

JOURNAL OF NEUROTRAUMA 32:1–8 (Month XX, 2015)  
 © Mary Ann Liebert, Inc.  
 DOI: 10.1089/neu.2014.3534

## Gray Matter Abnormalities in Pediatric Mild Traumatic Brain Injury

Andrew R. Mayer,<sup>1–3</sup> Faith M. Hanlon,<sup>1</sup> and Josef M. Ling<sup>1</sup>

### Abstract

Pediatric mild traumatic brain injury (pmTBI) is the most prevalent neurological insult in children and is associated with both acute and chronic neuropsychiatric sequelae. However, little is known about underlying pathophysiology changes in gray matter diffusion and atrophy from a prospective stand-point. Fifteen semi-acute pmTBI patients and 15 well-matched healthy controls were evaluated with a clinical and neuroimaging battery, with a subset of participants returning for a second visit. Clinical measures included tests of attention, processing speed, executive function, working memory, memory, and self-reported post-concussive symptoms. Measures of diffusion (fractional anisotropy [FA]) and atrophy were also obtained for cortical and subcortical gray matter structures to characterize effects of injury as a function of time. Patients exhibited decreased scores in the domains of attention and processing speed relative to controls during the semi-acute injury stage, in conjunction with increased anisotropic diffusion in the left superior temporal gyrus and right thalamus. Evidence of increased diffusion in these regions was also present at four months post-injury, with performance on cognitive tests partially normalizing. In contrast, signs of cortical atrophy in bilateral frontal areas and other left-hemisphere cortical areas only emerged at four months post-injury for patients. Current results suggest potentially differential time-courses of recovery for neurobehavioral markers, anisotropic diffusion and atrophy following pmTBI. Importantly, these data suggest that relying on patient self-report or standard clinical assessments may underestimate the time for true injury recovery.

**Key words:** diffusion tensor imaging; gray matter; pediatric; prospective; traumatic brain injury

### Introduction

**T**RAUMATIC BRAIN INJURY (TBI) is the leading cause of death in children,<sup>1</sup> with about half a million pediatric TBI patients visiting the emergency department (ED) each year.<sup>2</sup> Of these ED visits for TBI, 75% of injuries are classified as mild in nature (pediatric mild TBI [pmTBI]). A recent review of neuroimaging studies investigating the chronic effects (at least one year after injury) of TBI in children found neurodegenerative changes that include volume loss in a variety of brain structures, as well as decreased axonal integrity.<sup>3</sup> The effects of pmTBI are less understood, with only a single cross-sectional study suggesting long-term structural changes 10 years post-injury.<sup>4</sup> These underlying structural changes may result in cognitive symptoms, which have also been observed 12 months post-injury after mild TBI (mTBI) in children.<sup>5</sup>

Thus, the pathophysiology of pmTBI and the subsequent relationship to neurobehavioral sequelae remain actively debated. Diffusion tensor imaging (DTI) has emerged as a potential mechanism for non-invasively measuring traumatic injuries, with the majority of studies focusing on moderate-to-severe<sup>6–9</sup> or mixed<sup>10,11</sup>

pediatric injury cohorts at more chronic (e.g., months to years post-injury) time-points. The majority of these studies report decreased fractional anisotropy (FA) in the white matter of chronic patients. In contrast, reports of increased FA have also been observed in two other independent samples of semi-acutely injured pmTBI patients,<sup>12–15</sup> although these findings have not always been replicated.<sup>16</sup> No studies have prospectively examined diffusion abnormalities in gray matter following semi-acute pmTBI, in spite of recent reports of abnormalities in both animal<sup>17–19</sup> and adult human<sup>20–23</sup> studies.

Similarly, atrophic changes in gray matter have not been investigated in the semi-acute injury phase of pmTBI or prospectively. Neuronal degeneration (atrophy) occurs in the first few months of injury and continues into the more chronic injury phase in more severe TBI,<sup>24–26</sup> even in the absence of macroscopically detectable lesions.<sup>27,28</sup> Injury severity predicts the magnitude of both white and gray matter loss in severe pediatric TBI, with milder injuries also associated with long-term structural changes.<sup>4,29</sup> In adult mTBI, atrophy due to frank neuronal or neuropil loss may occur later in the course of mTBI,<sup>22,30</sup> may be limited to

<sup>1</sup>The Mind Research Network/Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico.

<sup>2</sup>Department of Psychology, University of New Mexico, Albuquerque, New Mexico.

<sup>3</sup>Department of Neurology, University of New Mexico School of Medicine, Albuquerque, New Mexico.

patients with lesions,<sup>31</sup> or may be limited to chronically symptomatic patients.<sup>32</sup>

The current study is the first to prospectively investigate both diffusion abnormalities and atrophic changes in gray matter after pmTBI. These potentially time-dependent biomarkers of gray matter injury were investigated in a cohort of semi-acutely injured pmTBI patients and matched healthy controls (HC). All pmTBI patients were evaluated with neuropsychological testing and brain imaging within 21 d of injury, with 11 patients returning for a second visit approximately four months post-injury. We hypothesized that pmTBI patients would show evidence of increased anisotropic diffusion in gray matter regions vulnerable to injury, such as the thalamus, hippocampus, and medial and lateral fronto-temporal cortices.<sup>27,33-35</sup> In contrast, we predicted that there would not be any evidence of atrophy during the semi-acute or early chronic (i.e., ~four months post-injury) phases of pmTBI.<sup>22</sup>

**Methods**

*Participants*

Sixteen patients with pmTBI and 16 age- and education-matched HC were recruited for the current study (Table 1) from local hospital EDs. Demographic data, details about each patient’s injury, inclusion and exclusion criteria, and white matter DTI data have been previously reported for this cohort and are only briefly repeated here.<sup>14</sup> All pmTBI were between 10 and 17 years of age and

experienced a closed-head injury with an alteration in mental status. Glasgow Coma Scale score was 13–15 at the time of injury, loss of consciousness (if present) was limited to 30 min, and post-traumatic amnesia (if present) was limited to 24 h.

Exclusion criteria for both groups included a positive history of neurological disease, psychiatric disturbance, prior closed-head injuries with more than 5 min of loss of consciousness, learning disorder, attention deficit hyperactivity disorder (ADHD), substance abuse, or alcohol abuse.

Clinical (mean days post-injury = 15.33 ± 4.58) and brain imaging (mean days post-injury = 15.87 ± 4.93) evaluations occurred within 21 d of injury and in close temporal proximity (mean day difference = 0.33 ± 1.09). Ten patients received a computed tomography scan as part of routine care, all of which were negative. Data from one patient and one control was eliminated secondary to excessive motion on diffusion scans.<sup>36</sup> Of the total 15 pmTBI patients included, four sustained injury from a fall, nine had a sports or activity-related injury, and two were in a motor vehicle accident. In addition, 10 of the 15 patients experienced loss of consciousness and the five that didn’t had symptoms of confusion that lasted longer than 15 min.

Informed consent was obtained from participants according to institutional guidelines at the University of New Mexico.

*Clinical assessment*

The Wide Range Achievement Test, Fourth Edition, provided an estimate of reading ability. Behavioral and emotional issues were

TABLE 1. DEMOGRAPHIC AND CLINICAL DATA FOR PEDIATRIC MILD TRAUMATIC BRAIN INJURY (pmTBI) AND HEALTHY CONTROL (HC) PARTICIPANTS

	<i>pmTBI</i>		<i>HC</i>		<i>Significance</i>	
	<i>Mean</i>	<i>SD (±)</i>	<i>Mean</i>	<i>SD (±)</i>	<i>p value</i>	<i>Effect Size</i>
<b>Demographic</b>						
Age	13.47	2.20	13.40	1.84	<i>p</i> > 0.10	0.03
Education (self)	6.87	2.23	7.27	1.87	<i>p</i> > 0.10	-0.19
Education (parent)*	13.18	2.59	15.62	3.71	<i>p</i> = 0.06	-0.76
Siblings	2.43	1.91	2.73	1.98	<i>p</i> > 0.10	-0.15
Gender	<i>M</i>	<i>F</i>	<i>M</i>	<i>F</i>	<i>p value</i>	<i>Phi</i>
	13	2	12	3	<i>p</i> > 0.10	-0.09
Handedness	<i>R</i>	<i>L</i>	<i>R</i>	<i>L</i>	<i>p value</i>	<i>Phi</i>
	13	2	15	0	<i>p</i> > 0.10	0.27
<b>Neuropsych</b>						
Attention*	52.31	5.40	56.54	3.65	<i>p</i> = 0.02	-0.92
Memory	52.82	9.94	58.11	7.61	<i>p</i> > 0.10	-0.59
WM	48.77	7.34	51.50	7.36	<i>p</i> > 0.10	-0.37
PS *	49.07	7.93	54.17	5.81	<i>p</i> = 0.05	-0.73
EF	53.37	7.54	52.78	5.04	<i>p</i> > 0.10	0.09
WRAT	51.87	11.69	52.84	5.82	<i>p</i> > 0.10	-0.11
<b>BASC-2 SRP</b>						
School	51.07	11.32	48.00	11.03	<i>p</i> > 0.10	0.27
Internalizing	46.47	9.58	43.07	8.31	<i>p</i> > 0.10	0.38
Inattention	53.47	14.63	47.07	11.49	<i>p</i> > 0.10	0.49
Emotional	45.33	9.25	43.67	9.16	<i>p</i> > 0.10	0.18
Adjustment	55.40	8.40	54.73	11.17	<i>p</i> > 0.10	0.07
<b>BASC-2 PRS</b>						
Externalizing	51.93	11.01	52.00	8.04	<i>p</i> > 0.10	-0.01
Internalizing	49.36	8.68	50.79	8.60	<i>p</i> > 0.10	-0.17
Behavioral	50.00	10.38	51.43	8.11	<i>p</i> > 0.10	-0.15
Adaptive	53.79	10.98	50.64	6.57	<i>p</i> > 0.10	0.35

Effect sizes (pmTBI - HC) are Cohen’s *d* unless denoted by Phi. Demographic data are raw scores (above the first solid line), whereas neuropsychological measures and BASC-2 measures are T-scores. \* denotes significant or trend findings.

SD, standard deviation; M, male; F, female; R, right; L, left; WM, working memory; PS, processing speed; EF, executive function; WRAT, Wide Range Achievement Test; BASC-2, Behavior Assessment System for Children, Second Edition; SRP, Self-Report of Personality; PRS, Parent-Rating Scales.

measured by both self-report and parent ratings from the Behavior Assessment System for Children, Second Edition. A modified version of the Rivermead Post-Concussion Scale captured previous history of head injuries and post-concussive symptoms. A battery of neuropsychological tests were administered,<sup>37</sup> with composite indices calculated for the domains of attention, memory, processing speed, working memory, and executive function.

#### Imaging protocol

High resolution T<sub>1</sub>-weighted (1×1×1 mm), T<sub>2</sub>-weighted (1.15×1.15×1.5 mm) and susceptibility weighted (1.0×0.9×1.5 mm) images were collected on a 3 Tesla Siemens scanner to investigate structural lesions. A single run of diffusion weighted images (b=800 sec/mm<sup>2</sup>; voxel size=2×2×2 mm) were acquired using a twice-refocused spin echo sequence with 30 diffusion gradients and the b=0 experiment repeated five times. Please see previous publication for further imaging details.<sup>14</sup>

#### Data analyses

DTI analyses involved a mixture of AFNI, FSL, and FreeSurfer software.<sup>14,22</sup> Image distortions caused by eddy currents or head motion were first corrected by registering all diffusion weighted images to the first b=0 sec/mm<sup>2</sup> image using an affine correction with mutual information as the cost function. Diffusion weighted images were anisotropically smoothed and tensors calculated using a non-linear method for tensor calculation. Final FA, axial diffusivity (AD), and radial diffusivity (RD) data were then registered to each individual's T<sub>1</sub> volume using a 12 degree of freedom affine transformation.

The FreeSurfer reconstruction pipeline (version 5.1) was used to generate cortical thickness values and regions of interest (ROIs) based on standard labels.<sup>38</sup> All processing results were visually inspected as part of routine quality assurance. The FreeSurfer longitudinal pipeline was used for all cortical data when comparing visits to maximize between visit image registration and account for variable time between visits.<sup>39</sup> Specifically, a "symmetrized" percent change (SPC) value was derived from the rate of change of FA or thickness, created by subtracting data from the first and second visits and normalizing by the amount of time between visits, using the formula  $\frac{(V2-V1)/(Time2-Time1)}{0.5(V1+V2)}$ . This effectively tests the Group×Time interaction. For cortical analyses, FA and cortical thickness were projected to surface-based vertices, smoothed with 10 mm full width at half maximum (FWHM) Gaussian kernel and then directly compared using diagnosis (pmTBI vs. HC) as the between-subjects factor. Results were corrected for multiple comparisons at  $p < 0.05$  (vertex-wise and cluster-wise) using Monte Carlo simulations.

Subcortical ROI were defined based on FreeSurfer segmentation labels<sup>38</sup> and multiplied by respective diffusion maps. Principal analyses examining subcortical diffusion (FA) and atrophy (normalized by estimates of total intracranial volume) were restricted to the thalami and hippocampi, and evaluated with a single multivariate analysis of variance (MANOVA) due to the moderate linear relationship that exists between structures. Secondary voxel-wise analyses were also conducted on the remainder of subcortical structures (see Supplementary Methods, online only).

## Results

#### Quality assurance and anatomical reviews

Previously published results<sup>14</sup> indicated no significant group differences in head motion or image quality between the pmTBI and HC group on DTI scans. An additional test was also conducted on total frame-wise displacement, which was also not significant ( $p > 0.10$ ). However, given the concerns with motion on diffusion<sup>36</sup>

and echo-planar<sup>40</sup> data, supplementary analyses were also conducted for any significant DTI findings using frame-wise displacement as a covariate (see Supplementary Results). A board-certified neuroradiologist who was blinded to diagnostic status did not report any trauma-related or other pathology on anatomical magnetic resonance imaging scans (T<sub>1</sub>, T<sub>2</sub>, and SWI).

#### Visit 1 clinical data

No significant group differences (all  $p$ 's > 0.10) existed for reading ability, self-reported behavioral deficits, parent-reported behavioral deficits, or history of self-reported previous mTBI (pmTBI=2/15; HC=4/15). There was a trend ( $t_{25} = -1.99$ ;  $p = 0.06$ ) for higher parental education for HC relative to pmTBI (data missing for three participants). All demographic and clinical data can be found in Table 1.

A MANOVA was performed to examine differences in cognition given correlational structure between domains. The multivariate effect of group was not significant ( $p > 0.10$ ). Uncorrected univariate tests of cognition suggested worse performance for pmTBI patients in the domains of attention (F<sub>1,28</sub>=6.34,  $p = 0.02$ ; Cohen's  $d = -0.92$ ) and processing speed (F<sub>1,28</sub>=4.04,  $p = 0.05$ ; Cohen's  $d = -0.73$ ), with moderate effect size also present for memory functioning (Cohen's  $d = -0.59$ ). However, performance was within the normal range (T-scores) for both groups across all cognitive measures.

#### Visit 1 imaging data

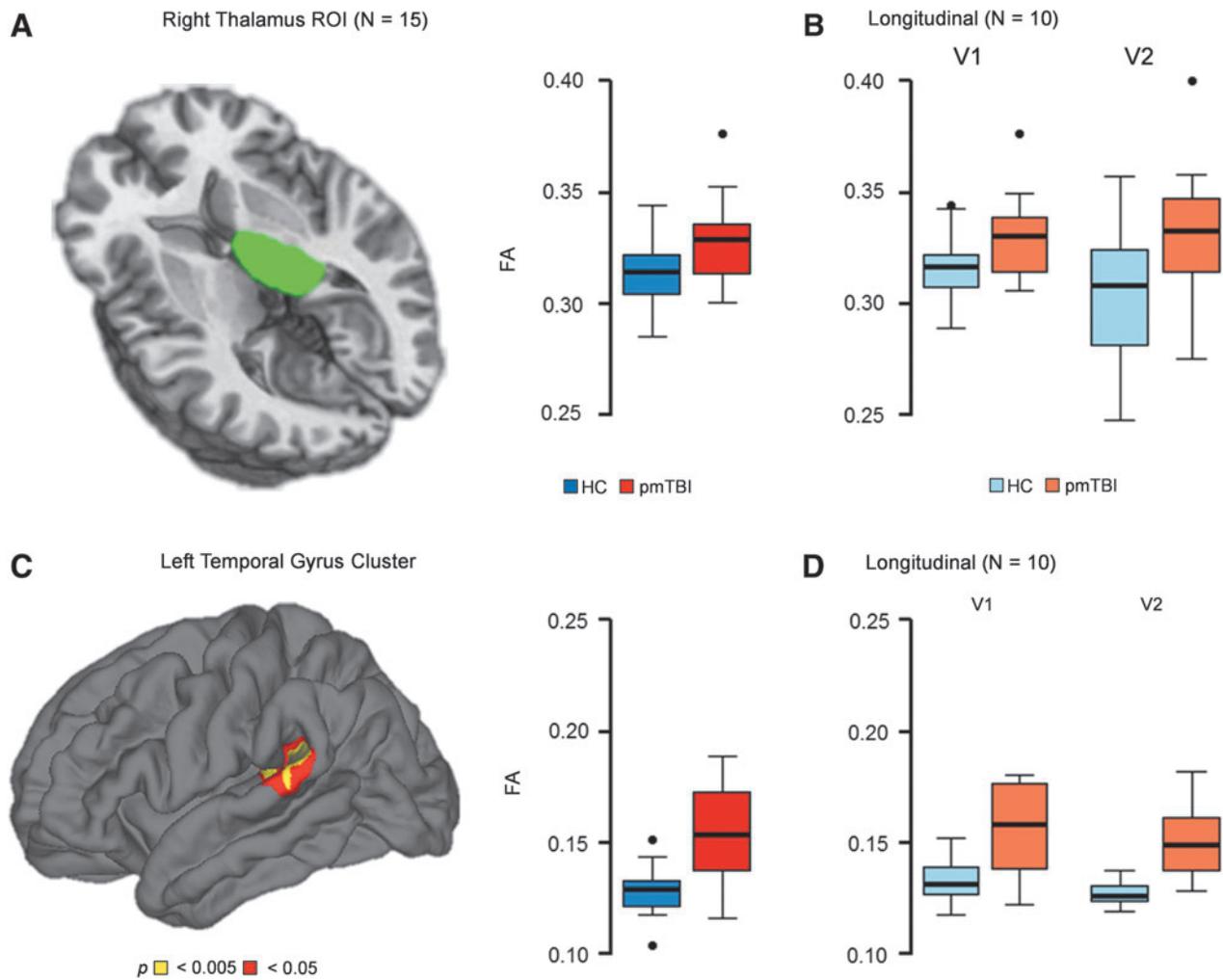
Results of the vertex-wise analysis of FA (Fig. 1) show a cluster of increased FA for pmTBI relative to HC within the left superior temporal gyrus extending to the posterior insula (Brodmann areas [BAs] 13/22/41; Montreal Neurological Institute [MNI] coordinates = -39.5, -30.8, 10.0; 1211 mm<sup>2</sup>). Follow-up tests indicated that RD was significantly decreased for pmTBI patients relative to controls (F<sub>1,28</sub>=6.20,  $p = 0.02$ ), with no group-wise differences in AD ( $p > 0.10$ ). There was no evidence of significant group differences (corrected  $p > 0.05$ ) in cortical thickness for Visit 1 data.

Results (Table 2) from the MANOVA examining diffusivity changes in a priori subcortical regions were not significant for the multivariate effect of group ( $p > 0.10$ ). However, a significant univariate effect was observed in the right thalamus (F<sub>1,28</sub>=4.94,  $p = 0.04$ ; Cohen's  $d = 0.81$ ) with pmTBI exhibiting increased FA (Fig. 1). Follow-up tests indicated that there were no significant differences in AD or RD ( $p > 0.10$ ). A moderate effect size was also observed within the left thalamus (Cohen's  $d = 0.51$ ; Table 2). There were no differences ( $p > 0.10$ ) in subcortical volume (hippocampi and thalami), with small effect sizes (Table 2). Supplementary analyses were also non-significant (see Supplementary Results).

Two multiple regressions examined the relationship between cognitive deficits (attention and processing speed) and regions exhibiting significant FA abnormalities (right thalamus and left temporal cortex) across both cohorts. FA in these regions did not predict processing speed performance ( $p > 0.10$ ). In contrast, the overall model was significant for attentional functioning (F<sub>2,27</sub>=5.91,  $p = 0.007$ ), with the left temporal gyrus ( $t = -3.34$ ,  $p = 0.002$ ) and right thalamus ( $t = 2.42$ ,  $p = 0.023$ ) both accounting for variance in the model. A significant relationship between FA and attentional measures was not present when the pmTBI group was run separately, which may have resulted from a lack of statistical power.

#### Visit 2 clinical data

Of the 30 participants included in Visit 1 analyses, 11/15 pmTBI and 12/15 HC returned at approximately four months (mean days



**FIG. 1.** (A) Displays a priori region of interest (right thalamus; green coloring) and box-and-whisker plots of fractional anisotropy (FA) for both patients with pediatric mild traumatic brain injury (pmTBI; red boxplot) and healthy controls (HC; blue boxplot) during the first visit ( $N = 15$ ). (B) Displays longitudinal changes in FA within the right thalamus for returning pmTBI (orange boxplots) and HC (light blue boxplots) at visits 1 (V1) and 2 (V2) ( $N = 10$ ). (C) Results of the vertex-wise analysis for V1 data, with magnitude of significance denoted by color (pmTBI > HC; yellow =  $p < 0.005$ ; red =  $p < 0.05$ ). Box-and-whisker plot displays FA for pmTBI and HC within the left temporal gyrus cluster during the first visit. (D) Displays longitudinal changes in FA within the left temporal cluster for returning pmTBI and HC at V1 and V2. Box-and-whisker plots are used to display the distributional properties of the data, with dots denoting values that were between 1.5 and 3.0 times the interquartile range.

post-injury =  $127.82 \pm 14.60$  for pmTBI patients). Among returning participants, data acquisition errors occurred for one HC, and two other participants exhibited extreme motion (one pmTBI patient and one HC), leaving a final sample of 10 pmTBI and 10 HC. Changes in performance on processing speed and attention across the two visits were investigated with  $2 \times 2$  (Group  $\times$  Time) mixed-measures analyses of variance (ANOVAs). Results indicated a significant main effect of time for measures of attention ( $F_{1,18} = 7.34, p = 0.01$ ; Visit 1 =  $54.61 \pm 3.50$ ; Visit 2 =  $57.48 \pm 5.18$ ) and processing speed ( $F_{1,18} = 5.55, p = 0.03$ ; Visit 1 =  $52.90 \pm 7.60$ ; Visit 2 =  $55.20 \pm 7.02$ ). In addition, a significant trend was present for the main effect of group in processing speed ( $F_{1,18} = 3.14, p = 0.09$ ; HC =  $56.68 \pm 5.10$ ; TBI =  $51.42 \pm 8.31$ ). The Group  $\times$  Time interaction was not significant for either cognitive domain ( $p > 0.10$ ). Effect sizes were reduced for both attention (Cohen's  $d = 0.03$ ) and memory (Cohen's  $d = -0.31$ ) at Visit 2, but remained in the moderate range for processing speed (Cohen's  $d = -0.64$ ).

#### Visit 2 imaging data

The  $2 \times 2$  mixed measures ANOVAs (Group  $\times$  Time) were used to evaluate significant changes in FA within the left temporal cortex and right thalamus as a function of time (Fig. 1). The main effect of group was at a trend level for the right thalamus ( $F_{1,18} = 3.57, p = 0.075$ ) and significant for the left temporal cluster ( $F_{1,18} = 16.44, p = 0.001$ ). The main effect of Time and the Group  $\times$  Time interaction were not significant ( $p > 0.10$ ) for either region. The FreeSurfer Longitudinal pipeline was also used to examine for different rates of change in cortical FA from Visit 1 to Visit 2 between pmTBI and HC in the remainder of the brain (Group  $\times$  Time interaction). Results for this analysis were not significant following correction for false positives.

In contrast, results from an identical FreeSurfer longitudinal analysis using the cortical thickness data (Fig. 2) showed significant atrophy (change from Visit 1 to Visit 2) within the left superior and medial frontal gyrus (BAs 10,11; MNI coordinates =  $-7.1,$

GRAY MATTER IN pmTBI

TABLE 2. SUBCORTICAL MEASUREMENTS AT VISIT 1

Region/H	pmTBI	HC	Significance			
<b>Fractional anisotropy</b>						
	Mean	SD (±)	Mean	SD (±)	<i>p</i> value	<i>d</i>
<b>Thalamus</b>						
L	0.344	0.025	0.333	0.015	<i>p</i> >0.10	0.51
R	0.329	0.020	0.314	0.017	<i>p</i> =0.04	0.81
<b>Hippocampus</b>						
L	0.185	0.014	0.183	0.019	<i>p</i> >0.10	0.09
R	0.176	0.011	0.181	0.019	<i>p</i> >0.10	-0.30
<b>Normalized volume</b>						
	Mean	SD (±)	Mean	SD (±)	<i>p</i> value	<i>d</i>
<b>Thalamus</b>						
L	0.0687	0.0027	0.0695	0.0028	<i>p</i> >0.10	-0.30
R	0.0699	0.0021	0.0694	0.0022	<i>p</i> >0.10	0.21
<b>Hippocampus</b>						
L	0.0519	0.0013	0.0519	0.0018	<i>p</i> >0.10	0.00
R	0.0522	0.0015	0.0520	0.0020	<i>p</i> >0.10	0.08

Effect sizes (pmTBI - HC) are Cohen's *d*.  
 H, hemisphere; pmTBI, pediatric mild traumatic brain injury; HC, healthy controls; SD, standard deviation; L, left; R, right.

55.8, -12.8; 621.37 mm<sup>2</sup>), left middle temporal gyrus (BA 19; MNI coordinates = -41.3, -79.0, 16.9; 566.18 mm<sup>2</sup>), left postcentral gyrus extending into inferior parietal lobule (BA 40; MNI coordinates = -43.0, -28.5, 43.2; 855.11 mm<sup>2</sup>), left inferior parietal lobule (BA 7; MNI coordinates = -37.0, -63.8, 41.3; 514.70 mm<sup>2</sup>), and left

cuneus and left middle occipital gyrus (BA 19; MNI coordinates = -7.4, -88.6, 30.4; 1027.24 mm<sup>2</sup>) for the pmTBI group. Significant atrophy for pmTBI patients was also observed within the right superior and middle frontal gyri (BA 10; MNI coordinates = 23.9, 49.3, 15.0; 971.31 mm<sup>2</sup>).

Finally, two 2×2 mixed measures ANOVAs (Group× Laterality) were conducted to assess for volume change (Visit 2 - Visit 1) for both the thalami and hippocampi. There were no significant differences in subcortical volume change as a function of group, laterality or the interaction (*p*>0.10).

Discussion

Although research examining gray matter changes following adult mTBI have recently been conducted,<sup>20-22</sup> there have been no studies prospectively examining both diffusion abnormalities and atrophic changes in gray matter pathology after pmTBI. Current preliminary results indicate subtle cognitive deficits in the domains of attention and processing speed during the semi-acute phase of injury relative to controls, with partial (processing speed) or full (attention) recovery of function at approximately four months post-injury. Although others have reported more striking long-term neurobehavioral sequelae,<sup>5</sup> current results are generally consistent with previous longitudinal studies suggesting that pmTBI patients exhibit cognitive impairments (ImPACT testing) that typically normalize in the first few weeks of injury.<sup>16</sup>

Similar to previous findings of increased FA in gray matter in adults,<sup>22</sup> pmTBI patients exhibited increased FA in the left temporal cortex and right thalamus relative to controls during the

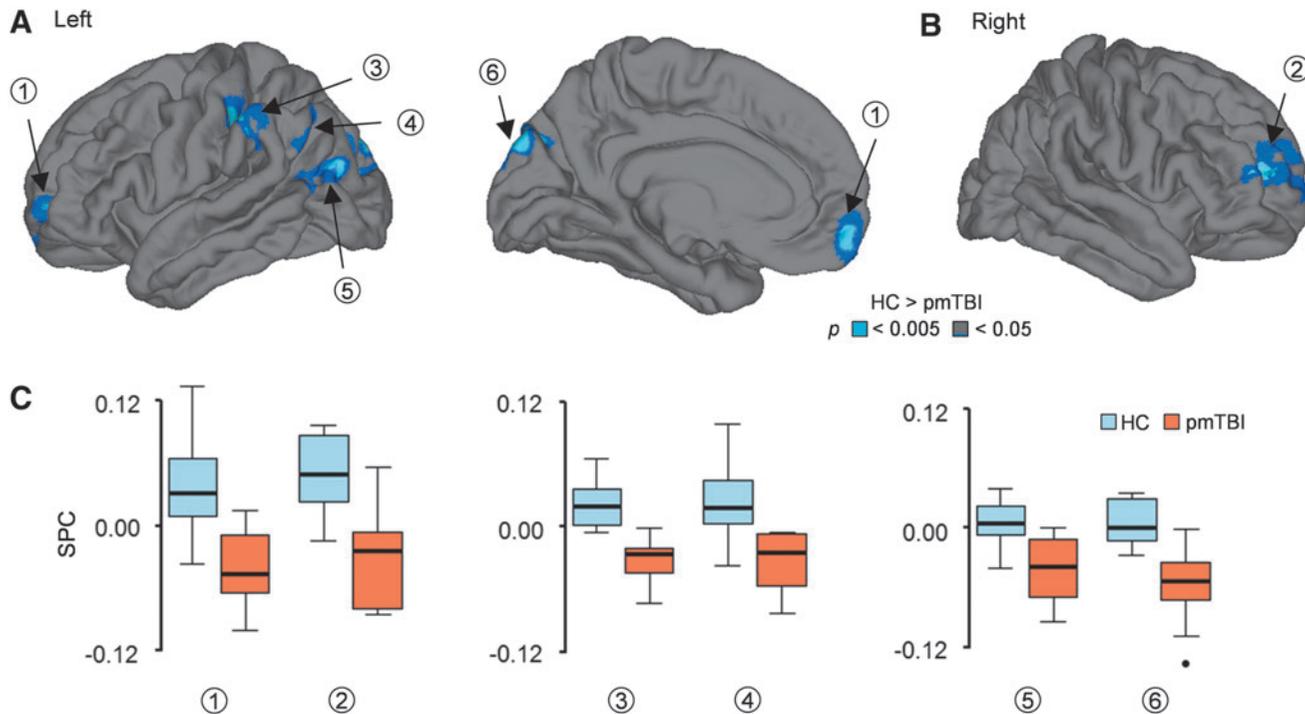


FIG. 2. Left (A) and right (B) hemisphere regions showing a significant decrease in cortical thickness from Visit 1 to Visit 2 in pediatric mild traumatic brain injury (pmTBI) patients relative to healthy controls (HC). Each significant cluster is denoted with the following numbers: 1) left superior and medial frontal gyrus; 2) right superior and middle frontal gyrus; 3) left inferior parietal lobule and postcentral gyrus; 4) left inferior parietal lobule; 5) left middle temporal gyrus; and 6) left cuneus and middle occipital gyrus. The magnitude of significance is indicated by color (light blue, *p*<0.005; dark blue, *p*<0.05). (C) Box-and-whisker plots denote the “symmetrized” percent change (SPC) value derived from the rate of change in cortical thickness for pmTBI (orange boxplot) and HC (light blue boxplot) for each of the numbered clusters. Box-and-whisker plots are used to display the distributional properties of the data, with dots denoting values that were between 1.5 and 3.0 times the interquartile range.

semi-acute injury phase, with the FA abnormalities associated with performance on attentional measures. FA remained increased within the left temporal cortex, with a trend seen for the right thalamus at approximately four months post-injury. Previous research indicates the thalamus is potentially more vulnerable to injury,<sup>35</sup> perhaps as a result of the accumulation of shear stresses in these regions.<sup>41</sup> Similarly, lateral frontotemporal cortices are also frequent injury sites as a result of the morphology of the skull.<sup>27,29,33</sup> Animal studies suggest that increased gray matter FA may result from the palisading of protoplasmic astrocytes and the subsequent formation of glial scars, occurring asymmetrically in the direction of the lesion.<sup>17,19</sup> Alternatively, increased FA and/or decreased apparent diffusion coefficient in gray matter have also been attributed to cytotoxic edema, reflecting alterations in the ratio of intracellular relative to extracellular water.<sup>18,42,43</sup> Future studies with increased angular resolution and multiple b-values may allow for the estimation of free intracellular versus extracellular water,<sup>44,45</sup> which may be helpful in differentiating cytotoxic edema versus reactive gliosis.

There was no evidence of gray matter atrophy (cortical thickness or sub-cortical volume changes) within the first few weeks of pmTBI. However, in contrast to a priori predictions, evidence of atrophy was present within bilateral frontal regions, as well as left temporal, parietal, and occipital cortex at four months post-injury. Gray matter volumes and functional connectivity have been shown to vary as a function of age,<sup>40,46,47</sup> and age at injury interacts with functional recovery in animal models.<sup>48</sup> However, a review of raw data confirmed that the reduction in cortical thickness occurred for the pmTBI cohort only over the four-month study interval and was not due to an increase for the controls. Similarly, in adult mTBI, atrophy has been reported to progress over time, possibly due to neuronal or neuropil loss later in the course of mTBI.<sup>27,30,32</sup> Previous reports of atrophy following pmTBI have been mixed, with one study reporting decreased hippocampal volume and increased cerebrospinal fluid 10 years post-injury.<sup>4</sup> In contrast, others have reported no evidence of atrophy at more than six months post-injury following complicated pmTBI.<sup>29</sup> Our use of a prospective design may have provided a more sensitive (i.e., within-subject) test of atrophy in frontal, temporal, and parietal regions as a direct function of injury progression relative to previous cross-sectional studies.

A particularly important topic for the field to address is the potential difference in the natural recovery courses for neurobehavioral (more rapidly resolving) and different physiological (more slowly resolving, evolving, or developing) signs of pmTBI. The current sample of pmTBI patients exhibited differential trajectories for neurobehavioral symptoms, increased anisotropic diffusion in both white<sup>14</sup> and gray matter, as well as atrophic changes in cortical thickness. Prospective studies in adult mTBI have shown a similar pattern of potentially faster resolution for self-reported symptomatology relative to diffusion abnormalities.<sup>22,49</sup> A similar process of cognitive improvement in conjunction with continuing or worsening brain pathology, potentially indicative of cerebral reorganization, is also common for more moderate to severe TBI patients.<sup>27</sup> In summary, similar to animal models of TBI,<sup>50</sup> the different signs and symptoms of pmTBI are likely to evolve differentially over time, which has important ramifications for determining when it is truly safe for a child to return to physical or cognitive activity.<sup>51</sup>

There are several limitations to the current study. First, our relatively small sample size may have limited our ability to detect meaningful group differences (i.e., moderate effect sizes in memory and FA for left thalamus) and increased the likelihood of

sampling bias. As a result, we have included effect size estimates for clinical measures and imaging measures where feasible. However, these results should be considered preliminary and require independent replication. Second, even though we did not observe significant neurobehavioral differences in returning versus not returning patients, the impact of sample attrition on longitudinal findings is unknown. Third, patients were assessed approximately 16 d post-injury, which may have compromised our ability to detect more rapidly-resolving neurobehavioral and pathological symptoms of mTBI.<sup>52,53</sup> Fourth, there are several factors other than trauma that can contribute to macroscopic measures of diffusion, such as head motion,<sup>36</sup> as well as individual differences in cognition<sup>54</sup> and emotional status.<sup>55</sup> We attempted to control for all of these factors through rigorous clinical assessment, as well as examining the effects of motion on current diffusion findings. Finally, we corrected for multiple comparisons in our imaging but not clinical data. Therefore, these results require future replication in independent samples.

In summary, pmTBI has been associated with an increased incidence of neuropsychiatric conditions,<sup>56</sup> delays in academic achievement, and decrease in overall quality of health.<sup>5</sup> Understanding the neuropathological underpinnings of these deficits is critical for both diagnostic considerations, clinical decisions about return to normal activity levels, and the development of novel psychiatric treatment strategies. Current preliminary results provide the first evidence of increased anisotropic diffusion and decreased cortical thickness as potential bio-markers of pmTBI. Importantly, our prospective design permitted the individual mapping of different timelines for these biomarkers of injury, as well as cognitive measures. The long-term consequences of a single episode of pmTBI on gray matter remain to be elucidated, as well as the potential disambiguation for single versus multiple temporally proximal pediatric brain injuries.

### Acknowledgments

This research was supported by grants from The Mind Research Network [DOE Grant No. DE-FG02-99ER62764] to A.R.M.

### Author Disclosure Statement

No competing financial interests exist.

### References

- Bell, M.J. and Kochanek, P.M. (2013). Pediatric traumatic brain injury in 2012: the year with new guidelines and common data elements. *Crit. Care Clin.* 29, 223–238.
- Faul, M., Xu, L., Wald, M.M., and Coronado, V.G. (2010). *Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002–2006*. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control: Atlanta.
- Keightley, M.L., Sinopoli, K.J., Davis, K.D., Mikulis, D.J., Wennberg, R., Tartaglia, M.C., Chen, J.K., and Tator, C.H. (2014). Is there evidence for neurodegenerative change following traumatic brain injury in children and youth? A scoping review. *Front. Hum. Neurosci.* 8, 139.
- Beauchamp, M.H., Ditchfield, M., Maller, J.J., Catroppa, C., Godfrey, C., Rosenfeld, J.V., Kean, M.J., and Anderson, V.A. (2011). Hippocampus, amygdala and global brain changes 10 years after childhood traumatic brain injury. *Int. J. Dev. Neurosci.* 29, 137–143.
- Yeates, K.O., Kaizar, E., Rusin, J., Bangert, B., Dietrich, A., Nuss, K., Wright, M., and Taylor, H.G. (2012). Reliable change in post-concussive symptoms and its functional consequences among children with mild traumatic brain injury. *Arch. Pediatr. Adolesc. Med.* 166, 615–622.

6. Babikian, T., Marion, S.D., Copeland, S., Alger, J.R., O'Neill, J., Cazalis, F., Mink, R., Giza, C.C., Vu, J.A., Hilleary, S.M., Kernan, C.L., Newman, N., and Asarnow, R.F. (2010). Metabolic levels in the corpus callosum and their structural and behavioral correlates after moderate to severe pediatric TBI. *J. Neurotrauma* 27, 473–481.
7. Hanten, G., Wilde, E.A., Menefee, D.S., Li, X., Lane, S., Vasquez, C., Chu, Z., Ramos, M.A., Yallampalli, R., Swank, P., Chapman, S.B., Gamino, J., Hunter, J.V., and Levin, H.S. (2008). Correlates of social problem solving during the first year after traumatic brain injury in children. *Neuropsychology* 22, 357–370.
8. McCauley, S.R., Wilde, E.A., Bigler, E.D., Chu, Z., Yallampalli, R., Oni, M.B., Wu, T.C., Ramos, M.A., Pedroza, C., Vasquez, A.C., Hunter, J.V., and Levin, H.S. (2011). Diffusion tensor imaging of incentive effects in prospective memory after pediatric traumatic brain injury. *J. Neurotrauma* 28, 503–516.
9. Oni, M.B., Wilde, E.A., Bigler, E.D., McCauley, S.R., Wu, T.C., Yallampalli, R., Chu, Z., Li, X., Hunter, J.V., Vasquez, A.C., and Levin, H.S. (2010). Diffusion tensor imaging analysis of frontal lobes in pediatric traumatic brain injury. *J. Child Neurol.* 25, 976–984.
10. Akpınar, E., Koroglu, M., and Ptak, T. (2007). Diffusion tensor MR imaging in pediatric head trauma. *J. Comput. Assist. Tomogr.* 31, 657–661.
11. Wozniak, J.R., Krach, L., Ward, E., Mueller, B.A., Muetzel, R., Schnobelen, S., Kiragu, A., and Lim, K.O. (2007). Neurocognitive and neuroimaging correlates of pediatric traumatic brain injury: a diffusion tensor imaging (DTI) study. *Arch. Clin. Neuropsychol.* 22, 555–568.
12. Wilde, E.A., McCauley, S.R., Hunter, J.V., Bigler, E.D., Chu, Z., Wang, Z.J., Hanten, G.R., Troyanskaya, M., Yallampalli, R., Li, X., Chia, J., and Levin, H.S. (2008). Diffusion tensor imaging of acute mild traumatic brain injury in adolescents. *Neurology* 70, 948–955.
13. Wu, T.C., Wilde, E.A., Bigler, E.D., Yallampalli, R., McCauley, S.R., Troyanskaya, M., Chu, Z., Li, X., Hanten, G., Hunter, J.V., and Levin, H.S. (2010). Evaluating the relationship between memory functioning and cingulum bundles in acute mild traumatic brain injury using diffusion tensor imaging. *J. Neurotrauma* 27, 303–307.
14. Mayer, A.R., Ling, J.M., Yang, Z., Pena, A., Yeo, R.A., and Klimaj, S. (2012). Diffusion abnormalities in pediatric mild traumatic brain injury. *J. Neurosci.* 32, 17961–17969.
15. Borich, M., Makan, N., Boyd, L., and Virji-Babul, N. (2013). Combining whole-brain voxel-wise analysis with in vivo tractography of diffusion behavior after sports-related concussion in adolescents: A preliminary report. *J. Neurotrauma* 30, 1243–1249.
16. Maugans, T.A., Farley, C., Altaye, M., Leach, J., and Cecil, K.M. (2012). Pediatric sports-related concussion produces cerebral blood flow alterations. *Pediatrics* 129, 28–37.
17. Budde, M.D., Janes, L., Gold, E., Turtzo, L.C., and Frank, J.A. (2011). The contribution of gliosis to diffusion tensor anisotropy and tractography following traumatic brain injury: Validation in the rat using Fourier analysis of stained tissue sections. *Brain* 134, 2248–2260.
18. Xu, S., Zhuo, J., Racz, J., Shi, D., Roys, S., Fiskum, G., and Gullapalli, R. (2011). Early microstructural and metabolic changes following controlled cortical impact injury in rat: A magnetic resonance imaging and spectroscopy study. *J. Neurotrauma* 28, 2091–2102.
19. Zhuo, J., Xu, S., Proctor, J.L., Mullins, R.J., Simon, J.Z., Fiskum, G., and Gullapalli, R.P. (2012). Diffusion kurtosis as an in vivo imaging marker for reactive astrogliosis in traumatic brain injury. *Neuroimage* 59, 467–477.
20. Bouix, S., Pasternak, O., Rathi, Y., Pelavin, P.E., Zafonte, R., and Shenton, M.E. (2013). Increased gray matter diffusion anisotropy in patients with persistent post-concussive symptoms following mild traumatic brain injury. *PLoS One* 8, e66205.
21. Grossman, E.J., Jensen, J.H., Babb, J.S., Chen, Q., Tabesh, A., Fieremans, E., Xia, D., Inglese, M., and Grossman, R.I. (2013). Cognitive impairment in mild traumatic brain injury: A longitudinal diffusional kurtosis and perfusion imaging study. *A.J.N.R. Am. J. Neuroradiol.* 34, 951–953.
22. Ling, J.M., Klimaj, S., Toulouse, T., and Mayer, A.R. (2013). A prospective study of gray matter abnormalities in mild traumatic brain injury. *Neurology* 81, 2121–2127.
23. Little, D.M., Kraus, M.F., Joseph, J., Geary, E.K., Susmaras, T., Zhou, X.J., Pliskin, N., and Gorelick, P.B. (2010). Thalamic integrity underlies executive dysfunction in traumatic brain injury. *Neurology* 74, 558–564.
24. Conti, A.C., Raghupathi, R., Trojanowski, J.Q., and McIntosh, T.K. (1998). Experimental brain injury induces regionally distinct apoptosis during the acute and delayed post-traumatic period. *J. Neurosci.* 18, 5663–5672.
25. Sato, M., Chang, E., Igarashi, T., and Noble, L.J. (2001). Neuronal injury and loss after traumatic brain injury: time course and regional variability. *Brain Res.* 917, 45–54.
26. Warner, M.A., Youn, T.S., Davis, T., Chandra, A., Marquez de la, P.C., Moore, C., Harper, C., Madden, C.J., Spence, J., McColl, R., Devous, M., King, R.D., and az-Arrastia, R. (2010). Regionally selective atrophy after traumatic axonal injury. *Arch. Neurol.* 67, 1336–1344.
27. Bigler, E.D., and Maxwell, W.L. (2012). Neuropathology of mild traumatic brain injury: Relationship to neuroimaging findings. *Brain Imaging Behav.* 6, 108–136.
28. Palacios, E.M., Sala-Llonch, R., Junque, C., Fernandez-Espejo, D., Roig, T., Tormos, J.M., Bargallo, N., and Vendrell, P. (2012). Long-term declarative memory deficits in diffuse TBI: Correlations with cortical thickness, white matter integrity and hippocampal volume. *Cortex* 49, 646–657.
29. Bigler, E.D., Abildskov, T.J., Petrie, J., Farrer, T.J., Dennis, M., Simic, N., Taylor, H.G., Rubin, K.H., Vannatta, K., Gerhardt, C.A., Stancin, T., and Owen, Y.K. (2013). Heterogeneity of brain lesions in pediatric traumatic brain injury. *Neuropsychology* 27, 438–451.
30. Zhou, Y., Kierans, A., Kenul, D., Ge, Y., Rath, J., Reaume, J., Grossman, R.I., and Lui, Y.W. (2013). Mild traumatic brain injury: longitudinal regional brain volume changes. *Radiology* 267, 880–890.
31. Hofman, P.A., Stapert, S.Z., van Kroonenburgh, M.J., Jolles, J., de, K.J., and Wilms, J.T. (2001). MR imaging, single-photon emission CT, and neurocognitive performance after mild traumatic brain injury. *A.J.N.R. Am. J. Neuroradiol.* 22, 441–449.
32. Ross, D.E., Ochs, A.L., Seabaugh, J.M., Demark, M.F., Shrader, C.R., Marwitz, J.H., and Havranek, M.D. (2012). Progressive brain atrophy in patients with chronic neuropsychiatric symptoms after mild traumatic brain injury: a preliminary study. *Brain Inj.* 26, 1500–1509.
33. McAllister, T.W., and Stein, M.B. (2010). Effects of psychological and biomechanical trauma on brain and behavior. *Ann. N. Y. Acad. Sci.* 1208, 46–57.
34. Maxwell, W.L., Dhillon, K., Harper, L., Espin, J., MacIntosh, T.K., Smith, D.H., and Graham, D.I. (2003). There is differential loss of pyramidal cells from the human hippocampus with survival after blunt head injury. *J. Neuropathol. Exp. Neurol.* 62, 272–279.
35. Maxwell, W.L., MacKinnon, M.A., Smith, D.H., MacIntosh, T.K., and Graham, D.I. (2006). Thalamic nuclei after human blunt head injury. *J. Neuropathol. Exp. Neurol.* 65, 478–488.
36. Ling, J., Merideth, F., Caprihan, A., Pena, A., Teshiba, T., and Mayer, A.R. (2012). Head injury or head motion? Assessment and quantification of motion artifacts in diffusion tensor imaging studies. *Hum. Brain Mapp.* 33, 50–62.
37. Yang, Z., Yeo, R., Pena, A., Ling, J., Klimaj, S., Campbell, R., Doezema, D., and Mayer, A. (2012). A fMRI Study of Auditory Orienting and Inhibition of Return in Pediatric Mild Traumatic Brain Injury. *J. Neurotrauma* 26, 2124–2136.
38. 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.
39. Reuter, M., Schmansky, N.J., Rosas, H.D., and Fischl, B. (2012). Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 61, 1402–1418.
40. 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.
41. Zhang, L., Yang, K.H., and King, A.I. (2004). A proposed injury threshold for mild traumatic brain injury. *J. Biomech. Eng.* 126, 226–236.
42. Newcombe, V.F., Williams, G.B., Outtrim, J.G., Chatfield, D., Gulia, A.M., Geeraerts, T., Manktelow, A., Room, H., Mariappen, L., Hutchinson, P.J., Coles, J.P., and Menon, D.K. (2013). Microstructural basis of contusion expansion in traumatic brain injury: Insights from diffusion tensor imaging. *J. Cereb. Blood Flow Metab.* 33, 855–862.
43. Van Putten, H.P., Bouwhuis, M.G., Muizelaar, J.P., Lyeth, B.G., and Berman, R.F. (2005). Diffusion-weighted imaging of edema following traumatic brain injury in rats: Effects of secondary hypoxia. *J. Neurotrauma* 22, 857–872.

44. Fieremans, E., Jensen, J.H., and Helpert, J.A. (2011). White matter characterization with diffusional kurtosis imaging. *Neuroimage* 58, 177–188.
45. Pasternak, O., Sochen, N., Gur, Y., Intrator, N., and Assaf, Y. (2009). Free water elimination and mapping from diffusion MRI. *Magn. Reson. Med.* 62, 717–730.
46. Paus, T. (2007). Maturation of structural and functional connectivity in the human brain, in: *Handbook of Brain Connectivity*. V. Jirsa and A.R. McIntosh (eds). Springer: Berlin Heidelberg, pps. 463–475.
47. Lenroot, R.K., Gogtay, N., Greenstein, D.K., Wells, E.M., Wallace, G.L., Clasen, L.S., Blumenthal, J.D., Lerch, J., Zijdenbos, A.P., Evans, A.C., Thompson, P.M., and Giedd, J.N. (2007). Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage* 36, 1065–1073.
48. Nemat, F. and Kolb, B. (2012). Recovery from medial prefrontal cortex injury during adolescence: implications for age-dependent plasticity. *Behav. Brain Res.* 229, 168–175.
49. Mayer, A.R., Ling, J., Mannell, M.V., Gasparovic, C., Phillips, J.P., Doezema, D., Reichard, R., and Yeo, R.A. (2010). A prospective diffusion tensor imaging study in mild traumatic brain injury. *Neurology* 74, 643–650.
50. Giza, C.C., and Hovda, D.A. (2001). The Neurometabolic Cascade of Concussion. *J. Athl. Train.* 36, 228–235.
51. Brown, N.J., Mannix, R.C., O'Brien, M.J., Gostine, D., Collins, M.W., and Meehan, W.P., III (2014). Effect of Cognitive Activity Level on Duration of Post-Concussion Symptoms. *Pediatrics* 133, e299–e304.
52. Nelson, L.D., Janecek, J.K., and McCrea, M.A. (2013). Acute clinical recovery from sport-related concussion. *Neuropsychol. Rev.* 23, 285–299.
53. 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.
54. Grieve, S.M., Williams, L.M., Paul, R.H., Clark, C.R., and Gordon, E. (2007). Cognitive aging, executive function, and fractional anisotropy: a diffusion tensor MR imaging study. *A.J.N.R. Am. J. Neuroradiol.* 28, 226–235.
55. Korgaonkar, M.S., Grieve, S.M., Koslow, S.H., Gabrieli, J.D., Gordon, E., and Williams, L.M. (2011). Loss of white matter integrity in major depressive disorder: evidence using tract-based spatial statistical analysis of diffusion tensor imaging. *Hum. Brain Mapp.* 32, 2161–2171.
56. McKinlay, A., Grace, R., Horwood, J., Fergusson, D., and MacFarlane, M. (2009). Adolescent psychiatric symptoms following pre-school childhood mild traumatic brain injury: evidence from a birth cohort. *J. Head Trauma Rehabil.* 24, 221–227.

Address correspondence to:

*Andrew R. Mayer, PhD  
The Mind Research Network  
Pete & Nancy Domenici Hall  
1101 Yale Boulevard NE  
Albuquerque, NM 87106*

*E-mail: amayer@mrn.org*

## Supplementary Material

### Supplementary Methods

Data were first smoothed with a 6 mm Gaussian full width at half maximum kernel. To reduce redundancy with surface based cortical analyses, a study-specific mask was first created based on the subcortical FreeSurfer labels. Individual participant subcortical masks were summed and only voxels in which there was 75% overlap in participants' masks were subjected to further analyses. General linear model analyses were then conducted on fractional anisotropy (FA) using diagnosis (pediatric mild traumatic brain injury [pmTBI] versus healthy controls [HC]) as a between-subjects factor, corrected for false positives at  $p < 0.05$ .

### Supplementary Results

#### *Visit 1*

Results of the subcortical voxel-wise FA analysis were non-significant following correction for false positives ( $p < 0.05$ ). The multivariate analysis of variance examining volumetric differences in the remainder of subcortical regions was not significant for both multivariate and univariate effects ( $p > 0.05$ ).

Supplementary analyses examining the effect of frame-wise displacement as a covariate did not significantly alter significant effects of increased FA in the right thalamus or left superior-temporal gyrus ( $p > 0.10$ ).