Brain Network Dysregulation, Emotion, and Complaints After Mild Traumatic Brain Injury

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Abstract: *Objectives:* To assess the role of brain networks in emotion regulation and post-traumatic complaints in the sub-acute phase after non-complicated mild traumatic brain injury (mTBI). *Experimental design:* Fifty-four patients with mTBI (34 with and 20 without complaints) and 20 healthy controls (group-matched for age, sex, education, and handedness) were included. Resting-state fMRI was performed at four weeks post-injury. Static and dynamic functional connectivity were studied within and between the default mode, executive (frontoparietal and bilateral frontal network), and salience network. The hospital anxiety and depression scale (HADS) was used to measure anxiety (HADS-A) and depression (HADS-D). *Principal Observations:* Regarding within-network functional connectivity, none of the selected brain networks were different between groups. Regarding between-network interactions, patients with complaints exhibited lower functional connectivity between the bilateral frontal and salience network compared to patients without complaints. In the total patient group, higher HADS-D scores were related to lower functional connectivity between the bilateral frontal network and both the right frontoparietal and salience network, and to higher connectivity between the right frontoparietal and salience network. Furthermore, whereas higher HADS-D scores were associated with lower connectivity within the parietal midline areas of the bilateral frontal network, higher HADS-A scores were related to lower connectivity within medial prefrontal areas of the bilateral frontal network. *Conclusions:* Functional interactions of the executive and salience networks were related to emotion regulation and complaints after mTBI, with a key role for the bilateral frontal network. These findings may have implications for future studies on the effect of psychological interventions. *Hum Brain Mapp* 37:1645–1654, 2016. © 2016 Wiley Periodicals, Inc.

Key words: mild traumatic brain injury; emotion regulation; post-traumatic complaints; fMRI; brain networks

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INTRODUCTION

Patients with mild traumatic brain injury (mTBI) frequently report post-traumatic cognitive and/or affective complaints during the first weeks after injury and these complaints may persist for months or even years in a small subgroup [Bazarian et al., 2005; Willer and Leddy, 2006]. Despite the presence of complaints, neuropsychological test results are most often in the normal range and conventional structural imaging reveals no abnormalities in most of the cases [Bazarian et al., 2006; Iverson et al., 2000]. Since post-traumatic complaints might be related to altered brain functioning, which can be visualized with functional MRI (fMRI), this technique could provide more insight into the underlying mechanisms of these complaints.

The human brain is organized into several intrinsic connectivity networks (ICNs), involved in various mental tasks [Seeley et al., 2007; van den Heuvel and Hulshoff Pol, 2010]. The default mode network (DMN) is the most iconic ICN and many fMRI studies focus on this particular network because of its central role in internally focused mental processes [Raichle et al., 2001]. In contrast to the DMN, the executive network(s) are activated during externally focused mental tasks [Cole et al., 2014; Seeley et al., 2007; Spreng et al., 2010; Sridharan et al., 2008]. The salience network, anatomically interposed between the DMN and executive networks, facilitates switching between these ICNs [Dosenbach et al., 2006; Seeley et al., 2007; Sridharan et al., 2008].

Altered functional network connectivity is associated with the presence of post-traumatic complaints in patients with mTBI [Mayer et al., 2011; Nathan et al., 2014; Sours et al., 2013; Zhou et al., 2012]. Disturbances in emotion regulation, reflected by anxiety and depression, are closely related to the presence of post-traumatic complaints [Silverberg and Iverson, 2011; van der Horn et al., 2013]. However, little is known about brain networks and emotion regulation after mTBI. Since the interaction between aforementioned ICNs appears to play a role in emotion regulation and the pathophysiology of mental disorders, such as anxiety and depressive disorders [Cole et al., 2014; Hamilton et al., 2011; Manoliu et al., 2014; Sylvester et al., 2012; Whitfield-Gabrieli and Ford, 2012], this is also likely to be the case for emotion regulation and post-traumatic complaints after mTBI [van der Horn et al., 2016]. In addition, few studies so far aimed to differentiate network function in patients with mTBI with complaints from that of patients without complaints in the sub-acute phase after injury. In the present study, these issues were investigated with resting-state fMRI in a large sample of patients with non-complicated mTBI in the sub-acute phase after injury.

MATERIALS AND METHODS

Study Participants

As part of a prospective follow-up study (UPFRONT study), 54 patients were enrolled in this fMRI study

between March 2013 and February 2015 at the University Medical Center Groningen (UMCG), The Netherlands (a level 1 trauma center). Diagnosis of mTBI was based on a Glasgow Coma Scale score of 13–15 and/or loss of consciousness ≤ 30 min [Vos et al., 2012]. Severity of trauma was measured using the Injury Severity Score (ISS) [Copes et al., 1988]. An ISS >15 indicates major trauma. For the assessment of non-head injury, ISS was corrected for mTBI (i.e., a score of four was subtracted from the total ISS).

At the emergency department (ED), patients received information regarding the UPFRONT-study that focuses on the course and outcome after mTBI by administering questionnaires at several intervals post-injury. Written consent was obtained at the ED or after discharge from the neurology ward when admitted (e.g., due to persistent post-traumatic amnesia (PTA)), by their treating physician. After filling in the first questionnaire at two weeks follow-up, patients (if aged between 18 and 65 years) were informed about the fMRI study. Exclusion criteria were: lesions on admission computed tomography (CT) scans, neurological or psychiatric co-morbidity, admission for prior TBI, drug or alcohol abuse, mental retardation, and contraindications for MRI (implanted ferromagnetic devices or objects, pregnancy or claustrophobia). Healthy controls (HCs) were recruited among social contacts and via advertisements, and matched with the mTBI group for age, sex, and educational level. All participants provided written informed consent.

The study was approved by the local Medical Ethics Committee of the UMCG and all procedures were carried out according to the declaration of Helsinki.

Patient Subgroups

Patients were selected based on self-reported complaints on a 19-item post-traumatic questionnaire [van der Horn et al., 2013], derived from the Rivermead Post-concussion Symptoms Questionnaire (RPQ) [King et al., 1995], administered at two weeks post-injury. Items of this questionnaire are listed in Supporting Information Table 1. Pre-injury and current complaints were measured on a scale from 0 to 2 (0 = never, 1 = sometimes, 2 = often), yielding a total complaint score and a severity score. Having post-traumatic complaints (PTC-present) was defined as ≥ 3 complaints (regardless of severity), with at least one complaint within the cognitive (including forgetfulness, poor concentration, slowness, fatigue, and drowsiness) or affective domain (including irritability, reduced tolerance for noise and anxiety). Having no complaints (PTC-absent) was defined as reporting <3 complaints.

Anxiety and Depression

To investigate emotion regulation, anxiety and depression were assessed with the Hospital Anxiety and Depression Scale (HADS) [Zigmond and Snaith, 1983], consisting

of seven anxiety (HADS-A) and seven depression (HADS-D) related items (each scale with a maximum of 21). Group analyses were performed on raw HADS-A and HADS-D scores. Patients with a score ≥ 8 on the HADS-A or HADS-D items were defined anxious or depressed, respectively [Bjelland et al., 2002].

Behavioral Data Analyses

The statistical package for Social Sciences (SPSS; version 22.0; Armonk, NY: IBM Corp) was used for behavioral data analyses. Shapiro–Wilk tests were used to assess normality. Group differences in age, interval from injury to scanning, ISS and HADS scores were examined using Kruskal–Wallis and Mann–Whitney *U* tests. Pearson’s chi-square tests were used for sex, education level, handedness, GCS score, PTA, and injury mechanism. Correlations between complaints and HADS scores were calculated using Spearman’s rank tests. Comparisons of HADS scores between male and female patients were performed using Mann–Whitney *U* tests.

MRI Acquisition Protocol

Image acquisition was done with a 3.0 T Philips Intera MRI scanner (Phillips Medical Systems, Best, The Netherlands) equipped with a 32-channel SENSE head coil. A high resolution transversal T1-weighted sequence image was made for anatomical reference (repetition time (TR) 9 ms; echo time (TE) 3.5 ms; flip angle (FA) 8°; field of view (FOV) 256 mm $\times$ 232 mm; voxel size 1 mm $\times$ 1 mm $\times$ 1 mm). During resting-state fMRI, participants were asked to close their eyes and to stay awake. Three-hundred volumes were acquired with slices aligned in the anterior commissure (AC)–posterior commissure (PC) plane and recorded in descending order (TR 2,000 ms; TE 20 ms; FOV 224 mm $\times$ 224 mm; voxel size 3.5 mm $\times$ 3.62 mm $\times$ 3.5 mm). To detect post-traumatic lesions the following sequences were used: coronal T2-gradient echo (TR 875 ms; TE 16 ms; FOV 230 $\times$ 183.28 mm; voxel size 0.40 $\times$ 1.12 $\times$ 4 mm) and transversal susceptibility weighted imaging (TR 35 ms; TE 10 ms; FOV 230 mm $\times$ 183.28 mm; voxel size 0.90 mm $\times$ 0.90 mm $\times$ 2 mm). Micro-hemorrhages (≥ 2 ; 2–10 mm) were observed in 35% of patients, with no differences in number and volume of lesions between patient subgroups.

fMRI Preprocessing

fMRI data was preprocessed using Statistical Parametric Mapping (SPM12 Wellcome Department, University College London, London, England) implemented in Matlab (version R2014a; MathWorks, Natick, MA). After slice timing correction, images were realigned to correct for head motion during acquisition, co-registered with individual participants’ T1-weighted images, normalized using diffeo-

morphic nonlinear registration tool (DARTEL) (isotropic voxels of 3 mm $\times$ 3 mm $\times$ 3 mm) and smoothed using an 8-mm full-width at half maximum Gaussian kernel.

fMRI Data Analyses

Independent component analysis (ICA) was performed using Group ICA of fMRI Toolbox (GIFT) version 3.0a implemented in Matlab [Calhoun et al., 2001]. The number of components was determined using Maximum Description Length (MDL) and Akaike’s Information Criterion. After intensity normalization and subject-specific PCA, group ICA was performed with 28 estimated components. Spatial-temporal regression was used for back-reconstruction and ICASSO was repeated 20 times [Himberg et al., 2004]. ICNs were identified visually (based on previously published literature) and by spatial regression of network templates provided in GIFT. Two components of the DMN, three components corresponding with the executive networks (left and right frontoparietal network (FPN) and bilateral frontal network) and the salience network were selected for further analyses (Fig. 1).

Different aspects of these ICNs were compared between the total mTBI and HC-group, and between PTC-present, PTC-absent, and HC subgroups. Group differences for within-network functional connectivity were investigated using a one-way ANOVA design in SPM. The total group of mTBI patients and HCs were compared using *t*-contrasts. Subgroups were compared using *F*-tests and post hoc *t*-contrasts were made if there was a significant group effect. Results were thresholded at uncorrected $P < 0.001$, $k > 10$, cluster-corrected at an estimated False Discovery Rate (qFDR) < 0.01 [Veer et al., 2010].

Static between-network functional connectivity was investigated using the Functional Connectivity Toolbox (FNC; version 2.3, MIALAB Software) [Jafri et al., 2008]. A band-pass filter of 0.013–0.15 Hz and a lag-shift of three seconds were applied. Both positive and negative correlations were taken into account and correlation values were Fisher Z-transformed. Statistical analyses were performed in Matlab. After normality testing (Shapiro–Wilk tests), comparisons between the total mTBI and HC groups were made using (*a priori*) independent two sample *t*-tests or Wilcoxon rank sum tests ($\alpha = 0.05$ with FDR-correction for 15 tested connections [Benjamini and Hochberg, 1995]). To analyze differences between subgroups, one-way ANOVA or Kruskal–Wallis tests were conducted ($\alpha = 0.05$ with FDR corrections for 15 tests), followed by post hoc tests ($\alpha = 0.05$ with FDR corrections for three groups) in case of significant group effects. Since the PTC-absent group contained a relatively high percentage of male patients, connections that were significantly different between PTC subgroups were also compared between male and female patients ($\alpha = 0.05$ with FDR corrections).

Dynamic between-network functional connectivity was examined using in-house Matlab scripts following the

Figure 1.

Spatial maps of selected independent components. Axial brain sections are displayed in neurological convention. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

methods by Allen et al. (2014). Time courses of the selected components underwent post-processing, including detrending, multiple regression of the six realignment parameters and their derivatives, and low-pass filtering at frequencies < 0.15 Hz. After variance normalization of the data, correlations were computed with a sliding-window approach (window of $20 \times TR$ (=40 seconds), steps of $1TR$, resulting in a total of 280 windows) and transformed with a Fisher's Z-transform. Subsequently, the standard deviation of these 280 correlation values was calculated. Group comparisons of standard deviations were conducted using non-parametric permutation testing in Matlab ($\alpha = 0.05$ with FDR corrections for 15 connections).

For within network functional connectivity, the relationship with anxiety and depression was analyzed in SPM using a one-way ANOVA design with inclusion of HADS-A and HADS-D scores as covariates ($P < 0.001$, $k > 10$, cluster $qFDR < 0.01$) [Veer et al., 2010]. For static between-network functional connectivity values and standard deviations of sliding window Z-scores, Spearman's rank correlations with HADS-A and HADS-D were calculated in Matlab ($\alpha = 0.05$ with FDR corrections for 15 correlations).

RESULTS

Participant Characteristics

Table I shows the participant characteristics. Besides the head injury, the majority of patients with mTBI had few

physical injuries in other regions (average ISS of 1.7 for the total mTBI group). The PTC-absent group contained more men than the PTC-present group ($\chi^2 = 7.78$, $P = 0.005$). For PTC-present patients, the average number and severity of complaints was 10 and 13, respectively. Prevalence of complaints is depicted in the Supporting Information Table 1. Twenty-nine percent of PTC-present patients had affective disorders: anxiety ($n = 3$), depression ($n = 4$), or both ($n = 3$). None within the PTC-absent group were anxious or depressed.

Associations Between Complaints and Anxiety/Depression

The number and severity of post-traumatic complaints were significantly related to HADS-A (Spearman's $\rho = 0.558$, $P < 0.001$; $\rho = 0.529$, $P < 0.001$) and HADS-D scores ($\rho = 0.754$, $P < 0.001$; $\rho = 0.760$, $P < 0.001$). Female patients reported higher HADS-D scores, but not HADS-A scores, than male patients ($U = 142$, $P = 0.004$).

Within-Network Functional Connectivity

For none of the six components, significant differences in within-network functional connectivity were found between the total group of mTBI patients and HCs, or between PTC-present, PTC-absent, and HC subgroups. In the mTBI group, lower functional connectivity of the posterior cingulate cortex and precuneus within the left FPN,

TABLE I. Participant characteristics

	PTC-present (<i>n</i> = 34)	PTC-absent (<i>n</i> = 20)	HC (<i>n</i> = 20)	<i>P</i> -value
Age, median (range), years	35 (19–63)	34 (20–64)	30 (18–61)	0.95 ^a
Sex, % male	53	90	70	0.02 ^b
Education level, median (range) ^c	6 (4–7)	6 (2–7)	6 (5–7)	0.25 ^b
Handedness, % right	91	80	85	0.50 ^b
Interval injury to MRI, median (range), days	33 (22–62)	33 (22–69)	N/A	0.44 ^d
GCS score, median (range)	14 (13–15)	15 (13–15)	N/A	0.09 ^b
Post-traumatic amnesia, % yes	91	74 ^e	N/A	0.09 ^b
Injury Severity Score, median (range) ^f	1 (0–13)	1 (0–13) ^g	N/A	0.16 ^d
Injury mechanism:				
Traffic, % of group	50	50	N/A	1 ^b
Falls, %	41	45	N/A	0.59 ^b
Sports, %	3	0	N/A	0.43 ^b
Assault, %	3	0	N/A	0.43 ^b
Other, %	3	5	N/A	0.69 ^b
HADS scores:				
HADS-A, mean (SD)	5.5 (4.0) ^h	2.5 (2.5)	N/A	0.004 ^d
HADS-D, mean (SD)	5.5 (4.0) ^h	1.0 (1.7)	N/A	<0.001 ^d

^aKruskal–Wallis test.^bPearson's chi-square test.^cEducation level was based on a Dutch classification system, according to Verhage (1964), ranging from 1 to 7 (highest).^dMann–Whitney *U* test.^ePost-traumatic amnesia was documented for 95% of the PTC-absent patients.^fInjury severity scores were corrected for mild traumatic brain injury.^gInjury severity scores were documented for 95% of the PTC-absent patients.^hHADS was completed by 91% of PTC-present patients.

GCS = Glasgow Coma Score; HADS = Hospital Anxiety and Depression Scale, A=anxiety, D=depression; MRI = Magnetic Resonance Imaging; N/A = not applicable; PTC = post-traumatic complaints.

and lower connectivity of the medial prefrontal cortex within the bilateral frontal network were related to higher HADS-A scores (Fig. 2). Lower functional connectivity of the right mid/posterior cingulate cortex and post-central gyrus within the bilateral frontal network was related to higher HADS-D scores.

Static Between-Network Functional Connectivity

Two sample *t*- and Wilcoxon rank sum tests showed no differences in static functional connectivity between the total mTBI group and HCs. Analyses of variance revealed that functional connectivity between the bilateral frontal network and salience network ($H = 12.76$; $P = 0.002$), between the right FPN and salience network ($F = 6.08$; $P = 0.004$) and between the left and right FPN ($F = 5.51$; $P = 0.006$) were significantly different between subgroups. Post hoc analyses showed that functional connectivity between the bilateral frontal network and salience network was significantly lower in PTC-present patients compared to PTC-absent patients ($W = 737$; $P < 0.001$; Fig. 3A), and that connectivity between the right FPN and salience network was significantly lower in PTC-absent patients compared to PTC-present patients ($t = 2.91$, $P = 0.005$) and HCs ($t = 3.16$; $P = 0.003$) (Fig. 3B). Functional connectivity

between the left and right FPN was also significantly lower in PTC-absent patients compared to PTC-present patients ($t = 2.81$, $P = 0.007$) and HCs ($t = 2.77$, $P = 0.009$). No significant group differences were revealed for connections involving DMN components.

Figure 4A shows a moderate negative correlation between static functional connectivity of the bilateral frontal network–salience network pair and HADS-D scores in the mTBI group. Functional connectivity between the right FPN and salience network was positively related to HADS-D scores (Spearman's $\rho = 0.472$, $P < 0.001$).

Within the mTBI patient group, female patients exhibited significantly lower functional connectivity between the bilateral frontal and salience network than male patients ($W = 267$, $P < 0.001$).

Dynamic Between-Network Functional Connectivity

Permutation tests showed no group differences in standard deviation of sliding window correlations for any of the functional connections. Within the total mTBI group, standard deviations of the left FPN–salience network pair (Spearman's $\rho = -0.370$, $P = 0.008$), bilateral frontal–right FPN pair ($\rho = -0.367$, $P = 0.008$), and bilateral frontal–salience

Figure 2.

Within-network functional connectivity related to anxiety and depression in patients with mTBI. **A:** Negative relationship between left frontoparietal network functional connectivity and HADS-A scores ($P_{\text{uncorrected}} < 0.001$, cluster $qFDR = 0.001$, peak MNI-coordinates: $-9 \ -57 \ 42$, $Z = 4.58$); **(B)** negative relationship between bilateral frontal network functional connectivity and HADS-A scores ($P_{\text{uncorrected}} < 0.001$, cluster $qFDR = 0.002$, peak MNI-coordinates: $9 \ 48 \ 24$, $Z = 4.37$); **(C)** negative relationship between bilateral frontal network functional connectivity and HADS-D scores ($P_{\text{uncorrected}} < 0.001$, cluster $qFDR < 0.001$, peak MNI-coordinates: $18 \ -36 \ 42$, $Z = 4.74$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

network pair ($\rho = -0.416$, $P = 0.002$; Fig. 4B) were negatively correlated with HADS-D scores. No significant correlations were found between dynamic functional connectivity and HADS-A scores.

DISCUSSION

In the current study, resting-state fMRI was used to assess intrinsic connectivity of brain networks in relation to emotion regulation and post-traumatic complaints in a large sample of patients with non-complicated mTBI. Differences in network interactions were found between patients with and without post-traumatic complaints, in particular for the salience network and the executive networks. Furthermore, functional connectivity within and between these networks was shown to be related to anxiety and depression in patients with mTBI.

One of the main research goals was to obtain more insight in the role of network function in the interplay between emotion regulation and post-traumatic complaints after mTBI. As expected, we found positive correlations between post-traumatic complaints and HADS-A and HADS-D scores, which is consistent with previous research [Silverberg and Iverson, 2011; van der Horn et al., 2013]. Regarding within-network functional connectivity, higher HADS-A and HADS-D scores in patients with mTBI were associated with weaker functional connectivity within prefrontal and parietal midline areas of the frontoparietal and bilateral frontal network. These findings are in line with previous studies that have shown that the medial prefrontal cortex and the posterior cingulate cortex (along with the precuneus) are affected in patients with mild [Eierud et al., 2014; Zhou et al., 2012] and mild to severe TBI [Bonnelle et al., 2011; Sharp et al., 2011]. These areas are important for emotion regulation [Coutinho et al., 2015], and switching between brain networks [Leech et al., 2011; Seeley et al., 2007]. It could be hypothesized that in patients with mTBI dysfunction of these areas may lead to impaired network interactions resulting in complaints, anxiety, and depression.

Interesting results were found with respect to interactions between the executive networks and salience network. Higher static functional connectivity between the bilateral frontal network and salience network was related to fewer complaints and lower HADS-D scores after mTBI, whereas higher connectivity between the right lateralized frontoparietal network and salience network was related to more complaints and higher HADS-D scores. Furthermore, stronger connectivity between the left and right frontoparietal network was found in patients with complaints compared to patients without complaints. Adequate function of the executive networks and the salience network is thought to be particularly important for emotion regulation and subsequent mental health [Cole et al., 2014] and dysfunction of these networks may result in anxiety and depressive

Figure 3.

Static between-network functional connectivity. **A:** Bilateral frontal network—salience network and **(B)** right frontoparietal network—salience network functional connectivity (FC) for healthy controls (HC), patients with complaints (PTC-present) and patients without complaints (PTC-absent). Asterisks indicate significance of $P < 0.05$ after FDR correction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

disorders [Sylvester et al., 2012; Whitfield-Gabrieli and Ford, 2012]. Although, one has to realize that mTBI is an entirely different condition, our results bear some resemblances to findings from studies on anxiety and depressive disorders. For example, anxiety disorders are thought to be related to excessive function of the salience network, which contains important structures for emotion processing such as the insula and amygdala [Craig, 2009; Seeley et al., 2007],

and impaired function of the executive networks [Sylvester et al., 2012]. Furthermore, in patients with a major depressive disorder, stronger connectivity between the executive networks and salience network appears to be associated with more adequate emotion regulation, in contrast to connectivity between salience network and DMN, which is associated with rumination [Belleau et al., 2015; Hamilton et al., 2011; Manoliu et al., 2014].

Figure 4.

Functional connectivity between the bilateral frontal and salience network related to depression in patients with mTBI. **A:** Static and **(B)** dynamic functional connectivity (FC) related to HADS-D scores in patients with (PTC-present) and without (PTC-absent) complaints after mTBI. Correlation coefficients (Spearman's ρ) were calculated for the total group of mTBI patients. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Since individuals constantly switch between brain networks and corresponding mental states, the strength of between-network connections is not static, but variable over time [Allen et al., 2014]. Therefore, we used a relatively new approach to examine this aspect of functional connectivity, i.e. dynamic functional connectivity. Recently, Mayer et al. were the first to use this method to investigate patients with mTBI; however, no significant findings were reported [Mayer et al., 2015]. With dynamic functional connectivity analyses, we have found that higher variability in functional connectivity between the bilateral frontal, frontoparietal, and salience network was associated with lower HADS-D scores in the total group of mTBI patients. To date, our study is the first to report that not only stationary interactions between networks, but also fluctuations in these correlations over time underline the role of emotion regulation after mTBI. Dynamic functional connectivity analysis seems a promising technique that offers the possibility to examine temporal aspects of network interactions in more detail. Future studies will have to confirm its value in patients with mTBI.

It is unclear whether our functional network findings are related to structural injury or to other factors. Recent studies have demonstrated that the influence of microstructural injury in the development of post-traumatic complaints after mTBI is debatable [Lange et al., 2015; Waljas et al., 2015]. We included a group of patients with complaints that reported at least one complaint within the cognitive or affective domain, because these complaints are more specific for mTBI as compared to somatic complaints [Dischinger et al., 2009; Ettenhofer and Barry, 2012; Lundin et al., 2006; Ponsford et al., 2011]. However, post-traumatic complaints are also reported by the general population and non-head injured patients, which suggests that other factors than structural injury play a dominant role in the development of (persistent) post-traumatic complaints [Cassidy et al., 2014]. Our functional network findings may also be related to non-injury factors rather than to the injury itself, especially since we found no differences between patients with complaints and healthy controls [van der Horn et al., 2016]. mTBI is a complex condition, because of its heterogeneous clinical and pathological nature [Rosenbaum and Lipton, 2012]. Pre-morbid mental health and personality characteristics seem to be strongly related to post-traumatic complaints, which suggest that network function in these patients may have already been different from healthy controls before injury [Lingsma et al., 2015]. Furthermore, inter-individual differences in coping styles, presumed to be stable personality characteristics [Nielsen and Knardahl, 2014], are of influence on the persistence of post-traumatic complaints [Anson and Ponsford, 2006; Bohnen et al., 1992]. Our network findings may indicate that patients without complaints have specific personality characteristics that prevent the development of post-traumatic complaints. More research is required to determine the relationship between coping and brain networks and the influence on long-term outcome after mTBI.

Consistent with other studies, a higher number of patients with post-traumatic complaints, anxiety, and depression were female [Bazarian et al., 2010; van der Horn et al., 2013]. In this study, we found a link between female sex, higher HADS-D scores, and weaker functional connectivity between the bilateral frontal and salience network. Recent research also indicates that sex differences are related to different patterns of brain activation during working memory performance after mTBI [Hsu et al., 2015]. Our findings may suggest that differences between male and female patients with mTBI could be partly attributed to differences in emotion regulation circuits, which has to be confirmed in other studies.

Remarkably, we did not find any significant results regarding the DMN, although previous research demonstrated DMN changes as a key feature in patients with post-traumatic complaints after mTBI [Mayer et al., 2011; Nathan et al., 2014; Sours et al., 2013; Zhou et al., 2012]. Stronger connectivity between the DMN and executive networks is thought to be associated with impaired switching between internally and externally focused mental state, possibly resulting in increased distractibility and mental fatigue in mTBI [Mayer et al., 2011; Sours et al., 2013]. The absence of DMN results could be attributed to several factors, such as timing and the fMRI paradigm. Recent literature suggests that most of the changes within the DMN occur within the first week after injury [Zhu et al., 2015], which could mean that in our study DMN function had already been normalized at the time of scanning. In addition, analogous to recent reports on depression, DMN functional connectivity alterations may be more pronounced during externally focused conditions than during resting or self-focused conditions, as used in our study [Belleau et al., 2015].

Other limitations need to be addressed in addition to those already discussed regarding our DMN findings. We did not administer the HADS to our healthy control subjects. Therefore, we were not able to determine differences in emotion regulation between patients and healthy controls. Furthermore, correlations between network measures and raw HADS scores might have been affected by the selection of patients, namely based on the presence or absence of post-traumatic complaints. Consequently, a relatively high percentage of patients without complaints also scored zero on HADS items, decreasing the variability. Notwithstanding, a challenging thought is whether it would be more informative for studies to select patients based on affective problems instead of post-traumatic complaints. At last, in mTBI research often an orthopedically injured control group is included in addition to a healthy control group. The lack of such a group may be considered a limitation of this study, because it impedes clear conclusions about the influence of somatic complaints, such as pain, on our findings. However, based on the relatively low ISS, indicating that our patients had few additional physical injuries, we have reasons to assume that the influence of pain as a result of physical injury on post-traumatic complaints is

negligible. Furthermore, according to our definitions it is possible that patients without complaints report one or two complaints. However, in our study 90% ($n = 18$) of the group without complaints reported zero complaints and the remaining 10% ($n = 2$) reported only one complaint; therefore, we have strong reasons to assume that this is a representative group of patients without serious risk for developing persistent complaints.

In summary, this study further supports the relationship between functional brain networks and post-traumatic complaints after non-complicated mTBI. The interplay between the executive networks and the salience network was shown to be closely related to anxiety and depression, underlining the putative role of these networks in emotion regulation after mTBI. In particular, functional connectivity of the bilateral frontal network appeared to play a modulating role in terms of fewer complaints and lower anxiety and depression scores. It may be worthwhile to further investigate the influence of psychological interventions to improve emotion regulation and the subsequent effect on (executive) network function in patients with post-traumatic complaints.

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