Chapter 6

Summary, general discussion & future perspectives
Summary & general discussion

The overarching aim of this thesis was to better understand cognitive decline in MS and its neural correlates, with an emphasis on information processing speed and memory. In order to do so, we investigated potential confounders of cognition, such as sleep-related problems and depression. To better understand the underlying neurobiological mechanisms of problems with information processing speed and memory, we used advanced MRI measures, including dynamics in brain communication. Finally, we predicted actual cognitive decline in patients with MS over a period of five years by using structural MRI.

In this chapter, I will summarize and discuss the main findings of the previous chapters and answer our research questions, which were:
1. Are sleep-related problems and depression important confounders of cognitive problems and what neurobiological mechanisms underlie these conditions?
2. What structural and functional brain measures explain the two most prominent cognitive problems in MS, namely information processing speed and memory, and what is the added value of dynamic functional connectivity?
3. Which MRI measures at baseline can accurately predict whether patients are at risk for cognitive decline over a period of five years?

This chapter will end with suggestions for future studies regarding better understanding of cognitive decline in MS.
Potential confounders of cognition and their neurobiological mechanisms
From previous studies we have learned that secondary symptoms of MS, such as sleep-related problems and depression, can negatively affect cognitive functioning and quality of life.1,2 With this in mind, in Chapter 2 we investigated the effect of sleep-related problems (Chapter 2.1) and depression (Chapter 2.2) on cognitive functioning in MS and also explored the role of (advanced) brain measures in better understanding of these symptoms. Ultimately, this could guide us in taking a next step, such as therapy to alleviate these secondary symptoms, and thereby potentially improve cognitive functions.

Sleep-related problems in MS
Sleep-related problems occur in approximately 50% of patients with MS.3,4 Therefore, in Chapter 2.1 we investigated the relationship between self-reported sleep disturbances with cognitive functioning and resting-state stationary functional connectivity of the hippocampus and thalamus (both shown to be related to poor sleep in healthy subjects).5–7 Based on scores on the Athens Insomnia Scale,8 23 out of 71 MS patients were categorized as sleep disturbed. Sleep disturbed and normal sleeping patients did not differ with respect to cognitive functioning and structural brain measures. Compared to normal sleeping patients, however, sleep disturbed patients reported more subjective cognitive problems and displayed lower stationary functional connectivity of the thalamus with several brain regions (middle and superior frontal gyrus, inferior frontal operculum, anterior cingulate cortex, inferior parietal gyrus, precuneus, and angular gyrus). Sixteen percent of variance in AIS score could be explained by stationary functional connectivity between the right thalamus and right inferior frontal operculum and hippocampal volume. For cognitive functioning, however, the most important predictors were educational level, hippocampal volume and total grey matter volume, explaining 27% of variance. Adding self-reported sleep problems to this model did not improve the prediction, suggesting not to be of importance in explaining cognitive functioning relative to the other predictors. Taken together, sleep-related problems were related to subjective cognitive problems in MS and coincided with a specific pattern of decreased thalamic stationary functional connectivity with especially frontal regions.

Depression in MS
In MS, the prevalence of depression is two to three times higher than in the general population.9–11 Psychological factors, such as coping with a chronic disease, may explain these symptoms.12 However, depression is more common in MS than in other chronic diseases such as rheumatoid arthritis, suggesting that MS pathology itself might trigger the onset of depressive symptoms.13 In major depressive disorder (without MS) structural and functional brain changes in frontal and limbic regions have been observed, suggestive of fronto-limbic disconnection.14–16
The fronto-limbic system, consisting of frontal regions (i.e. prefrontal cortex and cingulate cortex) and subcortical brain regions (i.e. amygdala, hippocampus, and thalamus), is an important network involved in emotion regulation. Hence, in Chapter 2.2 we investigated structural and functional disconnection of the fronto-limbic system and cognitive functioning in a sample of moderate-to-severe depressed MS patients, non-depressed MS patients, and healthy controls. Compared to non-depressed MS patients, depressed MS patients had a shorter disease duration (average of 15 and 8 years, respectively), but more severe white matter atrophy, decreased fractional anisotropy of the uncinate fasciculus (white matter tract connecting the temporal lobe with the prefrontal cortex), and decreased stationary functional connectivity between the amygdala and frontal cortex. Equal performance on the neuropsychological test battery was observed. Together with disease duration, the abovementioned structural and functional disconnection could explain 48% of variance in the severity of depression in MS. Our findings suggest that, relative to non-depressed MS patients, depressed MS patients have more pronounced structural and functional (MS) changes in temporo-frontal regions, suggestive of fronto-limbic disconnection.

Discussion
The findings of Chapter 2 with respect to the relationship between possible confounders (i.e. sleep-related problems and depression) and cognitive functioning were not entirely in line with previous literature. That is, in our studies we did not observe a direct relationship between sleep disturbances and depression and cognitive functioning in patients with MS. However, for sleep-related problems we did observe a relationship with subjective cognitive functioning, which is in line with previous studies on fatigue and depression. In Chapter 2.2, depressed patients performed equally well (or poor) as non-depressed patients despite their shorter disease duration. Although we did not have data on premorbid cognitive functioning of patients, we can speculate that the rate of cognitive decline in depressed patients was higher than in non-depressed patients. It would be interesting to follow patients with sleep-related problems and depression over time to see whether they are more prone to develop more severe cognitive problems over a period of several years. Furthermore, from a cognitive perspective, therapy aimed at reducing sleep-related problems and depression might not directly benefit objective cognitive functioning, but might help reduce the perceived cognitive deficits. Important to realize is that alleviating sleep-related problems or depression itself will nevertheless benefit a patient’s quality of life.
The MRI results of Chapter 2 revealed differences between patient groups with and without sleep-related problems and with or without depression, suggesting a combined fingerprint of MS and sleep-related problems or depression, respectively. Compared to normal sleeping patients, patients with sleep-related problems showed decreased resting-state stationary functional connectivity in thalamo-cortico connections. Previously, decreased stationary functional connectivity of the thalamus (and hippocampus) with frontal regions was observed in healthy subjects in relationship to sleep deprivation and daytime sleepiness.\textsuperscript{5,7,24} The biological explanation for this decrease in connectivity is not yet known, but it has been suggested to relate to a decrease in brain metabolism in mainly frontal regions and the thalamus (measured with positron emission tomography).\textsuperscript{25}

In depressed MS patients we observed more pronounced fronto-limbic disconnection compared to non-depressed MS patients, possibly suggesting a faster progressing MS phenotype. Our findings are in line with previous studies that linked, in isolation, structural and functional brain changes to depression in MS. These include: more severe white matter atrophy, decreased white matter integrity in the anterior temporal lobe, structural network alterations (including the amygdala and frontal regions), and lower stationary functional connectivity between the amygdala and prefrontal cortex during emotional processing.\textsuperscript{26–28} For both depression and sleep-related problems, it would have been informative if we were able to include a group of people without MS but with major depression disorder or sleep-related problems, respectively. This could have revealed whether the differences we observed in the brain in MS patients with comorbid depression or sleep-related problems might be similar to that of people with depression or sleep-related problems without MS, suggestive of a ‘primary’ depressive disorder, or that more pronounced differences in the brain can be observed, suggestive of an interaction effect.

Exploring the neural correlates of sleep-related problems and depression can be of interest when investigating the efficacy of novel interventions aimed at reducing these symptoms, by serving as a secondary or tertiary outcome measure (besides behavioral measures) in the (near) future. By answering our first research question, that is, confounders of cognition are of limited importance with respect to objective cognitive functioning in MS, but do relate to specific changes in the brain, new questions arise. One pressing question concerns the \textit{chicken or the egg} causality dilemma: do some MS patients have a \textit{predisposition} (i.e. specific brain alterations) to develop sleep-related problems or depression prior to MS, or are these problems a \textit{consequence} of MS pathology targeting specific brain regions? For now, we can only describe relationships between brain measures and behavior and speculate on the order of events. To deepening our understanding, longitudinal studies on this topic are necessary to disentangle the exact order of events.
Imaging information processing speed in MS

Information processing speed is one of the cognitive domains that is frequently affected already early in the disease.\textsuperscript{29,30} Furthermore, problems with information processing speed are thought to negatively affect other cognitive domains, such as memory, working memory, and executive functions.\textsuperscript{31} Therefore, in Chapter 3, we aimed to better understand this disabling symptom. Within the brain, there does not seem to be a clear locus for information processing speed, as for example the hippocampus is for memory encoding within the memory network.\textsuperscript{32} Rather, the whole-brain structural and functional network organization itself, both intertwined to a certain extent, seems to facilitate optimal information processing speed.\textsuperscript{33,34} In Chapter 3.1, we investigated the impact of different severities of whole-brain structural and functional damage on information processing speed. Additionally, in Chapter 3.2, we explored whether dynamic functional connectivity of the default mode network, which is important for cognitive functioning in health and disease,\textsuperscript{35,36} could add to the neurobiological understanding of information processing speed on top of other advanced MRI measures.

Structure versus function

With the whole-brain approach used in Chapter 3.1, we were the first to investigate the relative and joint impact of structural and functional brain changes on information processing speed in MS. Based on performance on the Symbol Digit Modalities Test (SDMT), 37 MS patients were categorized as information processing speed impaired ($n = 126$, Z-score $<-1.5$ relative to healthy controls, $n = 96$) or information processing speed preserved ($n = 204$). Next, both groups were compared on several measures obtained with 3T MRI, including lesion load, white matter volume, deep grey matter volume, cortical grey matter volume and whole-brain white matter integrity (diffusion tensor imaging). For each MS patient we calculated the severity of stationary resting-state functional connectivity changes relative to healthy controls. Compared to patients with preserved information processing speed, impaired patients had more white matter lesions, more pronounced white and grey matter atrophy, lower white matter integrity, and more increased whole-brain stationary functional connectivity. Older age, male sex, lower educational level, lower deep grey matter volume, loss of white matter integrity, and increased stationary functional connectivity were related to worse information processing speed (45% explained variance). Subsequently, using a median split approach, patients were divided into four groups based on structural (deep grey matter volume and white matter integrity) and functional (increase in stationary functional connectivity) brain damage. As expected, patients with only mild structural and functional damage showed the best information processing speed, whereas patients with severe structural and functional damage showed worst information processing speed. Additionally, patients with predominant structural damage (and mild functional damage)
had worse information processing speed than patients with predominant functional damage (and mild structural damage). With this study we showed that integrating whole-brain structural and functional changes enabled us to detect more subtle and gradual changes in information processing speed in MS.

Default mode network dynamics

In Chapter 3.2, we explored whole-brain structural measures in combination with functional measures of the default mode network in explaining information processing speed in patients with MS. More specifically, we focused on stationary and dynamic functional connectivity of the default mode network during resting-state, during task-state (i.e. information processing speed task), and the difference between resting-state and task-state, as these measures might reflect the level of functional network integration facilitating information processing speed.\textsuperscript{38,39} We hypothesized that dynamic functional connectivity would increase the explained variance on top of other (advanced) MRI measures, such as lesion load, atrophy, white matter integrity damage, and stationary functional connectivity of the default mode network. In all subjects, information processing speed outside the scanner was measured with neuropsychological tests. Using 3T MRI, structural and functional brain measures were obtained, including resting-state and task-state fMRI. During task-state fMRI, a modified version of the SDMT was performed.\textsuperscript{40} For both resting-state and task-state fMRI, stationary and dynamic functional connectivity of the default mode network, corrected for whole-brain stationary/dynamic functional connectivity, were obtained. Additionally, we calculated the difference between resting-state and task-state stationary/dynamic functional connectivity (i.e. subtracting resting-state stationary/dynamic functional connectivity from task-state stationary/dynamic functional connectivity) as a measure of task-induced 'responsivity' of the default mode network. Compared to healthy controls, MS patients had a lower information processing speed composite score, lower grey matter volume, and more white matter integrity damage. Furthermore, in both groups an increase in dynamic functional connectivity of the default mode network was observed during task-state relative to resting-state. No group differences were found for accuracy on the SDMT inside the scanner or stationary and dynamic functional connectivity of the default mode network. However, in MS better SDMT accuracy (inside the scanner) could be predicted by an increase in dynamic functional connectivity from resting-state to task-state of the default mode network (23% explained variance). Furthermore, higher information processing speed composite score in MS could be related to higher cortical grey matter volume, and adding dynamic brain measures doubled the explained variance to 52%, with the increase in dynamic functional connectivity from resting-state to task-state as a significant predictor. These findings suggest that the observed increase in dynamic functional connectivity from resting-state to task-state is relevant for information processing speed in MS.
on top of more conventional brain measures, which might reflect the ability of the default mode network to adapt upon task demands.

Discussion
The common thread of Chapter 3 is novelty in brain measures for better understanding of information processing speed in MS. Until now, well-established MRI techniques have been used to understand information processing speed, however, these might fall short as information processing speed is such a complex cognitive domain, especially with respect to underlying brain function. Therefore, we felt the need to introduce relatively novel ways of assessing brain function by combining whole-brain and dynamic measures.

The first novelty in Chapter 3 is the development of one single measure that captures the widespread stationary functional connectivity changes observed in MS, instead of focusing on only several connections. This nicely aligns with a more holistic view on the brain in relationship to cognition, but also with structural whole-brain measures that were already available (e.g. atrophy, white matter integrity). The whole-brain approach enabled us to investigate the joint impact of both structural and functional changes on information processing speed. At the same time, one should keep in mind that this approach comes at a cost of regional specificity. With respect to whole-brain stationary functional connectivity, we observed that an increase in connectivity was related to worse information processing speed. This is in line with previous studies linking increased stationary functional connectivity to worse cognitive performance. With respect to the joint impact of structural and functional changes on information processing speed, we found that structural damage, especially deep grey matter atrophy and loss of white matter integrity, had great impact (based on regression analyses). However, groups with a similar amount of structural damage had different degrees of functional damage, together with different levels of information processing speed. That is, the group with severe structural and functional damage had worse information processing speed than the group with severe structural and mild functional damage. This might suggest that in the latter group the functional network is less affected by structural damage, perhaps suggesting a more 'resilient' functional brain network. Furthermore, patients with predominant structural damage had worse information processing speed than patients with predominant functional damage, suggesting that structural damage might have more impact on information processing speed.

The second novelty introduced in Chapter 3 is the investigation of dynamic functional connectivity of the default mode network in different brain states (resting-state and task-state). The main reasons we focused on the default mode network is its relevance for cognition in health and disease, its suggested
role in information integration throughout the brain, and changes observed in MS.\textsuperscript{35,36,38,46,47} Additionally, previous studies have linked several dynamic default mode network measures in health and disease to cognitive functioning and/or symptoms.\textsuperscript{48,49} Although dynamic functional connectivity of the default mode network did not differ between MS patients and healthy controls in our sample, in MS the increase in dynamic functional connectivity in task-state relative to resting-state could predict information processing speed in- and outside the scanner on top of other advanced MRI measures (e.g. atrophy, white matter integrity or stationary functional connectivity). Adding dynamic default mode network measures doubled the explained variance from 26\% to 52\%. These findings suggest that our measure was able to pick up individual differences in patients regarding dynamic functional connectivity of the default mode network and information processing speed. This possible 'fingerprint' of brain dynamics in relationship to cognition has been described in healthy controls as well.\textsuperscript{50} It would be valuable to include measures of brain dynamics in future studies and explore its incremental value when explaining other types of cognitive problems on top of common MRI techniques.

We also investigated different brain 'states' with respect to stationary and dynamic functional connectivity. Interestingly, dynamic functional connectivity of the default mode network increased in MS and healthy controls when switching from resting-state to task-state. This suggests that the default mode network seems to change its connectivity pattern more often during information processing speed relative to resting-state, possibly reflecting increased information flow throughout the network.\textsuperscript{48,51,52} Our observation of behavioral relevance of this default mode network 'responsivity' regarding dynamic functional connectivity has been shown in a previous study, where the increase in dynamics between the default mode network and frontoparietal network from resting-state to task-state (i.e. cognitive flexibility paradigm) was related to better cognitive flexibility outside the scanner.\textsuperscript{53} Task-induced increases in stationary functional connectivity of the default mode network have also been described previously.\textsuperscript{38,46} Based on our results, we speculate that combining resting-state with task-state functional measures might enable to capture the ability of a patient's functional network to adapt upon task demands.

Next to structural whole-brain damage (grey matter atrophy and decreased white matter integrity), increased whole-brain stationary functional connectivity at rest negatively affects information processing speed in MS. Furthermore, by adding dynamic functional connectivity of the default mode network, we can explain even more variation in information processing speed. However, still not all variation can be accounted for. This will partly be explained by noise in our measurements (i.e. information processing speed and MRI). Furthermore, the brain is more than
the sum of its parts, especially in the light of information processing speed. With this in mind, we made the first steps by zooming out and focusing on the default mode network (in relationship to the rest of the brain) and obtaining whole-brain measures of structural and functional changes. Additionally, we used *multivariate* linear regression models to combine relevant brain measures and explain variance in information processing speed, with the assumption this approach explains more variance than *univariate* models. However, it is highly likely that relationships between brain measures, but also with cognitive functioning, are non-linear. These non-linear relationships are currently not captured with linear regression models. Therefore, more advanced statistical analysis methods should be explored in future studies.

**Imaging learning and memory in MS**

In *Chapter 4*, we investigated the neural correlates of learning and memory in MS. We did so by investigating verbal and visuospatial memory in *Chapters 4.1 and 4.2*, in which the hippocampus plays a prominent role.\(^{54}\) In the latter chapter, we explored the added value of dynamic functional connectivity in terms of explained variance next to more conventional hippocampal measures.

**Predictors of memory function**

Based on pathological and neuroimaging studies, the hippocampus in MS can display various abnormalities.\(^ {44,55–58}\) These include, amongst others, lesions, atrophy, altered task-related activation during memory encoding, and altered stationary resting-state functional connectivity, some of which were related to memory problems in MS.\(^ {44,55–58}\) Therefore, in *Chapter 4.1* we investigated the most important hippocampal measure(s) associated with hippocampus-dependent memory function in MS, including lesions, task-related brain activation (during encoding of an episodic memory paradigm), and resting-state stationary functional connectivity. Compared to healthy controls, MS patients had worse memory function and hippocampal atrophy. Although task-related hippocampal activation was not different between MS patients and healthy controls, MS patients did display increased stationary functional connectivity between the left hippocampus and right posterior cingulate cortex. In the MS group, worse memory function could be explained by male sex, lower task-related activation of the right hippocampus, and increased stationary functional connectivity between the hippocampus with the posterior cingulate cortex (adjusted \(R^2 = 0.27\)). Interestingly, hippocampal activation and connectivity were not correlated to each other, suggesting that both hippocampal activation and hippocampal connectivity contributed independently to memory problems in MS.
Hippocampal dynamics and memory

Although we identified important hippocampal changes relevant for memory function in patients with MS in the previous chapter, in Chapter 4.2 we investigated the added value of dynamic functional connectivity during memory encoding, on top of other hippocampal measures, for explaining verbal and visuospatial memory separately. Compared to healthy controls, MS patients performed worse on tests for verbal and visuospatial memory. Furthermore, patients had smaller hippocampi, but no difference in hippocampal activation and stationary or dynamic functional connectivity during memory encoding. In patients with MS, worse verbal learning and memory outside the scanner could be predicted by male sex, lower left hippocampal volume and higher left hippocampal dynamic functional connectivity (adjusted $R^2 = 0.53$). Additionally, worse visuospatial memory outside the scanner could be predicted by lower right hippocampal stationary functional connectivity and higher right hippocampal dynamic functional connectivity (adjusted $R^2 = 0.24$). For verbal and visuospatial memory, adding dynamic hippocampal measures to the ‘conventional’ hippocampal measures resulted in an increase in explained variance of 7% and 13%, respectively. These results suggest a potential role for hippocampal dynamic functional connectivity to maintain memory function in patients with MS.

Discussion

We identified various hippocampal measures important for verbal and visuospatial memory function. Worse overall memory function was related to lower hippocampal activation and increased resting-state stationary functional connectivity between the hippocampus and posterior cingulate cortex, suggesting that both functional measures might reflect independent processes contributing to memory (dys)function. Previously, it has been shown that the hippocampus can increase its activation that might relate to maintained cognitive functioning.\textsuperscript{58,59} This local effect might be beneficial, that is, preserving cognitive functioning. In contrast, the increase in resting-state stationary functional connectivity between the hippocampus and posterior cingulate cortex, both part of the default mode network, might reflect large-scale network alterations due to pathology that can be detrimental for cognition. Such increases in connectivity of the default mode network have previously been linked to cognitive problems in MS before.\textsuperscript{44,47}

Zooming in on verbal and visuospatial memory separately, and including dynamic functional connectivity of the hippocampus during memory encoding, revealed other, but not contradictory, hippocampal predictors for memory function. These slightly different findings are likely to be explained by methodological differences, such as operationalization of stationary functional connectivity (synchronization likelihood in Chapter 4.1 versus Pearson correlation coefficient in Chapter 4.2), different brain states (resting-state versus task-state) and combining
memory tests versus separate investigation of verbal and visuospatial memory. Worse verbal memory was related to lower left hippocampal volume and increased dynamic functional connectivity of the left hippocampus, whereas worse visuospatial memory was related to lower stationary functional connectivity of the right hippocampus and increased dynamic functional connectivity of the right hippocampus. With respect to dynamics, our results consistently suggest that low variability in functional connectivity of the hippocampus during memory encoding, interpreted as stable connectivity, benefits memory function. This seems to be in line with a previous study in healthy subjects that observed a whole-brain decrease in functional connectivity but increase in stationary functional connectivity during task engagement.\textsuperscript{60} However, other studies also linked increased dynamic functional connectivity to better performance on cognitive domains other than memory.\textsuperscript{51,53,61,62} On the one hand, this emphasizes the infancy of this particular field. On the other hand, it illustrates the complexity of brain dynamics in relationship to cognition. This relationship between both likely depends on the brain state in which it is measured (resting-state versus task-state) and the cognitive domain under investigation.

To conclude, both structural and functional hippocampal measures, including volume, task-related activation, resting-state and task-state stationary functional connectivity, and task-state dynamic functional connectivity, were related to memory function in MS. Future longitudinal studies should disentangle the interplay between different functional measures in relationship to memory. That is, does local hippocampal activation reflect a possible beneficial compensatory mechanism protecting against memory decline,\textsuperscript{58} whereas increased resting-state stationary functional connectivity reflects maladaptive large-scale network alterations?\textsuperscript{44} What is the order of events? And how exactly does dynamic functional connectivity fit into this picture?

**What are the most important predictors for cognitive decline in MS?**

Where we 'predicted' information processing speed and memory in Chapters 3 and 4 in cross-sectional studies, in Chapter 5.1, we actually predicted cognitive decline in the full sense of the word. That is, we identified what structural MRI (lesion load, white matter integrity, and grey and white matter atrophy), demographic, and clinical measures at baseline best predicted cognitive decline during a five-year follow-up period. A large sample of 234 MS patients was divided into cognitively declining patients (66 patients, 28%) and cognitively stable patients (168 patients, 72%). At baseline, cognitively declining patients had higher lesion volume, lower white matter integrity, and lower cortical and deep grey matter volume compared to cognitively stable patients. Logistic regression analyses showed that 76% of patients could be correctly classified based on cortical grey matter volume, MS-type (relapsing-remitting MS vs progressive MS), and age as
significant predictors. These findings illustrate, besides a more progressive disease type, the importance of cortical grey matter atrophy as a possible indicator for cognitive decline.

Discussion

The literature on MRI predictors for future cognitive decline in MS is scarce. A previous study in patients with clinically isolated syndrome found that lesion number at baseline could predict cognitive functioning seven years later.\textsuperscript{63} Additionally, in a group of early relapsing-remitting MS (within three years of disease onset), whole-brain atrophy during the first year was the main predictor for cognitive functioning after five years.\textsuperscript{64} Our results in a larger and more heterogeneous group of MS patients showed that especially cortical grey matter atrophy could predict cognitive decline in approximately five years. Furthermore, we found that deep grey matter atrophy, together with MS-type and level of education, were important predictors for cross-sectional cognitive impairment. This is in line with other cross-sectional studies and suggests that the current cognitive status of a patient might be well reflected by deep grey matter atrophy, however, this measure does not necessarily have much predictive value regarding cognitive decline over a five-year follow-up period.\textsuperscript{65,66} One explanation could be that a subset of our sample had longstanding MS (average disease duration of 15 years) and possibly already more severe cortical atrophy at baseline, which might have made them more prone for cognitive decline. This is supported by previous studies that have shown that deep grey matter atrophy can be affected early in the disease, whereas cortical atrophy is more pronounced in later stages.\textsuperscript{65,67–69} Longitudinal imaging analyses on this sample, that relate to changes in cognitive functioning, will provide additional insight into the underlying brain mechanisms of cognitive decline.

From a clinical perspective, the predictive value of cortical grey matter atrophy for cognitive decline is very valuable. On the one hand it can be taken into account in clinical trials as a potential outcome measure instead of normalized brain volume, while on the other hand it could help the neurologist paint a picture for the patient with respect to his/her cognitive decline (or not).\textsuperscript{70} This latter goal will be challenging to achieve because of a lack of validated rating scales (i.e. automatic processing of MRI data is not yet adopted in clinical care). A first step can be taken by exploring the potential of existing visual rating scales for cortical atrophy often used in dementia, such as the Koedam score (posterior atrophy),\textsuperscript{71,72} Pasquier scale (global cortical atrophy)\textsuperscript{73} or medial temporal lobe atrophy scale,\textsuperscript{74} in relationship to cognitive impairment (cross-sectional) and cognitive decline (longitudinal) in patients with MS. Ideally, after validation, these rating scales should be adopted by radiologists in the clinic.
In Chapter 5 we showed that especially cortical grey matter atrophy can predict whether MS patients show cognitive deterioration over a period of five years. However, there is still room to improve this prediction model, but also to better understand underlying brain mechanisms associated with cognitive decline over time. This might be achieved by including advanced functional MRI measures that have been explored in previous chapters. Pursuing this line of research eventually helps us to better understand the network collapse with respect to structural and functional MRI, and their possible interplay.
Conclusions

Importance of sleep-related problems and depression for cognition in MS
- Confounders of cognition might not directly impact objective cognitive functioning in MS, but relate to perceived cognitive functioning
- Sleep-related problems in MS are associated with decreased resting-state stationary functional connectivity of the thalamus with frontal regions
- Depression in MS is related to decreased white matter integrity of the uncinate fasciculus and decreased resting-state stationary functional connectivity between the amygdala and frontal regions, suggestive of fronto-limbic disconnection

Understanding information processing speed in MS
- Different levels of whole-brain structural damage (i.e. deep grey matter atrophy and decreased white matter integrity) and resting-state stationary functional connectivity (i.e. increased connectivity) relate to different levels of information processing speed in MS
- Next to higher cortical grey matter volume, an increase in dynamic functional connectivity of the default mode network from resting-state to task-state (information processing speed paradigm) can be related to better information processing speed in MS
- Adding dynamic functional connectivity of the default mode network with the rest of the brain increased the explained variance in information processing speed between the 23% and 26%

Understanding memory in MS
- Poor overall memory function in MS can be explained by lower task-related hippocampal activation and increased resting-state stationary functional connectivity of the hippocampus with the posterior cingulate cortex
- Worse verbal memory in MS can be related to lower left hippocampal volume and higher task-state (i.e. memory encoding paradigm) dynamic functional connectivity of the left hippocampus
- Worse visuospatial memory in MS can be related to lower task-state stationary functional connectivity, but higher dynamic functional connectivity of the right hippocampus
- Adding dynamic functional connectivity of the hippocampus increased the explained variance in memory function between 7% and 13%
Predicting cognitive decline in MS

- Cross-sectional correlates of cognitive impairment are MS-type, educational level, and deep grey matter atrophy
- Next to age and MS-type, cortical grey matter atrophy at baseline was the only structural imaging parameter to predict cognitive decline over a follow-up period of five years
Future perspectives: substrate of network efficiency

In the present thesis we aimed to better understand cognitive problems in MS using advanced MRI measures. As a framework for answering the main research questions, we used the network collapse hypothesis as introduced in Chapter 1. In short, the network collapse hypothesis arose from the observations of large heterogeneity in cognitive functioning between patients with MS and the relatively poor relationship between cognitive dysfunction and conventional MRI measures (e.g. lesion load or whole brain atrophy). It was argued that more sensitive and comprehensive brain measures are required (i.e. functional imaging), next to conventional structural brain measures, to help us better understand the heterogeneity in cognition. Moreover, not only local or widespread increases and decreases in brain activation and connectivity in isolation are informative for understanding cognitive decline in MS, but the entire brain's functional network should be assessed from a more holistic perspective. The network collapse hypothesis states that during the course of MS, the brain's functional network declines slowly in efficiency, probably triggered by structural damage (e.g. lesions and atrophy), which coincides with a decline in cognitive functions (see Figure 6.1). After a certain threshold is reached, that is, the 'tipping point', this decline in efficiency accelerates tremendously, possibly related to more widespread structural damage, eventually resulting in overt cognitive problems. To date, however, the translation from the concept of network efficiency to operationalization of this concept is still a matter of debate.

We therefore introduced an innovative way to assess brain function that might capture a certain aspect of network efficiency, namely task-induced changes in brain communication and its dynamics relative to a low cognitive demanding state (e.g. resting-state). From now on, these task-induced connectivity changes will be referred to as 'brain responsivity'. Note that this is different than task-related brain activation, which reveals local increases or decreases in brain activation upon task demands, and not in communication between brain regions. Furthermore, task-related functional connectivity studies only assess communication during a task, and usually not the changes in connectivity between different cognitive demanding states. In this section, I will briefly discuss the possible relevance of brain responsivity with respect to cognition, present an updated figure of the network collapse hypothesis, and end with some preliminary results exploring brain responsivity as a possible substrate of network efficiency.
Figure 6.1 The network collapse hypothesis

A gradual decline in network efficiency (green line) and increase in structural brain damage (blue line) can be observed in the early stages of the disease. After a certain threshold is reached (i.e. tipping point; indicated by the dotted line), network efficiency declines tremendously, resulting in overt cognitive problems (red line). Adapted from Schoonheim et al. (2015)77

Relevance of brain states

The brain is intrinsically organized into several large-scale networks, such as the default mode network and frontoparietal network, which can be identified with resting-state fMRI. This intrinsic functional organization, however, is also present across many different task-states in healthy subjects.78,79 A previous study that combined resting-state and a dozen different task-states (e.g. language, motor, and working memory) showed that this intrinsic functional organization is quite dominant and accounts for most of the brain's overall functional network architecture during individual tasks.80 Interestingly, subtle task-evoked connectivity changes do occur and are often similar to one another, suggesting a possible task-general network architecture with a prominent pattern of decreased connectivity within networks.80 Furthermore, this quite rigid intrinsic functional organization seems to enable the flow of activity (i.e. information) during task performance, thereby 'shaping' brain activation patterns during task performance that ultimately result in (complex) behavior, such as cognition.81,82

For both stationary and dynamic functional connectivity, brain responsivity has been observed. That is, the brain can occupy different functional network configurations that enable information integration. For example, with increasing
working memory load, the default mode network can increase its stationary and dynamic functional connectivity with other networks and frontal regions become more dynamic.\textsuperscript{38,39,46,51} This brain responsivity, which correlates with better task performance, is thought to reflect a higher level of functional network integration necessary to perform the task at hand.\textsuperscript{38,39,46,51} The widespread pathology in neurodegenerative disorders, such as MS or Alzheimer’s disease, can affect the brain's structural network and intrinsic functional organization, and thereby possibly also its responsivity (i.e. flow of information) during task-state.\textsuperscript{83,84}

We need to challenge the brain to expose its network efficiency, just as physical exercise exposes certain cardiac conditions

I therefore argue that by assessing the brain's functional network during a cognitively challenging task, and by comparing it to the intrinsic resting-state (or a low cognitive demanding task-state) organization, we might 'expose' network efficiency in the form of altered brain responsivity. In other words: by challenging the brain, information on the ability of a subject's functional network to adapt upon task demands will be brought to light and could serve as a proxy for network (in)efficiency, or metaphorically, by performing a challenging physical exercise, we are able to expose certain cardiac conditions more easily.\textsuperscript{85}

In the early phases of MS, brain responsivity and task performance might be similar to that observed in healthy controls, suggestive of an efficient network (see Figure 6.2). However, when the disease progresses, brain responsivity might become less pronounced, which is hypothesized to be a sign that the functional network is not able to adapt upon task demands optimally. Initially, this does not necessarily have to result in overt problems with task performance or cognitive functioning. However, when this diminished brain responsivity coincides with poor task performance or (mild) cognitive problems, it could be a sign that a subject is nearing or exceeding the 'tipping point' (i.e. inefficient network). Especially from the perspective of cognitive rehabilitation, an interesting question is whether repeated exposure to cognitively challenging tasks will slow down the decline in network efficiency over time (i.e. use it or lose it), or whether it will accelerate the decline in network efficiency (i.e. wear it and tear it).

Figure 6.2 displays a slightly updated graphical representation of the network collapse hypothesis. The general idea of this hypothesis has not changed. However, this figure now includes the ability of the brain to react upon external (cognitive) task demands, indicated by the green transparent plane above the green line representing network efficiency. This green plane represents all the possibilities
Summary, general discussion & future perspectives

When the disease progresses, the network becomes less efficient and the brain less responsive, being unable to adjust its functional network organization required for optimal task execution. Note that in Figure 6.2 the intrinsic functional organization (i.e. low cognitive demanding state) of the brain is similar before and after the tipping point, which is most likely not the case. That is, the decline in network efficiency will probably also be reflected in an altered intrinsic functional organization. However, based on the findings in this thesis, the exact changes in intrinsic functional organization are difficult to pinpoint.

Figure 6.2 Brain responsivity as a reflection of network efficiency

On top of the network efficiency curve (green line), a transparent green plane is added indicating the ability of the brain to respond upon task demands by changing its functional network (i.e. stationary and/or dynamic functional connectivity patterns). The plane represents the range of all the potential configurations of the functional network in order to perform the task at hand. This brain responsivity decreases with increasing structural damage and might be a substrate of network efficiency. In the bottom panel, as an example, brain responsivity of the default mode network is illustrated. On the left, a substantial change in connectivity within and between the default mode network is illustrated during the high cognitive demanding state relative to the low cognitive demanding state. On the right, this change in functional network architecture is less pronounced. Adapted from Schoonheim et al. (2015)77

of the brain to configure its functional network upon task demands (i.e. brain responsivity). When the disease progresses, the network becomes less efficient and the brain less responsive, being unable to adjust its functional network organization required for optimal task execution. Note that in Figure 6.2 the intrinsic functional organization (i.e. low cognitive demanding state) of the brain is similar before and after the tipping point, which is most likely not the case. That is, the decline in network efficiency will probably also be reflected in an altered intrinsic functional organization. However, based on the findings in this thesis, the exact changes in intrinsic functional organization are difficult to pinpoint.
With the updated hypothesis in mind, we performed a preliminary study in a small sample of MS patients and healthy controls that performed the N-back working memory task inside the scanner. We aimed to capture the responsivity of the default mode network with other cognitive-relevant large-scale networks, such as the frontoparietal network and executive control network, as these connections might be related to information integration throughout the brain. Furthermore, we explored the relevance of brain responsivity for task performance.

**Challenging the brain: task-evoked network integration as a substrate of network efficiency**

In this preliminary analysis, we investigated a group of 23 patients with MS (15 women, mean age: 43.0 ± 7.7 years, mean disease duration: 11.6 ± 7.1 years) and 15 healthy controls (9 women, mean age: 42.5 ± 10.3 years), who all underwent structural and functional MRI. By performing independent component analysis on resting-state fMRI data, we obtained resting-state networks. These networks included, amongst others, the default mode network, frontoparietal network, executive control network, and sensory networks (i.e. visual network, sensorimotor network, and auditory network). All subjects also performed a working memory task inside the scanner (N-Back), consisting of four difficulty levels (N0, N1, N2, N3; 60 trials per difficulty level). For each difficulty level, we calculated stationary functional connectivity, normalized for the mean and SD of stationary functional connectivity of the entire task (i.e. \( Z \)-score), of the default mode network with all other networks, with the frontoparietal network, and with the executive control network, but also between the visual network with the sensorimotor and auditory network (serving as a control connection). These connections reflect between-network stationary functional connectivity. Furthermore, we calculated the within-network stationary functional connectivity for the default mode network and visual network. Next, integration of the DMN and visual network was calculated for each N-Back difficulty level as follows: between-network stationary functional connectivity minus the within-network stationary functional connectivity. Hence, a positive value indicates relatively higher between- than within-network connectivity, which we defined as increased network integration. In this preliminary study, operationalization of brain responsivity is the change in network integration with increasing task load.

No group differences were observed regarding age, sex, and educational level. Regarding structural MRI, the MS group had lower deep grey matter volume (\( p = 0.016 \)) than healthy controls. However, no group differences were found with respect to white matter volume (\( p = 0.051 \)) and cortical grey matter volume (\( p = 0.883 \)).
In Figure 6.3, brain responsivity of the default mode network and visual network is displayed for patients with MS and healthy controls, by plotting the level of network integration for each difficulty level. No between-group differences in integration of the default mode network and visual network was observed for each task load. Within the MS group, an overall effect of task load on integration of the default mode network with all other networks and with the executive control network specifically was observed ($\chi^2 = 25.07, p < 0.001$ and $\chi^2 = 22.62, p < 0.001$, respectively). This increase in network integration was only observed between N1 and N2 (default mode network – all networks: $p = 0.023$; default mode network – executive control network: $p = 0.006$). In healthy controls, an overall effect of task load on integration of the default mode network with all other networks and with the executive control network was observed ($\chi^2 = 8.36, p = 0.039$ and $\chi^2 = 9.64, p = 0.022$, respectively). However, no significant increase in integration in consecutive difficulty levels was observed. These findings suggest that, in patients with MS, an increase in cognitive demands (i.e. N1 to N2) coincides with increased integration of the default mode network. In healthy controls, the increase in network integration seems to be more gradual.

For each difficulty level, no group differences in accuracy were found (see Figure 6.4). Within each group, a significant effect of task load on task accuracy was observed (MS: $\chi^2 = 47.82, p < 0.001$; healthy controls: $\chi^2 = 30.64, p < 0.001$). In both groups, each incremental increase in difficulty level resulted in a decrease in accuracy ($p < 0.035$ for all). Next, for each subject we calculated a regression line for the level of integration of the default mode network with all other networks and with the executive control network over all difficulty levels, yielding the overall change in network integration with increasing task load on subject level. Additionally, a regression line was calculated for the change in accuracy over all difficulty levels. Next, within each group we correlated the slope of the regression line of network integration with that of accuracy. In MS, no significant correlation was found between the slope of network integration and that of accuracy. However, in healthy controls, the slope of the default mode network with all other networks was positively correlated with the slope of accuracy ($\rho = 0.51, p = 0.050$). Furthermore, the slopes did not differ significantly between groups. These findings suggest that for healthy controls only, an increase in default mode network integration as a response of increasing cognitive demands is related to maintained task performance. Speculatively, this absent relationship in the MS group could suggest that the increase in default mode network integration is not sufficient to prevent a decline in task performance.
Figure 6.3 Brain responsivity of the default mode network

For each task load (N0, N1, N2, and N3), integration of the default mode network and visual network is plotted for healthy controls and patients with multiple sclerosis. MS: multiple sclerosis.
Figure 6.4 N-Back task performance

The percentage of correct responses for each task load (N0, N1, N2, and N3) is plotted for healthy controls and patients with multiple sclerosis.

HC: healthy controls; MS: multiple sclerosis
This initial exploration illustrates that when challenging the brain, its functional network adapts. This adaptation happens gradually in a healthy situation, but more pronounced in case of a neurological disease, such as MS. Although this brain responsivity was not related to maintained task performance in MS, in healthy controls such a relationship was observed. With this in mind, it would be interesting to pursue this line of research (i.e. brain responsivity) in future studies, as it could provide another layer of information that might capture one of the brain's most essential features: adaptation upon external demands, that is, plasticity. Ideally, future studies should include large sample sizes and different task-states, as the relationship between stationary and dynamic functional connectivity and cognition might depend on the state in which it is measured.

Furthermore, different neurodegenerative diseases should be compared with each other, possibly revealing common underlying mechanisms of cognitive decline (i.e. network collapse). Finally, longitudinal and cognitive rehabilitation studies are necessary to further elucidate the complex relationship between functional and structural brain networks, and their possibility to adapt upon effective therapy. Especially cognitive rehabilitation studies are of importance, as one can measure the effects of therapy on brain function and link this to changes on behavioral level. This way, we can better grasp complex brain measures, such as dynamic functional connectivity and brain responsivity, as it enables to determine whether functional changes are beneficial or adverse with respect to cognitive functioning. Luckily, in the past years, the number of cognitive rehabilitation studies in MS in combination with MRI has been growing, thereby producing lots of data in which these novel brain measures can be explored.

The abovementioned future perspectives are challenging to fulfill, especially regarding longitudinal studies across neurodegenerative disorders. In my opinion, in order to achieve all of the above, close collaboration between scientists is required. Not the type of collaboration where one plus one equals two. But the type of collaboration where the end result is more than the sum of the individual contributions.

“Unity is strength. When there is teamwork and collaboration, wonderful things can be achieved”

Mattie Stepanek
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