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Adaptation after mild traumatic brain injury

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8. The Default Mode Network as a Biomarker of Persistent Complaints After Mild Traumatic Brain Injury: A Longitudinal fMRI Study

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Abstract

The aims of this study were: (1) to investigate longitudinal functional connectivity of resting-state networks in patients with and without complaints after uncomplicated mild traumatic brain injury (mTBI), and (2) to determine the value of network connectivity in predicting persistent complaints, anxiety, depression and long-term outcome. Thirty mTBI patients with (≥ 3) post-traumatic complaints at two weeks post-injury, 19 without complaints, and 20 matched healthy controls were selected for this study. Resting-state fMRI was performed in patients at one month and three months post-injury, and once in healthy controls. Independent component analysis (ICA) was used to investigate the default mode, executive and salience networks. Persistent post-traumatic complaints, anxiety and depression were measured at three months post-injury and outcome was determined at one year post-injury. Within the group with complaints, higher functional connectivity between the anterior and posterior components of the default mode network at one month post-injury was associated with a higher number of complaints at three months post-injury ($\rho=0.59$, $p=0.001$). Minor longitudinal changes in functional connectivity were found for patients with and without complaints after mTBI, which were limited to connectivity within components of the default mode network. Furthermore, a minor difference in longitudinal connectivity within one of the components of the default mode network was found between patients with and without complaints. No significant results were found for the executive and salience network. In conclusion, current results suggest that the default mode network may serve as a biomarker of

persistent complaints in patients with uncomplicated mTBI.

Introduction

The vast majority of the traumatic brain injury (TBI) population comprises patients at the milder end of the injury severity spectrum (Roozenbeek et al. 2013; Cassidy et al. 2014). Patients with a mild TBI (mTBI) often report post-traumatic complaints in the acute phase post-injury (Cassidy et al. 2014). These acute complaints evolve into persistent complaints in a quarter of the patients, with complaints in the cognitive and affective domain being most persistent (Dischinger et al. 2009; Lundin et al. 2006; Ettenhofer & Barry 2012; Ponsford et al. 2011; de Koning et al. 2016; Cassidy et al. 2014). Despite these lasting complaints, neuropsychological performance has usually returned to normal levels one to three months after injury (Carroll et al. 2014; Rohling et al. 2011; Dikmen et al. 2016), and routine magnetic resonance imaging (MRI) scans often shows no intra- or extra-axial pathology (Hofman et al. 2001; Hughes et al. 2004; Yuh et al. 2013).

There is abundant evidence that (pre-injury) psychological factors have a strong influence on whether or not (sub-)acute complaints convert into chronic complaints. An important factor is coping, which is one's capacity to adapt to psychological stressors. In general, an active coping style, including positive thinking, is considered beneficial, whereas passive coping with worrying is viewed as maladaptive (Anson & Ponsford 2006; Linley 2012). A key aspect of coping is the ability to regulate negative emotions. The importance of emotion regulation is underlined by the close relationship between post-traumatic complaints, anxiety and depression after mTBI (van der Horn et al. 2013; Stulemeijer et al. 2007; Hou et al. 2012). Negative illness perception (e.g. the belief that symptoms will have long-lasting negative consequences) further contributes to the persistence of post-traumatic complaints (Whittaker et al. 2007; Hou et al. 2012). It seems likely that the interaction between maladaptive coping and negative illness perception increases attention to perceived symptoms and causes anxiety and depression in patients with mTBI, resulting in long-lasting complaints and disability. This process may be prevented by timely psychological interventions aimed at enhancing coping skills and reducing unrealistic illness perception and expectations (Quoidbach et al. 2015; Sheppes et al. 2015; Beutler et al. 2011; Bell et al. 2008; Silverberg et al. 2013).

In order to understand the neural substrate underlying the persistence of complaints after mTBI, more knowledge is needed regarding longitudinal changes in functional networks after mTBI (McDonald et al. 2012; Mayer et al. 2011; Harm J. van der Horn, Liemburg, Aleman, et al. 2016). Several longitudinal functional MRI (fMRI) studies have been conducted on functional networks in mTBI (Messe et al. 2013; Sours et al. 2014; Zhu et al. 2015; Meier et al. 2016; Sours et al. 2015). These studies have shown increases as well as longitudinal decreases in functional connectivity of brain networks in patients with mTBI, that are thought to reflect delayed injury effects or compensatory mechanisms. In particular, disruptions in

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functional connectivity have been found within and between the Default Mode Network (DMN), Executive Networks (EN) and Salience Network (SN), which appear to be related to the presence and scores of post-traumatic complaints, anxiety and depression (Mayer et al. 2011; Sours et al. 2013; Sours et al. 2014; Sours et al. 2015; Zhou et al. 2012). Whereas the DMN is primarily involved in internally focused mental processes, such as mind wandering, the EN are mainly switched on during externally directed mental processes, for instance when performing a cognitive task (Menon & Uddin 2010). The SN facilitates shifting between these networks and associated mental states (Seeley et al. 2007). Thus, network findings in mTBI so far indicate that disturbances of internally and externally directed mental processes could be underlying maladaptive behavior leading to persistent post-traumatic complaints and mood disturbances (Harm J. van der Horn, Liemburg, Aleman, et al. 2016). Further investigation into this matter is important for developing biomarkers that can be used for better identification of patients who are prone to suffer from persistent complaints, to monitor and improve psychological interventions, and to develop tailored treatment programs for patients with mTBI.

We conducted a longitudinal resting-state fMRI study in patients with and without post-traumatic complaints in the subacute phase after uncomplicated mTBI. We used independent component analysis (ICA) to examine longitudinal changes in functional connectivity within and between components of the DMN, EN and SN, and tested for group differences in connectivity between patients with and without complaints. An additional research goal was to assess whether functional network connectivity at one month post-injury was associated with persistent complaints and emotional distress at three months post-injury and outcome at one year post-injury.

Materials and methods
Study participants
This fMRI study was part of a multi-center cohort study (UPFRONT-study) on outcome after mTBI conducted between January 2013 and January 2016 in three level 1 trauma centers in the Netherlands (University Medical Center Groningen, St. Elisabeth Hospital Tilburg and the Medical Spectrum Twente). The definition of mTBI was based on the American Congress of Rehabilitation Medicine criteria: admission Glasgow Coma Score of 13-15, loss of consciousness ≤ 30 minutes and post-traumatic amnesia ≤ 24 hours (Kayd et al. 1993). Patients with mTBI were included if aged >18 years. The following exclusion criteria were applied: neurological or psychiatric co-morbidity, admission for prior TBI, drug or alcohol abuse and mental retardation.

Patients who suffered from post-traumatic complaints at two weeks post-injury (PTC-present) were asked to participate in a randomized controlled trial on the

effects of an early psychological intervention on recovery after mTBI (trial number: ISRCTN86191894) (Scheenen et al. 2017). The following inclusion criteria were used: age 18-65, no lesions on admission head computed tomography (CT) scans and having paid work or studying at time of injury. Patients were randomized for either cognitive behavioral therapy (CBT) or telephonic counseling (TC). Patients included in this trial were also asked to partake in the fMRI study. Contraindications for MRI (implanted ferromagnetic devices or objects, pregnancy or claustrophobia) were used as additional exclusion criteria. For the current study, only the fMRI data were used; the results of the total intervention study are subject of another paper (Scheenen et al., manuscript under revision). To control for a possible influence of treatment condition on our longitudinal fMRI results in the PTC-present group, we made additional comparisons between the CBT and TC groups.

In addition to PTC-present patients, a group of patients without post-traumatic complaints at two weeks post-injury (PTC-absent) was recruited for this fMRI study (age 18-65 years, no lesions on CT). Lastly, a group of 20 healthy controls (HC) was recruited, which was matched to the total mTBI group with respect to age, sex, education level and handedness. This group consisted of 70% male and 85% right handed subjects, with a median age of 30 (range: 18-61) and a median education level of 6 (range: 2-7) according to the Verhage classification system (Verhage 1964).

The study was approved by the local Medical Ethics Committee of the UMCG; written informed consent was obtained from all participants. All procedures were carried out according to the declaration of Helsinki.

Clinical measures

Self-reported complaints were measured with a 19 item Head Injury Symptoms Checklist (HISC) administered two weeks and three months post-injury (van der Naalt et al. 1999; de Koning et al. 2016). Patients had to rate presence of current and pre-injury complaints on a 3-point Likert scale ranging from 0 to 2 (0 = never, 1 = sometimes, 2 = often). Total number of complaints and severity of complaints (summation of all [current - pre-injury] scores) were calculated. Having post-traumatic complaints was defined as reporting ≥3 or more complaints (regardless of severity) at two weeks post-injury, with at least one complaint within the cognitive (including forgetfulness, poor concentration, slowness, fatigue and increased need for sleep) and/or affective domain (including irritability, reduced tolerance for noise and anxiety). Having no complaints was defined as reporting <3 complaints.

Feelings of anxiety and depression were measured at two weeks and three months post-injury using the Hospital Anxiety and Depression Scale (HADS) consisting of seven anxiety (HADS-A) and seven depression (HADS-D) related items (each item with a 4-point Likert scale ranging from 0 to 3) (Zigmond & Snaith

1983). Sum scores of HADS-A and HADS-D scores were used for analyses.

Outcome was determined at 12 months post-injury using the Glasgow Outcome Scale Extended (Wilson et al. 1998). This structured interview measures outcome on an eight-point scale: 8 = good recovery, 7 = suboptimal recovery, 6 = upper moderate disability, 5 = lower moderate disability, 4 = upper severe disability, 3 = lower severe disability, 2 = vegetative state and 1 = death. Outcome scores were dichotomized into: favorable (GOSE-score = 8) and unfavorable (GOSE < 8) outcome.

Behavioral data analyses

The Statistical Product and Service Solutions (SPSS; version 22, Released 2013, IBM Corp., Armonk, NY) was used for data analyses. Normality of data was assessed using Shapiro-Wilk tests. Testing for group differences was done with one-way analysis of variance (ANOVA) for normally distributed continuous variables and Kruskal-Wallis and Mann-Whitney U tests for non-normally distributed continuous variables. For nominal and ordinal variables, Pearson's chi-square tests were used.

MRI acquisition

Patients underwent scanning at four weeks (first visit) and three months (follow-up visit) post-injury. For PTC-present patients, scans were made before and after completion of either the CBT or TC sessions. The interval between scans for PTC-absent patients was matched to that of the PTC-present group. Healthy controls underwent scanning once.

A 3.0 T Philips Intera MRI scanner (Phillips Medical Systems, Best, The Netherlands) equipped with a 32-channel SENSE head coil was used for image acquisition. A high resolution transversal T1-weighted sequence image was made for anatomical reference (TR 9 ms; TE 3.5ms; flip angle 8°; FOV 256x232x170 mm; reconstructed voxel size 1x1x1 mm). For resting-state imaging, three-hundred T2*-weighted echo planar imaging volumes were acquired with slices aligned in the anterior commissure (AC)-posterior commissure (PC) plane and recorded in descending order (TR 2000 ms; TE 20 ms; FOV 224x224x136.5 mm; reconstructed voxel size 3.5x3.5x3.5 mm). Participants were instructed to close their eyes and to stay awake.

The following sequences were used to examine the presence of microhemorrhages: coronal T2-gradient echo (TR 875 ms; TE 16 ms; FOV 230x183x199 mm; reconstructed voxel size 0.45x0.45x4 mm) and transversal susceptibility weighted imaging (TR 35ms; TE 15 ms; FOV 230x183x150 mm; reconstructed voxel size 0.45x0.45x1 mm). These scans were assessed for traumatic lesions by an experienced neuroradiologist. Twelve patients (24.5%) had one or more microhemorrhages. There was no difference in number of microhemorrhagic

lesions between PTC-present (median = 0, range: 0 - 37) and PTC-absent (median = 0, range: 0 - 26) patients (U = 284, p = 0.967). None of the HC had lesions.

FMRI preprocessing
Statistical Parametric Mapping (SPM12 Wellcome Department, University College London, London, England) implemented in Matlab (version R2014a; MathWorks, Natick, MA, USA) was used for preprocessing, which consisted of slice timing correction, image realignment to the first functional image, co-registration of functional images with individual participants' T1-weighted images, normalization using diffeomorphic nonlinear registration tool (DARTEL) (isotropic voxels of 3x3x3 mm) to the MNI template and smoothing (8 mm full-width at half maximum (FWHM) Gaussian kernel).

Independent component analysis
Group ICA of fMRI Toolbox (GIFT) version 4.0a, implemented in Matlab, was used for spatial ICA (Calhoun et al. 2001). The mean number of independent components was estimated using Minimum Description Length (MDL) and Akaike's Information Criterion (Li et al. 2007). Following subject-specific PCA (1st step: 44 components, 2nd step: 29 components), group ICA was performed with 29 estimated components and ICASSO was repeated 20 times to establish stability of component decomposition (Himberg et al. 2004). Back-reconstruction was done using spatial temporal regression and results were scaled to the original data (i.e. % signal change). Identification of neural components was done by H.J.v.d.H. and E.J.L. independently, using previously published literature and spatial regression of network templates provided with GIFT. Differences were discussed until consensus was reached. Components corresponding with the DMN, EN and SN were selected for further analyses.

Within-component functional connectivity
Within-component functional connectivity (FC) was analyzed using the Statistical nonParametric Mapping (SnPM13) toolbox (Nichols & Holmes 2001; Eklund et al. 2016) in SPM12. A between-group ANOVA was used to test for FC differences between HC, PTC-present and PTC-absent groups during visit one and two. Longitudinal effects were examined for the PTC-present and PTC-absent groups using the paired t-test function. Differences in longitudinal effects were tested between the PTC-present and PTC-absent, and between the CBT and TC groups. To this end, spatial maps from scans obtained during the initial visit were subtracted from those obtained during the second visit using the imCalc function in SPM12. Subsequently, the resulting images (reflecting the slope of within-component FC) were entered into a two sample *t*-test. All analyses in SnPM were conducted using

10,000 random permutations, and results were deemed significant when surviving voxel-level family wise error (FWE) correction at α = 0.05. To facilitate future meta-analyses, unthresholded SnPM output files are available upon request.

Between-component functional connectivity
Between-component FC was computed using the MANCOVAN toolbox in GIFT (E. A. Allen et al. 2011). Prior to calculation of FC, component time-courses were detrended and despiked using 3dDespike (AFNI 1995), and filtered using a fifth-order Butterworth low-pass filter (<0.15 Hz). Functional connectivity values were extracted from the mancovan_results_fnc.mat output and further analyzed using in-house developed permutation scrips in Matlab. First, network connectivity during visit one and two was tested between HC, PTC-present and PTC-absent groups. Second, longitudinal FC effects were examined for the PTC-present and PTC-absent groups using tests for paired samples. Third, to investigate group differences in longitudinal effects of between-component FC, slopes of FC (i.e. FC at follow-up visit minus FC at first visit) were tested between the PTC-present and PTC-absent groups, and between CBT and TC groups. All permutation tests were conducted using 10,000 random permutations. Alpha was set at 0.05 and type I errors were controlled using the Simple Interactive Statistical Analysis (SISA) Bonferroni approach, similar to (Li et al. 2014). Contrary to traditional Bonferroni (α/number of tests or dependent variables), this method accounts for the covariance between dependent variables (i.e. component pairs), which was defined as the mean of absolute values in one triangular part of the component pair matrix. Effect sizes were calculated in Matlab using the common language effect size statistic (CL) (McGraw & Wong 1992). Tables containing uncorrected results were added as Supplementary Material.

Functional connectivity related to clinical measures
For PTC-present patients, associations between within-component FC during the first visit and complaint (number and severity), HADS-A and HADS-D scores at three months post-injury were examined using simple regression in SnPM. Also within the PTC-present group, differences in within-component FC during first visit between patients with a favorable and unfavorable outcome (based on GOSE) at 12 months post-injury were assessed using a two sample t-test in SnPM. Unthresholded SnPM output files are available upon request.

Regarding between-component FC, Spearman's rank correlations were calculated between FC values during visit one and complaint, HADS-A and HADS-D scores at three months post-injury using Matlab (α=0.05 with SISA Bonferroni adjustments). Confidence intervals (CI) were calculated using Fisher's z-transformation (95% CI=tanh (arctanh(r)± $\frac{1.96}{\sqrt{n-3}}$)) (Fisher 1915). Differences in

between-component FC during the first visit between patients with a favorable and unfavorable outcome were examined using permutation tests in Matlab. Uncorrected results were added as Supplementary Material.

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Results

Patient characteristics

Figure 1 shows the patient inclusion flowchart. Of the 91 PTC-present patients enrolled in the total intervention study, 30 were included for fMRI (30% of the total CBT-group and 36% of the total TC-group). Twenty PTC-absent patients were included, of whom one did not return for follow-up scanning. Patient characteristics are listed in Table 1.
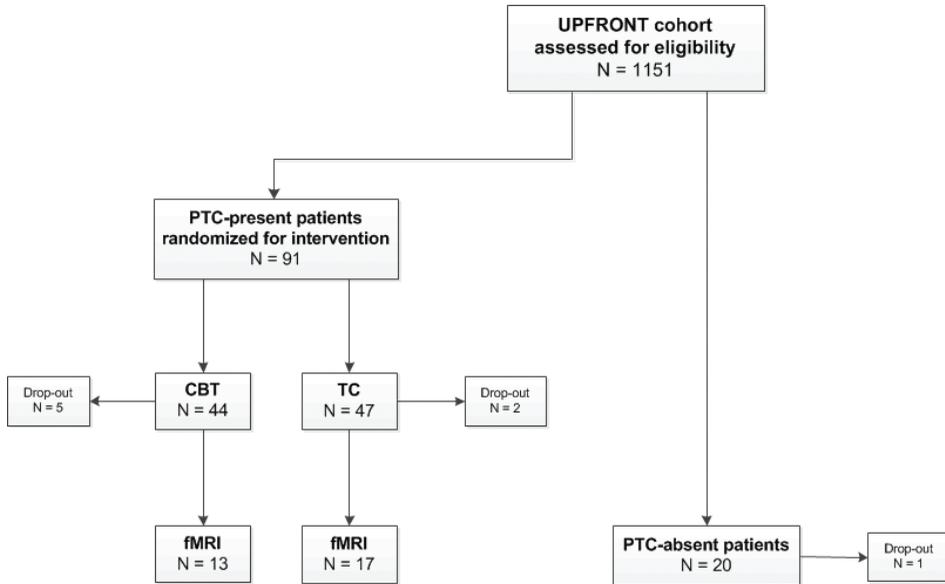

Figure 1: *Patient inclusion flowchart.*

Table 1: Patient characteristics.

	PTC-present (n=30)		(3) PTC-absent	Diff. 1-2-3	
	(1) CBT (n=13)	(2) TC (n=17)	(n=19)	Statistic (df)	P
Age, median (range), years	27 (19-54)	36 (21-61)	30 (20-62)	$K = 0.87$ (2)	0.647
Sex, % male	54	53	90	$X^2 = 6.912$ (2)	0.032
Education level, median (range)[a]	5 (4-7)	6 (5-7)	6 (2-7)	$X^2 = 9.882$ (8)	0.273
Handedness, % right	100	77	79	$X^2 = 3.034$ (2)	0.219
Interval injury to 1st MRI, median (range), days	34 (24-62)	32 (22-52)	32 (22-69)	$K = 1.204$ (2)	0.548
Interval injury to 2nd MRI, median (range), days	89 (76-207)	94 (61-114)	94 (77-126)	$K = 0.136$ (2)	0.934
GCS-score, median (range)	15 (13-15)	14 (13-15)	15 (13-15)	$K = 1.952$ (2)	0.377
Injury mechanism:					
Traffic, % of group	39	59	47	$X^2 = 1.254$ (2)	0.534
Falls, %	61	23	48	$X^2 = 5.548$ (2)	0.062
Sports, %	0	6	0	$X^2 = 1.922$ (2)	0.383
Assault, %	0	6	0	$X^2 = 1.922$ (2)	0.383
Other, %	0	6	5	$X^2 = 0.762$ (2)	0.683

[a] *Education level was based on a Dutch classification system (Verhage 1964), ranging from 1 to 7 (highest). Abbreviations: CBT = cognitive behavioral therapy; Diff. = difference; TC = telephone counseling; GCS = Glasgow Coma Scale; MRI = magnetic resonance imaging; PTC = posttraumatic complaints.*

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Longitudinal functional network connectivity in mTBI

Independent components

Twenty-nine components were extracted with ICA. Ten artifact components were discarded. Of the remaining 19 neural components, eight were selected for further analysis (Figure 2). Three of these components corresponded with parts of the DMN (DMN1 (posterior cingulate cortex and precuneus), DMN2 (medial prefrontal cortex, posterior cingulate cortex and inferior parietal cortex) and DMN3 (precuneus)); four components corresponded with the EN (left and right frontoparietal network (FPN), dorsal attention network (DAN) and bilateral frontal network); and one component reflected the SN.
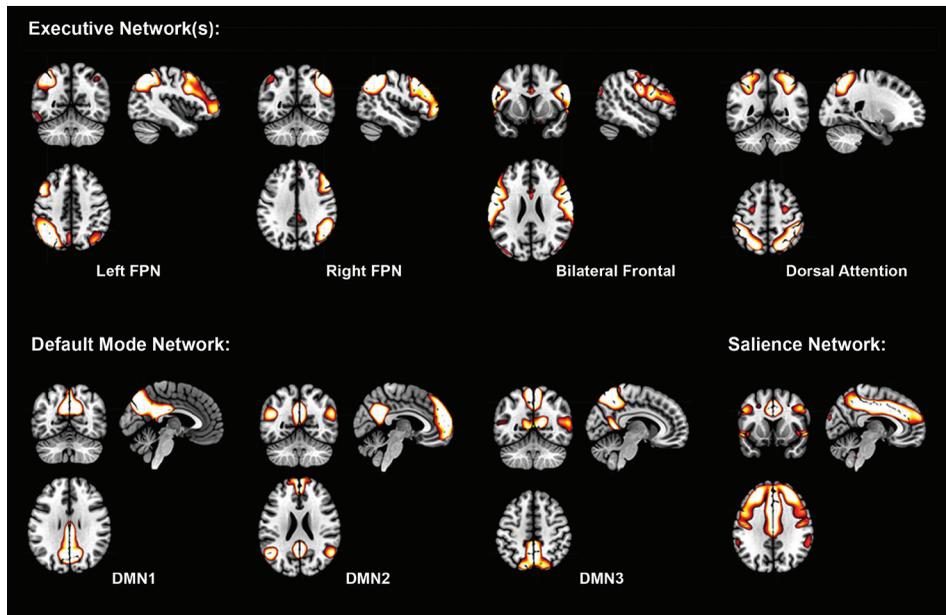

Figure 2: *Components of networks of interest.*

Within-component functional network connectivity

During visit one and two, FC of none of the components was found to be significantly different between HC, PTC-present and PTC-absent groups after multiple comparison corrections.

Small, but significant longitudinal changes in FC within the DMN were found for the PTC-present, PTC-absent and CBT group (Table 2). Furthermore, minor differences in longitudinal connectivity were found between PTC-present and PTC-absent patients, and between CBT and TC patients. There were no significant results with respect to the EN and SN.

Between-component functional connectivity

During the first and second visit, FC of none of the component pairs was found to be significantly different between HC, PTC-present and PTC-absent groups after correction for multiple comparisons. A trend toward significance was observed for FC between the bilateral frontal network and DAN, which was higher in PTC-absent patients than in HC (P_{uncorr} = 0.008; CL = 0.74) during visit one and visit two (P_{uncorr} = 0.005; CL = 0.75). Lastly, no significant changes in longitudinal between-component FC were found for either the PTC-present or PTC-absent group, and no differences were present between the PTC-present and PTC-absent groups, or between the CBT and TC groups.

Table 2: *Significant within-component functional connectivity results ($P_{FWE} < 0.05$).*

	Component	Contrast	Area	Cluster-level		Voxel-level			Peak coordinates		
				k	$P_{FWE\text{-}corr}$	$P_{FDR\text{-}corr}$	T	P_{uncorr}	x	y	z
Longitudinal effects within groups:											
PTC-present group	DMN3	Pre > post	Left hippocampus	1	0.0109	0.2529	5.71	0.0001	-12	-33	9
PTC-absent group	DMN3	Pre > post	Right middle frontal gyrus	1	0.0353	0.2428	6.02	0.0001	39	48	3
CBT-group	DMN2	Pre < post	Left middle temporal gyrus	3	0.0171	0.0484	7.55	0.0001	-45	-69	15
Longitudinal differences between groups:											
	DMN3	PTC-absent > PTC-present	Right middle frontal gyrus	1	0.0246	0.2428	5.11	0.0001	39	48	6
	DMN2	TC > CBT	Right middle temporal gyrus	2	0.0231	0.2970	5.64	0.0001	48	-69	12

Abbreviations: CBT = cognitive behavioral therapy; DMN = Default Mode Network; PTC = posttraumatic complaints; TC = telephonic counseling.

Functional connectivity related to clinical measures

For PTC-present patients, within-component FC during the first visit was not significantly related to number and severity of complaints, and HADS scores at three months post-injury. Also, no significant differences in within-component FC were found between PTC-present patients with unfavorable and favorable outcome at 12 months post-injury.

Regarding between-component FC, a significant positive correlation (at a SISA Bonferroni corrected $\alpha = 0.004$) was found between FC of the DMN1-DMN2 pair during the first visit and number of complaints at three months post-injury in the PTC-present group (Figure 3). Functional connectivity of this component pair was also significantly correlated with severity of complaints at three months post-injury ($\rho = 0.55$; $P_{uncorr} = 0.003$; 95% CI = 0.21-0.78). For none of the component-pairs, significant correlations were found between FC during the initial visit and HADS scores at three months post-injury. However, a negative correlation that approached significance was found between FC of the bilateral frontal network – SN pair during the first visit and HADS-A scores at three months post-injury ($\rho = -0.51$; $P_{uncorr} = 0.009$; 95% CI =[-0.14] – [-0.75]).

Between-component FC during the initial visit was not significantly different between PTC-present patients with favorable (n=17) and unfavorable (n=13) outcome at 12 months post-injury.
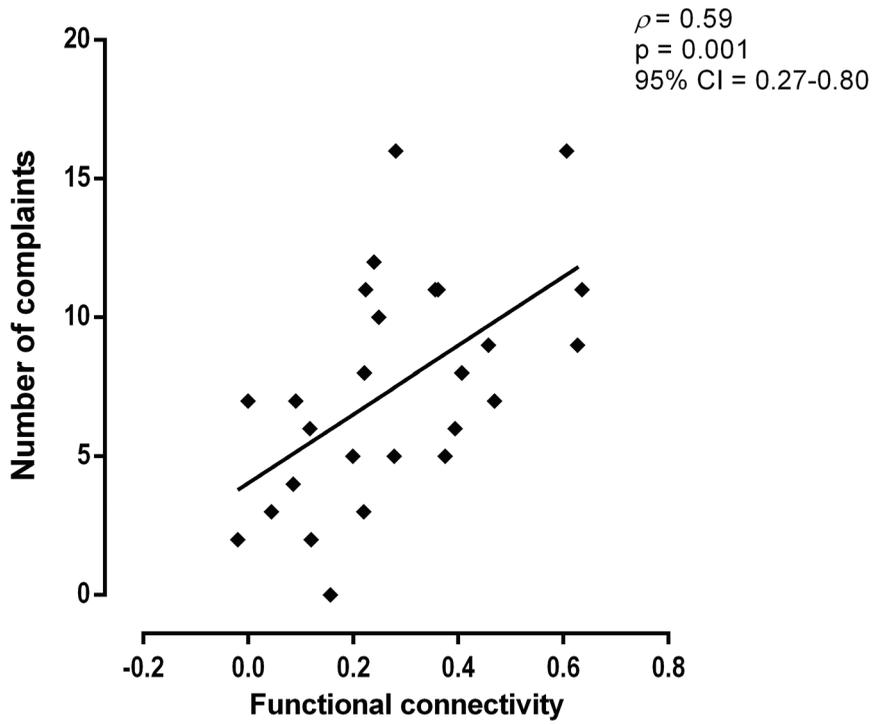
Figure 3: *Default mode network (DMN1-DMN2 pair) functional connectivity during the first visit was associated with posttraumatic complaints at follow-up three months post-injury.*

Discussion

In the current resting-state fMRI study, we aimed to investigate longitudinal network connectivity in patients with subacute uncomplicated mTBI. We examined patients with post-traumatic complaints, who were randomized for either cognitive behavioral therapy or telephone counseling, and a group without complaints who received no intervention. Independent component analysis was used to analyze functional connectivity within and between components of the default mode network, executive network and salience network. In addition, we assessed relationships between functional connectivity at one month post-injury and persistent post-traumatic complaints, anxiety, depression at three months post-injury, and long-term outcome one year after injury in the group of patients with complaints.

The clinically most relevant finding of our study was that in the PTC-present group, higher functional connectivity between the anterior and posterior part of the default mode network during the initial visit was related to a higher number of post-traumatic complaints at three months post-injury. This could indicate that the default mode network is involved in mechanisms underlying the persistence of complaints and/or response to treatment. Areas of the default mode network are strongly linked to spontaneous thought processes, such as mind wandering and envisioning past or future events (Andrews-Hanna et al. 2014; Fox et al. 2016). Based on our results, it could be hypothesized that relatively higher default mode network functional connectivity in patients with complaints is associated with ongoing thoughts about present complaints, sustained injury or its future consequences, and that this increased state of internally focused mental activity may impede recovery and/or possible treatment effects. In previous research it has been shown that reduced volume and higher activation of default mode network areas are associated with neuroticism, which is one of the Big Five personality traits (Servaas et al. 2014; Deyoung et al. 2011). Therefore, it would be interesting for future studies on mTBI to investigate if default mode network connectivity is associated with maladaptive pre-injury personality characteristics, and whether this is related to persistent post-traumatic complaints.

It has been consistently reported that the default mode network plays a pivotal role in psychopathology, such as anxiety disorders and major depressive disorder (Whitfield-Gabrieli & Ford 2012; Mulders et al. 2015; Sylvester et al. 2012). In the current study, we did not find any significant correlations between default mode network connectivity and anxiety or depression after mTBI. However, we did find a borderline significant negative correlation between FC of the bilateral frontal network – salience network pair at one month and anxiety scores at three months post-injury, which fits with the proposed role of the executive and salience networks in anxiety disorders (Sylvester et al. 2012).

Regarding within-component functional connectivity, we found significant

longitudinal decreases in small clusters of the default mode network in patients with and without complaints after mTBI. Previous studies have demonstrated longitudinal connectivity changes in areas of the default mode network in patients with mTBI and sports-related concussion (Meier et al. 2016; Messe et al. 2013). As has already been suggested by Meier and colleagues, the default mode network may be vulnerable to traumatic injury, because of the high level of functional connections in this network, and the susceptibility of the midline areas to shear strain injury (Meier et al. 2016; M. W. Cole et al. 2010; McAllister 2011). In theory, longitudinal decreases in default mode network functional connectivity could be interpreted as delayed injury effects or as fading compensatory increases in connectivity that might have occurred in the first days to weeks after injury. However, we did not find any significant differences in within-component functional connectivity between the patient groups and healthy controls, neither at measurement one nor at measurement two. Therefore, we have to conclude that the decreases in functional connectivity are not due to effects of injury.

With regard to between-component connectivity, significant longitudinal results were found for none of the patient groups. These findings match those of other studies, which have failed to demonstrate longitudinal changes in functional connectivity between the default mode network, executive network and salience network (Sours et al. 2015; Mayer et al. 2011). In addition to the possibility that there are in fact no significant longitudinal changes in network connectivity, our null findings may indicate that changes in network function could already have taken place and reached a plateau during the timeframe (i.e. first month post-injury) before the first scan. Other studies have demonstrated that longitudinal variations in resting-state functional connectivity are prominent within the first month (Meier et al. 2016; Sours et al. 2014; Sours et al. 2015) and may not occur between three weeks and five months post-injury (Mayer et al. 2011; Sours et al. 2015). Alternatively, functional network changes in our study groups may have been too subtle to be detected with current methods.

In the field of psychiatry, fMRI has been used frequently to study psychological treatments, particularly in patients with anxiety and depressive disorders (Ribeiro Porto et al. 2009; Yang et al. 2014; Frewen et al. 2008; Goldin et al. 2014). However, fMRI studies on the effect of psychological treatments in patients with mTBI are scarce (Roy et al. 2010; Laatsch et al. 2004). Of the two available studies, one investigated cognitive rehabilitation in a case series of five patients (Laatsch et al. 2004), and found within-subject changes in activation during cognitive tasks associated with language and visual processing. The other study investigated virtual reality and imaginal exposure therapy in veterans with blast-induced mTBI and (co-morbid) post-traumatic stress disorder and demonstrated pre- to post-treatment changes in amygdala and prefrontal activation during tasks involving emotional

stimuli (Roy et al. 2010). In the current study, patients receiving cognitive behavioral therapy were compared with patients receiving telephonic counseling as a means to control for possible influences of treatment condition on our longitudinal fMRI results in the PTC-present group. Only a minor difference in connectivity within the default mode network was found between these groups. This finding is of questionable relevance because cluster size was small (k=2 voxels), and paired tests revealed no longitudinal changes in functional connectivity of this particular cluster for neither of the groups. Interestingly, regarding the total intervention study, post-traumatic complaints have been shown to decrease significantly from two weeks to three months post-injury in the telephonic counseling group, but not in the cognitive behavioral therapy group (Scheenen et al., manuscript under revision). It is tempting to speculate that treatment effects are somehow mediated by the default mode network. However, the present fMRI study contained only one third of the total intervention study population and therefore lacks power to detect changes related to treatment. Future fMRI studies are required to further investigate the effects of psychological interventions on network connectivity in mTBI.

Despite the interesting results of our study, a major limitation is the lack of a group of patients with post-traumatic complaints that did not receive any treatment. Therefore, it was not possible to determine with certainty the natural course of network function in patients with complaints after mTBI. However, the group with complaints may be considered as a homogenous group since only a minor difference in network connectivity was found between the cognitive behavioral therapy and telephonic counseling group. Remarkably, most of the significant clusters that emerged in our within-component connectivity analyses contained only one voxel, and the maximum cluster size was three voxels. We performed permutation tests, which have been demonstrated to be superior to parametric methods with respect to the proportion of false positives (Eklund et al. 2016), and applied FWE-corrections for multiple comparisons. However, it is still possible that our within-component results are due to type I errors, considering that we did not correct for testing multiple components and clinical measures. Lastly, it also has to be realized that there is a possibility that longitudinal within-component connectivity changes reflect epiphenomena associated with non-injury related factors or test-retest manifestations without clinical significance (Skup 2010; Termenon et al. 2016).

To summarize, results from this resting-state fMRI study suggest that the default mode network may serve as a biomarker for selecting patients who are prone to develop persistent complaints. This finding, while preliminary, may hold implications for future development of tailored psychological interventions for patients with mTBI.

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