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ORIGINAL ARTICLE

IMAGING

## Longitudinal Patterns of Functional Connectivity in Moderate-to-Severe Traumatic Brain Injury

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### Abstract

Longitudinal neuroimaging studies aid our understanding of recovery mechanisms in moderate-to-severe traumatic brain injury (TBI); however, there is a dearth of longitudinal functional connectivity research. Our aim was to characterize longitudinal functional connectivity patterns in two clinically important brain networks, the frontoparietal network (FPN) and the default mode network (DMN), in moderate-to-severe TBI. This inception cohort study of prospectively collected longitudinal data used resting-state functional magnetic resonance imaging (fMRI) to characterize functional connectivity patterns in the FPN and DMN. Forty adults with moderate-to-severe TBI (mean  $\pm$  standard deviation [SD]; age =  $39.53 \pm 16.49$  years, education =  $13.92 \pm 3.20$  years, lowest Glasgow Coma Scale score =  $6.63 \pm 3.24$ , sex = 70% male) were scanned at approximately 0.5, 1–1.5, and 3+ years post-injury. Seventeen healthy, uninjured participants (mean  $\pm$  SD; age =  $38.91 \pm 15.57$  years, education =  $15.11 \pm 2.71$  years, sex = 29% male) were scanned at baseline and approximately 11 months afterwards. Group independent component analyses and linear mixed-effects modeling with linear splines that contained a knot at 1.5 years post-injury were employed to investigate longitudinal network changes, and associations with covariates, including age, sex, and injury severity. In patients with TBI, functional connectivity in the right FPN increased from approximately 0.5 to 1.5 years post-injury (unstandardized estimate = 0.19, standard error [SE] = 0.07,  $p = 0.009$ ), contained a slope change in the opposite direction, from positive to negative at 1.5 years post-injury (estimate =  $-0.21$ , SE = 0.11,  $p = 0.009$ ), and marginally declined afterwards (estimate =  $-0.10$ , SE = 0.06,  $p = 0.079$ ). Functional connectivity in the DMN increased from approximately 0.5 to 1.5 years (estimate = 0.15, SE = 0.05,  $p = 0.006$ ), contained a slope change in the opposite direction, from positive to negative at 1.5 years post-injury (estimate =  $-0.19$ , SE = 0.08,  $p = 0.021$ ), and was estimated to decline from 1.5 to 3+ years (estimate =  $-0.04$ , SE = 0.04,  $p = 0.303$ ). Similarly, the left FPN increased in functional connectivity from approximately 0.5 to 1.5 years post-injury (estimate = 0.15, SE = 0.05,  $p = 0.002$ ), contained a slope change in the opposite direction, from positive to negative at 1.5 years post-injury (estimate =  $-0.18$ , SE = 0.07,  $p = 0.008$ ), and was estimated to decline thereafter (estimate =  $-0.04$ , SE = 0.03,  $p = 0.254$ ).

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At approximately 0.5 years post-injury, patients showed hypoconnectivity compared with healthy, uninjured participants at baseline. Covariates were not significantly associated in any of the models. Findings of early improvement but a tapering and possible decline in connectivity thereafter suggest that compensatory effects are time-limited. These later reductions in connectivity mirror growing evidence of behavioral and structural decline in chronic moderate-to-severe TBI. Targeting such declines represents a novel avenue of research and offers potential for improving clinical outcomes.

**Keywords:** functional connectivity; longitudinal; magnetic resonance imaging; recovery; traumatic brain injury

## Introduction

Following moderate-to-severe traumatic brain injury (TBI), there is early resolution of neurological damage, with associated encephalomalacia, and presumed stability in the months and early years after.<sup>1</sup> However, contrary to still-prevailing assumptions, there is extensive evidence that deterioration persists during these early months and years post-injury.<sup>2–13</sup> For instance, longitudinal structural neuroimaging studies from our group have shown gray and white matter losses to cortical regions between 4.5 months and 2.5 years post-injury,<sup>4–6,9</sup> and subcortical regions between 5 and 20 months post-injury.<sup>2</sup> Colleagues have also observed longitudinal gray and white matter volume losses across 1 year post-injury,<sup>3</sup> white matter loss during the post-acute stage to at least 2 years post-injury,<sup>8</sup> as well as continued white matter integrity loss from 1 to 4 years post-injury.<sup>14</sup> Studies additionally demonstrate that white matter tends to be more susceptible to volume reductions than gray matter during the chronic stages of injury.<sup>3,15</sup>

Convergent with structural findings, longitudinal studies on cognition have demonstrated that a portion of patients show decline in some areas of functioning in the months and years post-injury.<sup>10–13</sup> Reviews have highlighted the importance of understanding functional connectivity changes in moderate-to-severe TBI to better delineate relationships between structure and behavior.<sup>16–18</sup> Further, understanding functional connectivity may provide insight into the mechanisms of how the brain recovers and deteriorates over the course of moderate-to-severe TBI, which is important for helping inform treatment and rehabilitation design.<sup>19</sup> However, there is a dearth of longitudinal research that examines functional connectivity in moderate-to-severe TBI. Only one longitudinal study has examined functional connectivity in moderate-to-severe TBI. Researchers found increases in connectivity from 3 to 6 months post-injury in the default mode network (DMN), central executive network, and salience network; but this study did not examine changes beyond 6 months.<sup>20</sup>

Cross-sectional studies of functional connectivity in the months and years post-injury show mixed findings as compared with healthy, uninjured participants. For example, resting-state functional magnetic resonance

imaging (fMRI) studies have observed hyperconnectivity in the DMN at 2.6 years post-injury<sup>21</sup> and at 4.1 years post-injury,<sup>22</sup> and in the DMN, central executive, sensorimotor, visual, and cerebellar networks at 6.7 years post-injury.<sup>23</sup> However, studies have also observed hypoconnectivity in TBI compared with healthy participants.<sup>21,24,25</sup> One such study examined patients with moderate-to-severe TBI (all male) at a range of 2–22.8 years post-injury, and found hypoconnectivity in brain regions associated with networks involved in cognition, including the precuneus, supramarginal gyrus, angular gyrus, superior parietal lobe, frontal pole, and middle and superior frontal gyri.<sup>25</sup>

Many cross-sectional studies in moderate-to-severe TBI have identified functional connectivity changes in brain regions and networks involved in various domains of cognition; however, to our knowledge, no study has examined networks involved in executive function in a hypothesis-driven manner. This domain of higher cognition is typically considered to include working memory, emotion regulation, cognitive flexibility, and inhibitory control.<sup>26–29</sup> There can be far-reaching effects of executive dysfunction after moderate-to-severe TBI, impacting inter-personal relationships, social cognition, reintegration into society, functional independence, and day-to-day functioning.<sup>30,31</sup> As such, executive dysfunction is the strongest predictor of functional and psychosocial outcomes post-injury.<sup>32–34</sup>

Two resting-state networks most exclusively linked to executive function are the frontoparietal network (FPN) and DMN.<sup>35</sup> Although the salience and dorsal attention networks have also been associated with executive function, they are more often associated with alerting and attention functions.<sup>36,37</sup> The FPN, sometimes referred to as the central executive network, primarily comprises the lateral prefrontal cortex, intraparietal sulcus, and ventral inferior temporal lobe.<sup>38</sup> It is crucial for multiple tests of executive function and cognitive control, including measures of task-switching, judgment, inhibitory control, and focal attention.<sup>38–40</sup> The DMN comprises the medial prefrontal cortex, inferior frontal and parietal cortices, posterior cingulate cortex, precuneus, and amygdala, and is engaged during internal thought processes, such as introspection, autobiographical recall, and daydreaming.<sup>41</sup>

Moreover, the DMN has increasingly been acknowledged for its involvement in executive function, including working memory and cognitive flexibility.<sup>42–46</sup>

This investigation therefore aimed to characterize longitudinal changes in resting-state functional connectivity in patients with moderate-to-severe TBI, focusing on the FPN and DMN because of their roles in executive function, which is often impaired in the chronic stages of moderate-to-severe TBI.<sup>10–13,20–24</sup> Patients were imaged at approximately 0.5, 1–1.5, and 3+ years post-injury, and we assessed change from the first to the second assessment, and from the second to the third assessment. Based on evidence that compensatory mechanisms can occur in the early stages of TBI recovery,<sup>19,21,22</sup> we hypothesized that patients would show functional connectivity increases in the FPN and DMN between the first and second assessment time-points. However, we reasoned that the high metabolic costs of hyperconnectivity would not be sustainable over time,<sup>47–49</sup> especially given evidence of neurodegeneration, which might reduce available metabolic resources.<sup>2–9</sup> As such, we also hypothesized that patients would show functional connectivity decreases in the FPN and DMN between the second and third assessment time-points.

## Methods

### Participants

Patients with moderate-to-severe TBI (i.e., who had a moderate TBI or a severe TBI) were referred to the inpatient neurorehabilitation program at the Toronto Rehabilitation Institute, University Health Network. This investigation was approved by the institutional research ethics board, and all participants provided written informed consent, in accordance with the Declaration of Helsinki.

This inception cohort study was derived from a larger prospective study, the Toronto Rehab TBI Recovery Study, which comprised 183 patient participants.<sup>2,4</sup> Inclusion criteria for the parent study were: (1) adults aged 18 or older; (2) fluency in English; and (3) clinical diagnosis of moderate-to-severe TBI, as determined by a Glasgow Coma Scale score less than 13,<sup>50</sup> length of post-traumatic amnesia greater than 1 day,<sup>51</sup> or positive findings on computerized tomography or MRI scan. An additional inclusion criterion for the current study was at least 1 resting-state blood-oxygen level dependent (BOLD) fMRI scan at 4 months and onwards. Exclusion criteria for the parent study were: (1) a history of TBI; (2) a history of psychotic disorders; (3) a history of neurological disorders mainly affecting the central nervous system; and (4) failure on the Test of Memory Malingering.<sup>52</sup> An additional exclusion criterion for this present study was the presence of lesions (i.e., contusions) in the frontal and parietal lobes, where the networks of

interest are found, and which affected normalization ( $n=$ three patients were excluded for this reason); this was assessed visually by a neuroradiologist.

Of the 42 patients who met inclusion and exclusion criteria, 1 had corrupted fMRI data and 1 had excessive motion warnings identified during image preprocessing. This resulted in a final sample of 40 patient participants and a total of 92 scans taken at approximately 0.5, 1–1.5, and 3+ years (range of 4 months to 6.7 years) post-injury (Table 1). Three of the scans at the third time-point were obtained later, at 4+ years post-injury, owing to procedural factors that precluded earlier testing. Of the 40 participants, 22 had scans at all three time-points, 15 had scans at two time-points, and 3 had scans at one time-point. Patients with all three scans did not differ from their counterparts (i.e., those with fewer than three scans) on any demographic, injury severity, or psychiatric characteristics, except for length of post-traumatic amnesia, where patients who did not have all three scans had a longer period of post-traumatic amnesia ( $p=0.042$ ,  $d=0.77$ ; see Supplementary Table S1). Given that our chosen statistical method, the linear mixed-effects model, allows data with missing points to be modeled overall by controlling for random effects, we included all eligible participants and scans in our analyses to maximize power and reduce type 2 errors.

Healthy, uninjured participants ( $n=25$ ) were included in the parent study based on the following inclusion criteria: (1) adults aged 18 or older; and (2) fluency in English. Exclusion criteria were: (1) history of TBI requiring hospitalization; (2) any disorder implicating the central nervous system; and (3) magnetic materials in the body. An additional inclusion criterion for this present study was that participants had at least one of two valid fMRI scans. This resulted in 17 healthy, uninjured participants who were scanned at two time-points, 5 of whom had only one scan (Table 1). Uninjured participants did not differ significantly from patients in age ( $p=0.897$ ,  $d=-0.04$  [small effect size]), years of education ( $p=0.185$ ,  $d=0.39$  [small effect size]), and time between first and second scans ( $p=0.069$ ,  $d=-0.61$  [medium effect size]), but differed in sex ( $p=0.004$ ,  $d=0.87$  [medium effect size]).

### Study design and procedures

This was an inception cohort study of prospectively collected, longitudinal data. Patients were followed longitudinally from approximately 5 months to 6.7 years post-injury and underwent one ( $n=38$ , approximately 5 months post-injury), two ( $n=37$ , approximately 1.1 years post-injury), or three ( $n=26$ , approximately 3 years post-injury) fMRI scans. Uninjured participants were scanned twice, with approximately 11 months

**Table 1. Demographic and Clinical Characteristics of Patients with TBI and Uninjured Participants**

Characteristic	Moderate-to-severe TBI patients (Mean ± SD)	Healthy Controls (Mean ± SD)	<i>p</i>	<i>d</i>
Age, years	39.53 ± 16.49 ( <i>n</i> = 40)	38.91 ± 15.57 ( <i>n</i> = 17)	0.897	-0.04
Sex (male: female)	28:12 ( <i>n</i> = 40)	5:12 ( <i>n</i> = 17)	0.004**	0.87
Education, years	13.92 ± 3.20 ( <i>n</i> = 39)	15.11 ± 2.71 ( <i>n</i> = 17)	0.185	0.39
Race	( <i>n</i> = 40)	ND	NA	NA
White	31			
Asian	6			
Other	3			
Learning disability history	( <i>n</i> = 39)	ND	NA	NA
No	36			
Yes	3			
Socioeconomic class	( <i>n</i> = 40)	ND	NA	NA
1 (major business and professional)	6			
2 (medium business, minor professional, technical)	10			
3 (skilled craftsmen, clerical, sales workers)	13			
4 (machine operators, semi-skilled workers)	6			
5 (unskilled labourers, menial service workers)	5			
Lowest recorded Glasgow Coma Scale score	6.63 ± 3.24 (severe) ( <i>n</i> = 35)	NA	NA	NA
Length of post-traumatic amnesia	( <i>n</i> = 36)	NA	NA	NA
1-7 days	9			
1-4 weeks	18			
>4 weeks	9			
Length of stay in acute care, days	33.35 ± 22.99 ( <i>n</i> = 37)	NA	NA	NA
Injury severity aggregate, z-score	0.0044 ± 0.83 ( <i>n</i> = 40)	NA	NA	NA
Time post-injury at scan 1	( <i>n</i> = 37)	NA	NA	NA
Months	4.97 ± 0.88			
Days	151.28 ± 26.67			
Time post-injury at scan 2 (patients), or Time between scans 1 and 2 (healthy, uninjured)	( <i>n</i> = 37)	( <i>n</i> = 12)	0.069	-0.61
Months	12.75 ± 1.50	12.13 ± 7.47		
Days	387.7 ± 45.72	350.29 ± 184.71		
Time post-injury at scan 3	( <i>n</i> = 32)	NA	NA	NA
Months	36.32 ± 13.97			
Days	1104.61 ± 424.86			
Injury Type	( <i>n</i> = 40)	NA	NA	NA
Motor vehicle accident	24			
Fall	12			
Assault	3			
Sports injury	1			
Baseline PAI Anxiety	46.46 ± 9.48 (average) ( <i>n</i> = 32)	ND	NA	NA
Baseline PAI Anxiety-Related Disorders	45.03 ± 9.79 (average) ( <i>n</i> = 32)	ND	NA	NA
Baseline PAI Depression	50.66 ± 11.87 (average) ( <i>n</i> = 32)	ND	NA	NA

Patients with moderate-to-severe TBI, *n* = 40; healthy, uninjured participants, *n* = 17.

\*\**p* < 0.01 between patients and controls.

A greater z-score of the injury severity aggregate represents higher injury severity. PAI scores are reported as T-scores.

Socioeconomic class categories were derived from Hollingshead and Redlich.<sup>53</sup>

SD, standard deviation; PAI, Personality Assessment Inventory; ND, no data; NA, not applicable.

between the baseline and follow-up scan. Linear mixed-effects modeling with a linear spline, which had a knot at 1.5 years post-injury, was employed to characterize FPN and DMN functional connectivity patterns in patients over time, as well as compared with uninjured participants.

### Image acquisition

Imaging data were acquired at Toronto General Hospital, University Health Network, using a General Electric Signa-Echospeed 1.5-T scanner (SIGNA EXCITE, GE Healthcare, Milwaukee, WI, USA), with an 8-channel receiver head coil configuration. Structural images were obtained first, with the following sequence: sagittal T1-weighted spin echo, repetition time of 300 msec, echo time of 13 msec, slice thickness of 5 mm without gap,

slice spacing of 2.5 mm, 256 × 128 matrix, and a 22-cm field of view. Resting-state, eyes-closed, BOLD fMRI data were obtained per the same geometric prescription but with the following gradient echo sequence: repetition time of 2000 msec, echo time of 40 msec, flip angle of 85 degrees. Participants were instructed to lie still in a supine position with their eyes closed and to maintain a rested state without falling asleep. The fMRI portion of the scan lasted 5 min.

### Image pre-processing and analysis

Pre-processing and analyses were conducted using the FMRIB Software Library (FSL version 5.0.1).<sup>54</sup> The first 5 volumes were removed to enable signal stabilization, and 145 volumes were analyzed. The pre-processing pipeline included: brain extraction with FSL BET,

motion correction with FSL MCFLIRT, spatial smoothing with a 5-mm Gaussian kernel, high pass temporal filtering with a frequency cutoff of 0.01 Hz using a Gaussian-weighted model, independent component analysis (ICA)-specific de-noising and reduction of head motion using FSL ICA\_AROMA (a validated data-driven approach for identifying and removing motion-related artifacts),<sup>55</sup> regression of white matter and cerebrospinal fluid signal with FSL FAST (threshold of 1 and 0.98, respectively), and linear registration with 12 degrees of freedom (df) to MNI152 space. Due to a logistical constraint, the information necessary for slice-timing correction was not available. Prior to analyses, all scans were visually reviewed to ensure lack of problematic contusions, artifacts, and/or excessive motion warnings.

A temporally concatenated group ICA with 25 dimensions was employed to identify major resting-state networks,<sup>22,56</sup> including our networks of interest, the DMN and right and left FPN. The number of dimensions chosen was determined based on pipelines used in the literature, and to capture all 10 major resting-state networks while preventing excessive capture of artifacts.<sup>23,56–59</sup> A dual regression was then performed (with 5000 permutations and threshold-free cluster enhancement, which comprised the following three stages: (1) utilizing the group ICA spatial maps to generate subject-specific time courses of connectivity; (2) utilizing these time courses to generate subject-specific spatial maps; and, (3) utilizing the subject-specific spatial maps in a group analysis to generate statistical maps for visualization and interpretation.<sup>60</sup> From the threshold-free cluster enhancement maps generated during stage 3 of the dual regression, significant clusters of the DMN and FPN ( $p < 0.01$ ) were extracted. These masks were then back-projected into stage 2 of the dual regression to obtain the mean parameter estimates of functional connectivity in significant regions of the DMN and FPN. These pre-processing and analyses steps were based on neuroimaging studies in the literature that have used similar pipelines for ICA.<sup>23,57–59</sup>

### Statistical analysis

Linear mixed-effects models were computed per network to characterize functional connectivity changes over time. This validated approach was chosen due to its ability to handle data missing at random, and to control for inconsistent variances over time, which can arise from an unequal number of follow-up scans per participant, and baseline and follow-up scans taken at different points in time.<sup>61,62</sup>

Statistical assumptions of data normality and homoscedasticity were checked for the dependent variable of mean parameter estimates of functional connectivity

using histograms and Q-Q plots.<sup>63</sup> Upon discovery that these data were not normally distributed, the data were log-transformed, which was consistent with a normal distribution.<sup>63,64</sup> Assumption tests were subsequently repeated and passed before model computation.

Uninjured participants were analyzed separately using a linear mixed-effects model to assess whether there was evidence of functional connectivity changes due solely to normal aging. The uninjured participant group was then analyzed in conjunction with the patients to provide a reference for possible effects of aging through the course of the longitudinal study. For each linear mixed-effects model, the fixed effects estimated population average functional connectivity and the relationship between healthy and patient groups, time post-injury, and covariates, including age, sex, and patient injury severity. All covariates were included as additive components in the models to control for their possible confounding effects.

Patient injury severity was determined by the calculation of an aggregate score, which comprised each patient's lowest Glasgow Coma Scale score, length of post-traumatic amnesia, and acute care length of stay. To place these characteristics on a comparable scale, they were transformed into z-scores. The three z-scores were then averaged; the averaged scores were again transformed into a z-score, which was then employed as our aggregate measure of injury severity. This procedure allowed us to incorporate all injury severity data into the model.

In addition to the aforementioned fixed effects, we accounted for three random effects in our models: (1) between-subjects variability of healthy, uninjured participants; (2) between-subjects variability of patients; and (3) the post-injury slopes over time in patients. A linear spline was also added to examine longitudinal functional connectivity in greater detail, where knot placement allowed for identification of directional changes at a specific time post-injury.<sup>65</sup> Knot placement was tested at 1, 1.5, 2, and 2.5 years post-injury for each network. These knots were chosen based on: our aim to characterize functional connectivity changes at both early and late chronic stages post-injury, previous findings of cognitive decline at 12+ months post-injury,<sup>10,11</sup> and the temporal proximity at which the second and third fMRI scans were performed (Table 1). This empirical approach was data-driven, and deemed appropriate given the unknown time course by which functional connectivity changes occur.

Model selection was then undertaken by comparing the log likelihood values between models corresponding to the same network, and choosing the position of the knot that produced the maximum log likelihood value.<sup>66</sup> Predicted estimates were then computed and used to visualize the group trajectories for each linear mixed-effects

model; this included data points up to 4 years post-injury only (and not up to the full range of 6.7 years post-injury), given the few number of observations beyond 4 years ( $n=3$ ). The models and associated Wald tests were used to estimate functional connectivity changes in the early and late chronic stages of injury, as well as the effects of different age groups on this relationship.

Model outputs included the following: (1) unstandardized estimates; (2) standard errors (SEs); (3) confidence intervals; (4) df; (5)  $t$ -values; and (6)  $p$ -values for fixed effects, which comprise the intercept, the slope of functional connectivity changes before and after the knot, change of slope (i.e., direction of functional connectivity patterns) at the knot, as well as covariate associations. Positive and negative estimates represent an increase and decrease in functional connectivity, respectively. The random effects are outputted as a correlation, where a value greater than zero suggests the magnitude of the effect for which the model has controlled. Variance inflation factor tests were performed to diagnose multicollinearity for the models.<sup>67</sup>

All analyses were computed in R v3.6.3.

### Exploratory analyses

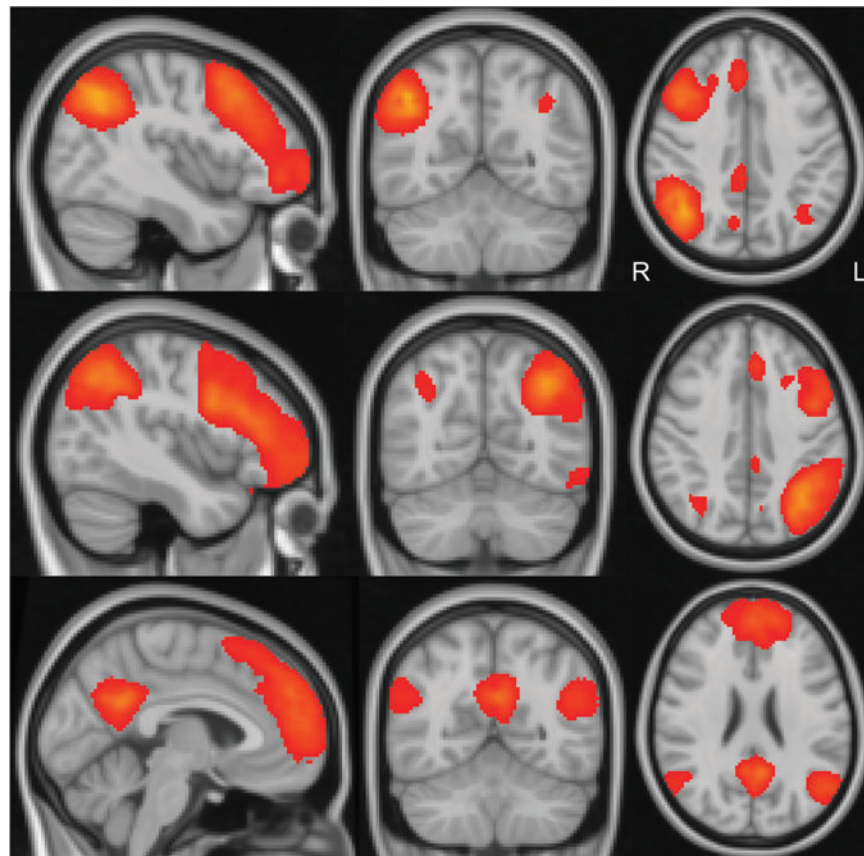
In addition to examining functional connectivity trajectories in the DMN and FPN, we explored trajectories in the salience network. The same imaging and statistical analysis parameters were used, except that significant clusters of the dual regression were thresholded at  $p < 0.05$  instead of  $p < 0.01$ .

Lastly, we computed correlations of the DMN-FPN time-series to explore inter-network interactions. We computed Pearson's correlation coefficients for the time-series between the DMN and left FPN, as well as between the DMN and right FPN. These correlation coefficients then underwent Fisher  $Z$ -transformation, and these normalized values were then used as responses in the linear mixed-effects models.

## Results

### Identification of resting-state networks

We obtained 25 components from our group ICA, and visually identified the major resting-state networks through comparisons to established literature.<sup>22,56</sup> Figure 1 illustrates the right FPN, left FPN, and DMN



**FIG. 1.** Group ICA identification of the three networks of interest. From top, to middle, to bottom: right FPN, left FPN, and DMN. Images are in radiological orientation. DMN, default mode network; FPN, frontoparietal network; ICA, independent component analysis; L, left; R, right.

**Table 2. Linear Mixed-Effects Model of Longitudinal Right FPN Connectivity Changes in Moderate-to-Severe TBI Patients**

<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>95% CI</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
(Intercept)	0.75	0.16	0.43, 1.07	75	4.61	0.000***
Group	-0.27	0.09	-0.45, -0.08	53	-2.82	0.007**
Age at assessment	0.002	0.002	-0.002, 0.006	75	1.16	0.250
Sex	0.08	0.07	-0.05, 0.22	53	1.23	0.225
Injury severity	-0.03	0.05	-0.12, 0.06	53	-0.70	0.487
Slope per year between 5 months and 1.5 years post-injury	0.19	0.07	0.05, 0.33	75	2.67	0.009**
Change of slope at 1.5 years post-injury	-0.29	0.11	-0.51, -0.07	75	-2.68	0.009**

<i>Random effects</i>	
<i>(Between-subject variability of participants)</i>	<i>SD</i>
Uninjured (between-control intercept)	0.22
Patients (between-patient intercept)	0.17
Years post-injury (between-patient slope)	0.15
Residual	0.21

-0.37 (with between-patient intercept)

Model is from approximately 0.5 to 3+ years post-injury.

Patients with moderate-to-severe TBI,  $n=40$ ; healthy, uninjured participants,  $n=17$ ; observations,  $n=135$ .

Numbers of observations per healthy control were 1 or 2 scans, and per patient were 1, 2, or 3 scans. Healthy controls were used as the reference group. Male was used as the reference sex.

\*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

CI, confidence interval; df, degrees of freedom; FPN, frontoparietal network; SD, standard deviation; SE, standard error; TBI, traumatic brain injury.

from the group ICA. The right and left FPN consisted of the inferior, middle, and superior frontal gyri, angular gyrus, posterior cingulate cortex, precuneus, and lateral occipital cortex. The DMN was defined by the precuneus, anterior and posterior cingulate cortices, paracingulate cortex, medial prefrontal cortex, inferior frontal cortex, and middle and inferior temporal gyri. Other key resting-state networks identified in our group ICA included the medial visual areas, lateral visual areas, occipital visual areas, sensorimotor network, cerebellar-brainstem network, salience network, and auditory network.<sup>22,56</sup>

### Model selection per network of interest

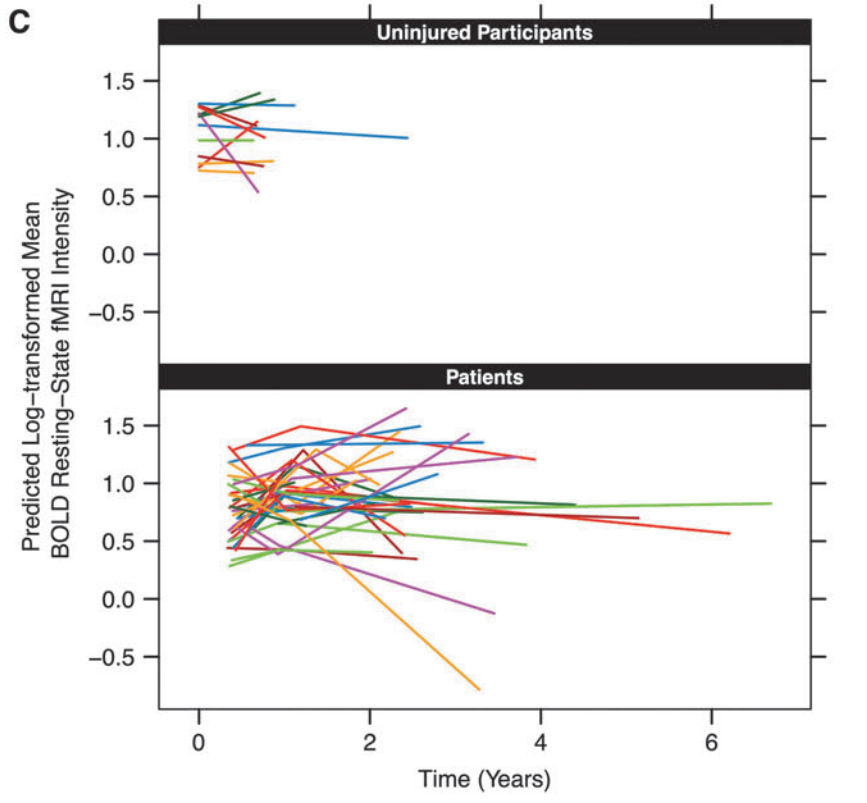
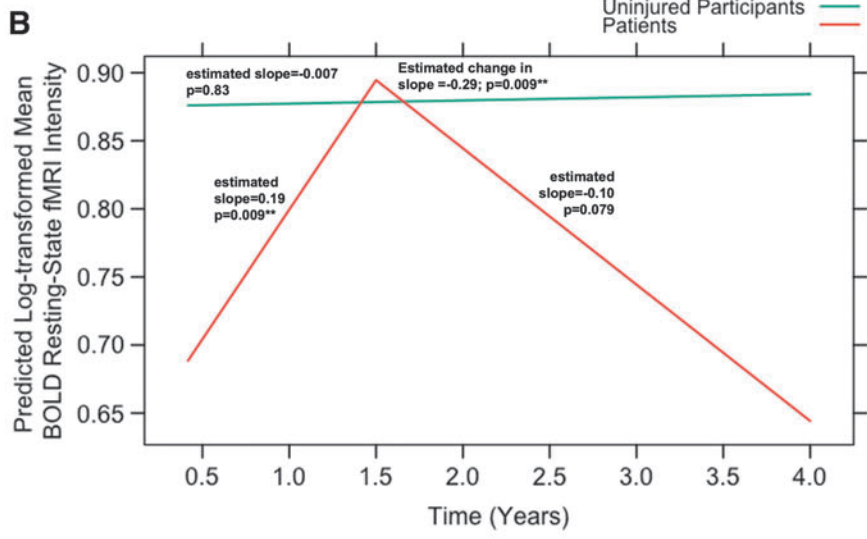
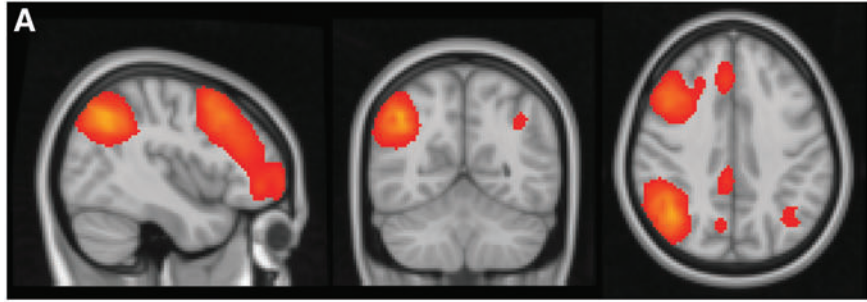
For each network of interest, four preliminary linear mixed-effects models were computed, one with each knot (1, 1.5, 2, or 2.5 years). The model that produced the largest log likelihood value per network of interest was then selected as the one of best fit. For all networks,

the DMN, and right and left FPN, knot placement was most optimal at 1.5 years post-injury, indicating that functional connectivity increased until 1.5 years post-injury and changed direction thereafter. See Supplementary Table S2 for a comparison of log likelihood values per model.

### Functional connectivity patterns in patients compared with healthy, uninjured participants from 0.5 to 1-1.5 years post-injury

In patient trajectories, we observed that functional connectivity increased in the right FPN, left FPN, and DMN between 0.5 and 1–1.5 years post-injury (first and second assessment time-points). Model results of longitudinal functional connectivity changes in the right FPN are reported in Table 2, and are visualized in Figure 2. In this network, there was an increase in intra-network functional connectivity from 0.5 years to 1.5 years post-injury

**FIG. 2.** Longitudinal functional connectivity changes of the right FPN from 0.5 to 3+ years post-injury. **(A)** Group ICA images of the right FPN in radiological orientation. **(B)** At the group level, patients showed hypoconnectivity at approximately 0.5 years post-injury compared with healthy, uninjured participants. The patient trajectories demonstrated functional connectivity increases between 0.5 and 1.5 years post-injury, a change in slope in the negative direction at 1.5 years post-injury, followed by marginal declines thereafter. Green represents the healthy, uninjured group; red represents the moderate-to-severe TBI patient group. **(C)** Each line represents the functional connectivity trajectory of an individual patient or healthy, uninjured participant. Predicted mean BOLD resting-state fMRI intensities were computed from the linear mixed-effects model of the right FPN with knot placement at 1.5 years post-injury. For patients, the x-axis of time is measured in years post-TBI. For healthy, uninjured participants, the first fMRI assessment (baseline assessment) lines up temporally and visually (in the graph) with approximately 0.5 years post-injury of patients (first assessment time-point); the x-axis of time for healthy, uninjured participants is therefore measured in years after baseline assessment. \*\* $p < 0.01$ . BOLD, blood-oxygen level dependent; fMRI, functional magnetic resonance imaging; FPN, frontoparietal network; ICA, independent component analysis; TBI, traumatic brain injury.



**Table 3. Linear Mixed-Effects Model of Longitudinal Left FPN Connectivity Changes in Moderate-to-Severe TBI Patients**

<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>95% CI</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
(Intercept)	0.98	0.12	0.75, 61.21	75	8.41	0.000***
Group	-0.20	0.07	-0.34, -0.06	53	-2.85	0.006**
Age at Assessment	0.00003	0.001	-0.003, 0.003	75	0.02	0.981
Sex	0.07	0.05	-0.02, 0.17	53	1.50	0.138
Injury severity	-0.01	0.03	-0.08, 0.05	53	-0.48	0.632
Slope per year between 5 months and 1.5 years post-injury	0.15	0.05	0.06, 0.24	75	3.26	0.002**
Change of slope at 1.5 years post-injury	-0.18	0.07	-0.32, -0.05	75	-2.72	0.008**

<i>Random effects</i>		
<i>(Between-subject variability of participants)</i>	<i>SD</i>	<i>Correlation</i>
Uninjured (between-control intercept)	0.17	
Patients (between-patient intercept)	0.10	
Years post-injury (between-patient slope)	0.05	0.074 (with between-patient intercept)
Residual	0.15	

Model is from approximately 0.5 to 3+ years post-injury.

Patients with moderate-to-severe TBI,  $n=40$ ; healthy, uninjured participants,  $n=17$ ; observations,  $n=135$ .

Numbers of observations per healthy control were 1 or 2 scans, and per patient were 1, 2, or 3 scans. Healthy controls were used as the reference group. Male was used as the reference sex.

\*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

CI, confidence interval; df, degrees of freedom; FPN, frontoparietal network; SD, standard deviation; SE, standard error; TBI, traumatic brain injury.

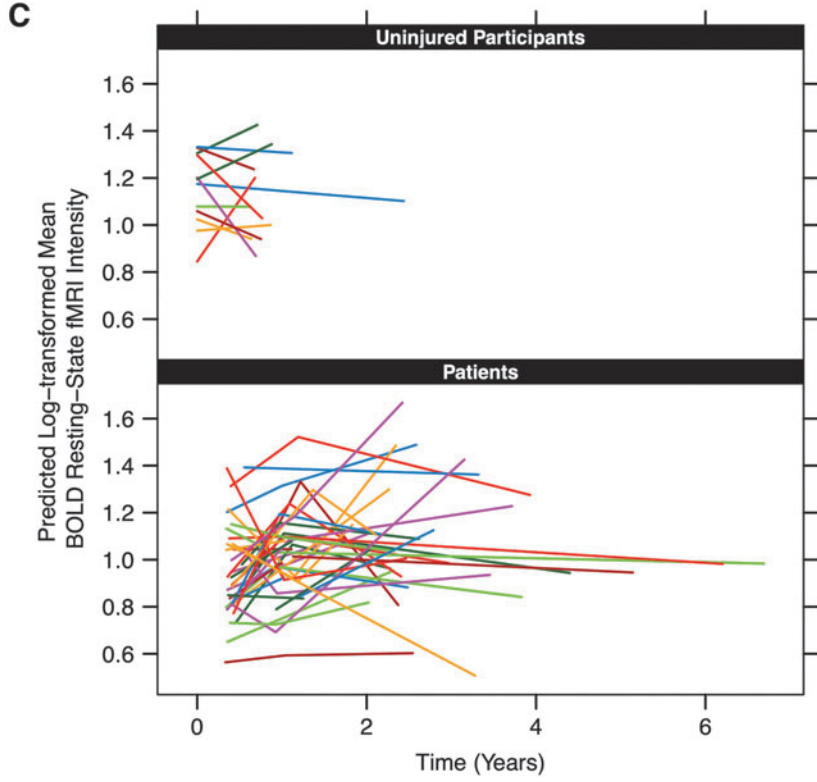
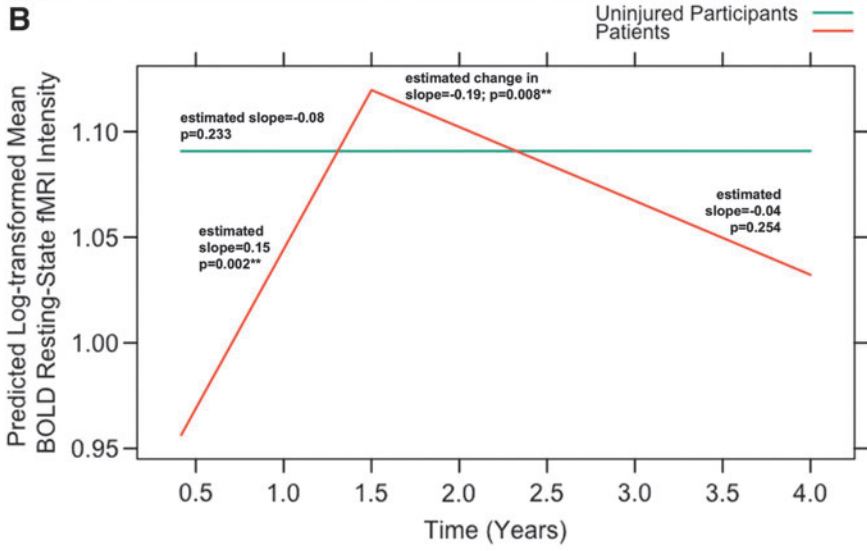
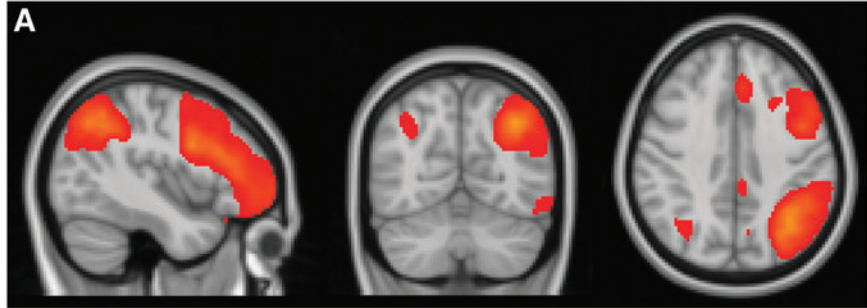
(estimate = 0.19, SE = 0.07,  $p = 0.009$ ), with evidence of change in slope in the opposite direction, from positive to negative at 1.5 years post-injury (estimate = -0.29, SE = 0.11,  $p = 0.009$ ). Figure 2 depicts right FPN trajectories (Fig. 2B) between groups, and (Fig. 2C) of individual patients and healthy, uninjured participants.

Linear mixed-effects modeling of the left FPN in patients revealed intra-network functional connectivity changes in the early-to-late chronic stages post-injury (Table 3, and Fig. 3). Functional connectivity increased from 0.5 years to 1.5 years post-injury (estimate = 0.15, SE = 0.05,  $p = 0.002$ ) and changed in slope in the opposite direction, from positive to negative at 1.5 years post-injury (estimate = -0.18, SE = 0.07,  $p = 0.008$ ). Figure 3 depicts left FPN connectivity trajectories (Fig. 3B) between groups, and of individual patients and healthy, uninjured participants (Fig. 3C).

Table 4 and Figure 4 summarize the longitudinal functional connectivity alterations in the DMN as predicted by a linear mixed-effects model with the knot of a linear spline at 1.5 years post-injury. Functional connectivity increased from 0.5 years to 1.5 years post-injury (estimate = 0.15, SE = 0.05,  $p = 0.006$ ) and changed in slope in the opposite direction, from positive to negative at 1.5 years post-injury (estimate = -0.19, SE = 0.08,  $p = 0.021$ ). Figure 4 depicts DMN connectivity trajectories (Fig. 4B) between groups, and of individual patients and healthy, uninjured participants (Fig. 4C).

Separate analyses of the healthy, uninjured participants demonstrated that functional connectivity did not change significantly over time in the right FPN (Supplementary Table S3A), left FPN (Supplementary Table S3B), or DMN (Supplementary Table S3C). Supplementary Table S4 shows that the trajectories of right

**FIG. 3.** Longitudinal functional connectivity changes of the left FPN from 0.5 to 3+ years post-injury. **(A)** Group ICA images of the left FPN in radiological orientation. **(B)** At the group level, patients showed hypoconnectivity at approximately 0.5 years post-injury compared to healthy, uninjured participants. The patient trajectories demonstrated functional connectivity increases between 0.5 to 1.5 years post-injury, a change in slope in the negative direction at 1.5 years post-injury, followed by estimated declines thereafter. Green represents healthy, uninjured group; red represents moderate-to-severe TBI patient group. **(C)** Each line represents the functional connectivity trajectory of an individual patient or healthy, uninjured participant. Predicted mean BOLD resting-state fMRI intensities were computed from the linear mixed-effects model of the left FPN with knot placement at 1.5 years post-injury. For patients, the x-axis of time is measured in years post-TBI. For healthy, uninjured participants, the first fMRI assessment (baseline assessment) lines up temporally and visually (in the graph) with approximately 0.5 years post-injury of patients (first assessment time point); the x-axis of time for healthy, uninjured participants is therefore measured in years after baseline assessment. \*\* $p < 0.01$ . BOLD, blood-oxygen level dependent; fMRI, functional magnetic resonance imaging; FPN, frontoparietal network; ICA, independent component analysis.



**Table 4. Linear Mixed-Effects Model of Longitudinal DMN Connectivity Changes in Moderate-to-Severe TBI Patients**

<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>95% CI</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
(Intercept)	0.90	0.13	0.63, 1.17	75	6.73	0.000***
Group	-0.21	0.08	-0.37, -0.06	53	-2.75	0.008**
Age at assessment	0.0003	0.002	-0.003, 0.004	75	0.16	0.870
Sex	0.08	0.06	-0.04, 0.19	53	1.38	0.173
Injury severity	-0.02	0.04	-0.10, 0.05	53	-0.64	0.525
Slope per year between 5 months and 1.5 years post-injury	0.15	0.05	0.04, 0.25	75	2.81	0.006**
Change of slope at 1.5 years post-injury	-0.19	0.08	-0.34, -0.03	75	-2.36	0.021*

<i>Random effects</i>		
<i>(Between-subject variability of participants)</i>	<i>SD</i>	<i>Correlation</i>
Uninjured (between-control intercept)	0.18	
Patients (between-patient intercept)	0.16	
Years post-injury (between-patient slope)	0.08	-0.36 (with between-patient intercept)
Residual	0.16	

Model is from approximately 0.5 to 3+ years post-injury.

Patients with moderate-to-severe TBI,  $n=40$ ; healthy, uninjured participants,  $n=17$ ; observations,  $n=135$ .

Numbers of observations per healthy control was 1 or 2 scans, and per patient were 1, 2, or 3 scans. Healthy controls were used as the reference group. Male was used as the reference sex.

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

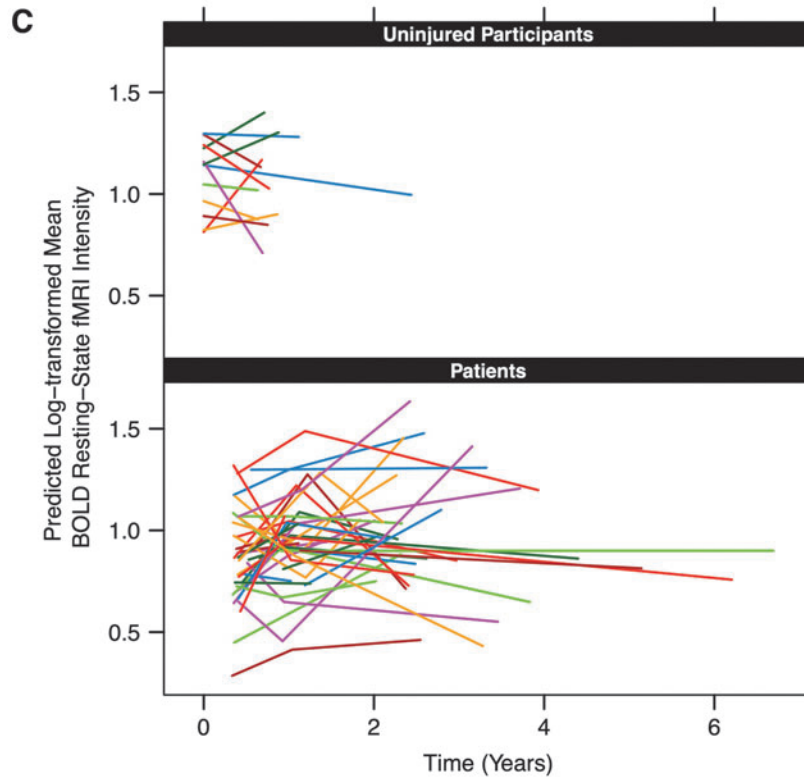
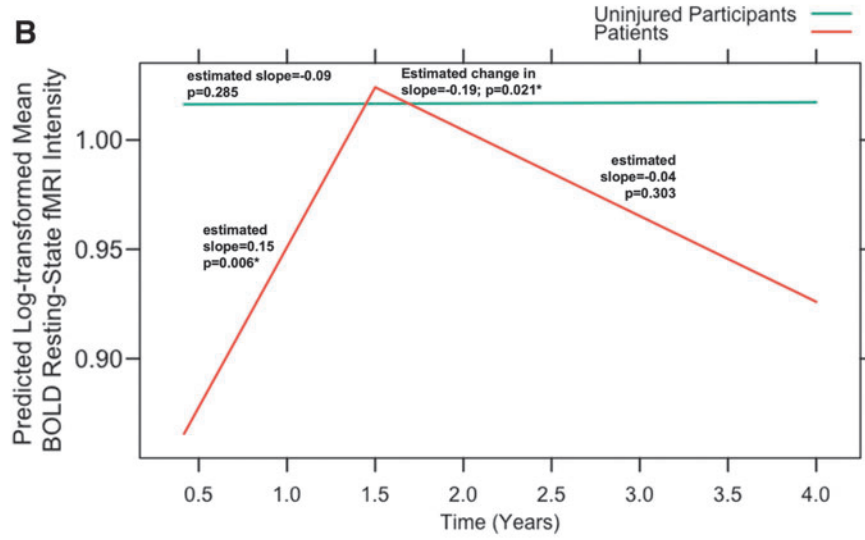
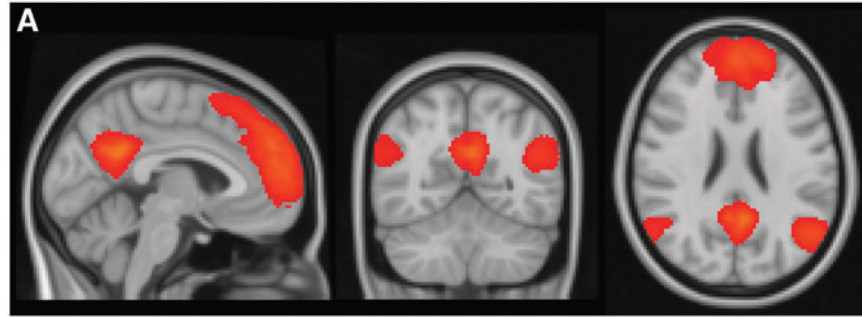
CI, confidence interval; df, degrees of freedom; DMN, default mode network; SD, standard deviation; SE, standard error; TBI, traumatic brain injury.

FPN functional connectivity are not identical for patients and healthy, uninjured participants ( $p=0.008$ ), with patients showing lower levels of intra-network connectivity at 0.5 years post-injury ( $p=0.026$ ). Similarly, Supplementary Tables S5 and S6 demonstrate that patients with moderate-to-severe TBI had lower levels of connectivity at 0.5 years post-injury in the left FPN ( $p=0.033$ ) and in the DMN ( $p=0.031$ ), respectively. Despite functional connectivity trajectories in patients demonstrating an increase between the first and second assessment time-points, there was no evidence of functional connectivity differences between groups in the right FPN at the second assessment time-point (Supplementary Table S4), left FPN (Supplementary Table S5), and DMN (Supplementary Table S6).

#### **Functional connectivity patterns in patients compared with healthy, uninjured participants from 1–1.5 to 3+ years post-injury**

In the right FPN of patients, functional connectivity showed marginal declines from 1.5 to 3+ years post-injury (Fig. 2 and Supplementary Table S4; estimate =  $-0.10$ , SE =  $0.06$ ,  $p=0.079$ ). Functional connectivity in the left FPN was estimated to decrease between 1.5 to 3+ years post-injury with respect to the trajectory before 1.5 years (Fig. 3 and Supplementary Table S5; estimate =  $-0.04$ , SE =  $0.03$ ,  $p=0.254$ ). Similarly, DMN connectivity was estimated to decrease in patients with respect to the trajectory before 1.5 years (Fig. 4 and Supplementary Table S6; estimate =  $-0.04$ , SE =  $0.04$ ,  $p=0.303$ ). However, compared with uninjured participants, there

**FIG. 4.** Longitudinal functional connectivity changes of the DMN from 0.5 to 3+ years post-injury. **(A)** Group ICA images of the DMN in radiological orientation. **(B)** At the group level, patients showed hypoconnectivity at approximately 0.5 years post-injury compared with healthy, uninjured participants. The patient trajectory demonstrated functional connectivity increases between 0.5 to 1.5 years post-injury, a change in slope in the negative direction at 1.5 years post-injury, followed by estimated declines thereafter. Green represents healthy, uninjured group; red represents moderate-to-severe TBI patient group. **(C)** Each line represents the functional connectivity trajectory of an individual patient or healthy, uninjured participant. Predicted mean BOLD resting-state fMRI intensities were computed from the linear mixed-effects model of the DMN with knot placement at 1.5 years post-injury. For patients, the x-axis of time is measured in years post-TBI. For healthy, uninjured participants, the first fMRI assessment (baseline assessment) lines up temporally and visually (in the graph) with approximately 0.5 years post-injury of patients (first assessment time-point); the x-axis of time for healthy, uninjured participants is therefore measured in years after baseline assessment. \* $p < 0.05$ . \*\* $p < 0.01$ . BOLD, blood-oxygen level dependent; DMN, default mode network; fMRI, functional magnetic resonance imaging; ICA, independent component analysis; TBI, traumatic brain injury.



was no evidence of functional connectivity differences in any of the three networks in patients at 3+ years post-injury (third assessment time-point).

### Addressing the effects of covariates

Comparing models with and without covariates demonstrated that the covariates, age, sex, and injury severity, were jointly not significant predictors of connectivity trajectories in the right FPN (chi-square = 3.66,  $df = 3$ ,  $p = 0.301$ ), left FPN (chi-square = 2.32,  $df = 3$ ,  $p = 0.509$ ), and DMN (chi-square = 2.24,  $df = 3$ ,  $p = 0.524$ ).

### Exploring functional connectivity patterns in the salience network

In healthy participants, functional connectivity in the salience network showed no evidence of change over the two time-points while controlling for the effect of aging ( $p = 0.4669$ ). In participants with moderate-to-severe TBI, connectivity in the salience network was not estimated to change from 0.5 to 1.5 years post-injury (estimate =  $-3.48$ ,  $SE = 5.92$ ,  $p = 0.559$ ). Similarly, change in slope was not estimated to occur at 1.5 years post-injury (estimate =  $3.44$ ,  $SE = 7.88$ ,  $p = 0.66$ ), or after 1.5 years post-injury (estimate =  $-0.04$ ,  $SE = 2.82$ ,  $p = 0.99$ ). Additionally, functional connectivity patterns of the salience network did not differ between groups ( $p = 0.0683$ ).

### Exploring correlations of the DMN-FPN time-series

The correlation coefficients of the time-series between the DMN and left FPN, as well as between the DMN and right FPN, were significantly different from 0. However, the correlation coefficients were not significantly related to the variables of group, age, sex, and time.

### Discussion

Using group ICA and linear mixed-effects modeling to examine longitudinal resting-state functional connectivity changes in the FPN and DMN, we found support for our hypothesis of functional connectivity increases between 0.5 and 1.5 years post-injury, and partial support for our hypothesis of functional connectivity decreases after 1.5 years post-injury. In line with the former hypothesis, patient trajectories of the DMN, left FPN, and right FPN demonstrated functional connectivity increases between 0.5 and 1.5 years post-injury, and also contained a slope change in opposite direction, from positive to negative at 1.5 years post-injury. Our latter hypothesis was partially supported, with the right FPN showing marginal decreases in functional connectivity after 1.5 years post-injury, whereas the DMN and left FPN were estimated to decline after 1.5 years post-injury. These findings are the first to characterize longitudinal functional

connectivity changes into the early and later chronic stages of injury in patients with moderate-to-severe TBI.

Previous functional connectivity studies in TBI have observed both hyperconnectivity and hypoconnectivity compared with healthy participants, although this literature was limited in sample size, had sex ratio imbalances, and was of retrospective and cross-sectional design.<sup>20,21,23–25</sup> However, the studies that observed hypoconnectivity compared with participants with TBI contained samples that were comparable to ours in demographics and clinical information.<sup>21,24,25</sup> For instance, one resting-state fMRI study of nine patients with moderate-to-severe TBI (all male,  $10.6 \pm 7$  years post-injury) observed hypoconnectivity in various brain regions implicated in the FPN and DMN, including the frontal pole, middle and superior frontal gyri, supramarginal gyrus, angular gyrus, superior parietal lobule, and precuneus.<sup>25</sup>

Konstantinou and colleagues<sup>25</sup> suggested that their findings were due to long-term structural brain connectivity and brain volume losses occurring as a result of TBI. Because our study examined functional connectivity changes at an earlier stage of injury (between 0.5, 1–1.5, and 3+ years post-injury), it is possible that our findings of hypoconnectivity at approximately 0.5 years post-injury reflect the initial neuropathological impacts of injury, such as cell death, inflammation, and cerebral edema.<sup>68</sup> More specifically, the loss of neurons and synaptic connections could result in reduced metabolic activity, which would be reflected by reduced BOLD signaling and hypoconnectivity compared with healthy, uninjured participants.<sup>49</sup>

Further, we observed connectivity increases in the FPN and DMN between 0.5 and 1.5 years post-injury in patients. These connectivity increases could be indicative of neural network inefficiency (without recovery) where the brain utilizes extra resources to compute the intended behaviors and functions.<sup>69</sup> Although this is possible, another common and accepted explanation in the literature for the phenomenon of hyperconnectivity is “compensation,” which involves a level of adaptation and optimization of brain connections.<sup>17,19,20,49</sup> Compensation postulates that neural networks adapt to loss of function by assuming the workload of missing network elements.<sup>19,70,71</sup> One mechanism of compensation is functional reorganization. This can involve both equipotentiality (where the non-damaged side of the brain supports lost function) and vicariation (where the brain rearranges regions nearby the site of damage to allow computation of the intended behaviors and functions).<sup>19,71–74</sup> Reviews on neuroplastic mechanisms underscore the importance of vicariation and equipotentiality during recovery after stroke,<sup>75,76</sup> and other forms of brain injury.<sup>77</sup>

Further, functional reorganization has been suggested to occur in human moderate-to-severe TBI.<sup>78,79</sup> For

example, in a study of brain blood flow during the completion of a memory retrieval task, positron emission tomography (PET) findings showed that adults with moderate-to-severe TBI showed hyperconnectivity in regions contralateral to those that compute memory functions, complemented by areas of hypoconnectivity compared with healthy participants.<sup>78</sup> Another study used resting-state magnetoencephalography to demonstrate that, compared with healthy participants, network strength (i.e., synchronicity) and network energetic costs were increased in patients with TBI at 5 months post-injury, before participating in neuropsychological rehabilitation.<sup>79</sup> However, these metrics decreased to a comparable level to healthy participants at 9.4 months post-injury, which was after rehabilitation, suggesting functional reorganization as a mechanism of action.<sup>79</sup>

More recent work in rat models also demonstrated that functional reorganization can occur after moderate-to-severe TBI, where studies have observed inter-hemispheric functional connectivity disruptions under resting-state fMRI,<sup>80</sup> and increased electroencephalographic (EEG) beta activity in the perilesional thalamocortical regions under optogenetic stimulation.<sup>81</sup> Taken together, the above results suggest that in our patient sample, compensatory functional reorganization may have led to an increased number or strength of connections within networks, resulting in increased functional connectivity from 0.5 to 1.5 years.

After the initial increases in functional connectivity, the trajectories of the FPN and DMN in patients showed significant change in direction at 1.5 years post-injury and marginal decline (in the right FPN) and estimated declines (in the DMN and left FPN) beyond 1.5 years post-injury. There are a number of possible explanations for the differences in rates between the early versus later time window, as discussed below. One explanation is the balance or competition between functional optimization (via compensatory functional reorganization and increased resource utilization) and chronically increased metabolic activity.<sup>48,49</sup> More specifically, it is now widely understood that higher levels of glucose metabolism correlates with greater amplitude signaling in resting-state fMRI and PET.<sup>82</sup> Studies have also correlated increased glutamatergic metabolism with increased BOLD activity in resting-state functional connectivity of the hippocampus,<sup>83</sup> and of the DMN.<sup>84</sup> Thus, increased BOLD activity likely reflects increased metabolic costs, and the estimated declines in functional connectivity we observed after 1.5 years post-injury may reflect neurons operating at or near maximum metabolic capacity.

With regards to the marginal decline observed in the right FPN, it is possible that progressive neurodegeneration may be implicated. As mentioned, both structural and cognitive deterioration have been observed in the chronic stages of moderate-to-severe TBI.<sup>2-13</sup> Cortical

and subcortical deterioration, as well as white matter losses have been observed from the early months post-injury out to at least 2.5 years.<sup>2-6,9,14,21</sup> Gray and white matter losses occurring in the first year after injury have also been associated with decreased performance on neuropsychological tests.<sup>3</sup> Similarly, white matter losses occurring up to 4 years post-injury have been associated with reductions in visuomotor speed.<sup>7</sup> Neurodegeneration would be expected to add further stress to the brain and could exacerbate the metabolic costs already required for recovery.<sup>49,85</sup> As Hillary and Grafman<sup>49</sup> argued in their review, the tipping point at which compensatory hyperconnectivity can no longer be maintained in many neurological diseases is attributable to neurodegeneration.

In our sample, it is likewise possible that 1.5 years post-injury marks the approximate time at which neurodegeneration begins to outcompete the brain's ability to maintain compensatory mechanisms of recovery. Although we did not observe hyperconnectivity compared with uninjured participants, we did observe early increases in the functional connectivity trajectories of patients. Interestingly, patterns of hyperconnectivity followed by hypoconnectivity compared with healthy participants have been reported in Alzheimer's disease, where hypoconnectivity occurs at a stage coinciding with relatively faster rates of neurodegeneration.<sup>86</sup>

The pattern of increased followed by marginally decreased connectivity also raises the question of whether overuse of neurons involved in early compensatory mechanisms may have caused harm or fatigue to the cells that have sustained chronically increased metabolic activity. For example, if the marginal and estimated declines indeed reflect neurons operating at or near maximum metabolic capacity, this could increase the risk of oxidative injury, whereby the mitochondria generate an increased quantity of reactive oxygen species that lead to apoptosis and chronic neuroinflammation.<sup>87</sup> To our knowledge, there has not been research on overuse injury conducted in human moderate-to-severe TBI.

However, reviews of mild TBI in animal models suggest that overuse injury can indeed occur; this involves a mismatch between energy supply and demand during the initial stages of recovery, leading to temporary states of hyperglycolysis and increased metabolic activity, followed by a state of hypometabolism, which ultimately impairs cognition.<sup>88</sup> Seminal work has also established that, in rats, excessive use of an injured limb can cause increased size of unilateral focal lesions in the injured sensorimotor cortex,<sup>89</sup> and that the rat brain is most vulnerable to overuse injury during the first 7 days post-injury, but not during the next 7 days.<sup>90</sup> If these concepts of overuse injury and fatigued neurons apply to human moderate-to-severe TBI, then that could be one reason for the marginal functional connectivity declines observed.

These functional connectivity findings and their proposed mechanisms have various clinical implications, including for therapeutic time windows. This concept is well-established for pharmacological treatment in moderate-to-severe TBI, where treating as early as 4 hours post-injury can be effective, but interventions with substantially longer therapeutic time windows are needed to maximize available options for all patients.<sup>91</sup> Broadening this concept, we speculate based on our findings that there are two specific therapeutic time windows for moderate-to-severe TBI recovery: (1) between 0.5 and 1.5 years post-injury, to capitalize on the greater potential for adaptive neuroplasticity and to augment the brain's natural recovery processes with cognitive or environmental enrichment; and/or (2) at 1.5 years post-injury (or later), with the aim of preventing or slowing brain deterioration. Interventions that may be useful include cognitive and environmental ones, which have been associated with experience-dependent adaptive neuroplasticity.<sup>61,92</sup> Our findings further suggest that functional connectivity trajectories has potential as a clinical biomarker to measure response to treatment, and to facilitate precision medicine research approaches.

### Limitations

We had fewer scans at the third time-point of assessment, which may have reduced our power to detect functional connectivity changes at 3+ years post-injury. Indeed, visual inspection after group ICA indicated that all networks at the third time-point were not as well defined as previous time-points. Although linear mixed-effects modeling is able to overcome the limitation of data missing at random, more data at the third time-point would have ensured greater stability in the model extrapolations. Our sample contained proportionately more males in the patient group—which is typical of TBI—as compared with the healthy, uninjured group. This may have affected the between-group comparison despite statistically controlling for sex as a covariate in the models, as females with TBI tend to show higher levels of connectivity than males with TBI.<sup>93</sup>

We also note that, due to our limited sample size, we were unable to include social determinants of health, such as years of education, or socioeconomic status, as covariates in our models and account for their potential effects on functional connectivity. Additionally, perhaps a healthy, uninjured brain is not the best comparator for determining magnitude of functional connectivity change in an injured brain. This is because the latter inherently starts with fewer cells and synapses, and what constitutes increased or decreased functional connectivity when a damaged brain begins with fewer cells and connections than a healthy one? This contrasts comparisons within the TBI group, which does not compare absolute values of activation and could thus obviate this issue. We addi-

tionally acknowledge that our study did not have another comparison group, such as an orthopedic injury group, which could otherwise help control for causes other than direct brain injury that may lead to substantial brain network changes.

Our study was also unable to determine the spatial extent of connectivity changes, which prevented testing for functional reorganization as a mechanism of recovery. Moreover, we have interpreted that our observed increasing connectivity represents an adaptive recovery mechanism (compensation), but further research is needed to verify this assumption. Like many resting-state BOLD fMRI studies, we also did not validate normal cerebrovascular reactivity in our sample of patients with moderate-to-severe TBI; it is therefore possible that dysregulated blood flow could have impacted the BOLD signals captured.<sup>94</sup> Lastly, our fMRI scans were obtained with a 1.5-T scanner (relatively lower signal-to-noise ratio than 3-T or 7-T scanners<sup>95</sup>), and we could not perform slice-timing correction due to logistical constraints; these factors may also have impacted our ability to detect connectivity differences across time and between groups.

### Future directions

Future studies with more data points at later stages post-injury (i.e., 3+ years) are needed to elucidate how long the marginal and estimated connectivity declines last, whether and when declines commence in all networks, and to what degree decline occurs (e.g., back to a level comparable to the acute or early chronic stages post-injury?). A better understanding of the mechanisms (i.e., compensatory effects, vicariation, equipotentiality, inefficient neural network functioning, metabolic cost increases, neurodegeneration, etc.) that underlie these functional connectivity alterations over time is also needed, such as using whole-brain inter-network connectivity analyses, and correlations with cognitive measurements. While controlling for dysregulated blood flow as a potential confounder, future studies can utilize task-based neuroimaging, as well as combine analytical approaches (i.e., group ICA, seed-based, and graph theory approaches) to determine the spatial extent of functional connectivity change and to test for functional reorganization. Our approach disregards how these changes are distributed within independent components, which precludes determining to what extent spatial heterogeneity plays a role or if there are common patterns of change across the networks. A valuable target of intervention research would be to reduce the metabolic costs of the early connectivity increases.

Future work could also examine the clinical utility of longitudinal functional connectivity as biomarkers of recovery to help track intervention progress and personalize treatment plans. Our study raises several important

questions that future studies can address. For instance, are functional connectivity increases an adaptive form of compensation, or could they signal the overtaking of networks? Could the marginal and estimated functional connectivity declines represent a form of maladaptive neuroplasticity? What does experience-dependent neuroplasticity mean for the types of therapeutic interventions needed at different times post-injury? Future research will be crucial to better understand recovery and decline mechanisms, and to optimize interventions to improve moderate-to-severe TBI outcomes.

## Conclusions

Previous cross-sectional studies have shown that functional connectivity changes occur after moderate-to-severe TBI, but did not examine the trajectory of these changes. Our study provides the novel longitudinal findings that functional connectivity increases from 0.5 to 1.5 years post-injury in the FPN and DMN in moderate-to-severe TBI, with evidence for marginal declines thereafter. These findings provide indirect evidence of time-limited compensatory mechanisms of recovery, and suggest a need to examine how to prolong compensatory mechanisms to enhance outcomes. They also raise the question of when to intervene to maximize clinical outcomes. Future research is needed to test the mechanistic hypotheses proposed in our study.

## Transparency, Rigor, and Reproducibility Summary

The study was not formally registered, as it was conceptualized as part of a larger prospective study, the Toronto Rehab TBI Recovery Study.<sup>2,4</sup> The analysis plan was not formally pre-registered, but the team members with primary responsibility for the analysis (lead author and statistician co-author) certify that the analysis plan was pre-specified. A sample size of 42 participants with TBI, and 25 healthy, uninjured participants, was planned based on inclusion and exclusion criteria; the actual sample size was 40 participants with TBI, and 17 healthy, uninjured participants, whose imaging data were successfully analyzed. All equipment and software used to perform imaging and preprocessing are widely available from commercial sources.<sup>54,55</sup> The key inclusion criteria and outcome evaluations are established standards.<sup>2,4,50–52</sup> Anonymized data and analytic code are not available in a public repository, but may be available by e-mailing the corresponding author.

## Acknowledgments

The authors thank the late Joanna Glazer for her immense contributions to the study data collection and processing, Julien Poublanc for advice during image preprocessing, Osvaldo Espin-Garcia for statistical advice, and Layan Elfaki for help with data transfer. The authors also

thank all participants for their involvement throughout the study. Chung Yan Isis So was supported by a CIHR Frederick Banting and Charles Best Canada Graduate Scholarship-Master's and an Ontario Graduate Scholarship. A version of this manuscript exists in the University of Toronto Thesis Repository, TSpace.

## Authors' Contributions

The authors contributed as follows. Chung Yan Isis So: conceptualization, methodology, software, formal analysis, data curation, investigation, visualization, writing—original draft; Liesel-Ann Meusel: methodology, data curation, formal analysis; Bhanu Sharma: methodology, data curation, formal analysis; Georges Monette: methodology, software, formal analysis; Brenda Colella: investigation, project administration of parent study, conceptualization of parent study; Anne Wheeler: conceptualization, methodology, supervision; Jennifer Rabin: conceptualization, methodology, supervision; David Mikulis: conceptualization, validation of pre-processed images, supervision; Robin Green: conceptualization, methodology, validation, investigation, project administration, writing—original draft, supervision, funding acquisition. All authors were involved in writing—review and editing.

## Funding Information

This study was funded by the Canadian Institutes of Health Research (#MOP-67072 and #MOP-86704), Physicians Services Incorporated Foundation (#12-43), and the Natural Sciences and Engineering Research Council of Canada (#UT458054).

## Author Disclosure Statement

No competing financial interests exist.

## Supplementary Material

Supplementary Table S1  
Supplementary Table S2  
Supplementary Table S3  
Supplementary Table S4  
Supplementary Table S5  
Supplementary Table S6

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