in mesial temporal lobe epilepsy. Brain Res 2010;1323:152–160.

54. Yount R, Raschke KA, Biru M, et al. Traumatic brain injury and atrophy of the cingulate gyrus. J Neuropsychiatry Clin Neurosci 2002;14(4):416–423.

55. Levine B, Kovacevic N, Nica EI, et al. The Toronto traumatic brain injury study: injury severity and quantified MRI. Neurology 2008;70(10):771–778.

56. Bazarian JJ, Zhong J, Blyth B, Zhu T, Kavcic V, Peterson D. Diffusion tensor imaging detects clinically important axonal damage after mild traumatic brain injury: a pilot study. J Neurotrauma 2007;24(9):1447–1459.

57. Inglese M, Makani S, Johnson G, et al. Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. J Neurosurg 2005;103(2):298–303.

58. Cicerone KD. Remediation of "working attention" in mild traumatic brain injury. Brain Inj 2002;16(3):185–195.

59. Halterman CI, Langan J, Drew A, et al. Tracking the recovery of visuospatial attention deficits in mild traumatic brain injury. Brain 2006;129(Pt 3):747–753.

60. Christodoulou C, DeLuca J, Ricker JH, et al. Functional magnetic resonance imaging of working memory impairment after traumatic brain injury. J Neurol Neurosurg Psychiatry 2001;71(2):161–168.

61. Scheibel RS, Newsome MR, Steinberg JL, et al. Altered brain activation during cognitive control in patients with moderate to severe traumatic brain injury. Neurorehabil Neural Repair 2007;21(1):36–45.

62. Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga-Barke EJ. Default-mode brain dysfunction in mental disorders: a systematic review. Neurosci Biobehav Rev 2009;33(3):279–296.

63. Greicius MD, Flores BH, Menon V, et al. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biol Psychiatry 2007;62(5):429–437.

64. Jones DT, Machulda MM, Vemuri P, et al. Age-related changes in the default mode network are more advanced in Alzheimer disease. Neurology 2011;77(16):1524–1531.

65. Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL. Functional-anatomic fractionation of the brain's default network. Neuron 2010;65(4):550–562.

66. Zuo XN, Kelly C, Adelstein JS, Klein DF, Castellanos FX, Milham MP. Reliable intrinsic connectivity networks: test-retest evaluation using ICA and dual regression approach. Neuroimage 2010;49(3):2163–2177.

67. Ma L, Wang B, Chen X, Xiong J. Detecting functional connectivity in the resting brain: a comparison between ICA and CCA. Magn Reson Imaging 2007;25(1):47–56.

68. van de Ven VG, Formisano E, Prvulovic D, Roeder CH, Linden DE. Functional connectivity as revealed by spatial independent component analysis of fMRI measurements during rest. Hum Brain Mapp 2004;22(3):165–178.

69. Tang L, Ge Y, Sodickson DK, et al. Thalamic resting-state functional networks: disruption in patients with mild traumatic brain injury. Radiology 2011;260(3):831–840.

JOURNAL OF NEUROTRAUMA 32:1031–1045 (July 15, 2015)

DOI: 10.1089/neu.2014.3610

Resting State Functional Connectivity in Mild Traumatic Brain Injury at the Acute Stage: Independent Component and Seed-Based Analyses

Armin Iraji,[1] Randall R. Benson,[2] Robert D. Welch,[3] Brian J. O'Neil,[3] John L. Woodard,[4] Syed Imran Ayaz,[3] Andrew Kulek,[3] Valerie Mika,[1,3] Patrick Medado,[3] Hamid Soltanian-Zadeh,[5] Tianming Liu,[6] E. Mark Haacke,[1,7] and Zhifeng Kou[1,7]

Abstract

Mild traumatic brain injury (mTBI) accounts for more than 1 million emergency visits each year. Most of the injured stay in the emergency department for a few hours and are discharged home without a specific follow-up plan because of their negative clinical structural imaging. Advanced magnetic resonance imaging (MRI), particularly functional MRI (fMRI), has been reported as being sensitive to functional disturbances after brain injury. In this study, a cohort of 12 patients with mTBI were prospectively recruited from the emergency department of our local Level-1 trauma center for an advanced MRI scan at the acute stage. Sixteen age- and sex-matched controls were also recruited for comparison. Both group-based and individual-based independent component analysis of resting-state fMRI (rsfMRI) demonstrated reduced functional connectivity in both posterior cingulate cortex (PCC) and precuneus regions in comparison with controls, which is part of the default mode network (DMN). Further seed-based analysis confirmed reduced functional connectivity in these two regions and also demonstrated increased connectivity between these regions and other regions of the brain in mTBI. Seed-based analysis using the thalamus, hippocampus, and amygdala regions further demonstrated increased functional connectivity between these regions and other regions of the brain, particularly in the frontal lobe, in mTBI. Our data demonstrate alterations of multiple brain networks at the resting state, particularly increased functional connectivity in the frontal lobe, in response to brain concussion at the acute stage. Resting-state functional connectivity of the DMN could serve as a potential biomarker for improved detection of mTBI in the acute setting.

Key words: functional connectivity; fMRI; functional MRI; mTBI; mild traumatic brain injury; resting state fMRI

Introduction

Traumatic brain injury (TBI) is a significant public health burden in the United States and worldwide.[1,2] Most patients with mTBI experience an injury of mild severity, often referred to as mild TBI (mTBI),[3] accounting for more than 1 million emergency department (ED) visits annually in the United States.[4] Most patients with mTBI usually stay in the EDs only for a few hours and then are discharged home without specific follow-up instructions, which is surprising given that a number of patients with mTBI experience acute and protracted neurocognitive symptoms. Further, even a mild injury can have a substantial impact on their quality of life and society.[3,5,6] Such rapid ED discharge is based on negative CT findings for most patients with mTBI.

The acute setting is a golden window of opportunity for impacting current treatment of patients with mTBI.[7] A constant challenge for emergency physicians is to detect neural abnormalities in patients with mTBI that may impact patients' protracted recovery or prolonged neurocognitive symptoms,[7] which may hold the best opportunity to improve the proactive treatment of patients with mTBI.

Advanced magnetic resonance imaging (MRI) holds great potential for mTBI injury detection and outcome prediction.[8,9] Examples include diffusion tensor imaging (DTI),[10–19] susceptibility weighted imaging (SWI),[20–22] perfusion imaging, and MR spectroscopy, among others.[8,9] Clinical and neurocognitive features of patients with mTBI include attention and memory deficits among a constellation of other physical and emotional symptoms. Given the

[1]Department of Biomedical Engineering, Wayne State University, Detroit, Michigan. [2]Center for Neurologic Studies, Novi, Michigan.
[3]Department of Emergency Medicine, Wayne State University, Detroit, Michigan.
[4]Department of Psychology, Wayne State University, Detroit, Michigan.
[5]Department of Radiology, Henry Ford Hospital, Detroit, Michigan.
[6]Department of Computer Science, University of Georgia, Athens, Georgia.
[7]Department of Radiology, Wayne State University, Detroit, Michigan.

EXHIBIT 6

fact that most patients with mTBI have no obvious damage on structural MRI, functional MRI (fMRI) may be a viable alternative for detecting injury-related abnormalities.[23–26]

Resting-state fMRI (rsfMRI), which reflects brain activity at rest while not performing any specific task, could reveal possible disruption in cognitive function from mild TBI.[27,28] rsfMRI has the advantage of detecting abnormalities in functional connectivity (FC) even in the presence of unremarkable structural imaging results. Numerous studies have reported disruptions of the resting-state networks (RSNs) and the brain's intrinsic low FC in various disorders, such as Alzheimer disease (AD), schizophrenia, attention deficit hyperactivity disorder (ADHD), and mTBI.[27–31]

Among all brain networks at the resting state, the Default Mode Network (DMN) is the most widely studied network in mTBI.[27,28,32,33] The DMN is assumed to control passive mental activities while persons are awake and alert but do not specifically perform any goal-directed task.[34] The posterior cingulate cortex (PCC) and the medial prefrontal cortex (MPFC) are two important components of the DMN. The PCC and MPFC are associated with self-referential and emotional processing, as well as semantic processing,[35] and their activity is attenuated when attention to a goal-directed task increases.[36] The reported important role of the DMN in several cognitive functions and its disruption in several neurocognitive disorders both highlight the importance of the connections of the DMN and possible alterations in its activity as a possible diagnostic marker of injury-related neural damage.

In terms of analysis of rsfMRI data, seed-based region of interest (ROI) analysis, also called seed-based analysis (SBA), is widely used. Mayer and associates[27] studied patients with mTBI at the subacute stage (within 2 weeks after injury) by choosing the rostral anterior cingulate gyrus as the seed region and demonstrated a reduction in connectivity within the DMN for patients with mTBI and an increase in connectivity within a task-related network (TRN) relative to matched controls. Similarly, Johnson and colleagues[28] scanned sports athletes with mTBI at the subacute phase (10 ± 2 days). By using both voxel-based and ROI-based analysis, they focused on DMN regions such as the PCC, MPFC, lateral parietal lobes, and the parahippocampal gyrus. They indicated that both the number and strength of connections decreased in the PCC and the lateral parietal cortices but increased in the MPFC.

For ICA analysis, Stevens and coworkers[32] explored different independent components analysis (ICA) components to find alterations between healthy subjects and patients with mTBI who underwent scanning 61 days after injury. They found diminished FC in the patient group in the DMN and many other networks, such as the primary visual processing circuit, the motor system, the left-lateralized frontoparietal circuit, the dorsomedial circuit, the frontoparietal executive system, and the frontostriatal network. They also found that the precuneus has greater connectivity with the DMN, while the connectivity between the cingulate and this network decreases.

Tang and colleagues[37] explored alterations in thalamus connectivity for patients with mTBI with a 22-day mean interval after injury. The thalamus contributes to communication between various cortical regions of the brain. In the seed-based method, the patients with mTBI demonstrated more widely distributed FC between thalamic and cortical regions. The ICA analysis indicated an increase in thalamocortical functional connectivity at the posterior cingulate and frontal regions in patients compared with healthy control subjects. They suggested an up-regulation of thalamocortical FC in response to disruption of thalamic RSNs in patients with mTBI.[37]

To date, most studies were conducted during the subacute or chronic stage, given that patients with mTBI were imaged between 10 and 60.9 days after injury or even longer. Few studies have investigated the alterations of RSN in mTBI at the acute stage, which is within 24 h post-injury or sooner. This time frame corresponds to the maximum amount of time that patients with mTBI typically stay within EDs, and rapid treatment of patients within this time frame can have a direct impact on their acute care.[7]

In addition, it is at the acute stage that most patients with mTBI report post-concussion symptoms (PCS) and neurocognitive problems. Although approximately 50% of patients' clinical and neurocognitive symptoms resolve during the subacute stage and most patients' symptoms have resolved by the chronic stage,[38] some patients with mTBI experience protracted symptoms. Detection of the neural basis of brain injury at the acute stage will be most likely to shed light on the link between early functional abnormalities and the possibility of protracted symptoms.

In addition to alterations in the DMN and thalamus connectivity, memory problems are one of the most prevalent neurocognitive symptoms experienced at the acute stage, in suggestion of likely disruption of the hippocampal functional networks.[39] In addition, emotional and behavioral symptoms in mTBI also suggest involvement of the limbic network, involving the amygdala. Along with the fusiform gyrus, the hippocampus is vulnerable because of its position within the middle cranial fossa and petrous bone, a region likely to directly sustain deformation injury, which in turn could affect connectivity. This also explains the functional network alterations seen when using the parahippocampal gyrus as a seed region as reported by Johnson and coworkers.[28]

In addition, the trauma incident is also an emotional event, as well as causing physical or physiological disruptions of the brain. Emotional disturbances such as anxiety and depression immediately after mTBI in the acute setting also suggest that the amygdala may play a role as well. Because of the location of the sphenoid bone in conjunction with the tentorium cerebelli, the amygdala is highly likely to be injured at all levels of injury severity, including mild.

In this prospective study, we have investigated RSNs in patients with mTBI and have assessed their clinical and neurocognitive symptoms within 24 h while they are still either at the ED or in the hospital. We used both ICA and ROI analyses by looking at both the DMN and thalamic regions as well as the hippocampus and amygdala to determine group differences between patients and controls. Our objective is to identify a possible neural basis of acute clinical and cognitive symptoms in mTBI by using rsfMRI. We hypothesized that the brain may demonstrate altered functional connectivity in the DMN and networks involving the thalamus, hippocampus, and amygdala in response to brain injury at the acute stage.

Methods

Subject recruitment

This study was approved by both the Human Investigation Committee of Wayne State University and the Institutional Review Board of Detroit Medical Center. Written informed consent was obtained from each subject before enrollment. All subjects were at least 18 years old and able to speak English. All patients with mTBI were recruited from the ED of Detroit Receiving Hospital (DRH), which is a Level-I trauma center. Patient eligibility was based on the mTBI definition by the American Congress of Rehabilitation Medicine.[40]

Only patients with a lowest-recorded Glasgow Coma Score (GCS) of 13–15 were considered. For a GCS of 15, there must be least one of the following: (a) loss of consciousness less than

30 min, (b) post-traumatic amnesia less than 24 h, or (c) an alteration in mental status (i.e., disoriented, dazed, or confused). Participants, both healthy controls and patients, with a history of a previous brain injury, neurological, neuropsychological, or psychiatric disorder, or concurrent substance abuse were excluded. Moreover, MRI exclusion criteria include metal and/or electronic implants, claustrophobia, pregnant or trying to become pregnant, and subjects weighing more than 300 pounds (136 kg; machine's capacity limit). If the MRI scan could not be performed in the acute setting in a patient, the patient was brought back for an MRI scan within a week of injury.

Cognitive assessment

In the acute setting, once a patient was conscious and medically stable and came out of post-traumatic amnesia, if any, they would be administered neurocognitive testing and surveyed about their PCS. A short instrument called the Standardized Assessment of Concussion (SAC)[41] was used as a brief assessment of neurocognitive function. The SAC was originally developed for field assessment of neurocognitive status after a sports concussion.[42] It has been reported that the SAC is sensitive to the acute changes after concussion and requires limited training to administer.[39]

The SAC is scored 0 to 30 and assesses four cognitive domains including orientation, attention, immediate memory, and delayed recall. The SAC has demonstrated sensitivity to brain injury in the acute setting, particularly in delayed recall.[39] The Emergency Room Edition of the SAC also includes a PCS questionnaire where symptoms were graded from 0 to 3 (i.e., none, mild, moderate, severe). The PCS score was the sum of symptom scores for the questionnaire.

Image acquisition

MRI data were collected on a 3-Tesla Siemens Verio scanner with a 32-channel radiofrequency head-only coil. A subject's head was fixed by foam pads to restrict motion. Resting state functional imaging was performed using a gradient echo echo-planar imaging sequence with the following imaging parameters: repetition time (TR) = 2000 msec, echo time (TE) = 30 msec, slice thickness = 3.5 mm, slice gap = 0.595 mm, flip angle = 90 degrees, pixel size = 3.125 mm in-plane, matrix size = 64 × 64, 240 volumes for whole-brain coverage, one average, acquisition time of 8 min. During resting-state scans, subjects were instructed to keep their eyes closed and to stay awake. In addition, structural high-resolution T1-weighted imaging was also performed by using the MPRAGE sequence with TR = 1950 ms, TE = 2.26 ms, slice thickness = 1 mm, flip angle = 9 degrees, field of view = 256 × 256 mm, matrix size = 256 × 256, and voxel size = 1 mm isotropic.

Image processing

Figure 1 demonstrates a pipeline of the comprehensive image processing. We used both ICA and SBA to analyze the resting state fMRI (rsfMRI). ICA was performed at both group and individual levels. Group ICA was performed first in both patient and control groups and then with cross-validation in subgroups. Individual ICA was performed by using two reported methods, "dual regression"[43,44] and "back-projection,"[43,44] and our new atlas-based ICA method to overcome the confounding factors of these two methods. ICA analysis was mainly performed in DMN and the basal ganglia network (BGN). Because the thalamus belongs to BGN, which is an independent network in ICA analysis in published data,[45] we investigated BGN to assess the thalamus in the ICA analysis.

After ICA analysis, SBA was also performed by calculating the Pearson correlation value between major network regions and the whole brain, and the correlation maps were used to compare healthy controls and patients. The network regions were selected for DMN, BGN, amygdala and hippocampus regions based on previous studies.[27,28,37] Specifically, the PCC, precuneus, angular, anterior prefrontal cortex (Brodmann area [BA] 10), and orbitofrontal cortex (BA 11) were selected for the DMN; and the putamen, pallidum, thalamus, and caudate were selected for the BGN. Below is a description of major steps.

Preprocessing. Preprocessing was performed using the FSL software (http://www.fmrib.ox.ac.uk/fsl/). First, the first five volumes were discarded to reach magnetization equilibrium. A high-pass temporal filtering (100 sec), motion correction, slice timing, brain extraction, and spatial smoothing (full width at half maximum = 6 mm) were applied. Grand mean scaling was also applied to the entire 4-dimensional data for each subject. The data were registered to the Montreal Neurological Institute standard space using nonlinear registration with 10 mm warp resolution and resampled to 3 mm isotropic voxel size. Variance normalization was used to rescale each time series. No intensity normalization was performed to prevent false anticorrelation. In addition, for SBA, the white matter (WM) and cerebrospinal fluid (CSF) signal were regressed out using the DPARSF toolbox (http://www.restfmri.net/forum/DPARSF).

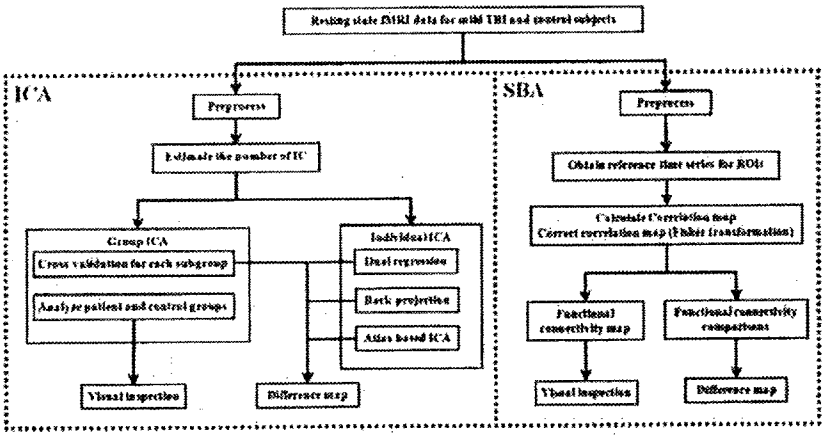


FIG. 1. Image processing pipeline. fMRI, functional magnetic resonance imaging; TBI, traumatic brain injury; ICA, independent component analysis; SBA, seed-based analysis; ROI, region of interest.

Group ICA (GICA) analysis. ICA was performed using the GIFT software package from MIALAB (http://mialab.mrn.org/software/gift/) and the MELODIC package, which is included in FSL (http://www.fmrib.ox.ac.uk/fsl/melodic). We chose 52 components for our analysis and applied an automated two-step approach for network selection to eliminate human errors (see Supplement 1 for further explanations; see online supplementary material at ftp.liebertpub.com). Briefly, the DMN and BGN were determined by using the spatial correlation similarity metric (Eq. 1) between ICA components and a template of the desired RSNs. The template includes 75 components built by Allen and coworkers[45] based on 603 subjects that contains 28 RSNs (see Supplement 1 for further explanations; see online supplementary material at ftp.liebertpub.com).

$$r = \frac{\sum_i X_i Y_i}{\sqrt{\sum_i X_i^2 \times \sum_i Y_i^2}}, \qquad \text{Eq. 1}$$

Where i is the number of voxels, X is a desired RSN, and Y is a component obtained from ICA.

The GICA was performed on the healthy control and patient groups. The group difference was measured between patients and controls in major network regions of both the DMN and BGN. The reliability and reproducibility of the results were measured by using a cross-validation method. Specifically, for each group (patient or healthy control), seven subgroups were randomly chosen with six subjects in each subgroup. GICA was performed for each subgroup, and the number of voxels in each network region associated with the related network and the voxel dependency were measured. Voxel dependency is a voxel's value of RSN map (the result of ICA) indicating its likelihood of belonging to the network—in other words, the extent to which a voxel belongs to the network.

Individual ICA. Individual ICA was performed using two reported methods—i.e., dual regression[43,44] and back-projection,[43,44] as well as a new atlas-based ICA approach to evaluate the alterations in patients with mTBI. The common space in both ICA methods was created by using the whole dataset of this study, because extracting the independent components of each group separately may overestimate the difference between groups. Given the relative small sample size in this study, however, the common space may still be different from that of the normal population. To overcome the potential confounding factor, we proposed an atlas-based ICA method.

Proposed atlas-based ICA. In the atlas-based ICA, independent components extracted from 603 subjects[45] were used as the common space, and a "spatially constrained ICA" method[46] was used to extract the individual components corresponding to the common space components.

Because the common space in the dual regression and back-projection methods is created by using the whole dataset of a particular study, the common spaces are different when the datasets of studies differ from each other. Consequently, RSNs of an individual could be changed depending on the group to which the individual belongs. On the other hand, in the temporal concatenation group ICA, we assume that there is a common space among all individuals; therefore, using the data of a study to find the common space is not ideal, especially when the number of samples is small, as in our study. Therefore, a template or atlas that indicates the common space of a big population is needed.

In the atlas-based ICA, unlike the dual regression and back-projection, instead of using the independent components of GICA of our dataset as a common space for individual ICA, the independent components from a large group of healthy subjects are considered as a true common space. In this case, (1) the RSNs extracted from an individual's rsfMRI data are more reliable because independent components were extracted from an atlas that is based on a large population instead of a small group of subjects; (2) independent components and subsequent analysis and results among various studies with different data sets only depend on individual differences rather than the whole data set in an study, because the common space is the same among all of this study. In other words, the difference between various subjects is only the result of the intrinsic variability between each pair of them.

Note that because this method uses atlas components to extract the individual components in individual subjects, it automatically solves the problem of finding corresponding RSNs across subjects. As previously explained, this method only requires the individual subject's data instead of a whole set of subjects' data, which makes it more applicable for clinical use.

SBA. SBA was performed using within-group and between-group analyses. A Pearson correlation coefficient was determined between the average of all voxels in a network region and all brain voxels to create a correlation map for each region.[37] The correlation maps were corrected by using the Fisher z-transformation to calculate a FC map and FC comparisons. The FC map was calculated by applying a one-sample t test on corrected correlation maps. In FC comparisons, a correlation mask was first applied to select voxels that are eligible for statistical analysis (see Supplement 1 for details regarding SBA; see online supplementary material at ftp.liebertpub.com). Then, statistical analysis was performed using two-sample t tests on corrected correlation maps that were further corrected by spatial thresholding to reduce the false positives and increase reliability.

Statistical analysis

Statistical analysis was performed using MATLAB software (http://www.mathworks.com/). Data normality was assessed by using the Kolmogorov–Smirnov test. Results for continuous variables are presented as mean (standard deviation [SD]) or median (interquartile range) as appropriate. Frequencies and percentages are presented for categorical variables. Unless specifically stated otherwise, a p value <0.05 was considered significant. For ICA, a two-sample t test was performed to compare RSNs between two groups when appropriate. For SBA, the correlation value was converted using the Fisher z-transformation to maintain the statistical analysis eligibility (see Supplement 1 for details; see online supplementary material at ftp.liebertpub.com).

A two-sample t test was performed for FC comparisons, and a one-sample t test was performed to determine the FC map for each network region. Statistical maps for both ICA and SBA were corrected with spatial (cluster) thresholding with the MonteCarlo simulation using Alphasim contained within resting-state fMRI software (http://www.restfmri.net/) to decrease false-positive error. In other words, to correct type I error without increasing the risk of type II error, dual thresholding (based on p value and cluster size) was performed to give a high reliability for statistical results.

Moreover, to increase the reliability of the between-group comparison, the healthy subjects were randomly divided into two groups, and a within-group analysis was performed by using the two-sample t test. The results were compared with the between-group analysis to improve reliability that the difference, which appears in between-group analysis, is generated from the group difference. Finally, the relationship between imaging analyses and the neurocognitive measurements, including SAC scores and scores of each SAC subcategory, were evaluated with bivariate correlations.

Results

Demographic and clinical characteristics are presented in Table 1. In the patient group ($n = 12$), 6 (50%) were men and 6 (50%)

TABLE 1. INDIVIDUAL DEMOGRAPHIC AND CLINICAL DATA OF THE 12 PATIENTS ENROLLED IN THE STUDY

Group	*Subject ID*	*Age/sex*	*Race*	*Mechanism of injury*	*MRI findings*	*Scan time delay*
Patients	PT-001	56/M	Indian	MVC	WMHI, IVH	26 h
	PT-002	35/M	Black	Fall	Negative	36 h
	PT-003	54/F	Black	MVC	Negative	5 h
	PT-004	31/F	Black	MVC	Negative	12 h
	PT-005	30/M	White	Sport	Negative	7 days
	PT-006	36/F	Black	MVC	Negative	9 h
	PT-007	19/M	Black	MVC	WMHI	3 h
	PT-008	30/F	Asian	MV vs. Ped.	Negative	8 h
	PT-009	51/M	Black	Assault	WMHI	13 h
	PT-010[a]	23/M	Black	MVC	Negative	9 h
	PT-011[a]	21/F	White	MVC	Negative	48 h
	PT-012[a]	73/F	Black	Fall	WMHI	6 h
	Summary	38 ± 17 (Mean ± SD) 6/6 (F/M)				13 h (Median) 3 h–7 days (Range)
Healthy controls	CTRL-001	52/F	White	N/A	Negative	N/A
	CTRL-002	44/M	White	N/A	WMHI	N/A
	CTRL-003	41/M	White	N/A	Negative	N/A
	CTRL-004	28/F	White	N/A	Negative	N/A
	CTRL-005[a]	27/F	White	N/A	Negative	N/A
	CTRL-006	29/M	Middle East	N/A	Negative	N/A
	CTRL-007	33/M	White	N/A	Negative	N/A
	CTRL-008	24/F	Asian	N/A	Negative	N/A
	CTRL-009	23/M	Indian	N/A	Negative	N/A
	CTRL-010	45/M	White	N/A	Negative	N/A
	CTRL-011	22/M	White	N/A	Negative	N/A
	CTRL-012	27/M	Asian	N/A	Negative	N/A
	CTRL-013	23/F	Asian	N/A	Negative	N/A
	CTRL-014	22/F	Asian	N/A	Negative	N/A
	CTRL-015	65/F	Asian	N/A	Negative	N/A
	CTRL-016	23/M	White	N/A	Negative	N/A
	Summary	33 ± 13 (Mean ± SD) 7/9 (F/M)				

MRI, magnetic resonance imaging; MVC, motor vehicle collision; WMHI, white matter hyperintensity; IVH, intraventricular hemorrhage; SD, standard deviation.

[a]Eliminated because of motion artifacts on their images.

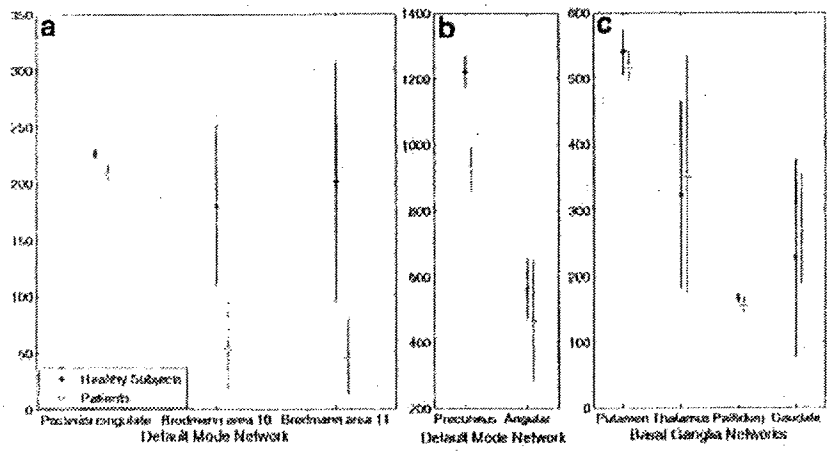


FIG. 2. Cross-validation results of the group independent component analysis on number of associated voxels to the resting state networks. The default mode network shows a difference between the two groups in (**a**) the posterior cingulate cortex, Brodmann area (BA) 10, BA 11 and (**b**) precuneus. (**c**) The basal ganglia network does not show any significant difference. Error bar is one standard deviation. Color image is available online at www.liebertpub.com/neu

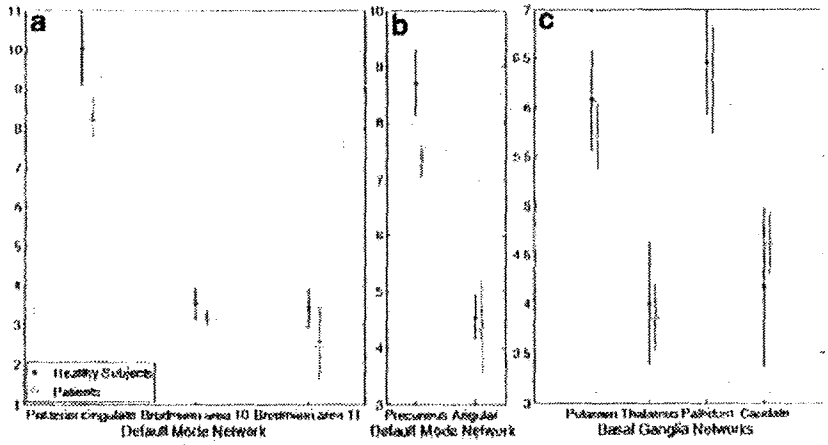


FIG. 3. Cross validation results of the group independent component analysis on voxel dependency to the resting-state networks. The default mode network shows a difference between the two groups in the (**a**) posterior cingulate cortex and (**b**) precuneus. (**c**) The The basal ganglia network does not show any significant difference. Error bar is one standard deviation. Color image is available online at www.liebertpub.com/neu

women. Their age was 38 ± 17 (mean ± standard deviation) years, and GCS scores were all 15 on admission to the ED. Mechanisms of injury included: seven motor vehicle accidents, two falls, one pedestrian struck by a car, one assault, and one sports concussion. All patients had negative CT findings that were diagnosed in the emergency setting. Four patients had "nonspecific WM hyperintensities" on FLAIR images, and one had a tiny intraventricular hemorrhage.

For the healthy controls ($n = 16$), 9 (56%) were men and 7 (44%) women, and the mean healthy subject age was 33 ± 13 years. There was no significant age or sex difference between patients with mTBI and healthy controls. Patients with mTBI underwent scanning in the acute setting (median of 13 h post-injury, range 5 h to 7 days). Except for the one patient who underwent scanning 7 days after injury, the rest underwent scanning within 48 h after injury. Three patients and one control subject were later eliminated from the study because of strong motion artifacts in the images, with a total of 9 patients and 15 healthy controls included in the final analysis.

Neurocognitive performance

The mean patient SAC score was 24.5 ± 2.3 (mean ± SD). We compared this mean with published normative data of more than 568 subjects[47] (mean ± SD, 26.3 ± 2.2). The patients' mean SAC score was significantly below this published mean score ($t(12) = -2.96$, $p = 0.015$). Among all subcategories of the SAC test, including orientation, immediate memory, concentration, and delayed recall, only delayed recall was significantly lower than published normalized data ($t(12) = -2.90$, $p = 0.016$).

ICA results

GICA. Figure 2 shows a significant decrease in number of associated voxels in the PCC and precuneus regions of the DMN in patients with mTBI compared with controls. Figure 3 further shows significantly decreased dependency of associated voxels in the same regions in patients with mTBI compared with controls. Table 2 further demonstrates results of the voxel-wise two-sample t-test on cross-validation GICA analysis ($p < 0.01$), showing group differences in the precuneus and PCC. The result shows a substantial reduction of voxel dependency to DMN for the precuneus and PCC, by 31.19% and 42.28%, respectively. The overall reduction for DMN is 10.51%.

By applying a two-sample t test with a more conservative p value of 0.001, cross-validation GICA results identified a cluster of 50 voxels in the PCC and a cluster of 442 voxels in the precuneus that have significantly lower voxel dependency to the DMN in patients compared with healthy control subjects (Fig. 4).

To sum up, our comprehensive GICA analysis demonstrates a consistent difference in PCC and precuneus between groups, suggesting that resting-state connectivity in these regions could discriminate between patients and healthy controls.

For the BGN, the cross-validation GICA did not reveal any changes in either voxel dependency or the number of voxels in the BGN.

TABLE 2. RESULTS OF VOXEL-WISE TWO-SAMPLE T TEST ON CROSS-VALIDATION OF GROUP INDEPENDENT COMPONENT ANALYSIS

Network	*Region*	*Number of voxels in the region*	*Number of voxels with group difference ($p < 0.01$)*	*% Difference*
DMN	Posterior cingulate cortex (PCC)	246	104	42.28%
	Precuneus	2026	632	31.19%
	Angular	885	0	0%
	Anterior prefrontal cortex (BA 10)	1397	0	0%
	Orbitofrontal cortex (BA 11)	2447	0	0%
	Overall	7001	736	10.51%
BGN	Thalamus	615	0	0%
	Putamen	606	0	0%
	Pallidum	176	0	0%
	Caudate	581	0	0%
	Overall	1978	0	0%

DMN, default mode network; BGN, basal ganglia network.

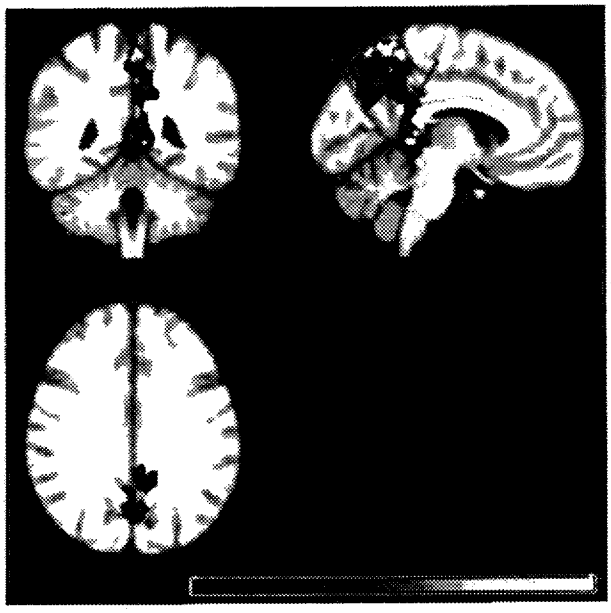


FIG. 4. Two-sample *t* test using a *p* value of 0.001 on the default mode network (DMN) extracted from the cross-validation of group independent component analysis. Results identified a cluster of 50 voxels in the posterior cingulate cortex (green arrow) and a cluster of 442 voxels in the precuneus (blue arrow) that have significantly lower voxel dependency to the DMN in patients compared with healthy control subjects. Color image is available online at www.liebertpub.com/neu

Individual ICA. Individual ICA was performed to investigate alterations of the DMN and BGN in patients compared with healthy controls. Dual regression, back-projection, and the atlas-based spatially constrained ICA have all been applied. Neither dual regression nor back-projection was able to discriminate between the two groups in individual analysis. The atlas-based spatially constrained ICA method, however, demonstrated an intriguing result.

Consistent with GICA results, a two-sample *t* test on the DMNs extracted from the atlas-based ICA method revealed a significant difference between the two groups in the PCC and surrounding areas including the precuneus. For a *p* value = 0.05, the two-sample *t* test map, which was corrected using the spatial threshold, manifested a cluster of 137 voxels with reduced voxel dependency to the DMN in the patient group compared with controls. This includes 55 voxels in the PCC (Fig. 5). This suggests reduced connectivity within the DMN, which is consistent with our GICA results. The ICA results are also consistent with results of Stevens and associates,[32] as discussed above.

SBA

The within-group analysis on healthy controls did not reveal any significant difference; however, the between-group analysis using the same parameters as that of the within-group analysis demonstrated several alterations in patients compared with healthy controls, including in connectivity maps generated by using the PCC, thalamus, amygdala, and hippocampus as seed regions.

PCC connectivity map. Table 3 demonstrates the one-sample *t* test of the FC map generated using the PCC as a seed region. It shows (a) stronger FC between the PCC and the precuneus in the healthy control group than that in patients, which suggests reduced connectivity within the DMN in consistency with the results of ICA; and (b) larger and more distributed FC in different regions of the brain in patients than in the healthy control group (also see supplementary Fig. S1; see online supplementary material at ftp.liebertpub.com). Specifically, patients showed stronger FC between the PCC and the frontal lobe regions than controls (see Table 3 and supplementary Fig. S1; see online supplementary material at ftp.liebertpub.com). For example, the FC map of patients with mTBI using $p = 0.01$ contains clusters with 108 voxels in the dorsolateral prefrontal cortex (BA 9) and 77 voxels in the anterior cingulate cortex (BA 32), while the FC map of the healthy control group does not include any voxels in these two regions. Moreover, the FC map of patients with mTBI contains 262 voxels in the anterior prefrontal cortex (BA 10), compared with the FC map of the healthy control group that has only 59 voxels. (see Table 3 and supplementary Fig. S1; see online supplementary material at ftp.liebertpub.com).

Figure 6 demonstrates further two-sample *t* tests of FC comparison for the PCC map (i.e., using the PCC as a seed region) between patients and controls. It shows significantly higher FC between the PCC and several regions of the frontal lobe in patients compared with controls, including the dorsolateral prefrontal cortex (BA 9) and adjoining voxels in BA 8 and the anterior cingulate cortex (BA 32) (Fig. 6). For *p* value < 0.01, the two-sample *t* test map showed two clusters of 117 and 223 voxels, in the dorsolateral prefrontal cortex (BA 9) with higher FC in patients than in controls. These clusters include 77 and 87 voxels, respectively. Along with a cluster containing 44 voxels in the dorsal anterior cingulate cortex (BA 32), these show the susceptibility of these regions to alterations

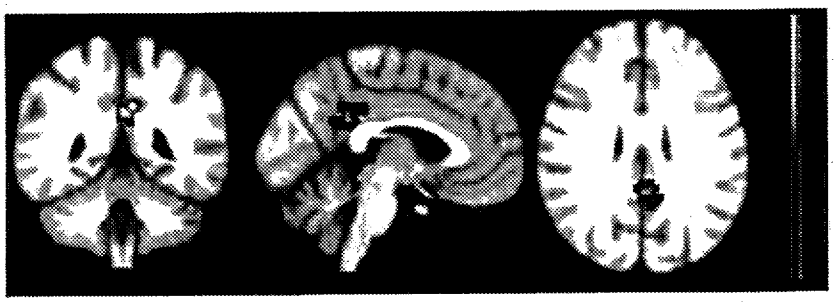


FIG. 5. Two-sample *t* test results demonstrate a difference in the default mode network (DMN) between the two groups in individual independent component analysis. Highlighted area shows the cluster is statistically significant ($p < 0.05$), which includes 55 voxels in the posterior cingulate cortex. The warm color labels the voxels with reduced DMN dependency in patients compared with healthy controls. Color image is available online at www.liebertpub.com/neu

TABLE 3. ONE-SAMPLE *T* TEST OF THE UNCTIONAL CONNECTIVITY MAP GENERATED USING THE POSTERIOR CINGULATE CORTEX AS A SEED REGION

	Number of voxels with p *value* = *0.01*		*Number of voxels with* p *value* = *0.001*	
Regions	*Control group*	*Patient group*	*Healthy group*	*Patient group*
Dorsolateral prefrontal cortex (BA 9)	0	108	0	0
Anterior prefrontal cortex (BA 10)	59	262	0	66
Anterior cingulate cortex (BA 32)	0	77	0	15
Cingulate posterior cortex	178	169	172	152
Precuneus	440	296	342	192

in patients with mTBI. Of particular note, these regions do not belong to the DMN.

Precuneus connectivity map. Using the precuneus as a seed region, the between-group analysis (two-sample *t* test) for $p<0.05$ shows stronger FC between the precuneus and two clusters in patients compared with healthy controls. The first cluster is in the supramarginal gyrus (BA 40) with 82 voxels; the other cluster has 85 voxels, which includes the BA 8 and the anterior cingulate cortex (BA 32) (Fig. 7). Using a cutoff of $p<0.01$ did not reveal any significant difference.

Thalamus connectivity map. A between-group comparison (two-sample *t* test) was performed using the thalamus as a seed region. For $p<0.01$, the patient group showed significantly higher FC with the thalamus than controls in several regions, including the anterior prefrontal cortex (BA 10) in two clusters of 83 and 123 voxels (Fig. 8a) and a cluster of 96 voxels in the supramarginal gyrus (BA 40) (Fig. 8b). This indicates an increased FC of the thalamus network with other regions of the brain.

Amygdala connectivity map. By using the amygdala as a seed region, a between-group comparison ($p<0.01$) demonstrates significantly increased FC with the left parietal superior cortex in the patient group (cluster size = 104) than the control group (Fig. 9). On the other hand, a one-sample *t* test shows that the healthy controls have higher connectivity within the amygdala. For $p<0.01$, the FC map of the healthy controls group includes 73 and 67 voxels, while for the patient group, these numbers decrease to 39 and 22 (Fig. 10).

Hippocampal connectivity map. A between-group analysis for the FC map with the hippocampus as the seed region demonstrates significant alteration in the FC of patients with mTBI. For $p<0.01$, three clusters (cluster sizes of 135, 61, and 52 voxels) are significantly different between the two groups. Figure 11 demonstrates increased FC in the fusiform gyrus and the precuneus (BA 7) and decreased FC in the inferior frontal gyrus in the patient group compared with controls (Fig. 11).

Relationship between FC and neurocognitive data. No significant correlation was found between the neurocognitive tests (SAC scores) and FC data. The largest Pearson correlation value (0.42) was found in the SBA for the PCC between the mean of the correlation value with the dorsal anterior cingulate and the delayed recall test, but it did not reach statistical significance.

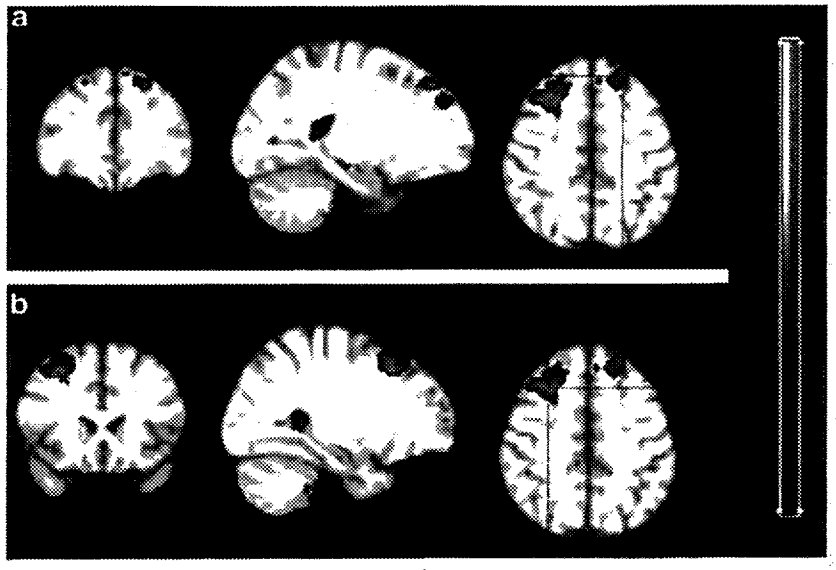


FIG. 6. Two-sample *t* test ($p=0.01$) for the posterior cingulate cortex functional connectivity (FC) map. The cold color labels the region that has more correlation with the posterior cingulate in the patient group than in the controls. The cross bar is located in different positions in the same FC map in images **a** and **b**. These regions do not belong to the default mode network. Color image is available online at www.liebertpub.com/neu

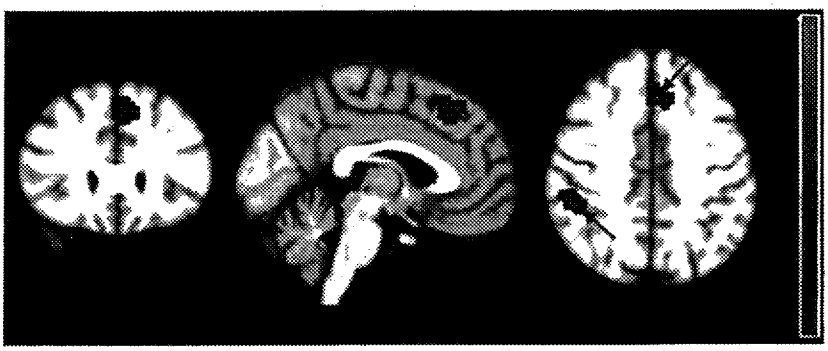


FIG. 7. Two-sample t test ($p<0.05$) of the precuneus functional connectivity map. The between group comparison using the precuneus as seed revealed differences in the supramarginal gyrus (red arrow) and the junction between Brodmann area (BA) 8 and the anterior cingulate cortex (BA 32) (green arrow). The cold color labels the regions that have more correlation with the precuneus in the patient group than in controls. Color image is available online at www.liebertpub.com/neu

Discussion

To our knowledge, this is the first investigation of resting-state functional networks in mTBI at the acute stage. Except for one patient who came back for an MRI scan at 7 days after injury, all patients were either still at the ED or in the hospital under observation at the time of scanning. MRI at this point might be the most clinically relevant. Except for one patient with one tiny intraventricular microhemorrhage seen on SWI but not on conventional structural imaging, anatomical imaging did not detect any obvious trauma-induced structural abnormalities in other patients. Both the ICA and SBA, however, revealed differences of brain communication across different brain regions between patients with mTBI and controls in the acute stage. Specifically, a comprehensive analysis showed reduced FC within the DMN and increased functional connectivity with extra-DMN regions in patients with mTBI. By using the thalamus, amygdala, and hippocampus as network regions, analyses revealed increased FC with other brain regions. Overall, our data suggest increased involvement of frontal regions after injury at the acute stage. This result confirmed our original hypothesis on altered FC in these functional networks. Further, our results also showed that independent component and seed-based analyses complement each other.

ICA vs. SBA

Both ICA and SBA are widely used methods in resting-state fMRI analysis. Instead of using one, we performed both in a comprehensive analysis of the DMN and the thalamus network at the resting state. Both our group and individual ICA analyses demonstrated reduced connectivity in the PCC and precuneus regions. This was further validated by our SBA results. It demonstrates that the PCC and precuneus connectivity changes in the DMN might be the most well-validated functional biomarkers of brain injury. Meanwhile, because of functional network remodeling in pathological conditions, analysis of the stereotyped networks in the ICA may not be

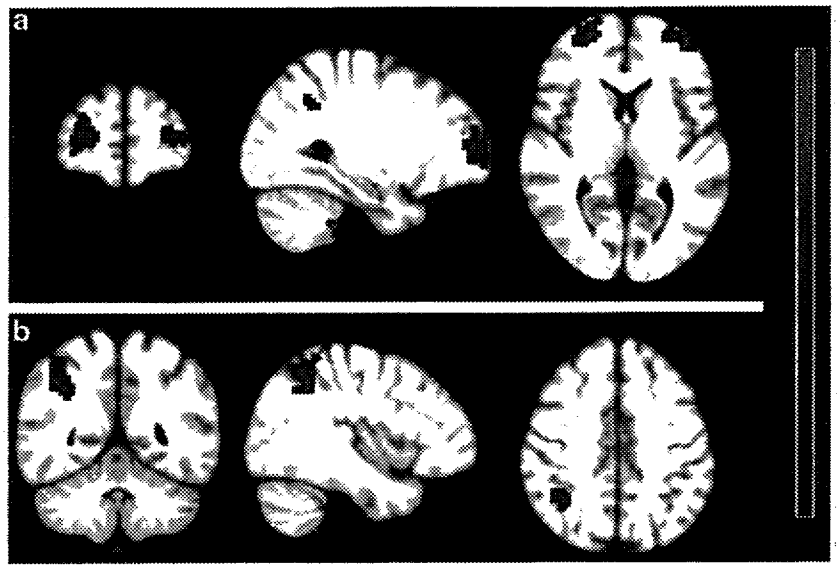


FIG. 8. Two-sample t test ($p<0.01$) of thalamus functional connectivity map. The seed is located in the thalamus and the cold color shows the regions that have higher correlation with seed point in the patient group compared with the healthy control group. (a) Shows a statistical difference between two groups at the anterior prefrontal cortex (Brodmann area [BA] 10) (green arrow) and (b) shows the difference in the supramarginal gyrus (BA 40) (red arrow). Color image is available online at www.liebertpub.com/neu

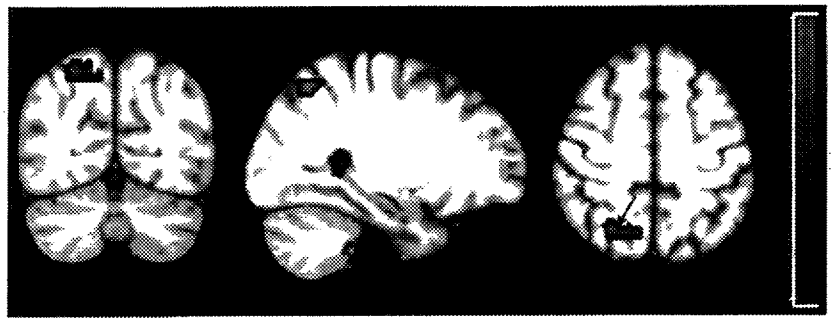


FIG. 9. Two-sample *t* test ($p<0.01$) for the amygdala correlation map. The cold color labels the region (the left parietal superior cortex) that has stronger correlation with the amygdala (red arrow) in the patient group than in controls. Color image is available online at www.liebertpub.com/neu

able to capture the dynamics of network alterations in mTBI. Instead, SBA demonstrated increased FC in major regions of the DMN, thalamus, amygdala, and hippocampus with other networks of the brain, and this finding further complements the ICA results.

DMN

Our data showed decreased strength of connections and number of voxels involved within the DMN and increased connectivity between the DMN and other brain regions. This result is, in large part, consistent with studies of mTBI at the subacute and chronic stages.[27,28,32] Mayer and associates[27] attributed this abnormality to the partial disruption of the putative balance between the DMN and task-related networks after mTBI, and Stevens and colleagues[32] interpreted this abnormality as a compensatory process of the brain. Palacios and coworkers[48] also attribute this to a compensatory process after microstructural damage to the brain, as detected by DTI. In addition, by considering the hyperconnectivity in thalamocortical network, hippocampal network, and amygdala network, our data also support the hypothesis by Stevens and colleagues[32] that multiple RSNs could have functional abnormalities after mTBI. The brain tends to respond to head injury by recruiting a cohort of networks in a global manner instead of just one or two networks, disrupting the putative balance between resting-state and task-related networks.

The PCC is an important part of the DMN and is also a central hub of the brain.[49] SBA has already reported various connections between the PCC and frontal regions, including the anterior cingulate cortex, orbital frontal cortex, and dorsolateral prefrontal cortex, which are involved in frontal and frontopartial networks.[49–51] Increases in the connectivity between the PCC and these regions were already observed during emotional, memory, and attentional processing.[52,53] A series of cognitive and emotional symptoms have been reported by patients with mTBI in the literature, including anxiety, stress, fear, depression, memory deficits, deficits in problem solving, and difficulty with attention and concentration.[54] Because the PCC receives strong afferent input from regions with functions related to emotional tasks, working memory, and attention including

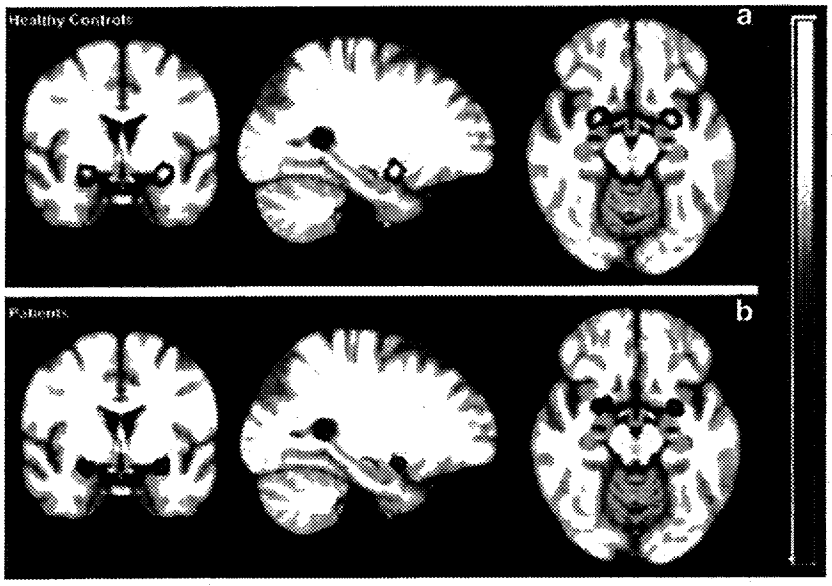


FIG. 10. One-sample *t* test of the amygdala map ($p<0.01$). (**a**) the functional connectivity (FC) map for the healthy control group and (**b**) the FC map of the patient group. The higher signal intensity on (a) shows higher intrinsic FC in the amygdala in the healthy group than that in patients. Color image is available online at www.liebertpub.com/neu

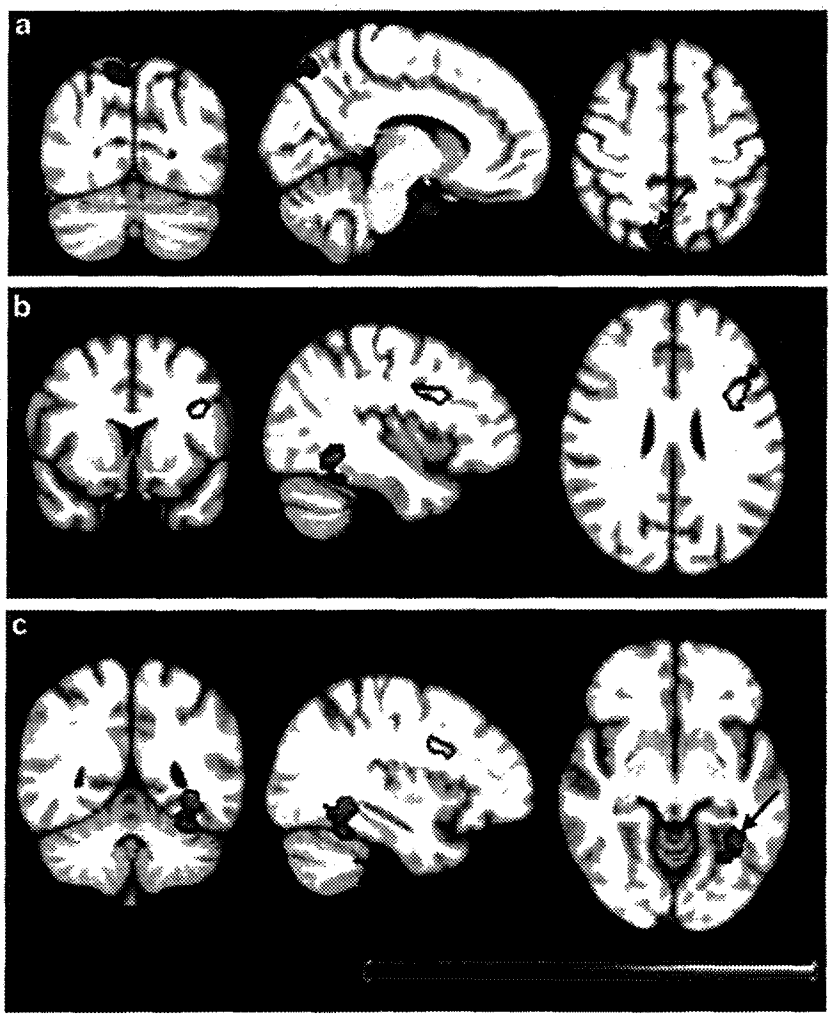


FIG. 11. Two-sample t test ($p < 0.01$) for the hippocampus functional connectivity (FC) map. The cold color labels the regions with more correlation with the hippocampus in the patient group compared with controls while the warm color labels the region with more correlation in the controls than in the patient group. The FC map was sectioned through (a) the precuneus association cortex (BA 7), red arrow; (b) the inferior frontal gyrus, green arrow; and (c) the fusiform gyrus, purple arrow.

the anterior cingulate cortex, orbital frontal cortex, and dorsolateral prefrontal cortex (areas 9 and 46),[52,55–59] the cognitive and emotional symptoms after injury could be related to changes in brain networks' activity and connectivity patterns, especially in PCC connectivity.

Our patient group demonstrated significant problems in delayed recall along with the increased connectivity between the PCC and these frontal regions. Further, our ICA results demonstrated a decrease in PCC activity in patients mostly in its ventral part, which is considered the predominant pattern of the DMN.[49] Our data are in line with the reported hypoactivity and hyperfrontality seen in retired National Football League players, as well.[60] This suggests that, right after injury, the PCC might gather information from different brain network regions as an acute compensatory response to bring the brain back to normal status.

One discrepancy between our data and previously reported work is the FC between the PCC and ACC. Previous seed-based studies of mTBI by Mayer and associates[27] and Johnson and coworkers[28] showed reduced FC within the DMN. Both groups showed decreases in FC between the PCC and ACC in the subacute stage while our results showed an increase in temporal correlation between these two regions in the acute stage. One reason for this could be the role of these regions in emotional processing and attention, in association with the temporal resolution. Studies from both groups were at the subacute stage while our study was in the acute stage. With the temporal resolution of patients' PCS, we might see different patterns in the connectivity between the PCC and ACC.

Thalamus FC

The abundance of gamma aminobutyric (GABA)ergic neurons has been thought to play an inhibitory role in the thalamocortical network at resting state.[61,62] Tang and colleagues[37] reported hyperconnectivities in the thalamocortical network in patients with mTBI in suggestion of the disruption of the GABAergic neurons after head injury. Particularly, they reported that the thalamus correlation map in the patient group in the subacute stage is more distributed compared with the healthy control group.[38] Their results show that the healthy subjects' map included areas of the bilateral

thalamus, the superior frontal gyrus, the middle frontal gyrus, the basal ganglia nuclei, the insula, and the cingulate gyrus while patients with mTBI demonstrated more widely distributed FC that extended to the bilateral middle temporal gyrus, the middle frontal gyrus, the precuneus, the inferior parietal gyrus, and the postcentral gyrus compared with the thalamic RSNs in the healthy control subjects ($p < 0.001$, corresponding to $R > 0.25$).

Although our results, unlike their results, showed that all of the active voxels are located in the thalamus for the control group, we got a similar result regarding more distributed correlation maps for the patient group compared with the healthy subject group. Their between-group comparison analysis for $p < 0.01$ shows that regions of the cingulate gyrus, temporal gyrus, and frontal gyrus show more correlation with the thalamus in the patient group compared with the control group. With the same p value, we also find increases in temporal correlation in the anterior prefrontal cortex and the supramarginal gyrus with the thalamus in patients compared with controls. In comparison with the data from Tang and associates,[37] our data also demonstrated an up-regulated thalamocortical network as a response to brain injury.

Memory and hippocampal FC

Our data showed memory deficits (in delayed recall) in patients with mTBI at the acute stage, which is in line with the published data that delayed recall is a sensitive index of neurocognitive measures for mTBI at this stage.[59] Our imaging data revealed that the patient group demonstrated decreased intrinsic connectivity in the inferior frontal gyrus and increased connectivity in the fusiform gyrus and precuneus (BA 7). The reduced connectivity in the inferior frontal region is similar to data from Johnson and coworkers,[28] which also reported reduced connectivity between these regions. Our findings of increased connectivity in the fusiform gyrus and precuneus, however, are in contrast with the report of Johnson and coworkers[28] of decreased connections between the parahippocampal gyrus and the left and right parietal lobes in comparison with controls.

Of particular note, our data are regarding patients who still have memory problems while the data of Johnson and associates[28] were collected after resolution of the symptoms. The difference in imaging findings between the two groups may represent the temporal recovery process of memory after mTBI. It also implies that, after suffering intrinsic connectivity problems of the memory network after a concussion, the brain is trying to recruit other networks to compensate.

Amygdala FC

The amygdala is an important structure of the limbic system, crucial to the processing of emotional fear, sadness, and depression, among others emotions.[63,64] Recent evidence suggests cytoarchitectural and functional subspecialization of its substructural nuclei.[65–68] Three major groups of subnuclei include the laterobasal, centromedial, and superfacial groups.[68] Bzdok and colleagues[64] also reported a relationship between functional subspecialization and its structural nuclei. Therefore, the conventional view of considering the amygdala complex as a single entity has been challenged.[69] After TBI, emotional symptoms are commonly reported as part of PCS constellation. In our study, 4 of 12 patients reported mild to moderate levels of sadness and depression after injury. The reduced connectivity inside the amygdala and increased connectivity of the amygdala with the left parietal superior cortex in our imaging findings might suggest that emotional fear and sadness after TBI could mediate the intranetwork connectivity among different subnuclei groups within the amygdala. As a consequence, the brain tends to recruit other network resources to compensate.

Limitation and future work

Several factors also set the limitation of this study. To draw a more statistically meaningful conclusion, this work should be performed in a large cohort with longitudinal follow-up instead of a handful of patients at one time point. This study demonstrates functional alteration in several brain networks in patients with mTBI in comparison with controls. All comparison was performed at group level instead of single subject level. For individual ICA analysis, the result still has to be compared at group level before we gain a better understanding of the variations in normal. Future work needs to be performed in a large cohort of patients and controls to identify the structural and functional connectivity damages at the single subject level. Further, the question of how RSN alterations affect the patients' neurocognitive performance as measured by PCS and a neuropsychological assessment battery still need to be determined.

Meanwhile, despite the normal findings on structural MRI in these patients, it does not mean they are normal on advanced MRI, including DTI. Mounting evidence demonstrates the microstructural damage in patients with mTBI.[8,70–73] Our pilot data suggest that, in response to microstructural damage on the cingulate bundle shown on DTI, other regions of the brain tend to have higher FC with the anterior cingulate cortex on resting state fMRI signal.[74] This suggests a compensatory mechanism of brain injury. A systematic investigation in a large cohort is warranted in this direction to delineate the brain compensatory mechanism. In addition, our data clearly demonstrate the multinetwork alterations after mTBI, which are in the same line as the work of Stevens and associates.[32] Further advanced DTI data also demonstrate that mTBI could render microstructural damages in multiple WM tracts.[70] This indicates the value of a future connectome-scale analysis of mTBI by combining both DTI and fMRI data to reveal a much more clear picture of brain injury.[74]

It is also worth mentioning that an increase in temporal correlation in different regions of a network does not necessarily indicate an increase in the network activity because the temporal signal of each brain's region consists of several independent time series, which indicate the connection of the region with various brain networks. For instance, although the PCC is well known as a main part of the DMN[75] and has a role in mind wandering, memory recollection, etc., it also has important roles in many other networks such as frontoparietal networks, a motor network, a sensory network, and an executive network.[49,76]

Therefore, it is possible that temporal correlations between the PCC and other brain regions increased while the activity of the DMN decreased, or vice versa, because the time series of each region can be the result of a combination of the temporal activity of several networks. For instance, Leech and associates[49] showed that the PCC consists of several subregions that have separate signals and each contribute to different networks. Although the average signal of these subregions showed high correlation between subregions, the correlation between the neural signal of these subregions was much lower, indicating that, despite having high temporal connectivity, they have tasks in different networks. Therefore, further investigation into the physiological underpinning of these functional network changes is still warranted.

Conclusion

We performed a comprehensive analysis of resting-state DMN and several other networks in patients with acute mTBI. Our study demonstrated multinetwork alterations in mTBI at the acute stage, including decreased intranetwork connectivity and increased internetwork connectivity of the DMN and amygdala network as well as changes in the thalamus and hippocampus networks. Particularly, the DMN alterations could be used as a biomarker for the diagnosis of functional deficit in mTBI in the acute setting. A longitudinal study of large-scale brain networks over a large cohort is also warranted to further reveal the physiological basis of the neurocognitive symptoms of mTBI.

Acknowledgments

This study was supported by the International Society for Magnetic Resonance in Medicine (ISMRM) seed grant and the Department of Defense (grant contract number: W81XWH-11-1-0493). The authors also thank Ms. Natalie Wiseman, MD, PhD candidate, for proofreading.

Author Disclosure Statement

No competing financial interests exist.

References

1. Kay, T. (1993). Neuropsychological treatment of mild traumatic brain injury. J Head Trauma Rehabil. 8, 74–85.
2. National Institutes of Health (1999). Rehabilitation of personswith traumatic brain injury. NIH Consensus Development Panel on Rehabilitation of Persons With Traumatic Brain Injury. JAMA 282, 974–983.
3. CDC (2003). *Report to Congress on Mild Traumatic Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem.* Centers for Disease Control and Prevention, National Center for Injury Prevention and Control: Atlanta, GA.
4. Bazarian, J.J., McClung, J., Cheng, Y.T., Flesher, W., and Schneider, S.M. (2005). Emergency department management of mild traumatic brain injury in the USA. Emerg. Med. J. 22, 473–477.
5. Bazarian, J.J., McClung, J., Shah, M.N., Cheng, Y.T., Flesher, W., and Kraus, J. (2005). Mild traumatic brain injury in the United States, 1998–2000. Brain Inj. 19, 85–91.
6. Ruff, R. (2005). Two decades of advances in understanding of mild traumatic brain injury. J. Head Trauma Rehabil. 20, 5–18.
7. Jagoda, A.S., Bazarian, J.J., Bruns, J.J., Jr., Cantrill, S.V., Gean, A.D., Howard, P.K., Ghajar, J., Riggio, S., Wright, D.W., Wears, R.L., Bakshy, A., Burgess, P., Wald, M.M., and Whitson, R.R. (2008). Clinical policy: neuroimaging and decisionmaking in adult mild traumatic brain injury in the acute setting. Ann. Emerg. Med. 52, 714–748.
8. Kou, Z., Wu, Z., Tong, K.A., Holshouser, B., Benson, R.R., Hu, J., and Haacke, E.M. (2010). The role of advanced MR imaging findings as biomarkers of traumatic brain injury. J. Head Trauma Rehabil. 25, 267–282.
9. Kou, Z., Benson, R.R., and Haacke, E. M. (2012). Magnetic resonance imaging biomarkers of mild traumatic brain injury, in: *Biomarkers for Traumatic Brain Injury.* S.A. Dambinova, R.L. Hayes, K.K. Wang (eds). Royal Society of Chemistry: Cambridge, UK.
10. Shimony, J.S., McKinstry, R.C., Akbudak, E., Aronovitz, J.A., Snyder, A.Z., Lori, N.F., Cull, T.S., and Conturo, T.E. (1999). Quantitative diffusion-tensor anisotropy brain MR imaging: normative human data and anatomic analysis. Radiology 212, 770–784.
11. Conturo, T.E., McKinstry, R.C., Akbudak, E., and Robinson, B.H. (1996). Encoding of anisotropic diffusion with tetrahedral gradients: a general mathematical diffusion formalism and experimental results. Magn. Res. Med. 35, 399–412.
12. Mac Donald, C.L., Dikranian, K., Song, S.K., Bayly, P.V., Holtzman, D.M. and Brody, D.L. (2007). Detection of traumatic axonal injury with diffusion tensor imaging in a mouse model of traumatic brain injury. Exp. Neurol. 205, 116–131.
13. Kou, Z., Shen, Y., Zakaria, N., Kallakuri, S., Cavanaugh, J.M., Yu, Y., Hu J., and EM, H. (2007). Correlation of fractional anisotropy with histology for diffuse axonal injury in a rat model. Proceedings of the Joint Annual Meeting ISMRM-ESMRMB: Berlin, Germany.
14. Ptak, T., Sheridan, R.L., Rhea, J.T., Gervasini, A.A., Yun, J.H., Curran, M.A., Borszuk, P., Petrovick, L., and Novelline, R.A (2003). Cerebral fractional anisotropy score in trauma patients: a new indicator of white matter injury after trauma. AJR Am. J. Roentgenol. 181, 1401–1407.
15. Huisman, T.A., Schwamm, L.H., Schaefer, P.W., Koroshetz, W.J., Shetty-Alva, N., Ozsunar, Y., Wu, O., and Sorenson, A.G. (2004). Diffusion tensor imaging as potential biomarker of white matter injury in diffuse axonal injury. AJNR Am. J. Neuroradiol. 25, 370–376.
16. Arfanakis, K., Haughton, V.M., Carew, J.D., Rogers, B.P., Dempsey, R.J., and Meyerand, M.E. (2002). Diffusion tensor MR imaging in diffuse axonal injury. AJNR Am. J. Neuroradiol. 23, 794–802.
17. Inglese, M., Makani, S., Johnson, G., Cohen, B.A., Silver, J.A., Gonen, O., and Grossman, R.I. (2005). Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. J. Neurosurg. 103, 298–303.
18. Benson, R.R., Meda, S.A., Vasudevan, S., Kou, Z., Govindarajan, K.A., Hanks, R.A., Millis, S.R., Makki, M., Latif, Z., Coplin, W., Meythaler, J., and Haacke, E.M. (2007). Global white matter analysis of diffusion tensor images is predictive of injury severity in traumatic brain injury. J. Neurotrauma 24, 446–459.
19. Mac Donald, C.L., Johnson, A.M., Cooper, D., Nelson, E.C., Werner, N.J., Shimony, J.S., Snyder, A.Z., Raichle, M.E., Witherow, J.R., Fang, R., Flaherty, S.F., and Brody, D.L. (2011). Detection of blast-related traumatic brain injury in U.S. military personnel. N. Engl. J. Med. 364, 2091–2100.
20. Kou, Z., Benson, R.R., and Haacke, E.M, Susceptibility weighted imaging in traumatic brain injury, in: *Clinical MR Neuroimaging,* 2nd ed. J. Gillard, A. Waldman, P. Barker (eds). Cambridge University: Cambridge, UK.
21. Paterakis, K., Karantanas, A.H., Komnos, A., and Volikas, Z. (2000). Outcome of patients with diffuse axonal injury: the significance and prognostic value of MRI in the acute phase. J Trauma 49, 1071–1075.
22. Reichenbach, J.R., Venkatesan, R., Schillinger, D.J., Kido, D.K., and Haacke, E.M. (1997). Small vessels in the human brain: MR venography with deoxyhemoglobin as an intrinsic contrast agent. Radiology 204, 272–277.
23. McAllister, T.W., Flashman, L.A., McDonald, B.C., and Saykin, A.J. (2006). Mechanisms of working memory dysfunction after mild and moderate TBI: evidence from functional MRI and neurogenetics. J Neurotrauma 23, 1450–1467.
24. Yang, Z., Yeo, R.A., Pena, A., Ling, J.M., Klimaj, S., Campbell, R., Doezema, D, and Mayer, A.R. (2012). An FMRI study of auditory orienting and inhibition of return in pediatric mild traumatic brain injury. J. Neurotrauma 29, 2124–2136.
25. Keightley, M.L., Saluja, R.S., Chen, J.K., Gagnon, I., Leonard, G., Petrides, M., and Puto, A. (2014). A functional magnetic resonance imaging study of working memory in youth after sports-related concussion: is it still working? J. Neurotrauma 31, 437–451.
26. Dettwiler, A., Murugavel, M., Putukian, M., Cubon, V., Furtado, J., and Osherson, D. (2014). Persistent differences in patterns of brain activation after sports-related concussion: a longitudinal functional magnetic resonance imaging study. J. Neurotrauma 31, 180–88.
27. Mayer, A.R., Mannell, M.V., Ling, J., Gasparovic, C., and Yeo, R.A. (2011). Functional connectivity in mild traumatic brain injury. Hum Brain Mapp 32, 1825–1835.
28. Johnson, B., Zhang, K., Gay, M., Horovitz, S., Hallett, M., Sebastianelli, W., and Slobounov, S. (2012). Alteration of brain default network in subacute phase of injury in concussed individuals: resting-state fMRI study. Neuroimage 59, 511–518.
29. Wang, L., Zang, Y.F., He, Y., Liang, M., Zhang, X.Q., Tian, L.X., Wu, T., Jiang, T.Z. and Li, K.C. (2006). Changes in hippocampal connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. Neuroimage 31, 496–504.
30. Zhou, Y., Liang, M., Tian, L.X., Wang, K., Hao, Y.H., Liu, H.H., Liu, Z.N., and Jiang, T.Z. (2007). Functional disintegration in

paranoid schizophrenia using resting-state fMRI. Schizophr. Res. 97, 194–205.
31. Tian, L.X., Jiang, T.Z., Wang, Y.F., Zang, Y.F., He, Y., Liang, M., Sui, M.Q., Cao, Q.J., Hu, S.Y., Peng, M., and Zhuo, Y. (2006). Altered resting-state functional connectivity patterns of anterior cingulate cortex in adolescents with attention deficit hyperactivity disorder. Neurosci. Lett. 400, 39–43.
32. Stevens, M.C., Lovejoy, D., Kim, J., Oakes, H., Kureshi, I. and Witt, S.T. (2012). Multiple resting state network functional connectivity abnormalities in mild traumatic brain injury. Brain imaging and behavior 6, 293–318.
33. Zhang, K., Johnson, B., Gay, M., Horovitz, S.G., Hallett, M., Sebastianelli, W., and Slobounov, S. (2012). Default mode network in concussed individuals in response to the YMCA physical stress test. J. Neurotrauma 25, 756–765.
34. Broyd, S.J., Demanuele, C., Debener, S., Helps, S.K., James, C.J., and Sonuga-Barke, E.J. (2009). Default-mode brain dysfunction in mental disorders: a systematic review. Neurosci. Biobehav. Rev. 33, 279–296.
35. Binder, J.R., and Desai, R.H. (2011). The neurobiology of semantic memory. Trends Cogn. Sci. 15, 527–536.
36. Gilbert, S.J., Dumontheil, I., Simons, J.S., Frith, C.D., and Burgess, P.W. (2007). Comment on "Wandering minds: The default network and stimulus-independent thought". Science 317, 43.
37. Tang, L., Ge, Y., Sodickson, D.K., Miles, L., Zhou, Y., Reaume, J., and Grossman, R.I. (2011). Thalamic resting-state functional networks: disruption in patients with mild traumatic brain injury. Radiology 260, 831–840.
38. McCrea, M.A. (2008). *Mild Traumatic Brain Injury and Postconcussion Syndrome*. Oxford University Press: New York, NY.
39. Naunheim, R.S., Matero, D. and Fucetola, R. (2008). Assessment of patients with mild concussion in the emergency department. J. Head Trauma Rehabil. 23, 116–122.
40. American Congress of Rehabilitation Medicine (1993). Definition of mild traumatic brain injury. J. Head Trauma Rehabil. 8, 86–88.
41. McCrea, M., Randolph, C., and Kelly, J.P. (2000). *Standardized Assessment of Concussion (SAC): Manual for Administration, Scoring and Interpretation*, 2nd ed. CNS Inc.: Waukesha, WI.
42. McCrea, M., Guskiewicz, K.M., Marshall, S.W., Barr, W., Randolph, C., Cantu, R.C., Onate, J.A., Yang, J., and Kelly, J.P. (2003). Acute effects and recovery time following concussion in collegiate football players: the NCAA Concussion Study. JAMA 290, 2556–2563.
43. Beckmann, C.F., Mackay, C.E., Filippini, N., and Smith, S.M. (2009). Group comparison of resting-state FMRI data using multi-subject ICA and dual regression. Neuroimage 47, S148.
44. Calhoun, V.D., Adali, T., Pearlson, G.D., and Pekar, J.J. (2001). A method for making group inferences from functional MRI data using independent component analysis. Hum. Brain Mapp. 14, 140–151.
45. Allen, E.A., Erhardt, E.B., Damaraju, E., Gruner, W., Segall, J.M., Silva, R.F., Havlicek, M., Rachakonda, S., Fries, J., Kalyanam, R., Michael, A.M., Caprihan, A., Turner, J.A., Eichele, T., Adelsheim, S., Bryan, A.D., Bustillo, J., Clark, V.P., Feldstein Ewing, S.W., Filbey, F., Ford, C.C., Hutchison, K., Jung, R.E., Kiehl, K.A., Kodituwakku, P., Komesu, Y.M., Mayer, A.R., Pearlson, G.D., Phillips, J.P., Sadek, J.R., Stevens, M., Teuscher, U., Thoma, R.J. and Calhoun, V.D. (2011). A baseline for the multivariate comparison of resting-state networks. Front. Syst. Neurosci. 5, 2.
46. Lin, Q.H., Liu, J., Zheng, Y.R., Liang, H., and Calhoun, V.D. (2010). Semiblind spatial ICA of fMRI using spatial constraints. Hum. Brain Mapp. 31, 1076–1088.
47. McCrea, M., Kelly, J.P., Randolph, C., Kluge, J., Bartolic, E., Finn, G., and Baxter, B. (1998). Standardized assessment of concussion (SAC): on-site mental status evaluation of the athlete. J. Head Trauma Rehabil. 13, 27–35.
48. Palacios, E.M., Sala-Llonch, R., Junque, C., Roig, T., Tormos, J.M., Bargallo, N., and Vendrell, P. (2013). Resting-state functional magnetic resonance imaging activity and connectivity and cognitive outcome in traumatic brain injury. JAMA Neurol. 70, 845–851.
49. Leech, R., Braga, R., and Sharp, D.J. (2012). Echoes of the brain within the posterior cingulate cortex. J. Neurosci. 32, 215–222.
50. Koechlin, E., Ody, C., and Kouneiher, F. (2003). The architecture of cognitive control in the human prefrontal cortex. Science 302, 1181–1185.
51. Koechlin, E., and Summerfield, C. (2007). An information theoretical approach to prefrontal executive function. Trends Cogn. Sci. 11, 229–235.
52. Maddock, R.J., Garrett, A.S., and Buonocore, M.H. (2003). Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. Hum. Brain Mapp. 18, 30–41.
53. Maratos, E.J., Dolan, R.J., Morris, J.S., Henson, R.N., and Rugg, M.D. (2001). Neural activity associated with episodic memory for emotional context. Neuropsychologia 39, 910–920.
54. Bergman, K., and Bay, E. (2010). Mild traumatic brain injury/concussion: a review for ED nurses. J. Emerg. Nurs. 36, 221–230.
55. Allison, T., Puce, A., and McCarthy, G. (2000). Social perception from visual cues: role of the STS region. Trends Cogn. Sci. 4, 267–278.
56. Carmichael, S.T., and Price, J.L. (1995). Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. J. Comp. Neurol. 363, 615–641.
57. Goldman-Rakic, P.S., Selemon, L.D. and Schwartz, M.L. (1984). Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. Neuroscience 12, 719–743.
58. Morris, R., Pandya, D.N., and Petrides, M. (1999). Fiber system linking the mid-dorsolateral frontal cortex with the retrosplenial/presubicular region in the rhesus monkey. J. Comp. Neurol. 407, 183–192.
59. Pandya, D.N., Van Hoesen, G.W. and Mesulam, M.M. (1981). Efferent connections of the cingulate gyrus in the rhesus monkey. Exp. Brain Res. 42, 319–330.
60. Hampshire, A., MacDonald, A., and Owen, A.M. (2013). Hypoconnectivity and hyperfrontality in retired American football players. Sci. Rep. 3, 2972.
61. Aron, A.R., Schlaghecken, F., Fletcher, P.C., Bullmore, E.T., Eimer, M., Barker, R., Sahakian, B.J., and Robbins, T.W. (2003). Inhibition of subliminally primed responses is mediated by the caudate and thalamus: evidence from functional MRI and Huntington's disease. Brain 126, 713–723.
62. Steriade, M. (2005). Sleep, epilepsy and thalamic reticular inhibitory neurons. Trends Neurosci. 28, 317–324.
63. Morris, J.S., Frith, C.D., Perrett, D.I., Rowland, D., Young, A.W., Calder, A.J., and Dolan, R.J. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. Nature 383, 812–815.
64. Bzdok, D., Laird, A.R., Zilles, K., Fox, P.T., and Eickhoff, S.B. (2013). An investigation of the structural, connectional, and functional subspecialization in the human amygdala. Hum. Brain Mapp. 34, 3247–3266.
65. Adolphs, R. (2008). Fear, faces, and the human amygdala. Curr. Opin. Neurobiol. 18, 166–172.
66. Adolphs, R. (2010). What does the amygdala contribute to social cognition? Ann. N. Y. Acad. Sci. 1191, 42–61.
67. LeDoux, J.E. (2000). Emotion circuits in the brain. Ann. Rev. Neurosci. 23, 155–184.
68. Amunts, K., Kedo, O., Kindler, M., Pieperhoff, P., Mohlberg, H., Shah, N.J., Habel, U., Schneider, F., and Zilles, K. (2005). Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. Anat Embryol (Berl) 210, 343–352.
69. Swanson, L.W., and Petrovich, G. (1998). What is the amygdala? Trends Neurosci. 21, 323–331.
70. Kou, Z., and VandeVord, P.J. (2014). Traumatic white matter injury and glial activation: from basic science to clinics. Glia 62, 1831–1855.
71. Kou, Z., Gattu, R., Kobeissy, F., Welch, R.D., O'Neil, B., Woodard, J.L., Ayaz, S.I., Kulek, A., Kas-Shamoun, R., Mika, V., Zuk, C., Hayes, R.L., Tomasello, F., and Mondello, S. (2013). Combining biochemical and imaging markers to improve diagnosis and characterization of mild traumatic brain injury in the acute setting: results from a pilot study. PLoS One 8, e80296.
72. Niogi, S.N., and Mukherjee, P. (2010). Diffusion tensor imaging of mild traumatic brain injury. J. Head Trauma Rehabil. 25, 241–255.
73. Niogi, S.N., Mukherjee, P., Ghajar, J., Johnson, C.E., Kolster, R., Lee, H., Suh, M., Zimmerman, R.D., Manley, G.T., and McCandliss, B.D. (2008). Structural dissociation of attentional control and memory in adults with and without mild traumatic brain injury. Brain 131, 3209–3221.

74. Kou, Z., and Iraji, A. (2014). Imaging brain plasticity after trauma. Neural Regen. Res. 9, 693–700.
75. Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., and Shulman, G.L. (2001). A default mode of brain function. Proc. Natl. Acad. Sci. U.S.A. 98, 676–682.
76. Pearson, J.M., Heilbronner, S.R., Barack, D.L., Hayden, B.Y., and Platt, M.L. (2011). Posterior cingulate cortex: adapting behavior to a changing world. Trends Cogn. Sci. 15, 143–151.

Address correspondence to:
Zhifeng Kou, PhD
Department of Biomedical Engineering
Wayne State University
Detroit, MI 48201

E-mail: Zhifeng_kou@wayne.edu

JOURNAL OF NEUROTRAUMA 33:977–989 (June 1, 2016)

DOI: 10.1089/neu.2014.3847

Is Traumatic Brain Injury Associated with Reduced Inter-Hemispheric Functional Connectivity? A Study of Large-Scale Resting State Networks following Traumatic Brain Injury

Arianna Rigon,[1] Melissa C. Duff,[1–3] Edward McAuley,[5,6] Arthur F. Kramer,[5] and Michelle W. Voss[1,4]

Abstract

Traumatic brain injury (TBI) often has long-term debilitating sequelae in cognitive and behavioral domains. Understanding how TBI impacts functional integrity of brain networks that underlie these domains is key to guiding future approaches to TBI rehabilitation. In the current study, we investigated the differences in inter-hemispheric functional connectivity (FC) of resting state networks (RSNs) between chronic mild-to-severe TBI patients and normal comparisons (NC), focusing on two externally oriented networks (i.e., the fronto-parietal network [FPN] and the executive control network [ECN]), one internally oriented network (i.e., the default mode network [DMN]), and one somato-motor network (SMN). Seed voxel correlation analysis revealed that TBI patients displayed significantly less FC between lateralized seeds and both homologous and non-homologous regions in the opposite hemisphere for externally oriented networks but not for DMN or SMN; conversely, TBI patients showed increased FC within regions of the DMN, especially precuneus and parahippocampal gyrus. Region of interest correlation analyses confirmed the presence of significantly higher inter-hemispheric FC in NC for the FPN ($p<0.01$), and ECN ($p<0.05$), but not for the DMN ($p>0.05$) or SMN ($p>0.05$). Further analysis revealed that performance on a neuropsychological test measuring organizational skills and visuo-spatial abilities administered to the TBI group, the Rey-Osterrieth Complex Figure Test, positively correlated with FC between the right FPN and homologous regions. Our findings suggest that distinct RSNs display specific patterns of aberrant FC following TBI; this represents a step forward in the search for biomarkers useful for early diagnosis and treatment of TBI-related cognitive impairment.

Key words: externally oriented networks; inter-hemispheric functional connectivity; internally oriented networks; resting state fMRI; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a global public health issue with the incidence of TBI rising worldwide.[1] In the United States alone, more than 1.7 million cases are reported annually. Although outcomes following TBI are highly variable, in about one out of five cases TBI marks the onset of long-term disabilities, evolving into persistent impairments in domains such as attention, memory, movement, sensation, executive functions and social conduct.[2,3] In an effort to design *ad hoc* effective rehabilitation protocols and to guarantee TBI patients a higher quality of life, interdisciplinary research that combines neuropsychology and neuroimaging has endeavored to obtain a better understanding of TBI outcomes on the brain and behavior. In particular, cognitive neuropsychology and brain imaging methods have been recruited with the aim of gaining insight into how specific profiles of behavioral impairment may correspond to specific patterns of functional impairment and/or anatomical brain damage.[4]

Individuals with TBI usually manifest a variety of co-existing complaints and complex cognitive and behavioral clinical outcomes; this observation hints at the fact that long-term impairment following brain injury parallel abnormalities not (or not entirely) due to damage in focal brain regions, but in the interactions between areas that participate in networks of functionally and/or anatomically connected neural systems.[5] Indeed, although TBI, especially in severe cases, may be accompanied by focal lesions

[1]Neuroscience Graduate Program, [2]Department of Communication Sciences and Disorders, [3]Department of Neurology, [4]Department of Psychological and Brain Sciences, University of Iowa, Iowa City, Iowa.
[5]The Beckman Institute for Advanced Science and Technology, [6]Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Illinois.

caused by cerebral contusion, it most frequently presents white matter microstructure changes and is characterized by diffuse axonal injury, a result of axon shearing due to the force of the angular and linear acceleration of the soft brain tissue inside the skull at the moment of impact.[6]

Resting state functional magnetic resonance imaging (rs-fMRI) is a widely used approach to explore the functional integrity of neural systems.[7] Resting state fMRI scans are collected as participants lie in the scanner without engaging in a specific task; the resulting patterns of synchronous activity at rest resemble functional networks typically observed during performance of cognitive tasks.[8,9] In this way, rs-fMRI offers a tool for a task-independent measure of functional integrity of large-scale cognitive systems. Analyses of rs-fMRI data often measure the level of synchrony, or functional connectivity (FC), by computing correlations between blood oxygen–level dependent (BOLD) signal fluctuations of brain regions of interest (ROIs) proposed to work together as parts of functional networks. The methodological advantages of measuring FC with this cognitive task-free data collection method, in contrast to task-related fMRI, include greater generalizability for predicting behavior, lack of practice or retest effects, and perhaps most importantly when dealing with a TBI population, the elimination of possible confounds due to task difficulty or floor/ceiling effects.[10]

Several studies have begun to characterize what aberrant patterns of FC at rest are associated with TBIs of different severities. In particular, research has focused on characterizing the consequences of TBI on FC between specific ROIs in three resting state networks (RSNs) that have been found to support some of the cognitive functions most commonly impaired in TBI populations: the default mode network (DMN), the fronto-parietal network (FPN), and the executive control network (ECN).[11–14] At a broader level it is useful to conceptualize the DMN as an internally oriented network (ION) and the FPN and ECN as externally oriented networks (EONs) based on the endogenous or exogenous quality of the information they manipulate, respectively.[15]

The DMN comprises anterior medial prefrontal cortex, posterior cingulate cortex (PCC), medial temporal lobe (MTL), and lateral parietal areas, and it is known to have a role in internally-directed cognition, such as autobiographical memory, theory of mind and future oriented thought.[16] FC in the DMN has been consistently found to be abnormal in both mild and severe TBI populations, albeit with seemingly contradictory findings. For instance, two studies noticed greater FC within the DMN for TBI, compared with healthy controls, particularly with the precuneus and posterior cingulate cortex[17,18]; however, others reported less FC within the DMN for TBI, compared with controls, especially between more posterior regions of the DMN.[19–21] These differences may be due to the heterogeneity of analytical frameworks employed, time elapsed since onset of injury, TBI severity, and extent of gray and white matter damage.

The FPN comprises the dorsolateral frontal and parietal cortices, and has traditionally been considered part of a broader "task positive" network due to its association with orienting attention to the environment rather than the self. It has been postulated that the FPN is involved in executive functions and externally-directed cognitive abilities, such as goal directed behavior—all higher level functions that consistently have been found to be impaired in TBI populations of all severity levels.[12,15,22–24] Similarly, the ECN includes mainly frontal regions involved in executive processes (goal oriented action and inhibition), emotion, and perception.[9] Not surprisingly, several studies on TBI samples have discovered deficits in FC between regions in the FPN, some additionally reporting associations between neuroimaging findings and behavioral performance.[18,25,26]

Interestingly, some recent task-related and rs-fMRI studies have demonstrated less inter-hemispheric FC in their TBI samples, compared with healthy controls, with reports of less FC between motor areas in a sample including mild, moderate, and severe TBI patients, between left and right hippocampi and anterior cingulate cortices in acute severe TBI patients, and between homologous prefrontal, parietal and hippocampal ROIs in acute mild TBI individuals.[27–32] In particular, Sours and colleagues[29] observed positive correlations between cognitive performance on subtests of a computerized cognitive assessment battery and inter-hemispheric FC in acute and sub-acute stages of TBI, while Marquez de la Plata and colleagues[28] found that bilateral hippocampal connectivity was associated with memory skills. These findings are perhaps not surprising, given that inter-hemispheric FC has been associated with, albeit not always in a linear fashion, white matter integrity, which often shows axonal degeneration following a TBI episode.[27–32]

To date, although some effort has been expended trying to identify the precise patterns of aberrant FC following TBI, no work has compared inter-hemispheric functional disconnection across distinct RSNs within a sample. Thus, the main purpose of the current study was to examine the differences in inter-hemispheric FC across large-scale brain networks. In particular, we explored the possible clinical significance of abnormal inter-hemispheric FC in EONs (FPN and ECN), an ION (DMN), and a fourth network (somato-motor network [SMN]) specifically selected to investigate the behavior of sensory-motor network FC following TBI damage. We obtained rs-fMRI data on a sample of 21 TBI individuals in the chronic stage ranging from mild to severe and 21 healthy comparison participants using ROIs from RSNs. We hypothesized that TBI individuals would show differences in FC between homologous areas of EON's, an ION, and a sensory network when compared with apparently healthy individuals matched for sex, education, age, and handedness.

Methods

Experiment 1

Participants. Individuals with TBI were recruited through the Traumatic Brain Injury Registry at the University of Iowa. All TBI patients were right handed and in the chronic stage of their injury (more than 6 months since injury onset, as in TBI most of the behavioral recovery is believed to occur during the first 6 months following injury; Table 1).[33,34] The Glasgow Coma Scale (GCS), in combination with available information on loss of consciousness (LOC), post-traumatic anterograde amnesia (PTA) and acute computed tomography (CT) findings, were employed to assess TBI severity in correspondence with the criteria described in the Mayo Classification System.[35,36] The Mayo Classification System was selected because of the opportunity it offers to maximally capitalize on the positive findings available for each individual TBI patient and their trauma-related history in order to retrospectively determine TBI severity.

Participants were classified as A) mild when GCS was 13–15, acute CT findings were unremarkable and no focal lesions were visible on a chronic MRI, LOC was 30 min or less and PTA was shorter than 24 h; or B) moderate-severe, when GCS was less than 12, positive acute CT findings or chronic intracranial abnormality defined as focal lesions visible on MRI were present, LOC was longer than 30 min, and PTA longer than 24 h. If only one of these criteria was met (i.e., GCS lower than 13, LOC longer than 30 min, PTA longer than 24 h or presence of any trauma-related abnormality), it was sufficient to exclude the patient from the mild TBI group and to classify them as moderate-severe (Table 1). As these

TABLE 1. DEMOGRAPHIC AND INJURY INFORMATION FOR PATIENTS WITH TRAUMATIC BRAIN INJURY

Subject	*Age*	*Sex*	*Education (years)*	*Etiology*	*GCS*	*Retrograde amnesia*	*PTA*	*LOC*	*Acute CT findings*	*Focal lesions (chronic MRI)*	*Severity*	*Chronicity (months)*
TBI1	71	F	12	Fall	3	2 h	22 days	2 weeks	SAH (required hemicraniotomy)	Left temporal lobe, Right Frontal lobe	Moderate-severe	8.6
TBI2	24	M	15	Fall	8	15 min	2 days	N/A	Bifrontal contusions (required craniotomy)	Bilateral frontal lobe, right temporal pole, left cerebellum	Moderate-severe	33.4
TBI3	25	M	15	Fall	N/A	A few minutes	1 day	~2 days	Basilar skull fracture	Bilateral frontal pole and orbital regions	Moderate-severe	55.4
TBI4	64	F	12	Fall	N/A	A few minutes	A few minutes	N/A	SAH	No	Moderate-severe	11.2
TBI5	70	M	20	Fall	15	A few minutes	A few minutes	N/A	Small SDH	No	Moderate-severe	145.7
TBI6*	50	M	12	Fall/MVA	N/A / 15	A few seconds / A few seconds	N/A / N/A	1 day/No	N/A / N/A	No	Moderate-severe	277.8/48.2
TBI7	48	F	16	MVA, unhelmeted	6	1 year	Duration unclear	Duration unclear	SAH	Left superior frontal gyrus	Moderate-severe	15.2
TBI8	41	F	13	Fall	N/A	No	No	A few minutes	SAH	No	Moderate-severe	22.5
TBI9	59	M	12	Fall	N/A	A few minutes	1 month	A few seconds	Negative	No	Mild	18.6
TBI10	53	F	13	Fall	15	A few minutes	A few minutes	A few minutes	Bifrontal hemorrhagic contusions	Bilateral frontal pole	Moderate-severe	44.5
TBI11	60	F	14	Fall	N/A	A few minutes	A few hours	N/A	SAH	No	Moderate-severe	12.0
TBI12	21	M	13	Fall	15	None	N/A	Duration unclear	EDH, right temporal bone fracture (required craniotomy)	No	Moderate-severe	12.7
TBI13	63	M	15	MVA	N/A	No	No	No	N/A	No	Mild	37.5
TBI14	43	F	16	Hit	N/A	A few minutes	2 months	5 min	SAH, occipital skull fracture	Right frontal lobe	Moderate-severe	18.9
TBI15	63	M	14	Fall	N/A	N/A	12 h	4–5 h	SAH	No	Moderate-severe	20.2
TBI16	42	F	16	MVA	N/A	N/A	N/A	Several hours	Intracranial hemorrhage (require craniotomy)	Right middle frontal gyrus	Moderate-severe	297.4
TBI17	69	F	12	UVA, unhelmeted	N/A	A few seconds	A couple of weeks	A few minutes	N/A	Left inferior temporal gyrus	Moderate-severe	374.8
TBI18	54	F	12	UVA, unhelmeted	N/A	None	A couple of weeks	20 min	Negative	No	Moderate-severe	29.5
TBI19	52	M	19	MVA, helmeted	13	N/A	N/A	3–5 min	SAH	No	Moderate-severe	17.5
TBI20*	60	M	14	Fall/MVA	N/A / 15	N/A / No	N/A / No	A few minutes/No	N/A	No	Mild	52.7/37
TBI21	55	M	13	MVA, helmeted	15	14 h	2 weeks	Duration unclear	SAH	No	Moderate-severe	37
TBI group (mean ± SD)	51.76 (±14.7)	11 (M)	14.19 (±2.27)									
NC group (mean ± SD)	52.19 (±14.6)	11 (M)	14.79 (±2.22)									

*Sustained two TBIs in two different occasions. All information was collected via patient/collateral report or, when available, via inspection of medical records.

GCS, Glasgow Coma Scale; PTA, post-traumatic amnesia; LOC, loss of consciousness; CT, computed tomography; MRI, magnetic resonance imaging; F, female; TBI, traumatic brain injury; SAH, subarachnoid hemorrhage; M, male; N/A, not available; SDH, subdural hemorrhage; EDH, epidural hemorrhage; UVA, unmotored vehicle accident.

are all common occurrences following TBI, perhaps not surprisingly 18 out of 21 participants were assigned to the moderate to severe group and only three to the mild group. However, it is important to mention that other classification systems would lead to different characterizations of the sample; in particular, those participants who are assigned to the moderate-severe group merely due to the presence of skull or intracranial abnormalities would be defined as complicated-mild TBI or high-risk mild TBI patients.[37,38] All TBI participants were cleared for contraindication to MRI scanning.

Normal healthy comparison participants (NC; $n=21$) were sampled from a large existing database of participants scanned at the University of Illinois on the same scanner model. Participants in this database were recruited from the community of Urbana-Champaign, Illinois. For NC participants, eligibility criteria included: 1) right handedness (at least 75% on the Edinburgh Handedness Questionnaire); 2) age between 18 and 80; 3) no previous history of psychiatric and neurological illness or traumatic brain injury; 4) a score >27 on the Mini-Mental State Exam; 5) normal or corrected-to-normal vision of at least 20/40 and no color blindness; and 6) suitability for MRI environment. Each TBI patient was matched pairwise with a NC participant for sex, age, education and handedness. A two-tailed t-test revealed that the TBI and NC groups did not significantly differ in age ($t=20$, $p>0.5$) or education ($t=20$, $p>0.5$; Table 1).

All participants signed a written informed consent and were compensated for their participation. The study was approved by the Institutional Review Boards at University of Iowa and the University of Illinois-Urbana Champaign.

Neuroimaging data. For both groups, all neuroimaging data were collected during a single session. Data for TBI participants were acquired at the University of Iowa on a 3T whole-body MRI scanner (Magnetom TIM Trio; Siemens Healthcare, Erlangen, Germany) operated with a 12-channel RF head receive coil. High resolution T1-weighted brain images were acquired using a three-dimensional (3D) Magnetization Prepared Rapid Gradient Echo Imaging (MPRAGE) protocol with 208 contiguous coronal slices, echo time (TE) = 3.04 msec, repetition time (TR) = 2530 msec, field of view (FOV) = 256 mm^2, voxel size = 1 mm^3, and flip angle = 10°. T2*-weighted resting state data were collected with a fast echo planar imaging (EPI sequence) with BOLD contrast (6 min, TR = 2000 msec, TE = 30 msec, 31 slices acquired in ascending order voxel size: 3.4 × 3.4 × 3.5mm, 64 × 64 matrix, flip angle = 75°).

MRI data for the NC group was collected using a 3T Siemens Trio Tim system at the University of Illinois-Urbana Champaign. High resolution T1-weighted brain images were acquired using a 3D MPRAGE protocol with 192 slices (TE = 3.32 msec, TR = 1900 msec, FOV = 230 mm^2, voxel size = 0.9 mm^3, and flip angle = 9°). Resting state images were acquired with a fast EPI sequence (6 min, TR = 2000 sec, TE = 25 msec, 35 slices acquired in ascending order, voxel size 3.4 × 3.4 × 4 mm, 64 × 64 matrix, flip angle = 80°). During resting state data collection, all participants were instructed to keep their eyes closed.

Pre-processing. Functional MRI data pre-processing was carried out using FSL 5.0.4 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl).[40] The investigator who conducted the analysis was not blinded to whether the data analyzed was collected from TBI or NC participants. High-resolution T1 images were skull stripped using BET and the resulting masks were further manually inspected and corrected.[41] EPI data were then motion corrected using MCFLIRT, and brain extracted and spatially smoothed using a full width at half maximum 6.0 mm Gaussian kernel. Single-subject independent component analysis (ICA) was computed with MELODIC and each component was visually inspected and manually classified as signal (components of interest) or noise (e.g., collection artifacts, signal of non-neural origin) by two independent raters using a custom-made graphic user interface (https://github.com/ktera/fmri-ica-gui).[42–44] A moderator, who made the final classification, further rated components on which the two raters did not achieve agreement. Inter-rater reliability analysis using Cohen's kappa statistics was performed to determine consistency among raters, and it was found that $\kappa=0.759$, with a disagreement of 9%, interpreted as substantial agreement.[45] The following steps included temporal filtering for frequencies below 0.008 and above 0.1 Hz, nuisance regression with FEAT (using nuisance ROIs placed in white matter and cerebrospinal fluid of the left ventricle and six motion parameters), global signal regression and volume censoring based on a conjunction of BOLD signal spikes and motion.[46] No volumes were scrubbed from any of the participants EPI data, likely due to ICA denoising and the use of the simultaneous nuisance regression approach suggested by Hallquist and colleagues.[47] Spatial normalization of the functional images to the Montreal Neurological Institute (MNI) 2-mm template brain was computed using the boundary based registration algorithm.[48] Registration from MPRAGE to MNI space was computed using FNIRT with the default 10 mm warp resolution. The two resulting transformations were concatenated and applied to the original EPI data to transform it into standard MNI space. This process allowed to better account for local anatomical variability due to atrophy and ventricle deformation.[49,50]

A research specialist with extensive experience in processing and analyzing neuroimaging data collected on neurological patients with focal lesions and blinded to diagnosis examined each participant's T1 for visible trauma-related focal lesions and identified eight participants who clearly showed distinct regions of atrophy possibly due to head contusion. Focal lesions were hand traced using FSLVIEW and the resulting masks were used during spatial normalization with FNIRT in order to increase the quality of the fit to the MNI brain.

Data for the TBI and NC groups were collected in separate scanners and with two slightly different protocols. In order to clarify the possible confounding effect of the multi-site acquisition, signal-to-noise (SNR) analysis was performed to determine if the marked differences between the two groups could be explained by different scanner properties (supplementary text and Tables S1 and S2; see online supplementary material at www.liebertpub.com).

Seed analysis. RSNs generated by Smith and colleagues[9] and available at http://fsl.fmrib.ox.ac.uk/analysis/brainmap+rsns were used as network ROIs (also known as "seeds") during the seed analysis. The RSNs were derived applying group ICA to extract 20 components from a 36 subjects rs-fMRI dataset, and were found to match considerably to 10 components resulting from group ICA carried out on the 29,671-subject BrainMap activation database.[9] In addition, these components highly resemble the ones generated by the Group ICA analysis performed by Stevens and colleagues[18] on a mild TBI sample, thus eliminating the potential concern in employing FC maps derived from a healthy sample to study a clinical population dataset.18 In particular, from the Smith database we selected as seeds RSN 420 (DMN), 620 (SMN), 820 (ECN), and 920 and 1020 (right and left FPN, respectively; Fig. 1A).[9] Both ECN and FPN have been found to be crucial for externally-focused attention, and thus were selected as examples of EONs, while the DMN served as the ION, and the SMN as a sensory network.[12,15] In addition, in order to better investigate inter-hemispheric FC differences between TBI patients and control participants, for each RSN, FSL was used to create symmetrical left and right lateralized seeds, only including network regions situated in one hemisphere (excluding the left and right FPNs, as they already showed strong lateralization; Fig. 1B). Lateralized maps were utilized for the seed analysis. All ROIs were generated from the Smith network maps with a threshold of $Z>2.33$.

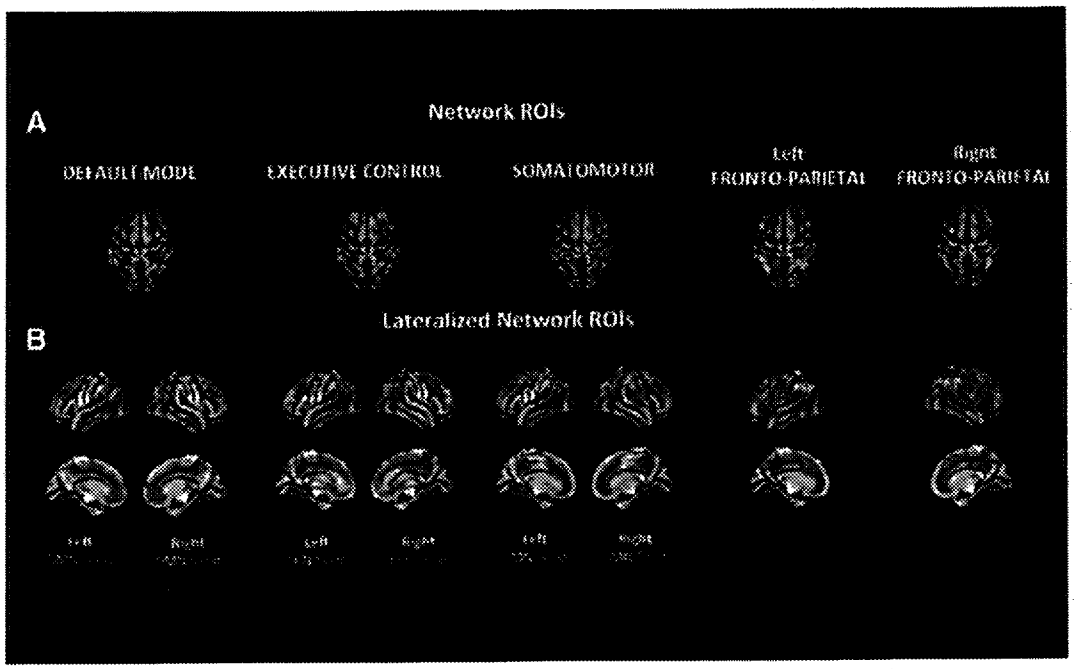


FIG. 1. Five resting state networks (RSNs)[9] were selected as regions of interest (ROIs) for the seed analysis. For each RSN, FSL was used to generate symmetrical left and right lateralized seeds, only including network regions situated in one hemisphere. Given their already strongly lateralized nature, left and right fronto-parietal network map were not modified before being used as ROIs. A threshold of $Z > 2.33$ was applied to all maps to create network-based ROIs. The networks are shown on the transverse plane (**A**) and on the sagittal plane (**B**). Color image is available online at www.liebertpub.com/neu

The concatenated transform obtained during pre-processing was applied to transform the seeds from MNI to native space and again to transform the seed correlation maps to MNI space. ROI correlation analysis included computing voxel-wise Pearson coefficients between the corrected average time series extracted from a given seed network with the corrected time series for each voxel in the brain; in order to ensure a normal distribution of the r values, MATLAB (2014a, The MathWorks, Inc., Natick, Massachusetts) was used to transform the resultant statistical maps to Fisher's z-scores.[51] A four-dimensional image file was created by concatenating individual network seed maps and FSL's flameo tool was used to perform a between participants ordinary least-square regression.[52]

To determine statistical significance and account for multiple comparisons, each network seed map was thresholded at $Z > 3.11$, with a cluster significance of $p < 0.05$. For each network seed, the resulting Z-statistic map represented a group average and a contrast of group differences. After visual inspection, anatomical labels were assigned by referencing the Harvard-Oxford Cortical and Subcortical Structural Atlases in the FSL analysis package.[53,54]

In order to obtain correlation values between different ROI pairs, the mean time series of all voxels contained in each ROI was extracted and correlated with the mean time series of all other ROIs. The resultant Pearson coefficient was then converted into a Fisher's z-score as was done for the voxel-wise analysis. Functional connectivity matrices and connectivity dendrograms were separately generated for each group using the obtained Fisher's z-values and Matlab's heatmap, linkage, and dendrogram functions. Further statistical analysis on these data was carried out using SPSS (released 2012, IBM SPSS Statistics for MacIntosh, Version 21.0; IBM Corp., Armonk, NY).

Lastly, in order to verify whether inter-hemispheric FC differences between groups were due to the presence of trauma-related focal lesions in the TBI sample, the same analyses were repeated eliminating the participants with lesions from the TBI sample and their match from the NC group.

Experiment 2

Participants and behavioral data. Participants included the same 21 TBI participants from experiment 1. Each of the TBI participants were administered a paper version of the Rey Osterrieth Complex Figure Test-Copy (ROCFT-C) and the Rey Osterrieth Complex Figure Test-Delayed Recall (ROCFT-DR). As no comparable neuropsychological behavioral data were collected for all of the NC group, TBI participants' ROCFT scores were z transformed in order to compare their performance with the population mean.

The ROCFT score collected for the TBI participants were z-transformed using internally generated normative data.[55] Inspection of the standard scores revealed that 75% of the sample placed below average ($Z < 0$), with 29% one SD below average ($Z < 1$) and 15% two deviation standards below mean ($Z < -2$; Table 2). For the subset of the TBI group for whom GCS information was available ($n = 10$), there was no correlation between GCS and performance on the ROCFT-C ($r = -0.211$, $p = 0.559$), nor between GCS and ROCFT-DR ($r = 0.254$, $p = 0.478$). As the ROCFT–DR has been found to be more strongly associated with executive functions than ROCFT-C, the former measure was regarded as more inclusive and globally representative of TBI patients' functioning, and used as a covariate of interest to investigate how inter-hemispheric FC may correlate with neuropsychological measures.[56]

Analysis of the relationship between FC and RCFT-delayed performance. FSL's flameo tool was used to perform an ordinary least square regression entering as a factor the demeaned z-scores for the ROCFT-DR.[52] The association between FC and performance of TBI participants in the ROCFT-DR was assessed by examining the correlation between normalized

TABLE 2. PERFORMANCE ON THE REY OSTERRIETH COMPLEX FIGURE TEST

	Rey Osterrieth Complex Figure Test (z-scores)	
	Copy	Delayed recall
Mean	−0.641	−0.705
SD	1.294	1.114
Z>1 (N)	2	2
−2<Z<−1 (N)	3	6
Z<−2 (N)	2	3

SD, standard deviation.

performance scores and FC in network seed maps. Z-statistic maps were thresholded at $Z>3.11$, with a cluster significance of $p<0.05$. All reported results are corrected for age, sex, and education.

Results

Experiment 1

Group differences in FC. Peaks for the FC maps obtained for each ROI network seed are reported in Table 3.

Left FPN

Based on the results of a t-contrast (NC > TBI), NC participants had more FC than the TBI patients between the left FPN lateralized seed and regions of the right FPN in the right frontal and parietal lobes, in particular the right superior and middle frontal gyrus, the right frontal pole, and the right angular gyrus (Table 2). In addition, NC participants showed more FC between the left FPN and bilateral regions in the thalamus (Fig. 2A). A second t-contrast (TBI > NC) revealed no regions of higher FC with the Left FPN in the TBI group compared with NC.

Right FPN. Similarly to the left FPN seed, for NC participants, the right FPN seed had more FC with the frontal and parieto-occipital lobe of the left hemisphere, with clusters in the medial portion of the superior frontal gyrus, middle and inferior frontal gyrus, superior parietal lobule (angular gyrus), dorso-medial part of the occipital cortex, and left thalamus. NC participants also showed higher FC with the right angular gyrus and right thalamus (Fig. 2B). TBI patients showed significantly more FC between the right FPN and the left inferior occipital cortex, a region not included in the left FPN (Table 2).

Left ECN. Compared with the TBI group, the NC group had significantly more FC between the left ECN lateralized seeds and three right hemisphere regions, the supramarginal gyrus, the occipital cortex and the middle frontal gyrus (Fig. 2C). Interestingly, none of these areas is homologous to regions comprised in the left ECN, nor overlaps with the right ECN. A t-contrast (TBI > NC) showed no clusters of significantly higher FC with the left ECN.

Right ECN. NC participants displayed significantly more FC between the right ECN seed and the left middle and inferior frontal gyrus, and some smaller clusters in the left superior parieto-occipital cortex (Fig. 2D). Conversely, TBI patients showed more FC with the right superior temporal gyrus and with a large cluster in the superior occipital cortex (Table 2).

Left DMN. NC participants displayed significantly more FC between the left DMN and the right postcentral gyrus and superior parietal lobule than TBI individuals (Fig. 2E). TBI patients had more FC between the left DMN lateralized seed and bilateral hippocampi and parahippocampal gyri, bilateral precuneus and PCCs (Fig. 3A).

Right DMN. NC showed higher FC with a cluster in the inferior frontal gyrus (Fig. 2F), while TBI exhibited significantly more FC between the right DMN seed and both left and right precuneus (Fig. 3B).

Left SMN. FC maps revealed more FC between the left SMN and clusters in bilateral precuneus, middle and superior frontal gyri, right angular gyrus and right middle temporal gyrus in NC participants (Fig. 2G); none of these regions is comprised in the right SMN (Table 2). The TBI group showed no regions of higher FC.

Right SMN. Comparably (but symmetrically) to the left SMN seed, NC participants displayed more FC between the right SMN seed and bilateral precuneus, the right middle and superior temporal gyrus, and the left angular and supramarginal gyrus. In addition, they had more FC with the anterior cingulate cortex and the left superior and middle temporal gyri (Fig. 2G; Table 2). TBI participants revealed more FC with the right superior temporal gyrus and the cerebellum (Table 2).

ROI analysis. Following analyses at the voxel-wise level, in order to better quantify the inter-hemispheric disconnection effect and its relative strength across RSNs, differences in inter-hemispheric FC between TBI and NC groups were investigated with an *a priori* ROI approach using the Smith and colleagues' network ROI seeds.[9]

Independent sample two-tailed *t*-tests were performed to assess between-group differences in FC between homologous ROIs. Statistical analyses revealed that TBI participants had significantly less ROI to ROI FC between lateralized seeds of the FPN ($t[40]=-4.134$, $p<0.01$) and the ECN ($t[40]=-2.313$, $p<0.05$), but not between left and right DMN seeds ($t[40]=0.860$, $p>0.05$) or SMN seeds ($t[40]=1.561$, $p>0.05$; Fig. 4). In order to examine the impact of the presence of mild TBI patients on these findings, we repeated the analysis removing the three mild TBI participants and their respective NC. A *t*-test revealed comparable results to the original sample: the TBI group had significantly less ROI to ROI FC between lateralized seeds of the FPN ($t[34]=-3.397$, $p<0.01$) and the ECN ($t[34]=-2.129$, $p<0.05$), but not lateralized DMN seeds ($t[34]=1.156$, $p>0.05$) or SMN seeds ($t[34]=1.433$, $p>0.05$).

To visualize the inter-relationships between all lateralized ROIs and how they differ between groups, FC dendrograms were generated separately for NC (Fig. 5A) and TBI (Fig. 5B). This approach allows us to explore where a given network ROI may have more FC for the TBI group, compared with the NC group, and examine this across all possible inter-ROI relationships. A visual inspection of the two dendrograms revealed marked differences in lateralized RSNs clustering, especially for the ECN and the FPN. In the NC group, lateralized ROIs for the two EONs are shown to be most strongly associated with their own homologous counterparts, and secondarily with the other EON. Conversely, TBI participants exhibited clustering seemingly more based on hemispherical lateralization than on RSN membership, with higher FC between right ECN and right FPN and left ECN with DMN seeds and left FPN.

Experiment 2

The map resulting from the addition of the ROCFT-DR performance as a covariate of interest revealed a positive linear relationship

TABLE 3. FUNCTIONAL CONNECTIVITY CLUSTERS AND PEAKS

	TBI > NC						NC > TBI					
		Peak (mm)						Peak (mm)				
Seed network	*Cluster size*	*x*	*y*	*z*	*Peak Z statistic*	*Anatomical location**	*Cluster size*	*x*	*y*	*z*	*Peak Z statistic*	*Anatomical location**
Left FPN							887	26	26	56	4.35	Right superior frontal gyrus
							566	26	50	12	4.36	Right frontal pole
							429	60	−52	38	4.91	Right angular gyrus
							298	−42	−64	−40	4.02	Left lateral superior occipital cortex
							235	8	−28	−14	4.35	Right brain stem
Right FPN	214	−36	−88	2	4.09	Left lateral inferior occipital cortex	1351	−22	26	54	4.58	Left superior frontal gyrus
							599	−6	−12	10	4.81	Left thalamus
							585	−44	−62	48	4.47	Left lateral superior occipital cortex
							285	52	−60	38	4.14	Right lateral superior occipital cortex
							245	−52	30	10	4.32	Left inferior frontal gyrus
							215	−34	−76	−42	4.6	Left cerebellum
Left ECN							354	46	−44	52	4.06	Right supramarginal gyrus
							202	40	−80	36	4.32	Right superior lateral occipital cortex
							169	46	52	4	3.77	Right frontal pole
Right ECN	779	−8	−84	44	5.21	Left superior lateral occipital cortex	570	−46	4	44	5.22	Left precentral gyrus
	308	58	−28	−2	4.76	Left posterior superior temporal gyrus	204	−48	36	2	4.3	Left inferior frontal gyrus
							186	−38	−78	48	3.85	Left superior lateral occipital cortex
							186	−36	−64	−42	4.51	Left Cerebellum
Left SMN							1259	10	−50	24	4.5	Right posterior cingulate gyrus
							286	58	−10	−20	4.11	Right middle temporal gyrus
							272	−22	24	48	4.23	Left superior frontal gyrus
							181	44	−56	22	3.8	Right angular gyrus
							179	20	32	46	4.56	Right superior frontal gyrus
Right SMN	395	6	−70	−16	4.56	Right lingual gyrus	1304	−14	−50	24	4.54	Left precuneus
	263	−34	46	12	3.81	Left frontal pole	534	−50	−24	−2	4.79	Left posterior superior temporal gyrus
							347	−48	−58	22	4.3	Left angular gyrus
							267	4	28	−10	4.3	Right subcallosal cortex
							266	60	−20	0	4.55	Right posterior superior temporal gyrus
Left DMN	1412	2	−66	38	4.71	Right precuneus	719	44	−30	64	4.53	Right postcentral gyrus
	400	−30	−24	−20	4.76	Left posterior parahippocampal gyrus						
	221	28	−16	−8	4.36	Right amygdala						
Right DMN	1132	8	−60	20	4.6	Right precuneus	267	−42	48	−4	4.01	Left frontal pole
							188	28	−36	58	4.19	Right postcentral gyrus

*Anatomical labels were assigned by imposing each peak on two probabilistic atlases, the Harvard-Oxford Cortical and Subcortical Structural Atlases in the FSL analysis package. For the cerebellum, labels were assigned following visual inspection. Cluster units are number of voxels in MNI space.

TBI, traumatic brain injury; NC, normal comparisons; FPN, fronto-parietal network; ECN, executive control network; SMN, somato-motor network; DMN, default mode network; MNI, Montreal Neurological Institute.

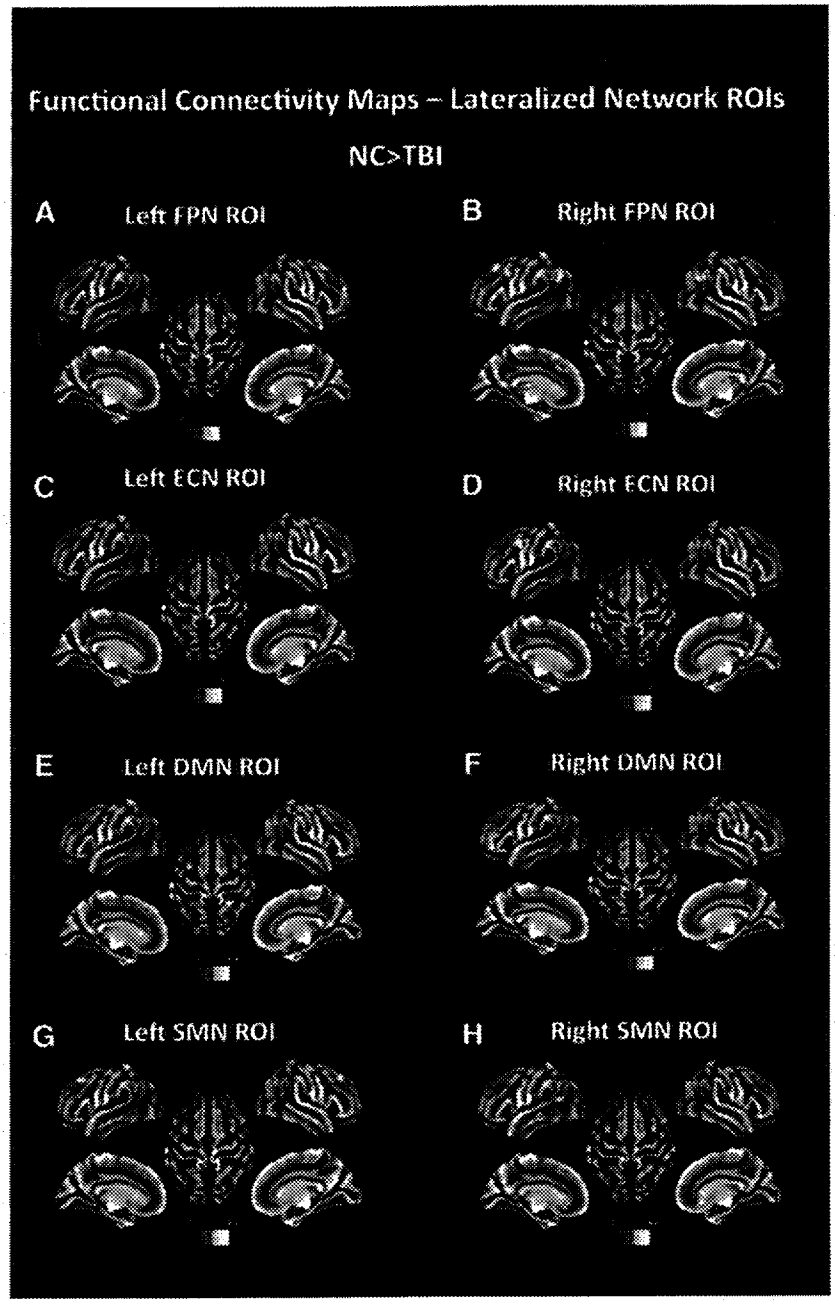


FIG. 2. Functional connectivity maps generated by performing a seed analysis using left fronto-parietal network (FPN; **A**), right FPN (**B**), left executive control network (ECN; **C**) and right ECN (**D**), left default mode network (DMN; **E**), right DMN (**F**), left SMN (**G**), and right DMN (**H**) as regions of interest (ROIs). The images are thresholded at $Z > 3.11$, with a cluster significance of $p < 0.05$ and show the regions displaying higher functional connectivity between the seeded ROIs and areas included in the maps in the normal comparison sample than in the traumatic brain injury (TBI) group. Color image is available online at www.liebertpub.com/neu

between behavioral data and the FC between the right FPN and a cluster extending from the pars opercularis of the left inferior frontal gyrus to the left ventral part of the precentral gyrus (peak at MNI coordinates (x,y,z) of (-54, 6, 16), $Z = 3.97$, 252 voxels in cluster), a region included in the homologous left FPN (Fig. 6A). No correlations were found for the left FPN, or the left and right ECN.

In order to visualize the association between the behavioral variables and FC and ensure that it was not driven by the presence of outliers, the average time series was extracted from ROIs defined based on the peaks in FC, and its correlation with said variables was calculated and plotted. FC between the right FPN network and the frontal ROI had a positive correlation of $r = 0.818$ ($p > 0.001$) with performance on the ROCFT-DR (Fig. 6B).

Discussion

Our analyses using both voxel wise and ROI correlation approaches revealed significantly less inter-hemispheric FC in TBI

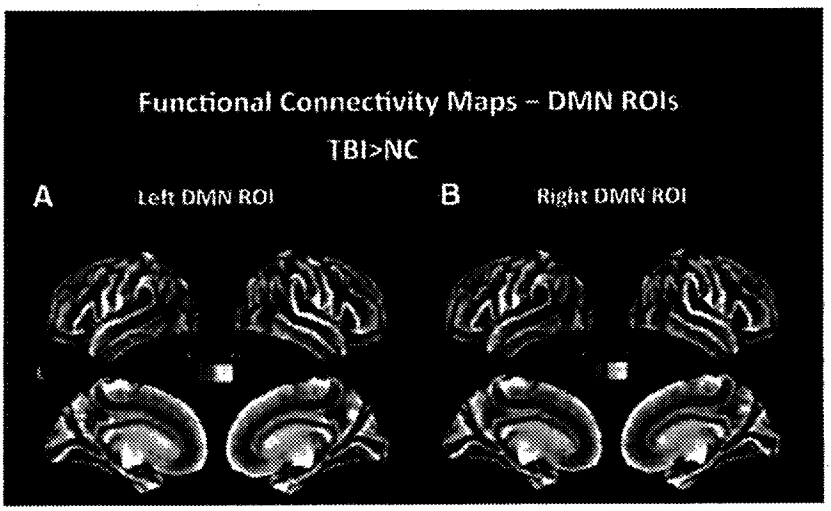


FIG. 3. Functional connectivity maps generated by performing a seed analysis using the left (A) and right (B) default mode network (DMN) as regions of interest (ROIs). The images are cluster thresholded for $p<0.05$ and for $Z>3.11$ and show regions displaying higher functional connectivity between the seeded ROI and areas included in the bilateral DMN. Color image is available online at www.liebertpub.com/neu

patients for EONs (FPN and ECN), but not for the DMN and the SMN. Given the selectively abnormal inter-hemispheric FC in the TBI group, we were interested in the clinical and behavioral significance of this pattern. As the ultimate goal of our research is to establish the utility of FC at rest as a potential rehabilitation-informing biomarker of cognitive impairment following TBI, we decided to investigate the parallels between FC at rest and specific behavioral impairments within TBI individuals.

To explore the relation between inter-hemispheric FC strength and behavioral performance, we examined performance of the TBI participants on a standardized neuropsychological test, the Rey-Osterrieth Complex Figure Test (ROCFT), administered outside of the scanner. We chose this measure as it is one of the four tests recommended by the American Brain Injury Consortium to assess neuropsychological outcome following TBI, and because the abilities measured by the ROCFT are commonly impaired in individuals with TBI, who often perform poorly on this task.[57,58] The ROCFT is a test sensitive to detection of impairment in visuo-spatial, visuo-perceptive and visuo-constructive abilities, memory, organizational skills, and highly associated with processing speed and executive functioning, and has the potential for providing insights into the mechanisms that underlie cognitive dysfunctions after TBI. We hypothesized that reduced inter-hemispheric FC would be associated with worse performance on the ROCFT within the TBI group.[56,59–61] As the aim of this analysis was exploring the relationship between inter-hemispheric connectivity and behavior, the addition of the ROCFT as a covariate of interest focused on the lateralized seeds of the two networks that had shown significantly less inter-hemispheric connectivity in the TBI group: the left and right FPN and the left and right ECN.

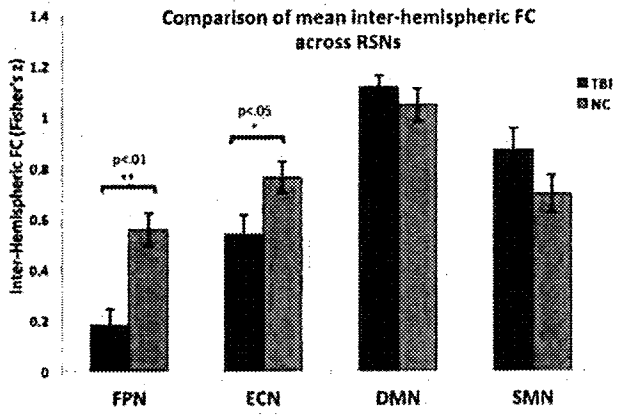


FIG. 4. Comparison of mean functional connectivity (FC; ± standard error) between lateralized resting state network (RSN) seeds in traumatic brain injury (TBI) and normal comparison (NC) participants.

To our knowledge, this is the first study to investigate how inter-hemispheric FC at rest may be altered with specificity at the level of functional networks. Our finding generalizes across a broad literature because we measured inter-hemispheric FC with lateralized RSN ROIs generated by meta-analytic ICA (i.e., derived from the literature as highly replicable functional networks). The use of such a template allowed us to maintain a network perspective throughout the analysis; in addition, in a high variability population such as the one analyzed in this study, where several changes in brain structure may occur following a traumatic injury, the use of more extensive seeds addresses some potential problems that might arise with the use of smaller ROIs (e.g., the sensitivity of resting state fMRI results to ROIs coordinates, size and shape, the difficulty of establishing the most representative ROIs for a given RNS and, specifically in the TBI population, the possible confounds due to changes and shifts in brain structure following head injury).[62,63]

Our results with chronic TBI patients are partially consistent with results on acute and sub-acute samples by Sours and colleagues[29] and Marquez de la Plata and colleagues[28] using more restricted frontal and parietal ROIs. In particular, we found that TBI participants display significantly less inter-hemispheric FC, but only between networks more involved in externally oriented cognition (FPN and ECN).[28,29] These RSNs repeatedly have been found to activate during tasks demanding cognitive flexibility, and to support cognitive functions necessary for successful interaction with the environment including goal directed action, set maintenance, attentional selection, encoding of salience, working memory

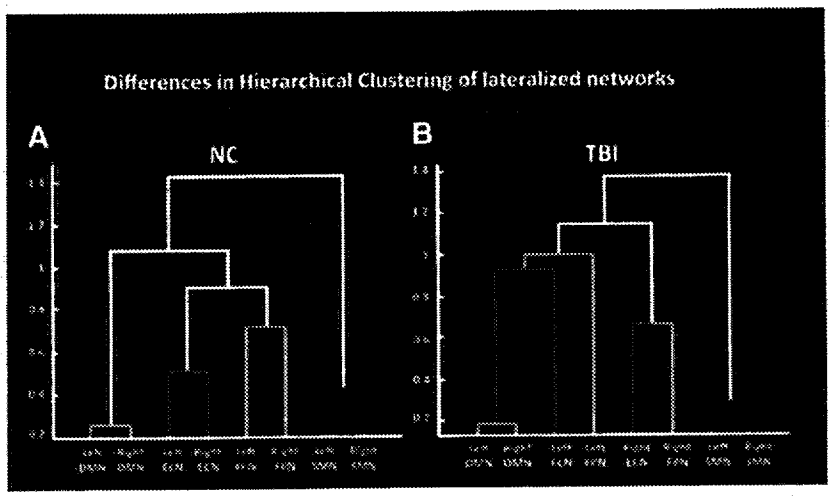


FIG. 5. Dendrograms showing differences in network clustering between the normal comparison NC **(A)** and traumatic brain injury TBI **(B)** groups.

and mental representations.[12,22,64,65] Given the across-the-board impairment in attention and cognitive functions found in TBI patients of all severities and the extensive neuropsychiatric, emotional, and behavioral problems displayed in everyday life, it is crucial to uncover how these complaints map onto specific aberrant patterns of FC in these large scale networks.[66,67] The marked inter-hemispheric disconnection found in our sample, coupled with the association between inter-hemispheric FC and performance on the ROCFT, has the potential of being informative about the mechanisms underlying TBI-related cognitive impairment. In particular, given the pattern of inter-hemispheric FC differences and the correlations with behavior found only in EONs, our results further our understanding of how impaired cognitive functions may selectively map onto specific attributes of specific functional systems; our findings reveal that not only the cognitive functions supported by a certain system (e.g., executive functions for the ECN), but also the specific nature of the aberrant connectivity patterns found in said system (e.g., reduced functional connectivity between homologous regions vs. globally reduced functional connectivity within a network) should be taken into consideration in the study of how behavioral dysfunction following TBI corresponds to neural activity at rest.

In our sample, the DMN inter-hemispheric FC was not reduced following TBI. We took a large-scale network-based approach and thus we did not directly use a hippocampal seed; however, the hippocampus is usually considered part of the MTL sub-network of the DMN.[16] Contrary to previous reports, which found less hippocampal connectivity for TBI, compared with controls, our analyses did not reveal less inter-hemispheric FC between lateralized DMN seeds for TBIs, compared with controls.[28,30] There are two possible explanations for this: first, the network maps we used as seeds might have led to different results, especially since when

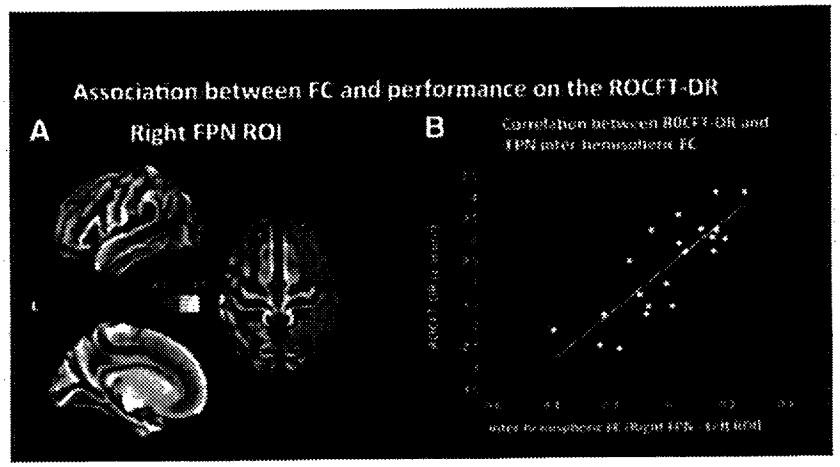


FIG. 6. Functional connectivity maps generated by performing a seed analysis using the right fronto-parietal network (FPN) as regions of interest (ROIs) and adding the demeaned performance on the Rey Osterrieth Complex Figure Test-Delayed Recall (ROCFT-DR) as a covariate. The maps (Fig. 6A) show regions displaying a positive temporal correlation with the seeded ROIs (in green), which is in turn associated with performance on the ROCFT-DR. Figure 6B was obtained by masking the areas included in the z-statistic maps shown in Figure 6A and using it to extract the time series; Figure 6B is only reported for visualization purposes. Figure 6A is thresholded at $Z > 3.11$, with a cluster significance of $p < 0.05$. Color image is available online at www.liebertpub.com/neu

thresholded at $Z > 2.33$ they did not include the hippocampus. Consequently, although the hippocampus is generally considered part of the DMN, our investigation focused on large-scale networks and not on small circular seeds, and this might have caused our discordant findings. Secondly, the failure to replicate might be related to the chronic stage of our TBI sample, whereas the mentioned studies focused mainly on acute TBI populations.

Although inter-hemispheric FC in the ION appeared unaffected, our TBI group did show greater FC between left DMN ROI and the right precuneus and the left parahippocampal gyrus and between the right DMN ROI and the right precuneus, replicating previous results on chronic samples.[17] Unfortunately, based on our findings it is impossible to conclude whether the stronger FC reflects an adaptive response to brain injury, as we did not administer tasks sensitive to DMN integrity to our TBI sample such as a sustained-attention task.[17,68] It is not surprising, then, that FC between the DMN and precuneus, PCC and hippocampi did not correlate with behavioral performance on the ROCFT. Recent reports indicate that focus on the DMN and the regions it comprises, as well as their disruption following disease or injury may be key to advancing the study of how different disorders can influence brain functions and cognition.[21,69,70] For this reason, evidence on the mechanisms through which DMN intra-network FC is associated with cognitive outcomes (inter-hemispheric FC vs. FC with nodes such as hippocampi and precuneus) can guide future research and inform targeted rehabilitation procedures.

Our findings regarding the SMN did not replicate the inter-hemispheric disconnection reported by Kasahara and colleagues using an event-related design, probably due to the different methodologies employed.[27] Although TBI patients tend to regain their gross neuro-motor skills over time, persistence of certain abnormalities, such as tandem gait problems and postural instability can cause severe distress and decrease quality of life.[27,71,72] The fact that no motor task performance was assessed for either group prevents us from making claims, but lack of a deficit in inter-hemispheric FC shown by NC, compared with screened healthy NC, suggest that subtle motor deficits following TBI may not be mediated by functional disconnection of homologous motor regions; future research should investigate this possibility by comparing FC during rest compared to tasks with respect to their predictive utility for TBI outcomes.

Finally, the differences reported in network clustering clearly demonstrate TBI can disturb network architecture and is associated with changes both within RSNs and in the relationship between RSNs.[29] Previous studies have highlighted the importance of characterizing the way networks interact with each other to reach a deeper understanding of the pathophysiological mechanisms of TBI outcome.[21,68] Notably, in our patient sample, FC between areas located in the same hemisphere was higher than in regions belonging to the same RSNs. Furthermore, as all our TBI patients were in the chronic stage of their injury, our results clearly demonstrate that these alterations in inter and intra network balance are long-term. Thus, our findings could serve as a starting point for future research that focuses on how specific changes in hierarchical clustering of RSNs may predict behavior following a TBI, and how FC between distinct networks and lateralized ROIs vary in their activation patterns during demanding cognitive tasks.

Limitations

The work presented here explored inter-hemispheric connectivity following TBI using an innovative large-scale network approach, which lead to robust findings important in the study of TBI, its sequelae and their biomarkers. Yet, some limitations should be noted. The first limitation concerns the structural and functional neuroimaging data being collected in different scanners for the TBI and NC groups. This could serve as a potential confound, although given the results of our SNR analysis and the nature of the results, we believe it is very unlikely that the specific group differences in inter-hemispheric connectivity could have been driven by differences in the hardware or scanning procedure. However, it is important to mention that at this stage we do not have the means to precisely quantify how much inter-scanner effects are contributing to the significant inter-group differences detected in the study. Furthermore, given the vast amount of resting state data that has and is still being collected by multicenter efforts such as the Human Connectome Project, and the growing necessity to produce studies with large effect sizes on patient groups, the comparison of data acquired in different sites and scanners to study different populations is soon to become a frequent reality; this situation will leave to each research team the responsibility of performing data analysis (e.g., SNR analysis) which can give insights on the possible confounds of different acquisition sites. The results of the SNR analysis we performed on our data clearly shows no significant differences between sites, thus increasing confidence in our findings.

While we found a significant relationship between FC and performance on a neuropsychological measure that captures the hallmark deficits of TBI, having a more extensive battery of neuropsychological tests and NC comparison data would strengthen the findings reported here. For this reason, the lack of extensive neuropsychological testing and behavioral indices for the NC poses another limitation: the two groups cannot be compared from a behavioral viewpoint, and conclusions drawn on whether the TBI group was cognitively impaired in one or more specific domains. Subsequently, without the possibility of investigating the association between behavior and FC profiles, it is difficult to interpret the clinical significance of our FC findings. However, the use of normative data allowed us to place our TBI participants below the mean, compared with a healthy population for the ROCFT, replicating previous work. The correlations found by adding behavioral measures as covariates are restricted to the TBI sample, precluding inter group comparison and begging the question of whether the relationship between cognitive abilities and FC changes qualitatively or quantitatively after TBI. Thus, future research should focus on expanding the present study's findings using a comparative framework. That said, the robust findings we reported represent a step forward in the investigation of the relationship between cognitive impairment and resting state FC in specific brain networks and in demonstrating the feasibility of a large scale network approach to the study of TBI.

Although our sample included three mild TBI participants, the vast majority was moderate to severe TBI patients. This invites caution in generalizing our findings to mild TBI populations. Similarly, all our participants were scanned over 6 months after their injury. Therefore, our findings cannot be generalized to TBI patients in the acute or sub-acute phase of their TBI, and no information on the evolution of the aberrant patterns of FC reported can be made.

Conclusion

The present study revealed that mild-to-severe chronic TBI is associated with network-specific aberrant patterns of FC: two externally oriented networks, the FPN and the ECN, showed

significantly less inter-hemispheric FC in TBI patients than in normal controls, while the DMN, an internally oriented network, had more FC with PCC and hippocampi in TBI patients. In addition, the fact that inter-hemispheric FC between regions of the FPN in TBI individuals who perform better in a task that measures visuospatial, memory and executive function points toward the clinical relevance of our findings and their potential usefulness. The robustness of our findings shows the usefulness of employing a network perspective in the study of the relationship between FC in brain systems and complex behavior and takes us further in the search for biomarkers that will serve as a diagnostic tool and allow more precise subtyping, classification and prediction of disorder trajectories following TBI.

Acknowledgments

This work was supported in part by start-up funds provided by the University of Iowa to MD and MV, and grant funding from NIH R37 AG025667 and NIH RO1 HD069381 supported image acquisition for the normal control participants. The authors would like to thank Joel Bruss for his advice during visual inspection of the anatomical images, Jocelyn Cole for the hand-tracing of the brain masks and Ruth Henson for logistic support in scheduling TBI participants.

Author Disclosure Statement

No competing financial interests exist.

References

1. Roozenbeek, B., Maas, A.I., and Menon, D.K. (2013). Changing patterns in the epidemiology of traumatic brain injury. Nature Rev. Neurol. 9, 231–236.
2. Selassie, A.W., Zaloshnja, E., Langlois, J.A., Miller, T., Jones, P. and Steiner, C. (2008). Incidence of long-term disability following traumatic brain injury hospitalization, United States, 2003. J. Head Trauma Rehabil. 23, 123–131.
3. Centers for Disease Control and Prevention. (2014). Injury Prevention and Control: Traumatic Brain Injury. www.cdc.gov/traumaticbraininjury/
4. McDonald, B.C., Saykin, A.J., and McAllister, T.W. (2012). Functional MRI of mild traumatic brain injury (mTBI): progress and perspectives from the first decade of studies. Brain Imaging Behav. 6, 193–207.
5. Ham, T.E. and Sharp, D.J. (2012). How can investigation of network function inform rehabilitation after traumatic brain injury? Current opinion in neurology 25, 662–669.
6. Sharp, D.J., Scott, G. and Leech, R. (2014). Network dysfunction after traumatic brain injury. Nature Rev. Neurol. 10, 156–166.
7. Fox, M.D. and Raichle, M.E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nature Rev. Neurosci. 8, 700–711.
8. Biswal, B., Yetkin, F.Z., Haughton, V.M., and Hyde, J.S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn. Reson. Med. 34, 537–541.
9. Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., Mackay, C.E., Filippini, N., Watkins, K.E., Toro, R., Laird, A.R., and Beckmann, C.F. (2009). Correspondence of the brain's functional architecture during activation and rest. Proc. Natl. Acad. Sci. U. S. A. 106, 13040–13045.
10. Fox, M.D. and Greicius, M. (2010). Clinical applications of resting state functional connectivity. Front. Syst. Neurosci. 4, 19.
11. Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., and Shulman, G.L. (2001). A default mode of brain function. Proc. Natl. Acad. Sci. U. S. A. 98, 676–682.
12. Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J., Glover, G.H., Kenna, H., Reiss, A.L., and Greicius, M.D. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 27, 2349–2356.
13. Spreng, R.N. and Grady, C.L. (2010). Patterns of brain activity supporting autobiographical memory, prospection, and theory of mind, and their relationship to the default mode network. Journal of cognitive neuroscience 22, 1112–1123.
14. Spreng, R.N., Stevens, W.D., Chamberlain, J.P., Gilmore, A.W., and Schacter, D.L. (2010). Default network activity, coupled with the frontoparietal control network, supports goal-directed cognition. NeuroImage 53, 303–317.
15. Spreng, R.N. (2012). The fallacy of a "task-negative" network. Front. Psychol. 3, 145.
16. Andrews-Hanna, J.R., Reidler, J.S., Sepulcre, J., Poulin, R., and Buckner, R.L. (2010). Functional-anatomic fractionation of the brain's default network. Neuron 65, 550–562.
17. Sharp, D.J., Beckmann, C.F., Greenwood, R., Kinnunen, K.M., Bonnelle, V., De Boissezon, X., Powell, J.H., Counsell, S.J., Patel, M.C., and Leech, R. (2011). Default mode network functional and structural connectivity after traumatic brain injury. Brain 134, 2233–2247.
18. Stevens, M.C., Lovejoy, D., Kim, J., Oakes, H., Kureshi, I., and Witt, S.T. (2012). Multiple resting state network functional connectivity abnormalities in mild traumatic brain injury. Brain Imaging Behav. 6, 293–318.
19. Mayer, A.R., Mannell, M.V., Ling, J., Gasparovic, C., and Yeo, R.A. (2011). Functional connectivity in mild traumatic brain injury. Hum. Brain Mapp. 32, 1825–1835.
20. Johnson, B., Zhang, K., Gay, M., Horovitz, S., Hallett, M., Sebastianelli, W. and Slobounov, S. (2012). Alteration of brain default network in subacute phase of injury in concussed individuals: resting-state fMRI study. NeuroImage 59, 511–518.
21. Arenivas, A., Diaz-Arrastia, R., Spence, J., Cullum, C.M., Krishnan, K., Boxworth, C., Culver, C., Kennard, B., and Marquez de la Plata, C. (2014). Three approaches to investigating functional compromise to the default mode network after traumatic axonal injury. Brain imaging and behavior 8, 407–419.
22. Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., and Raichle, M.E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc. Natl. Acad. Sci. U. S. A. 102, 9673–9678.
23. Krmpotich, T.D., Tregellas, J.R., Thompson, L.L., Banich, M.T., Klenk, A.M., and Tanabe, J.L. (2013). Resting-state activity in the left executive control network is associated with behavioral approach and is increased in substance dependence. Drug and alcohol dependence 129, 1–7.
24. McDonald, B.C., Flashman, L.A., and Saykin, A.J. (2002). Executive dysfunction following traumatic brain injury: neural substrates and treatment strategies. NeuroRehabilitation 17, 333–344.
25. Shumskaya, E., Andriessen, T.M., Norris, D.G., and Vos, P.E. (2012). Abnormal whole-brain functional networks in homogeneous acute mild traumatic brain injury. Neurology 79, 175–182.
26. Kasahara, M., Menon, D.K., Salmond, C.H., Outtrim, J.G., Tavares, J.V., Carpenter, T.A., Pickard, J.D., Sahakian, B.J., and Stamatakis, E.A. (2011). Traumatic brain injury alters the functional brain network mediating working memory. Brain inj. 25, 1170–1187.
27. Kasahara, M., Menon, D.K., Salmond, C.H., Outtrim, J.G., Taylor Tavares, J.V., Carpenter, T.A., Pickard, J.D., Sahakian, B.J., and Stamatakis, E.A. (2010). Altered functional connectivity in the motor network after traumatic brain injury. Neurology 75, 168–176.
28. Marquez de la Plata, C.D., Garces, J., Shokri Kojori, E., Grinnan, J., Krishnan, K., Pidikiti, R., Spence, J., Devous, M.D. Sr., Moore, C., McColl, R., Madden, C., and Diaz-Arrastia, R. (2011). Deficits in functional connectivity of hippocampal and frontal lobe circuits after traumatic axonal injury. Arch. Neurol. 68, 74–84.
29. Sours, C., Rosenberg, J., Kane, R., Roys, S., Zhuo, J., Shanmuganathan, K., and Gullapalli, R.P. (2014). Associations between interhemispheric functional connectivity and the Automated Neuropsychological Assessment Metrics (ANAM) in civilian mild TBI. Brain Imaging Behav. 9, 190–203.
30. Slobounov, S.M., Gay, M., Zhang, K., Johnson, B., Pennell, D., Sebastianelli, W., Horovitz, S., and Hallett, M. (2011). Alteration of brain functional network at rest and in response to YMCA physical stress test in concussed athletes: RsfMRI study. NeuroImage 55, 1716–1727.
31. Johnson, B., Zhang, K., Gay, M., Neuberger, T., Horovitz, S., Hallett, M., Sebastianelli, W., and Slobounov, S. (2012). Metabolic alterations in corpus callosum may compromise brain functional connectivity in MTBI patients: an 1H-MRS study. Neuroscience Lett. 509, 5–8.

32. O'Reilly, J.X., Croxson, P.L., Jbabdi, S., Sallet, J., Noonan, M.P., Mars, R.B., Browning, P.G., Wilson, C.R., Mitchell, A.S., Miller, K.L., Rushworth, M.F., and Baxter, M.G. (2013). Causal effect of disconnection lesions on interhemispheric functional connectivity in rhesus monkeys. Proc. Natl Acad. Sci. U. S. A. 110, 13982–13987.
33. Millis, S.R., Rosenthal, M., Novack, T.A., Sherer, M., Nick, T.G., Kreutzer, J.S., High, W.M. Jr., and Ricker, J.H. (2001). Long-term neuropsychological outcome after traumatic brain injury. J. Head trauma Rehabil. 16, 343–355.
34. Pagulayan, K.F., Temkin, N.R., Machamer, J., and Dikmen, S.S. (2006). A longitudinal study of health-related quality of life after traumatic brain injury. Arch. Phys. Med. Rehabil. 87, 611–618.
35. Teasdale, G. and Jennett, B. (1974). Assessment of coma and impaired consciousness. A practical scale. Lancet 2, 81–84.
36. Malec, J.F., Brown, A.W., Leibson, C.L., Flaada, J.T., Mandrekar, J.N., Diehl, N.N., and Perkins, P.K. (2007). The mayo classification system for traumatic brain injury severity. J. Neurotrauma 24, 1417–1424.
37. Williams, D.H., Levin, H.S., and Eisenberg, H.M. (1990). Mild head injury classification. Neurosurgery 27, 422–428.
38. Hsiang, J.N., Yeung, T., Yu, A.L., and Poon, W.S. (1997). High-risk mild head injury. J. Neurosurgery 87, 234–238.
39. Stonnington, C.M., Tan, G., Klöppel, S., Chu, C., Draganski, B., Jack, C.R., Jr., Chen, K., Ashburner, J., and Frackowiak, R.S. (2008). Interpreting scan data acquired from multiple scanners: a study with Alzheimer's disease. NeuroImage 39, 1180–1185.
40. Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., and Matthews, P.M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 23 Suppl 1, S208–S219.
41. Smith, S.M. (2002). Fast robust automated brain extraction. Hum. Brain Mapp. 17, 143–155.
42. Jenkinson, M., Bannister, P., Brady, M. and Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. NeuroImage 17, 825–841.
43. Beckmann, C.F. and Smith, S.M. (2004). Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans. Med. Imaging 23, 137–152.
44. Kelly, R.E., Jr., Alexopoulos, G.S., Wang, Z., Gunning, F.M., Murphy, C.F., Morimoto, S.S., Kanellopoulos, D., Jia, Z., Lim, K.O., and Hoptman, M.J. (2010). Visual inspection of independent components: defining a procedure for artifact removal from fMRI data. J. Neurosci. Methods 189, 233–245.
45. Landis, J.R. and Koch, G.G. (1977). The measurement of observer agreement for categorical data. Biometrics 33, 159–174.
46. Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L. and Petersen, S.E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 59, 2142–2154.
47. Hallquist, M.N., Hwang, K. and Luna, B. (2013). The nuisance of nuisance regression: spectral misspecification in a common approach to resting-state fMRI preprocessing reintroduces noise and obscures functional connectivity. NeuroImage 82, 208–225.
48. Greve, D.N. and Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. NeuroImage 48, 63–72.
49. Andersson, J.L.R., Jenkinson, M. and Smith, S. (2007). Non-linear optimisation. FMRIB technical report TR07JA1. Available at: www.fmrib.ox.ac.uk/analysis/techrep/tr07ja1/tr07ja1.pdf. Accessed March 18, 2016.
50. Andersson, J.L.R., Jenkinson, M., and Smith, S. (2007). Non-linear registration, aka Spatial normalisation FMRIB technical report TR07JA2. Available at: www.fmrib.ox.ac.uk/analysis/techrep/tr07ja2/tr07ja2.pdf. Accessed March 16, 2016.
51. J, Z. (1996). *Biostatistical Analysis*. Prentice-Hall: Upper Saddle River, NJ.
52. Beckmann, C.F., Jenkinson, M., and Smith, S.M. (2003). General multilevel linear modeling for group analysis in FMRI. NeuroImage 20, 1052–1063.
53. Desikan, R.S., Segonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., and Killiany, R.J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 31, 968–980.
54. Eickhoff, S.B., Paus, T., Caspers, S., Grosbras, M.H., Evans, A.C., Zilles, K., and Amunts, K. (2007). Assignment of functional activations to probabilistic cytoarchitectonic areas revisited. NeuroImage 36, 511–521.
55. Tranel, D. (1996). The Iowa-Benton School of Neuropsychological Assessment. Second ed. Oxford University Press: New York.
56. Schwarz, L., Penna, S., and Novack, T. (2009). Factors contributing to performance on the Rey Complex Figure Test in individuals with traumatic brain injury. Clinical Neuropsychol. 23, 255–267.
57. Bullock, M.R., Merchant, R.E., Choi, S.C., Gilman, C.B., Kreutzer, J.S., Marmarou, A., and Teasdale, G.M. (2002). Outcome measures for clinical trials in neurotrauma. Neurosurg. Focus 13, ECP1.
58. Ashton, V.L., Donders, J., and Hoffman, N.M. (2005). Rey Complex Figure Test performance after traumatic brain injury. J. Clin. Exp. Neuropsychol. 27, 55–64.
59. Lezack, M.D., Howieson, D.B., and David, W.L. (2004). *Neuropsychological Assessment. Fourth Edition ed.* Oxford University Press: New York.
60. Shukla, D., Devi, B.I. and Agrawal, A. (2011). Outcome measures for traumatic brain injury. Clin. Neurol. Neurosurg. 113, 435–441.
61. Diamond, B.J., DeLuca, J., and Kelley, S.M. (1997). Memory and executive functions in amnesic and non-amnesic patients with aneurysms of the anterior communicating artery. Brain : a journal of neurology 120 (Pt 6), 1015–1025.
62. Bramlett, H.M. and Dietrich, W.D. (2002). Quantitative structural changes in white and gray matter 1 year following traumatic brain injury in rats. Acta Neuropathologica 103, 607–614.
63. Irimia, A., Wang, B., Aylward, S.R., Prastawa, M.W., Pace, D.F., Gerig, G., Hovda, D.A., Kikinis, R., Vespa, P.M., and Van Horn, J.D. (2012). Neuroimaging of structural pathology and connectomics in traumatic brain injury: Toward personalized outcome prediction. NeuroImage. Clin. 1, 1–17.
64. Dosenbach, N.U., Fair, D.A., Miezin, F.M., Cohen, A.L., Wenger, K.K., Dosenbach, R.A., Fox, M.D., Snyder, A.Z., Vincent, J.L., Raichle, M.E., Schlaggar, B.L., and Petersen, S.E. (2007). Distinct brain networks for adaptive and stable task control in humans. Proc. Natl. Acad. Sci. U. S. A. 104, 11073–11078.
65. Ptak, R. (2012). The frontoparietal attention network of the human brain: action, saliency, and a priority map of the environment. Neuroscientist 18, 502–515.
66. Arciniegas, D.B., Topkoff, J., and Silver, J.M. (2000). Neuropsychiatric aspects of traumatic brain injury. Curr. Treat. Options Neurol. 2, 169–186.
67. Wortzel, H.S. and Arciniegas, D.B. (2012). Treatment of post-traumatic cognitive impairments. Curr. Treat. Options Neurol. 14, 493–508.
68. Bonnelle, V., Leech, R., Kinnunen, K.M., Ham, T.E., Beckmann, C.F., De Boissezon, X., Greenwood, R.J., and Sharp, D.J. (2011). Default mode network connectivity predicts sustained attention deficits after traumatic brain injury. J. Neurosci. 31, 13442–13451.
69. Zhang, D. and Raichle, M.E. (2010). Disease and the brain's dark energy. Nat. Rev. Neurol. 6, 15–28.
70. Leech, R. and Sharp, D.J. (2014). The role of the posterior cingulate cortex in cognition and disease. Brain 137, 12–32.
71. Walker, W.C. and Pickett, T.C. (2007). Motor impairment after severe traumatic brain injury: a longitudinal multicenter study. J. Rehabil. Res. Dev. 44, 975–982.
72. Pickett, T.C., Radfar-Baublitz, L.S., McDonald, S.D., Walker, W.C., and Cifu, D.X. (2007). Objectively assessing balance deficits after TBI: role of computerized posturography. J. Rehabil. Res. Dev. 44, 983–990.

Address correspondence to:
Arianna Rigon, MS
Neuroscience Graduate Program
W.IHC 250 Hawkins Drive
University of Iowa
Iowa City, IA 52242

E-mail: arianna-rigon@uiowa.edu

AJOB Neurosci. Author manuscript; available in PMC 2015 Mar 18.
Published in final edited form as:
AJOB Neurosci. 2014; 5(2): 41–43.
Published online 2014 Mar 18. doi: 10.1080/21507740.2014.884191

PMCID: PMC4223772
NIHMSID: NIHMS560931

Clarifying the Robust Foundation for and Appropriate Use of DTI in mTBI Patients

Michael L. Lipton, M.D., Ph.D., F.A.C.R.[✉] and Erin D. Bigler, Ph.D.

Michael L. Lipton, The Gruss Magnetic Resonance Research Center, Departments of Radiology, Psychiatry and Behavioral Sciences and The Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, Voice: (718) 430-3390, FAX: (718) 430-2185, michael.lipton@einstein.yu.edu;
Contributor Information.
[✉]Corresponding author.

Abstract

Go to:

As clinicians and scientists, we believe scientific evidence and prudent clinical practice form the proper basis for determining the utility of diagnostic measures, which should subsequently inform forensic use. The misleading and often entirely unsubstantiated opinions and positions of Wortzel et al., in opposition to DTI as a useful measure in mTBI, are at odds with the clear consensus of the scientific literature regarding mTBI, its clinical assessment and natural history. The authors' critique contains numerous errors. We will focus on four areas: (1) the clinical reality of mTBI (2) the true substance of the scientific evidence supporting use of DTI in mTBI, (3) the authors' erroneous and off-target opinions regarding DTI analysis and (4) critical appraisal and integration of clinical information for diagnosis of mTBI.

First, an underlying theme of the authors' arguments claims that lasting sequelae from mTBI is not a clinical reality. For example, "…best available evidence does not support notions that mTBI results in long-term cognitive impairments.". mTBI is a reality, which results in lasting sequelae in a substantial minority (Bigler, Farrer et al. 2013, McMahon, Hricik et al. 2013). mTBI is modeled in animals, yielding reproducible microstructural neuropathological and behavioral findings, as well as with finite biomechanical models of human mTBI. The impact of mTBI cannot be argued away by focusing on the majority who recover. Clinical, scientific and even forensic focus must be on the affected minority. Moreover, traditional neuropsychological approaches are problematic in assessing the cognitive effects of mTBI; they were never designed to assess subtle, but important deficits.

Second, the authors' critique of DTI challenges the "believability" of quantitative DTI findings, juxtaposing visual detection of spinal disc herniation and detection of microscopic mTBI pathology. They imply that because the microstructural abnormality cannot be "seen" without quantification its existence is in question. This "straw man" argument would also imply that other neuroimaging findings, which cannot be seen without quantification, such as spectroscopic and perfusion-based detection of tumor infiltration into normal appearing white matter, are not real or reliable. The substance and implications of the ASFNR guideline are mischaracterized to support the authors' position: "The guidelines… detailing the limitations in using DTI clinically, especially at the individual level and when

EXHIBIT 8

analyzed by voxel-based techniques" The guideline deals exclusively with clinical use in patients and does not isolate single patient assessment for scrutiny. Important cautions are listed, but the message is: When DTI is used in accordance with the guideline, reliable clinical use can be achieved. Assessment of DTI parameters is not singled out as most concerning; greater attention is paid to limitations of tractography and its misuse.

Third, the authors argue that method variance renders DTI research studies and clinical assessments inconclusive. Statements similar to "Numerous factors can influence results without current consensus as to the best parameters." recur throughout the paper, often without supporting citations, and imply that method variance across published studies undermines reliability and leads to (even willful) type 1 errors (i.e., false positives). The authors claim differences in acquisition, analysis, etc., preclude salient conclusions. This approach completely misses the point of a very large literature, which speaks with essentially one voice: low FA is characteristic of TBI patients, *despite significant variability across studies* (e.g., (Niogi and Mukherjee 2010, Aoki, Inokuchi et al. 2012, Shenton, Hamoda et al. 2012, Hulkower, Poliak et al. 2013)). Even if we *assume* that DTI metrics, such as FA, vary across scanners and institutions, we will not encounter bias in the identification of abnormalities in any individual; this issue is simply not germane to a properly conducted analysis. What *does* matter is that patient and control data are acquired, processed and analyzed in the same manner and that temporal variation be substantially less than the magnitude of the effect sought. This latter requirement, of course is out of concern for type 2 errors (i.e., false negatives). It is even more illogical to expect that method variance would yield regionally localized "abnormalities" that in fact represent type 1 errors. Bias due to acquisition and processing variation across subjects, if it were in fact a problem, would lead to a uniform bias at all brain locations. This would be the result, not the manufacture of lesions, if it were true that "technological parameters can be *manipulated* in ways that impact results.". Digging deeper into the authors' case for fatal variability of DTI metrics, we again note a void of supporting evidence. The authors conclude, "...unlike traditional MR sequences... the very existence of a lesion in any given single patient identified via DTI is fundamentally questionable...". The only relevant citation (Vollmar, O'Muircheartaigh et al. 2010), however, is completely misconstrued by the authors; it in fact documents the high degree of intra- and inter-institutional fidelity of FA measurements, also reported by others (Fox, Sakaie et al. 2012). Such misunderstanding of the science suffuses the discussion of technical issues. Glib citations such as "Not too surprisingly, when the same DTI data set was provided for analysis to nine different research groups..., nine different results were obtained." entirely misrepresent the substance of an unpublished abstract to suit the authors' bias. The study authors actually conclude: "This serves as a reminder of what is being tested under the null hypothesis, i.e. just because one method finds a particular difference, it does NOT mean that there were NO other differences -- a fact that can be easily overlooked." The concern is not that any of the findings are not "real", but that additional real findings may be missed in any analysis.

Another methods-specific argument is that abnormalities could occur simply by chance, "Statistical science also portends problems for the analysis of DTI...". After exaggerating the typical number of simultaneous comparisons by at least 50% and invoking a "...typical 5% chance of error...", the authors conclude "...statistical realities represent yet another potential avenue for abuse...". This rudimentary analysis does not acknowledge that 5% is not a typical threshold and that corrections should be and are made for multiple testing (not just that they "fortunately exist"). Most glaring is the authors' omission of spatial clustering, which dramatically reduces type 1 errors. DTI analyses do not seek individual voxel abnormalities, but ask "What is the likelihood that hundreds of voxels comprising a contiguous tissue volume several milliliters in size will all appear abnormal by mere chance alone?" Along these lines, the authors state, "Given that even carefully selected healthy controls will feature areas of "abnormality"..., it should be anticipated that *most unselected patients/litigants will feature areas of abnormality when compared to such normative databases.*". This is a gross misrepresentation

of Kraus et al. (Kraus, Susmaras et al. 2007), in the same way that Wortzel misused it previously (Wortzel, Kraus et al. 2011). The criterion for "abnormality" in Kraus et al. (1SD) is well within all concepts of normal. That some controls had some ROIs outside of 1SD is expected and does not bear on the finding that patients had significantly more ROIs outside of 1SD (Figure 5; Kraus et al.). This citation provides no basis whatsoever for inferring that normals will have "abnormalities", when reasonable thresholds for abnormality are employed. This sentence and especially its italicized emphasis have no basis in the cited paper or any scientific communication.

Fourth, diagnosis of mTBI, or any other disorder, is based on integration of clinical information, not the result of one diagnostic test. The authors offer another "straw man" argument that insinuates DTI should not be used as a standalone definitive diagnostic test, a use for which it has not been proposed. The realities of DTI use in the clinic entail weighing the strength of *all* clinical evidence. The authors argue that "...neuropsychiatric conditions are common in the general population, and are often present in individual litigants. The potential impact of common psychiatric conditions on DTI findings is well illustrated..." Placing these two sentences back to back is blatantly misleading. The authors cite White, et al. (White, Nelson et al. 2008) who reviewed studies of psychiatric patients, not of healthy people who unknowingly harbor as yet undiagnosed psychopathology. No literature exists to support that such individuals can be identified with DTI. Moreover, the authors do not consider that the literature on psychiatric diagnosis is comprised of studies detecting modest *group* differences, whereas TBI studies have specifically shown that *individuals*, though not all individuals, can differ from population norms to a degree that normals do not (see (Hulkower, Poliak et al. 2013)). It is not at all clear that DTI abnormalities in individuals with as yet undiagnosed psychiatric disease can be detected in the way that such abnormalities may be detected in TBI patients. Moreover, statements, such as "...early life stress and/or parental verbal abuse may result in differences in white matter integrity as measured by DTI.", give equal weight to single reports of small samples and fail to assess subject/control overlap and whether any inference at the individual level might even be supported. In stark contrast, the overwhelming consensus of a substantial body of scientific inquiry supports DTI for detecting pathology in mTBI patients.

Acknowledgements

Go to:

The authors wish to acknowledge the critical review and helpful suggestions of Jacqueline A. Bello, M.D., F.A.C.R.

Contributor Information

Go to:

Michael L. Lipton, The Gruss Magnetic Resonance Research Center, Departments of Radiology, Psychiatry and Behavioral Sciences and The Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, Voice: (718) 430-3390, FAX: (718) 430-2185, michael.lipton@einstein.yu.edu.

Erin D. Bigler, Magnetic Resonance Imaging Research Facility, Department of Psychology, Brigham Young University, 1001 Kimball Tower, Provo, Utah 84602, Voice: (801) 422-3407, FAX: (801) 422-0602, erin_bigler@byu.edu.

References

Go to:

1. Aoki Y, Inokuchi R, Gunshin M, Yahagi N, Suwa H. Diffusion tensor imaging studies of mild traumatic brain injury: a meta-analysis. J Neurol Neurosurg Psychiatry. 2012;83(9):870–876. [PMC free article] [PubMed]
2. Bigler ED, Farrer TJ, Pertab JL, James K, Petrie JA, Hedges DW. Reaffirmed limitations of

meta-analytic methods in the study of mild traumatic brain injury: a response to Rohling et al. Clin Neuropsychol. 2013;27(2):176–214. [PubMed]

3. Fox RJ, Sakaie K, Lee JC, Debbins JP, Liu Y, Arnold DL, Melhem ER, Smith CH, Philips MD, Lowe M, Fisher E. A validation study of multicenter diffusion tensor imaging: reliability of fractional anisotropy and diffusivity values. AJNR Am J Neuroradiol. 2012;33(4):695–700. [PubMed]
4. Hulkower MB, Poliak DB, Rosenbaum SB, Zimmerman ME, Lipton ML. A Decade of DTI in Traumatic Brain Injury: 10 Years and 100 Articles Later. AJNR. American journal of neuroradiology. 2013 [PubMed]
5. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain. 2007;130(Pt 10):2508–2519. [PubMed]
6. McMahon P, Hricik A, Yue JK, Puccio AM, Inoue T, Lingsma HF, Beers SR, Gordon WA, Valadka AB, Manley GT, Okonkwo DO, Casey SS, Cooper SR, Dams-O'Connor K, Menon DK, Sorani MD, Yuh EL, Mukherjee P, Schnyer DM, Vassar MJ. Symptomatology and Functional Outcome in Mild Traumatic Brain Injury: Results from the Prospective TRACK-TBI Study. J Neurotrauma. 2013
7. Niogi SN, Mukherjee P. Diffusion tensor imaging of mild traumatic brain injury. J Head Trauma Rehabil. 2010;25(4):241–255. [PubMed]
8. Shenton M, Hamoda H, Schneiderman J, Bouix S, Pasternak O, Rathi Y, Vu MA, Purohit M, Helmer K, Koerte I, Lin A, Westin CF, Kikinis R, Kubicki M, Stern R, Zafonte R. A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. Brain Imaging and Behavior. 2012;6(2):137–192. [PMC free article] [PubMed]
9. Vollmar C, O'Muircheartaigh J, Barker GJ, Symms MR, Thompson P, Kumari V, Duncan JS, Richardson MP, Koepp MJ. Identical, but not the same: intra-site and inter-site reproducibility of fractional anisotropy measures on two 3.0T scanners. Neuroimage. 2010;51(4):1384–1394. [PMC free article] [PubMed]
10. White T, Nelson M, Lim KO. Diffusion tensor imaging in psychiatric disorders. Top Magn Reson Imaging. 2008;19(2):97–109. [PubMed]
11. Wortzel HS, Kraus MF, Filley CM, Anderson CA, Arciniegas DB. Diffusion tensor imaging in mild traumatic brain injury litigation. J Am Acad Psychiatry Law. 2011;39(4):511–523. [PubMed]

Diao v. S. Cal. Gas Co.

Court of Appeal of California, Second Appellate District, Division One

January 8, 2016, Opinion Filed

B258840

Reporter
2016 Cal. App. Unpub. LEXIS 109 *

PENGXUAN DIAO, Plaintiff and Respondent, v. SOUTHERN CALIFORNIA GAS COMPANY, Defendant and Appellant.

Prior History: [*1] APPEAL from a judgment of the Superior Court of Los Angeles County, No. BC481312, Elizabeth R. Feffer and Richard E. Rico, Judges.

Disposition: Affirmed.

Core Terms

injuries, trial court, homeowners, damages, deposition, pain, brain, burns, gas leak, scars, car accident, examinations, garage, psychiatrist, skin, natural gas, neurological, discovery, scan, neurologist, radiologist, designated, cognitive, memory, traumatic brain injury, awarding damages, comments, contends, interrogatory response, substantial evidence

Counsel: Willenken Wilson Loh & Delgado, Paul J. Loh, Jason H. Wilson; Gibson, Dunn & Crutcher, Julian W. Poon, and Lauren M. Blas for Defendant and Appellant.

Law Offices of Martin N. Buchanan, Martin N. Buchanan; Panish Shea & Boyle, Kevin R. Boyle, Rahul Ravipudi, and Robert S. Glassman for Plaintiff and Respondent.

Judges: CHANEY, J.; ROTHSCHILD, P. J., JOHNSON, J. concurred.

Opinion by: CHANEY, J.

Opinion

Defendant Southern California Gas Company (SoCalGas) appeals from a judgment entered after a jury returned a special verdict awarding plaintiff Pengxuan Diao $19,786,818 in compensatory damages for injuries he sustained as a result of a natural gas leak and fire at his residence. Prior to trial, SoCalGas stipulated its employee negligently caused the leak and fire. On appeal, SoCalGas contends (1) the trial court abused its discretion in declining to exclude evidence of Diao's traumatic brain injury—an injury SoCalGas argues Diao concealed until shortly before trial; (2) there is insufficient evidence demonstrating the gas leak or fire caused a traumatic brain injury; and (3) the [*2] "damages award is punitive, excessive, and the result of passion and prejudice, as well as improper argument and misconduct by [Diao]'s counsel." For the reasons explained below, we reject SoCalGas's contentions and affirm the

judgment.

BACKGROUND

The Natural Gas Leak and Fire

In January 2011, Diao, then 23 years old, rented and lived in a detached, converted garage in San Gabriel he shared with a roommate under a sub-tenancy arrangement. On January 19, 2011, Simon Youde, then a SoCalGas employee, was dispatched to the property where Diao lived to restore gas service to the main house after an earthquake shutoff valve automatically shut off gas service to the property. As Youde was attempting to relight the pilot light on the water heater in the main house, he reached around the back of the water heater and twisted a valve he felt but could not see, inadvertently opening up a gas line which led to the garage. The gas line, which was installed before Diao moved into the garage and was no longer in use, was uncapped at the end where it terminated behind some cabinets in the garage. After Youde restored gas service, he left the property without performing the required leak check. He did **[*3]** not realize gas was flowing freely into the garage where Diao was then sleeping.

Diao slept for another two hours after Youde opened the uncapped gas line to the garage. Diao's bedroom in the garage filled with about 300 cubic feet of natural gas (one-quarter to one-third the volume of his bedroom, which measured 11 feet, 7 inches by 11 feet, 9 inches by 8 feet). Diao awoke at approximately 11:00 a.m., sat up on the edge of his bed, and attempted to light a cigarette. When Diao sparked the lighter, he heard a boom, saw fire engulf his room, and felt a force pushing against him as he ran out of the garage. His entire body felt hot so he ran into the main house and took a cold shower. Afterward, he noticed the skin on his left arm was falling off. An ambulance transported Diao to the hospital.

Burns and Treatment

Diao sustained second and third degree burns on 18-20 percent of his body. He remained in the burn unit at Los Angeles County-USC Medical Center for weeks as he underwent painful treatments, including (1) dressing changes once or twice per day during which the remaining skin in the area of the burns was scraped away to prevent infection, (2) surgical debridement during which the **[*4]** dead tissue on Diao's left forearm was removed with a long knife, and (3) skin grafting during which healthy skin from Diao's thigh was removed and placed on his left forearm.

When Diao was released from the hospital, he still had open wounds all over his body. He went to live with his father. A nurse came to the home daily to clean Diao's wounds, which was a very painful process. The skin in the area of the burns would stick together, so every day Diao had to pull it apart and stretch it. Skin fell off Diao's face. He would wake with his pillow covered in blood due to his face bleeding. Diao received follow-up treatment at the burn center. He was in extreme pain every day.

This Lawsuit

In March 2012, Diao filed this negligence action against SoCalGas. He alleged SoCalGas's negligence caused "catastrophic and permanent injuries" to him, including third degree burns, "shock and injury to his nervous system and person," and "physical and mental pain and suffering." SoCalGas answered the complaint with a general denial and assertion of affirmative defenses.

In September 2012, SoCalGas stipulated to negligence and that its negligence was a substantial factor in causing Diao's harm, but denied **[*5]** its negligence caused the extent of harm Diao claimed. Thereafter, SoCalGas cross-complained against the homeowners and sub-landlord, alleging they were negligent in making or allowing illegal modifications to the garage where Diao lived and in failing to cap the gas line that ran into the garage. The homeowners, in turn, cross-complained against SoCalGas and the sub-landlord for equitable apportionment and indemnification.

Discovery Issues and Disputes Pertinent to This Appeal

In July 2012, in response to SoCalGas's form interrogatories, Diao listed the following injuries he attributed to the natural gas leak and fire: "Plaintiff [Diao] suffered multiple serious injuries in the subject incident, including second and third degree burns to his face, neck, both upper extremities including hands and digits, right lower back and right lower extremity. Plaintiff underwent surgery for debridement and skin grafting. Plaintiff now has severe hyperpigmentation and areas of severe burn scarring. Plaintiff further suffers from chronic pain; numbness; itching, tightness, weakness; limited range of motion; frequent bouts of diarrhea; sexual dysfunction; difficulty falling to sleep; and emotional [*6] distress and depression." Diao further stated in his response that discovery and investigation regarding his injuries were not complete.

At his deposition in June 2013, Diao testified, in or about July 2011, his friends began commenting that his memory and reflexes were worse than they were before the gas leak and fire. For example, after he returned to work at the hot pot restaurant where he had worked before the fire, his former girlfriend stated it took him longer to understand instructions such as the type of vegetables to purchase for the restaurant. Diao testified, "before the accident, I would just go out and make the purchase, but after the accident, I would have to think about it and then repeat it, what she told me to confirm, what I was understanding correctly, and then went out to make the purchase."[1] Diao explained his memory, comprehension and reaction time were slower than before the fire. Diao also testified, when he returned to community college after the fire, he found he had problems with his short-term memory. He would learn new vocabulary words in his English as a second language (ESL) class and then forget them shortly thereafter.[2] In the fall of 2012, he failed [*7] his ESL class.

On July 30, 2013, the homeowners' counsel sent a letter to Diao's counsel requesting Diao submit to a neurological examination (in addition to a psychiatric examination) based on Diao's deposition testimony which, in the words of the homeowners' counsel, "raised for the first time a potential brain injury claim." Diao's counsel initially refused to submit to any mental examinations, absent the homeowners' coordination with SoCalGas, because Diao did not want to submit to multiple defense examinations. SoCalGas refused to participate in the examinations requested by the homeowners. The homeowners filed motions to compel the examinations. Before the motions were heard, [*8] Diao agreed he would submit to an examination by the homeowners' neurologist, Dr. Michael Gold, and the homeowners' psychiatrist, Dr. David Rudnick.

Diao retained his own neurologist, Dr. Fisk. On November 14, 2013, Dr. Fisk conducted a neurological examination of Diao and prepared a seven-page report. After interviewing Diao through a Mandarin interpreter and examining him, Dr. Fisk stated the following "Assessment": "Following the injury, the patient has manifested persistent impairment of cognition of uncertain etiology at this point. Given the persistence of these symptoms and their impact in terms of his academic performance, he should be evaluated neuropsychologically, in Mandarin. A 3.0 Tesla brain MRI scan is recommended. The need for a neuropsychiatric evaluation remains to be determined. [¶] The patient's right ankle pain should be evaluated orthopedically. [¶] The patient's headaches are largely the consequence of stress with which the patient is contending. He will

[1] Diao quit his job at the hot pot restaurant because he was making so many mistakes and was fearful the cooking would lead to a gas explosion or fire. He quit a subsequent job at a tea house because he could not remember the recipes, was in pain when he stood on his right ankle for long periods (due to the manner in which the burns had healed), and his skin was too sensitive when he came in contact with water.

[2] Diao immigrated to the United States from China in 2009, and was studying ESL at the time of the fire.

be started on a seven-step cervical range of motion stretching sequence. These exercises were explained and demonstrated in detail. They were written down for the patient so he would not forget them. [¶] Appropriate [*9] therapeutic recommendations will be made following completion of the patient's neurodiagnostic evaluation." As one of six "Impressions," Dr. Fisk listed: "Posttraumatic headaches in the context of a possible traumatic brain injury."

Diao's attorneys have maintained they did not receive Dr. Fisk's report until Dr. Fisk was deposed on May 1, 2014. Thus, they did not provide this report to the defense at the time expert witness disclosures were made in February 2014, as explained in more detail below.

On December 10, 2013, Diao submitted to the brain MRI Dr. Fisk ordered and the result was normal.

On December 11, 2013, Dr. Gold, the homeowners' neurologist, conducted a neurological examination of Diao and prepared a report. Dr. Gold reviewed documents, including the transcript of Diao's deposition, interviewed Diao through a Mandarin interpreter about his complaints, including "[m]emory impairment," and examined Diao. Dr. Gold concluded Diao's neurological examination was normal, "except for mildly decreased sensation over the dorsum of the left arm and hand." SoCalGas did not participate in this examination.

On December 13, 2013, Dr. Rudnick, the homeowners' psychiatrist, conducted a neuropsychiatric [*10] examination of Diao and prepared a report. Dr. Rudnick reviewed Diao's medical records, interviewed Diao through a Mandarin interpreter about his complaints, including memory impairment, and conducted a mental status examination of Diao. Dr. Rudnick concluded Diao had "chronic Posttraumatic Stress Disorder" and "Major Depression" and "should have full recovery from his psychiatric condition" with treatment, including psychotherapy, medication, and cosmetic treatments for his scarring. SoCalGas chose not to participate in this examination.

On January 3, 2014, Diao voluntarily supplemented his responses to SoCalGas's form interrogatories. Regarding injuries he attributed to the gas leak and fire, Diao added: "[A]s Plaintiff testified to in his deposition, as a result of the subject incident, Plaintiff suffers from: trouble with attention skills, concentration and with memory, nightmares, flashbacks, poor appetite, phobic reactions, and hypervigilance." Diao further stated in his supplemental response that discovery and investigation regarding his injuries were not complete.

On February 4, 2014, Diao's retained neurologist, Dr. Fisk, ordered a functional brain MRI (fMRI) and brain SPECT scan [*11] for Diao, indicating on the forms "DX:TBI" (a diagnosis of traumatic brain injury).

On February 5, 2014, SoCalGas's counsel sent a letter to Diao's counsel requesting Diao submit to another mental examination. The letter states, in pertinent part, "your client has made mental injury an issue by alleging in the complaint, discovery responses and deposition that he suffers from sexual dysfunction, difficulty falling to sleep, emotional distress and depression, trouble with attention skills, concentration and with memory, nightmares, flashbacks, poor appetite, phobic reactions, hypervigilence, impaired academic abilities, a phobia of cooking anything that requires a flame, the inability to be exposed to common household products due to irritation caused by the chemicals, and mental pain and suffering." Apparently, Diao's counsel declined this request for another defense mental examination.

Also in February 2014, the parties served their expert witness designations. The homeowners designated, among other experts, Dr. Gold (neurologist) and Dr. Rudnick (psychiatrist), both of whom had conducted mental examinations of Diao in December 2013, as discussed above. SoCalGas designated, among other [*12] experts, Dr. Barry Ludwig (neurologist) and Dr. Mark

Kalish (psychiatrist), neither of whom had examined Diao. Diao designated, among other experts, Dr. Fisk (neurologist), Dr. Johnny Wen (neuropsychologist), Dr. Wei-Chin Hwang (psychologist), and Dr. Monte Buchsbaum (psychiatrist/radiologist). As stated above, Diao did not provide the defense with Dr. Fisk's November 13, 2013 report. His attorneys claim they were unaware of it until Dr. Fisk's deposition on May 1, 2014.

Sometime in February 2014, Dr. Fisk received a report indicating Diao's brain SPECT scan was abnormal. Fisk also learned the results of neuropsychological testing by Dr. Wen were abnormal. Fisk diagnosed Diao with traumatic brain injury. In March 2014, Dr. Buchsbaum (Diao's retained psychiatrist/radiologist) examined the SPECT scan and report, the neuropsychological report, Diao's medical records, and Diao's deposition to opine about the status of Diao's brain. Buchsbaum diagnosed Diao with traumatic brain injury.

On March 4, 2014, SoCalGas filed an ex parte application to continue the April 8, 2014 trial date due to the unavailability of the sub-landlord. The court continued the trial to May 20, 2014. The non-expert **[*13]** discovery cutoff date of March 8, 2014 remained unchanged.

Also on March 4, 2014, SoCalGas filed an ex parte motion for leave to conduct psychiatric and neurological examinations of Diao. On April 2, 2014, after considering Diao's opposition, the trial court (Judge Richard E. Rico) denied the motion, finding SoCalGas (1) had not demonstrated why the proposed examinations were necessary given the neurological and psychiatric examinations already conducted in December 2013 by the homeowners' experts, and (2) had not shown the proposed examinations would not be duplicative of the prior examinations. In its minute order, the court explained: "In light of the examinations already conducted, the examinations requested by [SoCalGas] constitute unnecessary intrusions and invasions of plaintiff's privacy and are burdensome, harassing, and oppressive."

The parties conducted expert witness depositions in April and May 2014. On April 23, 2014, Dr. Buchsbaum (Diao's retained psychiatrist/radiologist) testified about his opinion that Diao suffered from a traumatic brain injury (TBI). According to SoCalGas, this was the first time Diao's "TBI claim was revealed," "just 27 days before trial and over **[*14]** one month after the close of fact discovery."[3]

On May 1, 2014, Dr. Fisk (Diao's retained neurologist) also testified at his deposition that Diao suffered from TBI. This is also the date Diao produced Dr. Fisk's November 14, 2013 examination report to defendants, two-and-one-half months after the February 18, 2014 expert witness disclosure and production deadline. As set forth above, Dr. Fisk's "Assessment" in the November 14, 2013 report, did not include a diagnosis of TBI, but he listed "Posttraumatic headaches in the context of a possible traumatic brain injury" as one of six "Impressions." SoCalGas's counsel examined Dr. Fisk about the report.

On May 1, 2014, Dr. Ludwig (SoCalGas's neurologist) was deposed and presented his opinion Diao did not suffer from TBI and provided the basis for his opinion. At the time of his deposition—which occurred the same day Dr. Fisk produced his November 14, 2013 report—Dr. Ludwig already was aware Dr. Wen (Diao's retained neuropsychologist) and Dr. Buchsbaum (Diao's retained radiologist/psychiatrist) **[*15]** had diagnosed Diao with TBI. On May 8, 2014, Dr. Kalish (SoCalGas's psychiatrist) was deposed and presented his opinion Diao did not suffer from a TBI and provided the basis for his opinion.

On May 13, 2014, SoCalGas filed an ex parte application, seeking a five-week trial continuance

[3] As stated above, nearly nine months earlier, the homeowners' counsel recognized "a potential brain injury claim" based on Diao's deposition testimony.

and permission to augment its expert witness list to include a radiologist and a neuropsychologist. The homeowners joined the ex parte application. SoCalGas argued there was good cause for the relief requested because Diao provided "willfully false discovery responses." In his January 2014 voluntary supplemental responses to SoCalGas's form interrogatories, Diao did not disclose his TBI claim or his November 14, 2013 neurological examination with Dr. Fisk. According to SoCalGas, Diao was required to disclose the examination because Dr. Fisk was acting as a treating physician at the time of the examination and not an expert witness.[4] On May 14, 2014, the trial court (Judge Rico) denied the ex parte application.

On May 19, 2014, SoCalGas filed a motion in limine to exclude evidence of and argument about TBI and anoxia (lack of oxygen from the gas leak and/or fire) based on arguments (1) Diao provided willfully false discovery responses, and (2) there was a lack of evidence of TBI due to trauma or anoxia. The homeowners filed a similar motion in limine, which SoCalGas joined. Plaintiff filed an opposition to these motions in limine.

The trial court (Judge Elizabeth R. Feffer who was assigned to the case for trial) heard oral argument on the motions in limine on May 23, 2014. The court faulted Diao's counsel for not producing Dr. Fisk's November 14, 2013 report in a timely manner, commenting that "there were games being played" and "it's all problematic." The court noted, however, that "ultimately, it [the report] was disclosed and the defense did have an opportunity to explore it." The court declined to exclude evidence of and argument about TBI and anoxia. The court did allow **[*17]** SoCalGas to augment its expert witness designation to include a radiologist (Dr. Alan Waxman), concluding it was unclear from Diao's designation that Dr. Buchsbaum would be offering opinions as a radiologist in addition to his opinions as a psychiatrist. The court rejected SoCalGas's request to designate a neuropsychologist, finding Diao had disclosed Dr. Wen as his neuropsychologist and SoCalGas could have designated its own neuropsychologist at that time.

Trial

Trial commenced on May 29, 2014. Diao testified extensively about his injuries (past and present). As discussed above, Diao underwent excruciatingly painful treatments for his burns in the months following the fire. He was extremely upset about the way he looked. He felt his life had "become black, all black."

By the time of trial, more than three years after the fire, Diao still experienced physical pain due to his injuries. Pain in his right ankle prevented him from standing for long periods of time. Because he suffered from neuropathic pain syndrome on his left arm and hand, a light touch caused intense pain. He had weakness in his left hand. He also experienced numbness, tightness, pain, dryness, and itching in the areas **[*18]** around his burn injuries.

Diao continued to feel extremely upset and self-conscious about the scarring on his body. (Fortunately the burns on his face had healed quite nicely by all accounts.) He had areas of hypertrophic scars which were thick and cordlike and caused him physical discomfort. He also had areas of hyperpigmentation where his skin was much darker and areas of hypopigmentation where his skin was much lighter because there was no pigment. Diao tended to cover his scars with clothing, including long sleeves, because he believed people would perceive him as gross and deformed if they saw his scars. Diao rarely socialized with friends and did not date. He avoided exposure to the sun due to his scarring.

[4] Dr. Fisk testified at his deposition that he "started out" as Diao's treating physician. In or about November 2013, after the date of the neurological examination, Diao signed an agreement giving Dr. **[*16]** Fisk a lien on Diao's recovery in the litigation, which indicates Dr. Fisk was a treating physician. At trial, however, Dr. Fisk testified that he was retained by Diao's counsel in 2013, before the neurological examination.

Diao presented evidence regarding future surgeries and treatments to improve the look of his scars and to alleviate some of the discomfort associated with them, including steroid injections and a balloon expansion to stretch normal skin over the scar. There were no treatments, however, that could eliminate the scars entirely.

Diao testified about his cognitive impairments after the fire, including his problems with memory and comprehension at school and on-the-job. He remained **[*19]** unemployed and out of school.

Diao presented evidence that neuropsychological testing, the SPECT scan and the fMRI of his brain all demonstrated TBI. He also presented evidence that the TBI most likely was caused by the force of the blast when the natural gas ignited, but could also have been caused by trauma to the head during the explosion, lack of oxygen to his brain (anoxia) while he was sleeping in a room filled with natural gas, carbon monoxide released during the explosion, or a combination of these causes.

At the time of trial, Diao was 26 years old. He presented evidence indicating he would likely develop early-onset dementia by age 55 as a result of his brain injury, requiring assisted living and later 24-hour care.

Diao's father testified on cross-examination that, before the fire, Diao was involved in a car accident which required him to replace his car. Later, when Diao's direct examination resumed, Diao testified he was involved in three car accidents shortly after he came to the United States in 2009. SoCalGas extensively cross-examined Diao regarding the car accidents and impeached him with documents it gathered mid-trial showing an attorney submitted an insurance claim **[*20]** on behalf of Diao and his father after one of the accidents and a chiropractor indicated in a report that Diao had sustained a concussion in the accident. Diao did not disclose an insurance claim or a prior head injury in his interrogatory responses. Diao testified he was unaware of the insurance claim his father's attorney submitted and he disputed the characterization of his alleged injuries as stated in the claim and the chiropractor's report.

None of SoCalGas's experts attributed the car accidents to TBI because they testified Diao did not suffer from TBI either before or after the gas leak and fire. The defense experts disputed the results of the neuropsychological testing, the SPECT scan and the fMRI of Diao's brain indicated TBI.

SoCalGas also cross-examined Diao about his educational history in China. SoCalGas argued to the jury that Diao was not truthful about his academic performance in China and that his pre-fire cognitive abilities were not as superior as he had indicated.

Mid-trial, SoCalGas moved for nonsuit, mistrial and terminating sanctions, arguing Diao had suppressed evidence of TBI and his prior car accidents and had failed to prove TBI causation. The trial court (Judge **[*21]** Feffer) denied the motions, explaining, among other things, (1) it already had remedied any belated TBI disclosure by allowing SoCalGas to designate a radiologist, (2) any failure to disclose the car accidents did not warrant wholesale exclusion of TBI evidence, but was a credibility issue for the jury to weigh, and (3) there was sufficient evidence of TBI causation to go to the jury, including testimony by SoCalGas's experts that asphyxiation is a risk of breathing natural gas.

On June 25, 2014, the jury returned a special verdict awarding Diao $186,718 in past economic damages, $2,600,100 in future economic damages, $8,500,000 in past non-economic damages, and $8,500,000 in future non-economic damages, for a total damages award of $19,786,818. The jury found the homeowners were negligent, their negligence was a substantial factor in causing harm to Diao, and SoCalGas's negligence was not a superseding cause of the harm to Diao. The jury further found the sub-landlord was not negligent. The jury assigned 90 percent of Diao's harm to SoCalGas and 10 percent to the homeowners. On July 2, 2014, the trial court entered judgment

consistent with the special verdict.

Post-Trial Motions

SoCalGas [*22] moved for a partial judgment notwithstanding the verdict and for a new trial on several of the grounds raised on appeal. SoCalGas argued Diao committed discovery abuses related to his TBI claim and presented insufficient evidence of TBI. SoCalGas also argued Diao's counsel made improper arguments which resulted in an excessive and punitive damages award. SoCalGas requested the trial court enter a remittitur and reduce the damages award to no more than $6,000,000. The homeowners also made a motion for new trial, arguing for a reduction in the damages award. At the hearing on the post-trial motions, SoCalGas requested the trial court reduce the damages award to a total of $3,486,718.

In connection with the post-trial motions, SoCalGas submitted new evidence related to the car accidents and Diao's educational history. Diao objected to all of the new evidence on multiple grounds (hearsay, lack of foundation) and the trial court sustained all of Diao's objections.

The trial court (Judge Feffer) issued a 28-page ruling addressing defendants' arguments and denying the post-trial motions. In discussing SoCalGas's claim of discovery abuses related to TBI, the court explained the voluminous exhibits [*23] submitted by the parties indicated SoCalGas did not exercise reasonable diligence in investigating the cause of Diao's alleged cognitive defects which were revealed during Diao's June 2013 deposition.

DISCUSSION

I. Discovery Issues Regarding TBI

A. Dr. Fisk

SoCalGas contends the trial court erred in declining to exclude Dr. Fisk's TBI testimony under *Code of Civil Procedure section 2034.300* based on Diao's failure to disclose Dr. Fisk's November 14, 2013 report in a timely manner.

Code of Civil Procedure section 2034.300 states, in pertinent part, "on objection of any party who has made a complete and timely compliance with *Section 2034.260*, the trial court shall exclude from evidence the expert opinion of any witness that is offered by any party who has unreasonably failed to do any of the following: [¶] (a) List that witness as an expert under *Section 2034.260*. [¶] (b) Submit an expert witness declaration. [¶] (c) *Produce reports and writings of expert witnesses under Section 2034.270*. [¶] (d) Make that expert available for a deposition under Article 3 (commencing with *Section 2034.410*)." (Italics added.)

Prior to or during trial, SoCalGas never asked the trial court to exclude Dr. Fisk's testimony under this provision. Instead, SoCalGas asked the court to exercise its discretion and issue an evidence sanction—excluding *all* evidence [*24] of TBI—for misuse of the discovery process under section 2023.030, subdivision (c). SoCalGas should not fault the trial court for declining to grant relief SoCalGas never requested.

Had SoCalGas asked the trial court to exclude Dr. Fisk's testimony under this particular provision and the court declined, we would not disturb the court's ruling on appeal. Assuming SoCalGas could demonstrate Diao "unreasonably failed" to produce the report—although Diao's attorneys maintain they were unaware of the report until the date they produced it—SoCalGas cannot demonstrate prejudice. There was substantial evidence of TBI, separate and apart from Dr. Fisk's testimony, including the results of the neuropsychological testing, the SPECT scan and the fMRI of Diao's brain, and substantial evidence the gas leak and fire caused the TBI, as set forth in more detail below in response to SoCalGas's challenge to the sufficiency of the evidence.

B. Interrogatory responses

SoCalGas contends the trial court erred in declining

to grant any relief for Diao's "false" interrogatory responses, and further contends it should have been "able to rely on [Diao's] discovery responses to set at rest significant issues, and remove those issues from the case." **[*25]**

First, SoCalGas points to the fact Diao did not disclose TBI in his interrogatory responses. The evidence shows Diao had not been diagnosed with TBI at the time he responded to SoCalGas's form interrogatories in June 2012 and at the time he voluntarily supplemented his responses to the form interrogatories in January 2014. The results of Diao's initial MRI in December 2013 were normal. Notwithstanding that, Diao disclosed his cognitive impairments at his June 2013 deposition—which alerted the homeowners' counsel to a potential brain injury claim—and in his January 2014 supplemental responses to the form interrogatories. Moreover, Diao stated in his interrogatory response that discovery and investigation regarding his injuries were not complete, which is borne out by the record of subsequent medical testing Diao underwent. Accordingly, Diao's interrogatory responses regarding his injuries were not false, and his TBI claim is not barred because he did not specifically identify "traumatic brain injury" in interrogatory responses served before TBI was diagnosed.

Second, SoCalGas points to the fact Diao did not disclose the three 2009 car accidents in response to form interrogatories asking **[*26]** him to identify injuries to the same parts of his body injured in the gas leak and fire and asking him to identify prior claims or demands for compensation for personal injuries. Diao testified at trial that he was unaware his father's attorney had made an insurance claim on his behalf regarding one of the car accidents, and he disputed he sustained a head injury in any of the car accidents. SoCalGas impeached Diao's credibility at trial with documents relating to one of the car accidents. An evidence sanction was not warranted.

Third, SoCalGas argues Diao misrepresented his academic history in his discovery responses. SoCalGas cites the new evidence it submitted in connection with its post-trial motions. The trial court sustained all of Diao's numerous evidentiary objections to this new evidence. SoCalGas does not properly challenge the trial court's evidentiary rulings on appeal. It merely states in a couple of footnotes that the new evidence was not offered for the truth of the matter asserted. Hearsay is not the only ground on which the trial court sustained Diao's objections to the new evidence. Because SoCalGas has not demonstrated the court abused its discretion in sustaining **[*27]** objections on multiple grounds, we may not consider this evidence on appeal.

Finally, SoCalGas faults Diao for not identifying Dr. Fisk as a treating physician when he voluntarily supplemented his interrogatory responses in January 2014. As addressed above, the evidence regarding whether Dr. Fisk was acting as a treating physician or a retained expert at the time he conducted Diao's neurological examination in November 2013 was conflicting. Given that, we need not address this issue further.

II. Sufficiency Of Evidence Of TBI Causation

SoCalGas contends there is insufficient evidence demonstrating the gas leak or fire caused Diao's TBI.

"In a personal injury action, causation must be proven within a reasonable medical probability based on expert testimony; a mere possibility is insufficient. [Citation.] A possible cause becomes 'probable' when, in the absence of other reasonable causal explanations, it is more likely than not that the injury resulted from its action." (*Sparks v. Owens-Illinois, Inc. (1995) 32 Cal.App.4th 461, 476.*) "[A]n expert's opinion based on assumptions of fact without evidentiary support [citation], or on speculative or conjectural factors [citation], has no evidentiary value . . . ." (*Jennings v. Palomar Pomerado Health Systems, Inc. (2003) 114 Cal.App.4th 1108, 1117.*)

Substantial evidence presented at trial **[*28]**

demonstrated, after SoCalGas's employee uncapped the gas line to the garage, Diao slept for another two hours as one-quarter to one-third the volume of his room filled with natural gas. When Diao awoke and sparked the lighter, he heard a boom, saw fire engulf his room, and felt a force pushing against him as he ran out of the garage. At trial, Diao testified the force of the explosion knocked him to the ground, although he denied falling when he testified earlier at his deposition.

According to Diao's experts, results of neuropsychological testing, a SPECT scan and an fMRI of Diao's brain indicated TBI.

Regarding causation, substantial evidence demonstrated Diao's friends and family noticed a decline in his cognitive functioning after the gas leak and fire. His memory, comprehension and reaction time were slower after the fire than they were before the fire.

Dr. Buchsbaum (Diao's retained radiologist/psychiatrist) testified about TBI causation. He gave his opinion the TBI did not predate the gas leak and fire based on the severity of the damage shown on the brain scans, and the substantial evidence of Diao's cognitive decline after the gas leak and fire and absence of these cognitive **[*29]** impairments before.

Like Dr. Fisk, Dr. Buchsbaum testified that four factors could have contributed to Diao's TBI: the blast that occurred when the natural gas ignited, trauma to Diao's head in connection with the blast (striking his head on an object or the ground), anoxia (a lack of oxygen), and inhalation of carbon monoxide. Based on his review of the brain scans, and the "asymmetry" of the brain injury, Dr. Buchsbaum opined the blast or head trauma during the blast caused Diao's TBI. Dr. Buchsbaum had recently worked on and completed a study supported by the veterans administration regarding blast injuries in veterans from Iraq. He explained how and why a blast, "just in and of itself, can shake and distort the brain" causing TBI without head trauma or a skull fracture.

Based on the foregoing, substantial evidence presented at trial demonstrated it is more likely than not that the gas leak and fire caused Diao's TBI and that there is an absence of other reasonable causal explanations.

III. Damages Award

A. Diao's counsel's conduct

SoCalGas contends Diao's counsel committed misconduct in arguments to the jury. According to SoCalGas, Diao's "counsel delivered an inflammatory closing rebuttal **[*30]** in which he vilified SoCalGas, invited the jury to punish the company for defending itself at trial and for unproven injuries to *other* hypothetical victims, and referred extensively to SoCalGas's wealth and resources, despite the court having largely granted SoCalGas's Motion in Limine No. 8, precluding such references."

SoCalGas takes issue with the following types of arguments Diao's counsel made: Diao's counsel referred to SoCalGas as a "bully" in condemning SoCalGas's litigation tactics (e.g., cross-examining Diao for more than 50 pages of transcript regarding the prior car accidents); indicated SoCalGas would celebrate and move on to the next case, the next burn victim if the jury awarded Diao the insufficient amount SoCalGas suggested ($1.6 million in total damages); commented that it is difficult to fight the gas company given its "unlimited resources;" and highlighted the cumulative amount SoCalGas spent on expert witness fees.

Below, SoCalGas did not object to any of counsel's arguments it now takes issue with on appeal. SoCalGas did not afford the trial court an opportunity to evaluate any of these claims of misconduct and provide an admonition if necessary. Accordingly, these **[*31]** claims of misconduct are forfeited on appeal. (*Cassim v. Allstate Ins. Co. (2004) 33 Cal.4th 780, 794-795.*)

SoCalGas asserts an objection was not necessary to

preserve these claims for appeal because counsel's misconduct was repetitive and multiple objections "would only have exacerbated the prejudice." SoCalGas's counsel did not make any effort to stop the alleged misconduct with even one objection. Indeed, "'[o]ne of the primary purposes of admonition at the beginning of an improper course of argument is to avoid repetition of the remarks and thus obviate the necessity of a new trial.'" (*Cassim v. Allstate Inc. Co., supra, 33 Cal.4th at p. 795*.) The types of arguments SoCalGas belatedly complains about are precisely the types of arguments that could have been ameliorated with an admonition, to the extent SoCalGas believed at the time of trial that the arguments were improper (e.g., comments about its resources, and comments about other potential victims).

We do not condone, and in fact strongly disapprove, Diao's counsel's conduct during argument in making inflammatory and inappropriate comments to the jury. But even if SoCalGas had preserved these claims for appeal, we would not reverse the judgment based on the portions of counsel's argument with which SoCalGas now takes issue. None of the comments **[*32]** SoCalGas plucks from counsel's argument, alone or in combination, constitute reversible misconduct. SoCalGas cannot demonstrate it is reasonably probable the judgment would have been more favorable to SoCalGas if Diao's counsel had not made these comments. (*Cassim v. Allstate Ins. Co., supra, 33 Cal.4th at p. 802*.) The award is not excessive in light of the evidence presented, as discussed in more detail below.

B. Propriety of amount of award

SoCalGas contends the $19,786,818 damages award is excessive, disproportionate to other awards for comparable injuries, punitive and violates due process.

The standard of review in cases in which a defendant "attacks the jury verdict on the ground of excessive damages . . . is well established." (*Westphal v. Wal-Mart Stores, Inc. (1998) 68 Cal.App.4th 1071, 1078*.) "The amount of damages is a fact question, committed first to the discretion of the jury and next to the discretion of the trial judge on a motion for new trial. [Citations.] All presumptions favor the trial court's ruling, which is entitled to great deference because the trial judge, having been present at trial, necessarily is more familiar with the evidence and is bound by the more demanding test of weighing conflicting evidence rather than our standard of review under the substantial evidence rule. [Citations.] **[*33]** [¶] We must uphold an award of damages whenever possible [citation] and 'can interfere on the ground that the judgment is excessive only on the ground that the verdict is so large that, at first blush, it shocks the conscience and suggests passion, prejudice or corruption on the part of the jury.' [Citations.] [¶] In assessing a claim that the jury's award of damages is excessive, we do not reassess the credibility of witnesses or reweigh the evidence. To the contrary, we consider the evidence in the light most favorable to the judgment, accepting every reasonable inference and resolving all conflicts in its favor." (*Ibid.*)

As outlined above, the jury awarded Diao $186,718 in past economic damages, $2,600,100 in future economic damages, $8,500,000 in past non-economic damages, and $8,500,000 in future non-economic damages, for a total damages award of $19,786,818. SoCalGas addresses its arguments to the total damages award as a whole and does not analyze each separate category of damages awarded and explain why it believes that particular amount is too high.

SoCalGas views the award in light of a universe of injuries that does not include TBI, based on its arguments the trial court should **[*34]** have excluded evidence of TBI and Diao presented insufficient evidence of TBI causation at trial. We have rejected these arguments and accordingly include TBI in our calculus of the propriety of the award. SoCalGas maintains, even if TBI is included, the award is still too high. We disagree.

Substantial evidence presented at trial demonstrates: Diao suffered through the trauma of a natural gas explosion and fire in his bedroom. He sustained burns on 18-20 percent of his body. He underwent excruciatingly painful treatments and suffered through a lengthy and painful healing process. He has extensive scarring on his body that continues to cause him great physical and mental discomfort. He will undergo additional surgeries and treatments to alleviate some of his discomfort and to improve the look of the scars, but the scars will always be visible.

As a result of the gas leak and fire, Diao suffered TBI. His cognitive functioning declined in a manner noticeable to his friends and family. He could not succeed in college or in his employment due to troubles with memory and comprehension. He will likely develop early-onset dementia by age 55 and be unable to take care of himself.

Diao suffers **[*35]** from depression and his outlook on life has changed. He rarely socializes and does not date.

In light of these severe and debilitating injuries, we do not find the award is excessive. We are not persuaded by SoCalGas's comparison of this award to awards in other cases. "Each case must be determined on its own facts." (*DiRosario v. Havens (1987) 196 Cal.App.3d 1224, 1241.*) The award in this case is supported by the evidence.

"The mere fact that the judgment is large does not validate an appellant's claim that the verdict is the result of passion or prejudice of the jury." (*DiRosario v. Havens, supra, 196 Cal.App.3d at p. 1241.*) The jury deliberated for one and one-half days during which the jury requested and received both sides' written breakdowns of suggested damages for each category of injuries. The jury did not award Diao the entire amount he requested—$36,500,000. It awarded about half. Based on the record before us, we do not find the award is punitive or based on passion or prejudice.

DISPOSITION

The judgment is affirmed. Respondent is entitled to recover costs on appeal.

CHANEY, J.

We concur:

ROTHSCHILD, P. J.

JOHNSON, J.

End of Document

LaMasa v. Bachman

Supreme Court of New York, Appellate Division, First Department

November 20, 2008, Decided; November 20, 2008, Entered

4608, 129996/93

Reporter

56 A.D.3d 340 *; 869 N.Y.S.2d 17 **; 2008 N.Y. App. Div. LEXIS 8686 ***; 2008 NY Slip Op 9162 ****

[****1] Salvatore LaMasa et al., Respondents, v John K. Bachman, Appellant.

Prior History: *Lamasa v Bachman, 2007 NY App Div LEXIS 9134 (N.Y. App. Div. 1st Dep't, July 26, 2007)*

Core Terms

plaintiffs', discrepancies, stationary, collision, distance, injuries, roadway, willful, tests, safe, wet

Headnotes/Syllabus

Headnotes

Motor Vehicles--Collision.--Court correctly directed verdict in plaintiffs' favor; defendant saw plaintiff's car stopped at red light, braked hard and shifted to low gear, but his truck skidded on wet roadway and hit rear of plaintiff's car; rear-end collision with stationary vehicle created prima facie case of negligence, and wet roadway did not suffice as nonnegligent explanation for defendant's failure to maintain safe distance.

Witnesses--Expert Witness

Counsel: [***1] Conway, Farrell, Curtin & Kelly, P.C., New York (Jonathan T. Uejio of counsel), for appellant.

Flomenhaft & Cannata, LLP, New York (Benedene Cannata of counsel), for respondents.

Judges: Lippman, P.J., Mazzarelli, Buckley, McGuire, DeGrasse, JJ.

Opinion

[*340] [**17] Judgment, Supreme Court, New York County (Martin Shulman, J.), entered August [**18] 11, 2006, after a jury trial, in favor of plaintiffs and against defendant in the total amount of $ 2,774,460, unanimously affirmed, without costs.

On the issue of fault, the trial court correctly directed a verdict in plaintiffs' favor based on defendant's own testimony that he saw the injured plaintiff's car stopped at a red light, braked hard and shifted to low gear, but his pick-up truck skidded on the wet roadway and hit the rear of plaintiff's car. A rear-end collision with a stationary vehicle creates a prima facie case of negligence requiring a judgment in favor of the stationary vehicle unless defendant proffers a nonnegligent explanation for the failure to maintain a safe distance (*Mitchell v Gonzalez, 269 AD2d 250, 251, 703 NYS2d 124 [2000]*). A wet roadway is not such an explanation. A driver is expected to drive at a sufficiently safe speed and to maintain enough distance between [***2] himself and cars ahead of him so as to avoid collisions with stopped vehicles, taking into account weather and road conditions (*id.*). On the issue of serious injury, plaintiffs' experts, relying on objective medical tests, testified to brain damage and other injuries that they attributed to trauma, and the conflicting medical

EXHIBIT 10

evidence and opinions of defendant's experts concerning the permanence and significance of plaintiff's injuries simply raised issues of fact for the jury (*see* Noble v Ackerman, 252 AD2d 392, 395, 675 NYS2d 86 [1998]). Concerning defendant's motion to preclude expert testimony, with respect to the nonproduction of raw data produced in tests conducted by the experts, defendant fails to show either prejudice or willful and contumacious conduct. With respect to the experts whose designations were made shortly before trial, CPLR 3101 (d) (1) (i) **[*341]** does not require a party to retain an expert at any particular time, and the court allowed defendant appropriate additional disclosure. With respect to the discrepancies between the trial testimony of some of plaintiffs' experts and their reports, defendant did not show a willful attempt to deceive or prejudice, and such discrepancies, which **[***3]** defendant was free to raise on cross-examination, go only to the weight, not the admissibility, of the testimony (*see* Hageman v Jacobson, 202 AD2d 160, 161, 608 NYS2d 180 [1994]; Dollas v Grace & Co., 225 AD2d 319, 321, 639 NYS2d 323 [1996]). On the issue of foundational support for expert opinion, while some of plaintiffs' experts relied on new technology or methodologies, the same experts also opined based on well-established and recognized **[****2]** diagnostic tools, and we find that they provided reliable causation opinions (*see* Parker v Mobil Oil Corp., 7 NY3d 434, 447, 857 NE2d 1114, 824 NYS2d 584 [2006]). We have considered defendant's other arguments and find them unavailing. Concur--Lippman, P.J., Mazzarelli, Buckley, McGuire and DeGrasse, JJ.

End of Document

PROOF OF SERVICE
(C.C.P. 1013A, 2015.5)

STATE OF CALIFORNIA

I am employed in the county of Los Angeles, State of California. I am over the age of eighteen years and not a party to the within action; my business address is 100 Wilshire Boulevard, 21st Floor, Santa Monica, California 90401.

On May 8, 2017 I served the foregoing document, described as **PLAINTIFFS' OPPOSITION TO DEFENDANTS' MOTION *IN LIMINE* NO. 3; MEMORANDUM OF POINTS AND AUTHORITIES; DECLARATION OF TAYLOR RAYFIELD, ESQ.; EXHIBITS** on the interested parties in this action.

___ by placing the true copies thereof enclosed in sealed envelopes addressed as stated on the attached mailing list.

__X__ by placing ___ the original __X__ a true copy enclosed in sealed envelopes addressed as follows:

__X__ **BY MAIL.**

___ I deposited such envelope in the mail at Santa Monica, California. The envelope was mailed with postage thereon fully prepaid.

__X__ As follows: I am "readily familiar" with the firm's practice of collection and processing correspondence for mailing. Under that practice it would be deposited with U.S. postal service on that same day with postage thereon fully prepaid at Santa Monica, California in the ordinary course of business. I am aware that on motion of the party served, service is presumed invalid if postal cancellation date or postage meter date is more than one day after date of deposit for mailing in affidavit.

Executed on May 8, 2017 at Santa Monica, California.

___ **BY PERSONAL SERVICE.** I delivered such envelope by hand to the offices of the addressee.

___ **BY OVERNIGHT DELIVERY.** I caused such envelope to be deposited with a delivery service (Federal Express) in Santa Monica, California, for overnight delivery to the addresses set forth on the attached mailing list.

__X__ **BY E-MAIL OR ELECTRONIC TRANSMISSION.** I caused the document(s) to be sent to the person(s) at the e-mail address(es) listed on the Service List. I did not receive, within a reasonable time after transmission, any electronic message or other indication that the transmission was unsuccessful.

Executed on May 8, 2017 at Santa Monica, California.

__X__ **(State)** I declare under penalty of perjury under the laws of the State of California that the above is true and correct.

Robert Gersten _____
Name **Signature**

SERVICE LIST

Androlia v. Entertainment Center, et al.

Case No. BC534479 (Los Angeles Superior Court)

BY MAIL & EMAIL Roy Goldstein, Esq. SKEBBA, ISAAC, BISHOP & HENDERSON 777 South Figueroa Street, Suite 1507 Los Angeles, CA 90017 Tel: (213) 689-3625 Fax: (213) 226-4019 Email: roy.goldstein@aig.com	Attorneys for Defendants/Cross-Complainants/Cross-Defendants: ENTERTAINMENT CENTER, LLC and CBRE, INC.
BY MAIL & EMAIL J. Alan Frederick, Esq. Karla Baer, Esq. Paul Whitfield, Esq. MARRONE, ROBINSON, FREDERICK & FOSTER 111 North First Street, Suite 209 Burbank, CA 91502-1851 Tel: (818) 841-1144 Fax: (818) 841-0746 Email: alanfrederick@mrfflaw.net karlabaer@mrfflaw.net paulwhitfield@mrfflaw.net mickeyvalenti@mrfflaw.net	Attorneys for Defendant/Cross-Defendant/Cross-Complainant (DOE 2): AREF & ASSOCIATES, INC.
BY MAIL & EMAIL Mary Childs, Esq. Timothy McCormick, Esq. YOKA & SMITH 445 S. Figueroa Street, 38th Floor Los Angeles, CA 90071 Tel: (213) 427-2300 Fax: (213) 427-2330 Email: mchilds@yokasmith.com tmccormick@yokasmith.com ANaylor@yokasmith.com SVillamarin@yokasmith.com	Attorneys for Defendant/Cross-Defendant (DOE 1): TASLIMI CONSTRUCTION CO., INC.
BY MAIL & EMAIL Susan Oliver, Esq. Tracey Angelopoulos, Esq. Jessica G. Heppenstall, Esq. TYSON & MENDES 5661 La Jolla Blvd. San Diego, CA 92037 Tel: (858) 459-4400 Fax: (858) 459-3864 Email: SOliver@TysonMendes.com TAngelopoulos@TysonMendes.com jheppenstall@tysonmendes.com VFennema@TysonMendes.com SWeidemann@TysonMendes.com	Attorneys for Defendant TRAMMELL CROW COMPANY, LLC