9. General Discussion and Future Perspectives

Mild traumatic brain injury (mTBI) is often considered to be a relatively innocuous condition; however, long-term findings suggest otherwise as a quarter of these patients suffer from persistent post-traumatic complaints which interfere with daily functioning (Cassidy et al. 2014). Thus, mTBI imposes a significant healthcare problem and economic burden on society. The etiology of persistent complaints is complex, and the exact contribution of neurobiological and psychological factors is still poorly understood (Silverberg & Iverson 2011). More importantly, imaging biomarkers are not available to accurately predict who will or will not develop persistent complaints after mTBI. It is important to know whether certain patterns of structural or functional brain network connectivity, as explored by imaging, are associated with complaints, and to ascertain whether these patterns are related to injury, or rather to premorbid characteristics. These biomarkers might also aid in the development of targeted (psychological) treatments. In this dissertation, we examined brain networks after mTBI from a structural and functional perspective in the hopes of gaining new insight into the multifaceted nature of complications resulting from mTBI.

The role of structural injury in mTBI

Currently, the clinical value of conventional imaging modalities mostly pertains to ruling out gross pathology in the acute setting after mTBI, since no clear correlations have been found between lesions and post-traumatic complaints or outcomes (Jacobs et al. 2010; Lannsjö et al. 2013; Hughes et al. 2004; Hofman et al. 2001; Yuh et al. 2013). Although microhemorrhagic lesions on T2*-GRE and SWI were significantly more frequent in patients than in healthy controls, we found no clear differences in number of lesions between patients with and without complaints in the subacute phase post-mTBI, which is in accordance with the current literature (Hughes et al. 2004; Hofman et al. 2001). In fact, three out of four patients with mTBI showed no lesions. This could imply that microhemorrhaging in mTBI does not adequately reflect axonal injury, rendering it clinically irrelevant. Alternatively, only a sufficient burden of microhemorrhagic lesions and/or specific anatomical distribution may cause complaints which interfere with daily activities (Yuh et al. 2013). Furthermore, we have found that most lesions were located within the prefrontal cortex, but no differences in anatomical localization of lesions were found between patients with and without complaints, and depth was not related number of complaints.

The availability of advanced neuroimaging techniques, such as functional and diffusion MRI, have made it possible to examine candidate neural mechanisms underpinning post-traumatic sequelae (Mayer et al. 2015). As alterations in functional network connectivity can be present even in asymptomatic patients who sustained sub-concussive head traumas, network dysfunction may be influenced by the injury itself (Johnson et al. 2014), although it seems that the clinical consequences of such a trauma are negligible (Belanger et al. 2016). Other studies have demonstrated that mTBI and post-traumatic complaints are unrelated to diffusion MRI abnormalities, which could indicate that it is not the injury itself which matters (Ilvesmaki et al. 2014; Wäljas et al. 2014; Lange et al. 2015). Excluding findings demonstrating lower connectivity within the left temporal pole in patients with mTBI, our research did not provide indisputable evidence that the structural connectome was different from healthy controls in the subacute phase after sustaining an mTBI (Harm Jan van der Horn et al. 2016). Nonetheless, interesting differences in the connectome were found within the patient group, i.e. between patients with and without complaints. Patients with complaints showed higher global and local efficiency than patients without complaints, which may be associated with an increased tendency to worry, or with compensatorily increased mental effort to adequately perform daily activities. Altogether, we have reasons to suspect that the influence of the brain injury itself on the integrity of the structural connectome in patients with mTBI is minor. Instead, inter-individual differences in structural network configuration among patients with mTBI may be explained by pre-existent psychological factors, which determine the likelihood of suffering from a large number of long-term complaints (Silverberg & Iverson 2011).

Adaptation and post-traumatic complaints

Although it is likely that post-traumatic complaints in the acute post-injury stages are related to the brain injury itself, the persistence of complaints over time is probably determined by inadequate emotion or stress regulation capacities which prevent the patient from adequately coping with the situation (Miller & Mittenberg 1998; Silverberg & Iverson 2011; Anson & Ponsford 2006; Iverson 2005). Thus, it is not the brain injury which explains the persistence of complaints, but rather pre-existent psychological factors which determine individual adaptive abilities. This hypothesis is strengthened by a significant recurrent finding throughout this dissertation, which is that functional and diffusion MRI findings were similar for both patients with post-traumatic complaints and healthy controls; although it must be noted that the absence of evidence is not evidence of absence. Actually, patients with complaints and healthy controls may not be so different, as post-traumatic complaints reported by patients with mTBI are also found in the general population (Cassidy et al. 2014; Wäljas et al. 2014). It could be hypothesized that individuals become more aware

of these complaints after stressful events, such as an mTBI. The *good-old-days bias*, which indicates the overestimation of pre-injury level of functioning as being better than after injury (Lange et al. 2010; Iverson et al. 2010), in addition to erroneous expectations and beliefs about the injury and long-term consequences, may play a role in further intensifying symptomatology after mTBI (Whittaker et al. 2007; Edwards et al. 2012). Studies have succeeded in demonstrating neural substrates for personality characteristics, such as neuroticism (Servaas et al. 2014; Servaas et al. 2015; Deyoung et al. 2011). An interesting consideration is whether the same personality characteristics and corresponding neural features of patients with complaints in our study were also, to some extent, present in the healthy control group, explaining the absence of brain network differences between these groups. It has been shown that approximately 80% of patients with mTBI report at least one post-traumatic complaint up to 12 months after injury, with a mean of seven complaints overall (McMahon et al. 2014; Wäljas et al. 2014). Furthermore, over 50% of healthy controls report between one and five post-traumatic complaints (Wäljas et al. 2014). Therefore, the group of patients without complaints (defined as reporting less than three complaints two weeks post-injury) which was studied in this dissertation is rather extraordinary, considering that ninety percent of this group reported no complaints at all. From a psychological standpoint, it could be argued that patients in this group are different from the average population, making them less prone to developing problems after mTBI, perhaps due to a lower-than-average tendency to worry and ruminate. Noteworthy is that the groups of patients with and without complaints were similar regarding all injury severity measures (i.e. Glasgow Coma Scale score, post-traumatic amnesia, and injury mechanism). Therefore, studying patients without complaints may lead to a better understanding of the causative mechanisms in those who do develop persistent complaints.

Functional brain networks and adaptation after mTBI

Adaptive behavior relies on adequate functioning of the prefrontal cortex and associated brain networks (Cole et al. 2014; Tops et al. 2014; Frank et al. 2014; Ochsner et al. 2012; Ochsner & Gross 2005). An important aspect in this process is the ability to effortlessly switch between the default mode network and executive network(s), which are involved in internally and externally focused mental activity, respectively (Cole et al. 2014; Tops et al. 2014; Menon & Uddin 2010; Greicius et al. 2003). The default mode network is important for self-reflection and future event-planning (Andrews-Hanna et al. 2014). However, for optimal cognitive performance it is crucial that the engagement of executive networks during an externally-directed task coincides with suppression of activity within the default mode network. In case this mechanism is impaired, default mode interference might occur, which may result in cognitive impairments and complaints (Sonuga-Barke & Castellanos 2007;

Bonnelle et al. 2011). Located within the prefrontal midline and insulae, the salience network modulates the interactions between the default mode- and executive network in response to salient internal or external stimuli (Menon & Uddin 2010; Seeley et al. 2007). Interestingly, a recent meta-analysis has shown that not only areas of the default mode network, but also areas of the executive and salience networks are involved in internally focused mental processes (Fox et al. 2016). This finding accentuates the importance of the executive networks in regulation of internally generated emotional stimuli (Cole et al. 2014). Therefore, it seems likely that the interaction between networks within the prefrontal cortex plays a prominent role in adaptation after mTBI.

To gain insight into brain network function and adaptation after mTBI, it is possible to use fMRI to study patients during cognitive tasks or resting conditions. A meta-analysis has shown increased activation of prefrontal executive network areas during performance of working memory tasks in patients with mTBI compared to healthy controls; this could be explained as either neural compensation or poor regulation of cognitive resources (Bryer et al. 2013). In addition, higher functional connectivity of the executive network after mTBI has been reported during resting conditions (Shumskaya et al. 2012). Other resting-state fMRI studies have shown lower connectivity within the default mode network, and higher connectivity between the default mode and the executive and salience networks in patients with mTBI compared to healthy controls; this was determined to be related to post-traumatic complaints (Mayer et al. 2011; Sours et al. 2013; Sours et al. 2015). It has been suggested that these findings could be explained as compensatory network reorganizations which function to achieve adequate deactivation of the default mode network when in cognitively-challenging situations; this could eventually lead to mental fatigue.

In addition to our structural diffusion MRI findings, our functional MRI studies also showed few differences in brain network function between patients with mTBI and healthy controls, which again indicates that it is highly likely that the injury itself has no direct consequences in terms of network function. However, we did identify clues which could help us understand the role of functional brain networks in developing (persistent) post-traumatic complaints after mTBI. In our working memory study, patients without complaints showed stronger deactivation of the default mode network compared to patients with complaints and healthy controls, especially during difficult conditions (van der Horn et al. 2015). Patients who are more capable of suppressing the default mode network may require less effort for performing cognitive tasks, which may lead to fewer cognitive complaints and mental fatigue. Furthermore, patients without complaints exhibited lower functional connectivity between the default mode network and a frontal executive network compared to patients with complaints, which suggests that patients

without complaints need less top-down control of the executive network to prevent default mode interference (Sonuga-Barke & Castellanos 2007; Sours et al. 2013). Remarkably, differences in executive network activation between patients with and without complaints were absent, which may be consistent with the fact that cognitive test scores in patients with mTBI are often found to be within normal range, which we also found to be true for our working memory experiment (Carroll et al. 2004; Carroll et al. 2014; Rohling et al. 2011; Dikmen et al. 2016). In contrast to our working memory study, one of our resting-state fMRI studies showed no differences between patients with and without complaints with regard to functional connectivity of the default mode network (Harm J. van der Horn, Liemburg, Scheenen, et al. 2016). Interestingly, a study by Belleau and colleagues revealed higher default mode network functional connectivity in patients with major depressive disorder during externally- instead of internally-focused task conditions, as compared to healthy controls (Belleau et al. 2015). Furthermore, almost two decades ago, Raichle and colleagues already demonstrated in their hallmark paper *A Default Mode of Brain Function* that areas which are deactivated during an attention-demanding non-self-referential task are not activated during rest (Raichle 2015; Raichle et al. 2001). Thus, our results could suggest that a cognitively-challenging circumstance — in addition to, or instead of resting conditions — is required to detect subtle problems with modulating default mode network activity and/or connectivity after mTBI.

The aforementioned dynamics of prefrontal networks during cognitive performance are also related to regulation of emotion (Cole et al. 2014; Anticevic et al. 2012). As such, disturbances in these dynamics are often found during emotional distress and mental illness. For example, studies on patients with major depressive disorder have shown increased activity and connectivity within the default mode network, decreased connectivity between the default mode network and the executive network, and increased connectivity between the default mode network and the salience network (Hamilton et al. 2011; Manoliu et al. 2014; Belleau et al. 2015; Mulders et al. 2015). Anxiety disorders are proposedly characterized, among other things, by increased functioning of the salience network and diminished functioning of the default mode and executive network (Sylvester et al. 2012). In patients with mTBI, higher activity and functional connectivity of the default mode network were found to be related to higher anxiety and depression scores (Nathan et al. 2015; Chen et al. 2008). Other research has shown that higher functional connectivity within the anterior default mode network was correlated with fewer feelings of anxiety and depression after mTBI, although the authors have suggested that increased usage of the medial prefrontal cortex may, in time, lead to persistent emotional distress (Zhou et al. 2012). Our results demonstrate that post-traumatic complaints, anxiety, and depression after mTBI were related to higher functional connectivity in the posterior midline of the default mode network, and

lower functional connectivity within medial and lateral prefrontal and parietal areas of the executive networks. These findings were accompanied by lower functional connectivity between the lateral prefrontal and salience networks. Also, higher default mode network connectivity one month following injury predicted a higher number of complaints three months after injury. It can be assumed that an imbalance in the intrinsic connectivity between the default mode, executive, and salience networks leads to impaired network switching involved in cognitive performance and emotion regulation, resulting in the persistence of complaints. These network dynamics may be related to specific pre-injury personality characteristics which become apparent after a traumatic brain injury.

Future perspectives

A difficult but challenging aspect of mTBI research is the heterogeneity or 'chaos' in clinical, pathophysiological and pre-injury characteristics (Rosenbaum & Lipton 2012). The mTBI spectrum comprises patients who sustained their injuries by various mechanisms: civilian-, sports- and blast-related injuries, for example; patients with single or multiple head injuries; and patients with uncomplicated or complicated mTBI. These factors may explain part of the diversity in results of neuroimaging studies on mTBI (Mayer et al. 2015; Harm J. van der Horn, Liemburg, Aleman, et al. 2016). To develop a more thorough view of the mechanisms underlying long-term complications of mTBI, it is of paramount importance for future studies to focus on inter-individual differences in adaptive capacities, despite the fact that patients report similar complaints. For example, studies must take into account inter-individual differences in emotion regulation strategies and associated coping styles (Ochsner & Gross 2005). Furthermore, fMRI paradigms consisting of emotion regulation (e.g. reappraisal or suppression of emotional content) and non-regulation conditions (e.g. passive viewing of emotional content) can be used to discern brain network dysfunction related to anxiety and depression after mTBI (Frank et al. 2014). In addition to patients with (persistent) complaints, we recommend further investigation of patients without any complaints, because they may be one of the "missing links" in the mechanisms underlying (persistent) post-traumatic complaints. It may be informative to investigate whether emotion regulation strategies and associated brain network function in these patients are different from patients with lasting complaints and/or healthy controls.

Another interesting topic for future research on mTBI is mindfulness meditation (Link et al. 2016). The practice of mindfulness meditation is derived from Buddhism and involves focusing one's attention to the present moment, maintaining awareness and control of present thoughts, feelings and sensations, and observing them without judgment. A commonly-used 10-week training program is Mindfulness-Based Stress Reduction (MBSR), initially developed by John Kabat-

Zinn to treat patients with chronic pain (Kabat-Zinn et al. 1985; Kabat-Zinn 1982). Reviews have demonstrated that MBSR has several beneficial effects on mental health (Gu et al. 2015; Khoury et al. 2015). In a pilot study, MBSR also improved quality of life and perceived self-efficacy in patients with mTBI in the chronic phase after injury (Azulay et al. 2013). It would be worthwhile to study this method in an early phase after mTBI, and to perform pre- and post-treatment scans to measure effects on neural networks. Mindfulness meditation has been shown to reduce default mode network activity, which may explain the positive effects of meditation on excessive negative self-referential processes (Simon & Engström 2015). In addition, there are some indications that mindfulness meditation alters the balance between the default mode, executive, and salience networks; this could possibly explain the mindfulness-induced improvements of attentional control (King et al. 2016; Tops et al. 2014; Simon & Engström 2015). MBSR has also been shown to improve symptoms in veterans with post-traumatic stress disorder (Polusny et al. 2015). Considering that post-traumatic complaints after mTBI are related to post-traumatic stress symptoms, it could be hypothesized that mindfulness may also be beneficial for patients with mTBI (Lagarde et al. 2014). Future work is required to examine whether practicing mindfulness prevents the persistence of complaints after mTBI, and whether this will be accompanied by reductions in default mode network activity/connectivity and changes in the interactions with the executive and salience networks.

A body of literature shows that women report post-traumatic complaints, anxiety and depression more often than men after mTBI (Bazarian et al. 2010; Cassidy et al. 2014; Dischinger et al. 2009; van der Horn et al. 2013). Specific (pre-injury) neural organizations underlying emotional processing could make women more prone to reporting complaints or developing affective problems than men (Tunç et al. 2016; Ingalhalikar et al. 2014; Whittle et al. 2011). Although the role of structural injury in mTBI can be considered minor, it could be questioned whether the female brain is more susceptible to injury and/or post-injury pathological processes leading to more complaints (Bazarian et al. 2010). Our study was not designed to find explanations for sex differences in mTBI, and more research is undeniably needed to clarify this topic.

Advanced neuroimaging is only in its infancy, and therefore many caveats are involved in the interpretation of current results. Direct comparison of network results with those of other studies may, at times, be difficult due to differences in analysis techniques (D. M. Cole et al. 2010). Studies using independent component analysis (ICA) cannot be directly compared with studies using other techniques, such as seed-based analysis; however, comparisons strictly between ICA studies may also be challenging. Large-scale brain networks, such as the default mode network, may be found divided into one or more components, depending on the dataset and the model order that is either estimated in a data-driven manner, or

manually selected. Another issue is the nomenclature of networks. For example, *the frontoparietal network* (Corbetta 1998) described in our study has also been referred to as the *central executive network* (Sridharan et al. 2008), *executive control network* (Seeley et al. 2007), *frontoparietal attention system* (Ptak 2012), *frontoparietal control network* (Spreng et al. 2010), and the *frontoparietal control system* (Cole et al. 2014). We realize that functional brain networks are, to some extent, specific for a single study; nevertheless, future neuroimaging studies may benefit from more consistent nomenclature for describing network results and theories about network interactions. Abovementioned topics form a major challenge in the search for clearly delineated and uniform imaging biomarkers in neurologic and psychiatric diseases, including mTBI.

Conclusion

In this dissertation, we used structural and functional MRI to investigate the underlying mechanisms of post-traumatic complaints and emotional distress after mTBI. Structural and functional brain networks were different between patients with and without complaints, while networks in patients and healthy controls appeared strikingly similar. Our findings indicate that the influence of emotion regulation and adaptation is likely to outweigh that of structural injury in the development and persistence of post-traumatic complaints. Our work offers a starting point for new research that focuses on brain network function regarding non-injury related factors in patients with mTBI.