Chapter 1

General introduction

This chapter is partly based on Cognition and MS: The role of MRI
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DeLuca & Sandroff (Eds), Cognition and Behavior in Multiple Sclerosis (2018)
Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and neurodegenerative disease of the central nervous system. This debilitating disease affects more than 2.3 million people worldwide with approximately 17,000 patients in the Netherlands.\(^1\)\(^2\) MS is one of the most common neurological disorders among young adults (i.e. age of onset is usually between the 25 and 35 years) and has a great impact on a person’s life.\(^2\) Furthermore, there is a huge socioeconomic burden in terms of health care costs and indirect costs (i.e. costs related to short-term absence, long-term absence, invalidity, and early retirement), resulting in annual average costs between €23,100 and €50,500 per patient in the Netherlands depending on the level of disability.\(^1\) The exact cause of MS remains unknown, although the disease is most likely multifactorial, as genetic factors (e.g. immune-associated genes) but also environmental factors (e.g. Epstein-Barr viral infection) have been linked to the disease.\(^3\)\(^4\)

**MS pathology**

The central hallmark of MS pathology is demyelination in both the white and grey matter (cortex and deep grey matter structures) and axonal loss.\(^5\) The loss of myelin, serving as an electrically insulating layer facilitating signal transmission throughout the nervous system, can be related to immune cells that enter the brain parenchyma (i.e. auto-immune response).\(^5\) In early and acute phases of MS, an autoimmune reaction occurs, where macrophages and leukocytes (e.g. T-cells, B-cells) enter the brain via a leaking blood brain barrier and recognize and ingest myelin, resulting in (further) demyelination, also known as lesions.\(^6\)\(^7\) Lesions can be (partly) remyelinated by oligodendrocytes, thereby preserving axons and neurons to some extent in the early phases of the disease.\(^6\) As the disease progresses, axonal damage becomes more pronounced, which is thought to be mediated by mitochondrial dysfunction through production of reactive oxygen species.\(^8\) At this phase of the disease, profound white and grey matter atrophy (i.e. tissue loss) can be observed.\(^7\) The observation of grey matter involvement with respect to lesions and atrophy and disease progression despite the use of (relatively) effective disease modifying treatment (i.e. immunomodulatory medication), have led to the hypothesis that MS might not primarily be an autoimmune disease, but instead a neurodegenerative disease.\(^9\) This discussion is important, as it might lead to novel insights and development of more effective medication.
**Clinical course and manifestation**

The clinical course of MS is extremely unpredictable; some patients progress rapidly, while others remain relatively stable over the course of the disease. The largest part of patients (approximately 85%) present with relapsing-remitting MS (RRMS) with the onset typically in early adulthood (between 25 and 35 years). Characteristic for this phenotype are episodes of acute symptom worsening, followed by partial or complete recovery. Approximately two decades after onset, nearly half of the RRMS patients will develop secondary progressive MS. In this phase of the disease, symptoms will become progressively worse without relapses or (partial) recovery. The remaining 10 to 15% of patients that do not have relapse-onset MS, present with a rapidly progressing disease course, called primary progressive MS. The age of onset for this progressive-onset MS is usually around 40 years. Finally, on average more women than men (2:1) are affected in relapse-onset MS, but not in progressive-onset MS.

Depending on the location of lesions and type of MS onset, the first symptoms of patients with relapsing-onset MS usually include acute unilateral optic neuritis, partial myelitis or a brainstem syndrome. In contrast, for progressive-onset MS patients, symptoms progress slowly over the course of months or years, such as asymmetric paraparesis, hemiparesis or cerebellar ataxia. Other physical symptoms that can occur during the disease are numbness, tingling, body weakness, imbalance, gait impairment, spasms, and vision loss.

**Treatment**

In MS, several treatment options exist, which target the inflammatory component and are therefore only available for patients with RRMS. These therapies aim to prevent the occurrence of new inflammatory episodes (i.e. relapses) and slow down disease progression. Discrimination can be made between first-line therapy and second-line therapy. First-line therapy aims to reduce the inflammatory response and consists of injectable and oral drugs, such as interferon beta and glatiramer acetate (injectable), and more recently registered, dimethyl fumarate and teriflunomide (oral). These therapies reduce relapse rate by approximately 30%. Second-line therapies are more effective, but usually have more severe side effects, as they aim to block the immune response completely. Currently, two types of second-line treatment are used in regular clinical practice: natalizumab and fingolimod. Natalizumab prevents the passage of immune cells through the blood-brain barrier, whereas fingolimod prevents the departure of immune cells from the lymph nodes. Both therapies reduce the relapse rate with approximately 60% to 70%. Recently, the efficacy of several new therapies has been investigated in phase III clinical trials, such as ocrelizumab and laquinimod, showing varying results. Ocrelizumab was found to reduce relapse rate with approximately 45% compared to interferon beta, whereas laquinimod did not.
significantly reduce relapse rate compared to placebo or interferon beta.\textsuperscript{23,24} Finally, hematopoietic stem cell therapy has recently been investigated in several clinical trials.\textsuperscript{25,26} As stem cell therapy is such an aggressive treatment option, these trials include only small groups of patients with a very active disease barely responding to regular MS medication. Nevertheless, the results of these trials seem promising for this particular group of patients (i.e. very active RRMS), as most of the patients showed activity-free survival for several years (approximately three to five years).\textsuperscript{25,26}

Cognitive problems

Besides well-described physical problems, between the 40\% and 70\% of MS patients suffer from cognitive problems, irrespective of their disease phenotype.\textsuperscript{27,28} The term cognition comes from the Latin verb \textit{cognoscere}, which means \textit{to know} or \textit{to learn}, and refers to mental actions or processes of acquiring knowledge and understanding through thought, experience and senses. More precisely, it refers to complex behavior, such as, amongst others, information processing speed, learning and memory, attention, and executive functions (i.e. cognitive domains). In MS, cognitive domains that are often affected include information processing speed, learning and memory, and executive functions.\textsuperscript{29}

The performance on different cognitive domains can be assessed with a neuropsychological test battery. Several well-established neuropsychological test batteries are widely used in a research and clinical setting. These include the \textit{Brief Repeatable Battery of Neuropsychological} tests (BRB-N) and the \textit{Minimal Assessment of Cognitive Function in MS} (MACFIMS).\textsuperscript{30,31} More recently, a brief version of the MACFIMS, the \textit{Brief International Cognitive Assessment for MS} (BICAMS), has been developed as a screening tool for cognitive impairment.\textsuperscript{32}

Various factors can influence cognitive functioning, such as fatigue, sleep-related problems and depression, which are called confounders of cognition.\textsuperscript{33–35} Sleep-related problems, such as obstructive sleep apnea, insomnia, and sleep disturbances, occur in approximately 50\% of MS patients.\textsuperscript{36,37} Furthermore, it has been shown that it can explain up to a quarter of variance in fatigue.\textsuperscript{38} Self-reported sleep problems usually correlate with the severity of perceived cognitive problems, whereas objective measures of sleep quality (using polysomnography) have been associated with objective cognitive functioning.\textsuperscript{33,39–41} The prevalence of depression in MS is approximately 25\% to 30\%, which is two to three times higher than in the general population.\textsuperscript{42–44} Several studies have shown a relationship between depression and worse cognitive functioning on several domains, including information processing speed, visuospatial memory, working memory, and executive functions.\textsuperscript{35,45}
As one can imagine, these psychological and cognitive problems have a great impact on a patient’s quality of life.\textsuperscript{46} Hence, there is a great need for better understanding of these problems from a neurobiological perspective, hopefully resulting in novel, and (more) effective, therapies. But how can we visualize the brain and link abnormalities to cognitive decline?

**Radiology: in vivo visualization of brain abnormalities in MS**

Since the 1980s, magnetic resonance imaging (MRI) has been used to visualize and study the brain in neurological disorders (see Box 1.1 for an introduction on MRI physics). In MS, MRI has become an invaluable tool for diagnosing the disease.\textsuperscript{47} Diagnostic criteria state that a person must fulfill two criteria in order to be diagnosed with MS: *dissemination in space* and *in time*. *Dissemination in space* can be proven by having a lesion in at least two out of five areas of the central nervous system (i.e. periventricular, infratentorial, juxtacortical, optic nerve, and spinal cord). *Dissemination in time* is proven by the presence of at least one new T2 or gadolinium-enhancing lesion on follow-up MRI (with reference to a baseline scan) or the simultaneous presence of gadolinium-enhancing and non-enhancing lesions at any time.\textsuperscript{47}

For researchers, MRI can provide insight into the neural correlates of cognitive dysfunction. The MRI techniques applied in MS research vary greatly from relatively simple (i.e. conventional) MRI sequences that visualize brain lesions or atrophy, often used in clinical setting as well, to advanced measures that quantify the integrity of brain tissue and network characteristics. Several MRI techniques and their relationship with overall cognitive functioning in MS will be introduced below. Next, we will present a selection of (functional) MRI studies investigating problems with information processing speed and learning and memory, as these are common cognitive problems in MS and the main topics covered in this thesis.

**White and grey matter lesions**

The white matter lesions that can be typically seen in MS patients, can be visualized *in vivo* with conventional MRI techniques such as T2-weighted or fluid attenuated inverse recovery sequences (FLAIR; see Box 1.2 for more information on MRI sequences). However, both clinicians and researchers have struggled with the poor correlation between white matter lesions and clinical manifestation of the disease. That is, some patients only have few white matter lesions in the central nervous system but are seriously affected in terms of clinical disability (i.e. physical and cognitive problems), while other patients can have a large amount of white matter lesions and are only mildly affected. This apparent contradiction is also referred to as the *clinico-radiological paradox* of MS.\textsuperscript{48}
As white matter lesions cannot fully explain MS-symptoms, including those related to cognition, researchers have developed new imaging techniques to better visualize the grey matter that is most likely to play a role in cognitive functioning. Additionally, in post-mortem tissue of patients, grey matter lesions were observed, which led to the development of specialized MRI sequences to visualize the grey matter, and its lesions, in vivo. One of the most often used techniques to detect this type of lesion, with relatively high specificity verified post-mortem, is the double inversion recovery (DIR) sequence. Although grey matter lesions relate stronger to cognitive problems than white matter lesions, correlations are still moderate.

Brain atrophy
Next to lesions, brain atrophy was already observed in the late 1970s with computerized tomography (CT) in the form of widening of the ventricles. Soon, MRI replaced CT allowing for more accurate detection as it can better distinguish between brain tissue types. Yearly whole-brain atrophy in MS is estimated to be approximately three times higher than in the general (healthy) population with a 0.7% loss per year (compared to 0.1% to 0.3% per year in healthy individuals). This whole-brain atrophy is the combined result of white and grey matter loss. When MS progresses, especially the rate of grey matter atrophy is thought to increase and relates quite strongly to physical and cognitive problems.

Advanced MRI techniques
Lesions and atrophy are profound brain abnormalities that occur in MS, but unfortunately not sufficient to fully understand the neurobiological mechanisms of cognitive decline. More subtle structural brain alterations also occur that are highly relevant for optimal functioning of the brain. For example, reduced integrity of (normal appearing) white and grey matter tissue can be observed in MS, by using diffusion tensor imaging (DTI; see Box 1.2). The reduction in integrity of white matter tracts is thought to hamper signal transduction between brain regions and thereby resulting in various (cognitive) problems.

The abovementioned MRI techniques have enabled us to (partly) capture structural brain abnormalities. However, this does not necessarily tell us anything about (changes in) brain function that might be affected by MS. The development of functional MRI (fMRI) has enabled us to assess activation of brain regions during a specific (cognitive) task or at rest (resting-state; intrinsic activity in the absence of any sensory or cognitive stimulus), by mapping the change in the level of blood oxygenation (see Box 1.2). Compared to healthy subjects, in MS various brain regions show differences in the level of activation when performing cognitive tasks. For example, cognitively impaired patients usually display a widespread decrease in brain activation compared to healthy controls, whereas cognitively impaired patients...
preserved patients can show increased brain activation compared to healthy subjects. This observation has puzzled many researchers and its interpretation still causes much debate in the field: do patients display some kind of functional compensation mechanism early in the disease that prevents overt cognitive problems, is the observation of increased task-activation prior to overt cognitive problems a sign of maladaptation, or can it be both?

Connectomics: the brain as a network
In the past decade, the investigation of focal changes in brain activation has shifted to more holistic network-based approaches. That is, considering the brain as a complex network of interconnected brain regions that communicate dynamically with each other, which ultimately underlies the emergence of complex behavior (e.g. cognitive functions). For example, in resting-state fMRI, patterns of simultaneous brain activation can be extracted by using independent component analysis, which has led to the identification of various resting-state networks. Some of these networks, such as the default mode network and frontoparietal network, are highly relevant for information processing throughout the brain and relate to cognitive functions in health and neurological disorders, such as MS.

At the same time, neuroscience has embraced the methods of network science to capture the brain's structural (i.e. white matter connections between brain regions) and functional (i.e. communication between brain regions) organization (also termed connectomics). Brain networks can be investigated using graph theory in which the network is represented by nodes (or vertices) that are interconnected by links (or edges; see Figure 1.2). In a functional brain network, nodes can be predefined brain regions and the links the statistical dependence between the activity of brain regions (i.e. functional connectivity). Various network measures can be applied to such a graph in order to quantify its topology and certain characteristics, such as its efficiency or level of integration and segregation. In MS, the characteristics of structural and functional networks are different compared to that of healthy subjects. The observations of widespread network changes in MS have led to a novel and more holistic hypothesis for cognitive decline in MS, namely that of a network collapse. That is, the brain's functional network of patients becomes increasingly less efficient. At a certain moment in the disease, a "tipping point" is reached, after which the entire brain network "collapses", thereby resulting in overt cognitive impairment. To date, much effort is put into ways to measure (aspects of) network efficiency and to determine the (individual) tipping point.
Box 1.1 The basics of magnetic resonance imaging: countless shades of grey

Magnetic resonance imaging (MRI) is a medical imaging technique that allows in vivo visualization of different tissues types and features of the brain, such as wiring and brain activation. An MRI scan is built up of voxels (three-dimensional pixels) with certain dimensions (e.g. 1 × 1 × 1 mm) that display grey values (i.e. intensities). The MRI scanner is a large magnet in which the patient is positioned. Due to the strong magnetic field, protons in the patient’s body are aligned in the direction of the magnetic field (which is called the preferred direction; see Figure 1.1). Next, a radio frequency pulse is emitted, which flips the alignment of protons. However, the protons want to realign to their preferred direction (i.e. parallel to magnetic field). During this realignment, energy is emitted from the protons, which can be measured by the receiving coil of the MRI scanner. Eventually, the amount of energy emitted is translated into a certain grey value for each voxel. However, the time it takes to realign to the preferred direction varies across tissue types (i.e. relaxation time), resulting in differences in emitted energy and thus grey values. Hence, different shades of grey fill up the image, enabling to distinguish tissue types. Sequence parameters can be adjusted to image different properties of tissue, pathology, or brain features.

Figure 1.1 A schematic representation of image acquisition using magnetic resonance imaging

In the magnetic resonance imaging (MRI) scanner, hydrogen protons are aligned with the magnetic field (A). The radio frequency pulse flips the alignment of protons (B). However, the protons want to realign parallel to the magnetic field (i.e. preferred direction), thereby emitting energy. Depending on the tissue type, the time it takes to realign to the preferred direction, and thus the emitted level of energy, varies (C). Finally, the emitted energy is translated into grey values by the scanner, resulting in an image (D).

RF: radio frequency
Figure 1.2 A graph-theoretical representation of the brain

The brain can be represented as a network in which nodes (or vertices) are connected by links (or edges). The nodes can be individual voxels or brain regions. Links can represent the strength in functional connectivity at rest or during task performance (the amount of communication between brain regions; see Box 1.3 for more information) or structural connectivity (the strength of physical connections). Based on this network representation of the brain, various measures can be obtained, such as the average strength of connectivity of each node.
Box 1.2 Conventional and advanced MRI sequences

Various conventional (i.e. "standard") and advanced MRI sequences have been developed and used to investigate neural correlates of cognitive functioning in MS. Below we will briefly describe the main MRI techniques and their application that have been used in this thesis.

T1- and T2-weighted imaging

With a T2-weighted sequence, MS lesions can be seen as focal hyperintensities on the image (see Figure 1.3). The volume of all lesions together is often referred to as lesion load. However, these T2-weighted lesions are rather unspecific in terms of underlying pathology. On a T1-weighted sequence, the anatomy of the brain can be visualized. In addition, a subset of the lesions can be seen as focal hypointensities, also known as black holes, indicating axonal loss (see Figure 1.3). To visualize acute inflammatory processes, during a T1-weighted scan a contrast agent (e.g. gadolinium) can be administered, which contains iron particles that can "leak" into the brain where the blood brain barrier is disrupted, resulting in hyperintense “spots” on T1-weighted images. Because of the abovementioned features, T1- and T2-weighted images are often acquired in routine clinical practice for diagnosing and monitoring MS. In a research setting, T2-weighted images can be used to calculate the total lesion load, indicative for overall brain damage, whereas T1-weighted images are used to segment white and grey matter (cortical and subcortical) in order to calculate brain volumes.

![Figure 1.3 T2- and T1-weighted image of a patient with multiple sclerosis](image)

A T2- (left) and T1-weighted image (right) in which white matter lesions are indicated by red and blue circles. The blue circles indicate black holes.
Fluid attenuated inverse recovery imaging

A fluid attenuated inverse recovery (FLAIR) sequence shows MS lesions as hyperintense areas, while suppressing (i.e. darkening) the signal coming from the cerebrospinal fluid (see Figure 1.4). Hence, lesions in the white matter, but also cortical and juxtacortical lesions, can be nicely visualized. FLAIR imaging seems to be superior to T2-weighted imaging for detection of MS lesions, and is therefore often used in research setting.76

Figure 1.4 FLAIR image of a patient with multiple sclerosis

A FLAIR image in which several white matter lesions are indicated by red circles.

Diffusion tensor imaging

With diffusion tensor imaging (DTI), the diffusion of protons in the brain is measured. With this imaging technique, the integrity of the white matter, but also that of the grey matter, can be quantified. The diffusivity of water molecules can be expressed in different measures, namely:

- Axial diffusivity: diffusivity along the axon
- Radial diffusivity: diffusivity perpendicular to the axon
- Mean diffusivity: average diffusion
- Fractional anisotropy (FA): a summary measure that quantifies the degree of anisotropy (i.e. directionality) of diffusion

Intact white matter (i.e. in the healthy brain) restricts water diffusion along the axons, resulting in high directionality of water diffusion (i.e. high FA) along the axons. However, when myelin is damaged, the diffusion of water molecules also occurs in other directions (e.g. perpendicular to the axon). This results in decreased directionality of water diffusion and thus lower FA values (but higher radial diffusivity values), indicative for decreased white matter integrity.
Information processing speed deficits on MRI

The exact definition of the construct ‘information processing speed’ is debatable. However, a recent review on this topic identified three common definitions: (1) the amount of time a person needs to execute a cognitive task, (2) a complex construct that is the result of the interaction of multiple factors (i.e. it is not a unitary construct), and (3) from a physiological level, it is the speed with which one can process information in the brain. As information processing speed is quite an elementary cognitive function, it is thought to influence "downstream" cognitive processes, such as learning and memory, working memory, and executive functions. Hence, it has been suggested that measuring information processing speed in MS provides a quite reliable estimation of overall cognitive performance.

Approximately 50% of patients with MS suffer from information processing speed problems. From a neurobiological perspective, information processing speed intuitively relies on the ability of the brain to rapidly communicate between remote brain regions (i.e. process information). With this in mind, intact white matter connections seem to be very important for optimal signal transduction and processing. In MS, damage to the white matter (lesions, atrophy and reduced tissue integrity) has indeed been linked to problems with information processing speed. However, cortical and subcortical atrophy (e.g. thalamus) has also been linked to degraded information processing speed. On a functional level, brain activation during an information processing speed task was found to be lower in MS patients compared to healthy controls in various regions, including the anterior cingulate cortex, inferior and middle frontal gyri, and parietal cortex.
Additionally, altered patterns of effective connectivity (i.e. the direction of functional connectivity) have been found in MS patients compared to healthy controls, but also between MS patients with and without information processing speed problems, with alterations in regions that belong to the frontoparietal network.\cite{85,86} Finally, better information processing speed in MS has been linked to stronger functional connectivity between medial prefrontal and frontal pole regions at rest.\cite{87}

### Learning and memory deficits on MRI

Memory is a complex trait that can be divided into different subtypes, such as *implicit* (non-declarative) and *explicit* (declarative) memory. Implicit memory refers to the ability of people to express acquired information via performance in the *absence of conscious awareness of memory* (i.e. no reference to learning episodes), whereas explicit memory is characterized by *awareness of memory retrieval*.\cite{88,89} Especially explicit memory, including verbal and visuospatial memory, seems to be disturbed in MS, with problems mainly in memory encoding, but not so much in retrieval.\cite{28,29,90,91}

From a neurobiological perspective, the hippocampus and its connections to the (frontal) neocortex (i.e. the medial temporal memory system) play a crucial role in (explicit) memory formation.\cite{89,92} From pathology studies we learned that MS-pathology is present in the hippocampus in the form of focal demyelination and atrophy.\cite{93,94} *In vivo* MRI studies have confirmed these abnormalities and linked them to poor performance on memory tests. That is, hippocampal lesions, atrophy and reduced quality of important white matter tracts (e.g. cingulum) have been associated with worse verbal and/or visuospatial memory performance.\cite{95–99} Functionally, the hippocampus showed less activation during memory encoding in cognitively impaired versus cognitively preserved patients and healthy controls.\cite{61} Additionally, increased functional connectivity between the hippocampus and especially frontal regions has been observed in memory impaired compared to memory preserved patients and healthy subjects.\cite{100} However, another study showed that on a network level memory impaired MS patients have lower connectivity of the default mode network than memory preserved patients.\cite{101}

To summarize, previous studies have shown that advanced MRI can help to better understand problems with information processing speed and memory in MS. As MS is such a complex disease, and cognitive functioning a complex trait, we argue that multimodal MRI studies, in which conventional and advanced MRI techniques are combined, are essential for increasing our understanding of cognitive decline.\cite{102,103} Hence, in the present thesis a multimodal approach will be adopted in order to better understand problems with information processing speed and memory.
Shifting toward dynamics

In this thesis, the term dynamics refers to two different concepts, namely brain communication and cognitive deterioration. First, and most important: the brain is not a static organ, but highly dynamic in terms of communication within and between brain networks. These dynamics in communication are thought to underlie (complex) behavior such as cognition. In neurological disorders, brain dynamics can be affected, thereby possibly explaining a part of the variance in cognitive (dys)functioning. The second concept refers to cognitive decline: this is per definition a process that occurs over time. However, many studies conducted in the past decades only investigated the neural correlates of cognitive functioning in a cross-sectional manner. In this thesis, we investigated both concepts of dynamics.

From connectomics to chronnectomics

The focus on the brain's connectome (i.e. functional connections between brain regions) has recently shifted towards the brain's chronnectome: the changes in functional connectivity that occur during performance of a task or at rest. On a small scale, neuronal signaling is highly dynamic as it adapts to external input (e.g. task performance). On a larger scale this can also be observed, as the entire brain's functional network adapts upon task demands. These brain dynamics are thought to underlie the emergence of cognition and are therefore of interest in neurological disorders.

From a methodological perspective, functional connectivity has usually been quantified by averaging the correlation coefficient between brain activation of remote regions over an entire time series (i.e. during a task or at rest; from here on referred to as stationary functional connectivity). In contrast, brain dynamics refers to the variability in functional connectivity during a time series (from here on referred to as dynamic functional connectivity). Although different methods exist to quantify dynamic functional connectivity, in this thesis we used a sliding window approach (see Box 1.3 for a detailed description of stationary and dynamic functional connectivity).

Various studies in healthy subjects have shown the behavioral relevance of dynamic functional connectivity. For example, dynamic functional connectivity of several brain regions and networks during executive functioning, working memory, and attention tasks have been linked to cognitive performance. Resting-state dynamic functional connectivity has also been linked to performance on various domains, such as executive functions, episodic memory, and attention. Moreover, it has been shown that dynamic functional connectivity can explain more than twice the variance in different behaviors (e.g. alertness, cognition, and emotion) as compared to stationary functional connectivity. However, a big
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Box 1.3 Stationary and dynamic functional connectivity

With task-related and resting-state fMRI we acquire a series of signals over time for each voxel in the brain. These so-called *time series* represent levels of activation for each brain region (see Figure 1.5A; in this figure the time series for region A and B are displayed) over the entire duration of the scan. With the time series of region A and B we can calculate the synchronicity between both time series using Pearson correlation coefficients (see Figure 1.5B), which indicate the average strength of functional connectivity (i.e. stationary functional connectivity) that can be visualized as a graph (see Figure 1.2). However, as one can see in the Figure 1.5A, there is variation in synchronicity between brain activation over the entire time series. With stationary functional connectivity this information is lost due to averaging over the entire time series.

With dynamic functional connectivity, the *variation* in functional connectivity over the time series is captured. One of the methods to do so, and used in this thesis, is the *sliding window approach*. With this method, the entire time series is divided into partly overlapping windows of approximately 60 seconds and shift of approximately 10 seconds (see Figure 1.5C). For each window, stationary functional connectivity is calculated. Next, the absolute change in stationary functional connectivity between each consecutive window is calculated and summed, resulting in one value of dynamic functional connectivity for each connection (e.g. between region A and B). The higher the value, the more fluctuations in functional connectivity occur during task performance or at rest.

challenge is the interpretation of the correlation between dynamic functional connectivity and cognition, which are both positive and negative, depending on the cognitive domain that is investigated and the operationalization of brain dynamics.

In other neurological disorders, changes in dynamic functional connectivity have been observed. For example, in Parkinson’s disease, increased dynamic functional connectivity at rest was found compared to healthy subjects, which was related to more severe motor problems. However, in major depressive disorder and epilepsy, decreased dynamic functional connectivity has been observed. In the latter disease, epilepsy patients with overt memory problems displayed lower dynamic functional connectivity than patients without memory problems. The heterogeneity of findings with respect to dynamic functional connectivity between patient groups, its link with symptoms, but also the link between dynamic functional connectivity and cognition in healthy subjects, are most likely explained by different types of pathology, operationalization of dynamic functional
Figure 1.5 Overview of stationary and dynamic functional connectivity analysis

Each voxel, or brain region, displays different levels of brain activation during a cognitive task or at rest, which is called a time series (A). Stationary functional connectivity is calculated by averaging the synchronicity in brain activation for region A and B over the entire time series (indicated by the transparent yellow box; B). This can be calculated with Pearson correlation coefficient. With a sliding window approach, the time series are divided into partially overlapping windows (indicated by the transparent colored boxes) for which stationary functional connectivity is calculated (C). Next, the absolute change in stationary functional connectivity between each consecutive window is calculated and summed, resulting in a measure indicating the variability in connectivity over the entire time series.

dFC: dynamic functional connectivity; sFC: stationary dynamic functional connectivity
connectivity and the state in which it is measured (i.e. during task performance or at rest). This heterogeneity and difficulty regarding interpretation of findings highlight that the field of chronnectomics is still in its infancy, although emerging rapidly. Nevertheless, dynamic functional connectivity seems to capture certain aspects of the brain, such as the ability to adjust to constantly changing demands, that may have not been captured with other advanced MRI measures before. So far, the relevance of dynamic functional connectivity for cognitive functioning in MS has not been studied. Therefore, in this thesis we will explore the added value of this novel measure on top of other conventional and advanced MRI measures in understanding problems with information processing speed and memory.

**Predicting cognitive decline in MS**

Short-term cognitive changes (< 5 years) in MS seem to be very subtle; perhaps even too subtle to detect with most neuropsychological tests.\(^{117}\) Although relatively scarce, studies with a longer follow-up duration (more than 5 years) have yielded interesting insight into the "dynamics" of cognitive decline. Early studies showed that patients remained stable over a period of 8.5 years on tests for attention and visuospatial memory, but not for verbal memory, which was unrelated to the increase in lesion load, whereas in another study with follow-up duration of ten years a substantial part of patients (20 out of 37) remained cognitively unchanged.\(^{118,119}\) More recent studies show similar results. For example, studies with follow-up duration between five and 18 years showed a decline in several domains (e.g. information processing speed, attention, episodic memory, and executive function), or less specific, an increase in overall cognitive impairment between 18% and 28%.\(^{120,121}\) Interestingly, cognitive decline was associated with lesion accumulation in all lobes, except the occipital lobe, and whole-brain atrophy of white and grey matter.\(^{120,121}\) Another study explored the ability of baseline MRI to predict cognitive decline over seven years, and showed that diffuse brain damage (measured with magnetic transfer ratio) could predict memory decline, while deterioration in information processing speed was related to baseline whole-brain atrophy.\(^{122}\) Unfortunately, all aforementioned studies included relatively small groups of patients (12 to 66), thereby limiting the generalizability of the results. Hence, in a large cohort of 234 patients we investigated cognitive deterioration over a period of five years and what MRI measures at baseline could predict this decline.
Thesis aim and outline

The overarching aim of this thesis was to better understand the neural correlates of cognitive decline in MS, with emphasis on information processing speed and memory. This was achieved using a multifaceted and dynamic approach, namely: investigating confounders of cognition, exploring dynamic brain measures with respect to cognitive (dys)functioning, and investigating cognitive decline over a period of five years. This thesis is divided into chapters that address more specific research questions that contribute to the overarching aim:

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, that is, 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?

The studies that were performed in order to answer the abovementioned research questions are assigned to four chapters. In Chapter 2 we will investigate potential confounders of cognition (i.e. sleep-related problems and depression) and their association with advanced structural and functional brain measures. Structural and functional brain measures that underlie problems with information processing speed and memory will be investigated in Chapter 3 and 4, respectively. Both chapters contain multimodal imaging studies in which we will explore the added value of dynamic functional connectivity on top of conventional and advanced MRI measures in explaining variance in these cognitive domains. In Chapter 5 we will investigate cognitive decline over a period of five years in a large cohort of 234 patients and identify which MRI measures at baseline best predict cognitively declining versus non-declining patients. Finally, in Chapter 6 we will summarize and discuss the findings of all performed studies. Furthermore, we will make recommendations for future studies on cognition in MS, supported by pilot data which illustrates a possible method to operationalize network efficiency.
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