

**Organizational principles of  
spontaneously arising  
functional connectivity in the human brain**

- Insights from pathological alterations  
in multiple sclerosis

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# Abstract

The brain is a complex network consisting of an astonishing number of cells that interact with one another in a manifold manner. On different spatial and temporal scales, various aspects of this system can be described analytically, including the arrangement and nature of its structural elements, the topology of emerging networks of functional communication, or dynamic changes of such structural or functional features. The macroscopic examination of functional patterns that arise in the absence of external stimuli, so-called resting state, is considered to be a particularly useful and convenient tool, and has become exceptionally popular in both basic and clinical neuroscience for this reason. Over the last few decades, a wide range of methodological and conceptual approaches have been developed for this purpose and applied in studies with most divergent research questions. Nonetheless, the overall understanding of the underlying organizational principles of such spontaneously arising functional connectivity in the human brain is still limited. A promising approach towards a more comprehensive picture is the investigation of alterations in neurological patients. It enables the inquiry into effects of structural impairment on functional connectivity in humans and the exploration of associations with symptoms on the behavioral level. Multiple sclerosis (MS) is a multifaceted chronic neurological disorder, that is characterized by inflammatory and demyelinating pathological processes in the central nervous system, leading to circumscribed white matter (WM) lesions. Studying this clinical population offers therefore the unique opportunity to explore specifically the effect of local WM impairment, distinguishing MS from other neurological disorders.

For the present doctoral thesis, resting state functional connectivity based on functional magnetic resonance imaging (fcMRI) was explored cross-sectionally and longitudinally in 40 patients suffering from relapsing remitting MS and a group of individually matched healthy controls. The work is divided into four analyses that focus on different aspects. Each analysis has a short introduction and a discussion section on the significance of the findings and the specific methodological limitations. In the first analysis, cross-sectional group differences in fcMRI, structure, and behavior were described in detail. As for all analyses, functional connectivity was defined as the Pearson's correlation between blood oxygen level-dependent (BOLD) signal time-series of pairs of regions of interest (ROIs), and computed between a set of ROIs covering the whole brain. This basic analysis is followed by a systematic evaluation of connection-specific relationships between fcMRI and both WM integrity and cognitive performance. Each functional connection was classified by its qualitative association with these two variables, and various features of the resulting distribution of connection-types were described. The third analysis focused on group differences in the variability of fcMRI over time, captured with a sliding-window approach, and on how this feature relates to performance in neuropsychological testing and clinical status. The final analysis of this doctoral thesis concentrated on the longitudinal study part, hence alterations that arose over the observation period of one year in 38 of the original 40 MS patients and their matched controls. To distinguish between functional, structural, and behavioral changes that occur in the course of natural aging and those that reflect disease progression, the analysis focused on interaction effects between time-points and study groups. Another aim of this part was to explore which parts of the functional connectome would correlate with the individual changes in fatigue severity, a particularly burdening symptom pattern that affects the majority of MS patients. In addition, the available literature on fcMRI in MS was reviewed systematically to complement the empirical work and to provide an adequate base for its interpretation within the scientific context.

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The findings of the conducted analyses indicated considerable inter-individual variability with respect to the location, strength, and the effect of the structural impairment on the functional connectome and the manifestation of behavioral symptoms in MS. Statistical evidence for group differences in fcMRI and behavioral variables was therefore weak, despite substantial alterations on the individual level. The largest cross-sectional differences and longitudinal changes in fcMRI, however, revealed both local increase and decrease of functional connectivity in MS patients. The exploration of connection-specific associations suggested a bias towards negative relationships between fcMRI levels and both cognitive performance and structural integrity in patients. In healthy controls, on the other hand, lower structural integrity was more often associated with lower fcMRI, which in turn was related to worse cognitive performance. In addition, the whole-brain association patterns of structure and behavior were shown to differ considerably from each other, indicating that WM impairment and behavioral symptoms are mediated by functional mechanisms that cannot be explained by local effects alone. The third analysis revealed an increase of the variability of fcMRI over time in MS with a beneficial effect on the severity of the overall disability, fatigue, memory, and attention capacity. Last, the longitudinal analysis disclosed distinct interrelation patterns between changes in fcMRI and motor, respectively cognitive fatigue. Most importantly, worsening of fatigue was related to increasing fcMRI in both cases.

The results of this explorative investigation confirm the occurrence of both increase and decrease of fcMRI in MS. The outcome furthermore suggests that the manifestation of associated behavioral symptoms is substantially shaped by the influence of indirect and secondarily arising functional alterations, hence the spread of the impact of primary disturbance within the functional connectome. Together, these interpretations introduce a new perspective on compensational effects in MS that complements but also integrates previous contradicting findings. In addition, no clear evidence emerged for a specific role of a single network for MS, the impact of WM impairment, cognitive disturbance, or fatigue severity. Instead, the findings indicate a highly relevant role of static and dynamic characteristics of functional connections that link classical resting state networks.

The thesis closes with a general discussion on the significance of these findings for the development of a comprehensive concept of functional compensation, the insight into longitudinal alterations of fcMRI in the healthy brain, the understanding of the neuropathology of MS, and finally the translation of neuroimaging findings into clinically useful biomarkers and upcoming challenges in this field.

# Zusammenfassung

Das Gehirn ist ein komplexes Netzwerk, das aus einer großen Anzahl einzelner Zellen aufgebaut ist, die in unterschiedlichster Art und Weise miteinander interagieren. Verschiedenste Aspekte dieses Systems, wie die Anordnung und Beschaffenheit der strukturellen Elemente, die Topologie funktioneller Netzwerke, oder dynamische Veränderungen, können mit diversen räumlichen und zeitlichen Auflösungen analytisch untersucht werden. Die makroskopische Betrachtung funktioneller Muster, die sich ohne externe Stimulation in einem so genannten Ruhezustand ergeben, hat sich dabei als besonders nützliche Herangehensweise für die Untersuchung von Grundlagen- wie auch klinischer Forschungsfragen etabliert. Im Laufe der letzten Jahrzehnte wurde eine Reihe methodischer und konzeptueller Ansätze hierfür entwickelt und auf unterschiedlichste Fragestellungen angewandt. Das Verständnis grundlegender Prinzipien der spontanen Organisation funktioneller Konnektivität ist trotz allem nach wie vor begrenzt. Ein vielversprechender Ansatz hin zu einem umfassenderen Gesamtbild ist die Exploration von Veränderungen, die in neurologischen Erkrankungen auftreten. Dies ermöglicht einerseits die Erforschung von Effekten struktureller Schädigung auf funktionelle Interaktion im menschlichen Gehirn und erlaubt andererseits die Untersuchung von Zusammenhängen mit Symptomen auf der Verhaltensebene. Multiple Sklerose (MS) ist eine facettenreiche chronische neurologische Erkrankung, deren pathologisches Korrelat entzündliche und demyelinisierende Prozesse sind, die zu umschriebenen Läsionen in der weißen Substanz (white matter, WM) führen. Die Untersuchung dieser klinischen Population bietet daher die Möglichkeit, im Vergleich zu anderen neurologischen Erkrankungen, spezifisch Effekte lokaler Schädigungen der WM zu erforschen.

Im Rahmen dieser Doktorarbeit wurde mithilfe von funktioneller Magnetresonanztomographie funktionelle Konnektivität (fcMRI) im Ruhezustand quer- wie auch auch längsschnittlich in 40 Patienten mit schubförmig verlaufender MS und individuell zugeordneten Kontrollprobanden untersucht. Die Arbeit ist unterteilt in vier Analysen, die unterschiedliche Aspekte im Fokus haben. Jede Analyse hat eine kurze Einleitung und eine Diskussion der Ergebnisse sowie der spezifischen methodischen Limitierungen. In der ersten Analyse wurden zunächst querschnittliche Gruppenunterschiede in fcMRI, Struktur und Verhalten im Detail beschrieben. Wie für alle Analysen, wurde funktionelle Konnektivität dabei definiert als die Pearson Korrelation zwischen den blood oxygen level-dependent (BOLD)-Zeitreihen von jeweils zwei definierten Regionen, und für ein Set von Regionen berechnet, welches das gesamte Gehirn abdeckt. Dieser basalen Untersuchung folgt eine systematische Evaluierung verbindungsspezifischer Korrelationen zwischen fcMRI und der Integrität der WM sowie der kognitiven Performanz. Jede Verbindungen wurde dabei anhand ihrer qualitativen Beziehungen mit diesen beiden Variablen klassifiziert und verschiedene Aspekte der daraus resultierenden Verteilung der Verbindungstypen beschrieben. Die dritte Analyse fokussierte auf Gruppenunterschiede in der Variabilität der fcMRI über die Zeit, ermittelt mit einem sogenannten Sliding Window Ansatz, und auf Zusammenhänge zwischen diesem Maß und der kognitiver Leistungsfähigkeit sowie dem klinischen Zustand. Die letzte Analyse dieser Doktorarbeit konzentrierte sich auf den längsschnittlichen Studienteil, also die Veränderungen, die sich im Laufe von einem Jahr in 38 der ursprünglich 40 MS Patienten und deren Kontrollpartnern ergeben haben. Um natürlich auftretende funktionelle, strukturelle und Verhaltensänderungen von solchen zu unterscheiden, die das Fortschreiten der Erkrankung widerspiegeln, lag der Fokus dabei auf den Interaktionseffekten zwischen Zeitpunkten und Gruppenzugehörigkeit. Ein weiteres Ziel dieser Analyse war zudem, die Teile des funktionellem Konnektoms zu identifizieren, die mit der individuellen Veränderung des Schweregrades der Fatigue korrelierten, einem besonders belastendem Symptom

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der MS. Um die empirische Arbeit zu ergänzen und eine adequate Basis für deren Interpretation zu gewährleisten, wurde zudem die Literatur zu fcMRI in MS systematisch evaluiert.

Die Befunde der durchgeführten Analyse deuteten an, dass es bezüglich der Lokalisierung, der Stärke und des eigentlich Effektes von struktureller Schädigung auf das funktionelle Konnektom und die Manifestierung von Symptomen eine erhebliche inter-individuelle Variabilität zwischen MS Patienten gibt. Die statistische Evidenz für Gruppenunterschiede in fcMRI and in Verhaltensvariablen war daher schwach trotz wesentlicher Veränderungen auf der individuellen Ebene. Die deutlichsten Befunde der quer- und längsschnittlichen Analysen zeigten dabei sowohl lokale Zunahmen wie auch Abnahmen der Stärke funktioneller Verbindungen in der Patientengruppe. Die Untersuchung verbindungsspezifischer Assoziationen ergab für MS Patienten eine Tendenz zu negativen Beziehungen zwischen fcMRI und sowohl Struktur wie auch Verhalten. In der Gruppe der Gesunden war niedrigere strukturelle Integrität dagegen häufiger mit niedriger fcMRI assoziiert, und diese wiederum mit schlechterer kognitiver Leistung. Die Assoziationsmuster der beiden Variablen wichen außerdem stark voneinander ab, was nahelegt, dass Schädigungen der WM und Symptomatik auf der Verhaltensebene über funktionelle Mechanismen vermittelt werden, die sich nicht alleine aus lokalen Effekten erklären lassen. Die dritte Analyse zeigte eine erhöhte Variabilität der fcMRI in MS, was zudem einen günstigen Effekt auf die Schwere der Behinderung, Fatigue, Gedächtnis und Aufmerksamkeit zu haben schien. Zuletzt konnten anhand der Longitudinalanalyse unterscheidbare Assoziationsmuster identifiziert werden zwischen längsschnittlichen Veränderungen der fcMRI und motorischer bzw. kognitiver Fatigue. Eine Verschlechterung ging dabei in beiden Fällen mit steigender fcMRI einher.

Die Ergebnisse dieser explorativen Untersuchung bestätigen vorherige Befunde von stärkerer, als auch schwächerer fcMRI in MS im Vergleich zu gesunden Kontrollprobanden. Die Resultate legen weiterhin nahe, dass die Manifestation von Symptomen substanziell durch den Einfluss indirekter und sekundär auftretender funktioneller Veränderungen geprägt ist, also der Ausbreitung des Primäreffektes über das funktionelle Konnektom. Zusammengefasst eröffnen diese Interpretationen eine neue Perspektive auf kompensatorische Effekte in MS, die frühere widersprüchliche Befunde ergänzt, aber auch deren Integration ermöglicht. Es konnte zudem keine klare Evidenz für eine spezifische Rolle eines bestimmten Netzwerkes für die MS, Effekte von Läsionen in der WM, kognitive Defizite oder des Schweregrades der Fatigue gefunden werden. Stattdessen weisen die Ergebnisse auf eine höchst relevante Rolle statischer und dynamischer Aspekte funktioneller Kommunikation zwischen bekannten Netzwerken im Ruhezustand hin.

Die Arbeit schließt mit einer allgemeinen Diskussion der Bedeutung der Befunde für die Weiterentwicklung eines Konzepts für funktionelle Kompensation im Kontext von Läsionen der WM, Einblicke in längsschnittliche Veränderungen von fcMRI im gesunden Gehirn, das Verständnis der Neuropathologie der MS sowie die klinische Anwendung derartiger Ergebnisse und zukünftige Herausforderungen dieses Feldes.

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"I hadn't recognized it until that reminder from my friends, but it's true:  
We get to do cool things, and it's not just playing with torches.  
Whenever I decide that my work is getting boring, I can go and learn a new technique.  
I'm encouraged to argue with my lab mates and my adviser,  
and I win praise for making things up - that is,  
for coming up with ideas and solving problems in creative ways.  
We make things.  
We play.  
And the people we work with are great."

- by Cathy Walker (2014) published in Science

In the last three and a half years, I have experienced all the ups, seen all the great, done all the cool stuff, felt all the passion. I have gone through all the downs, have suffered from the worst despair, have fought the toughest frustration, and have been disappointed in all possible ways.

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**Part I.**

# **General Introduction**





# 1. Functional connectomics

The brain consists of an enormous number of single cells that interact continuously with one another in divergent ways, realizing small- and large-scale flow of information in space and time. Studying the organization of neurons that underlies and facilitates the precise interplay between the central nervous system and its environment is the subject of a scientific field called connectomics (Sporns et al., 2005; Sporns, 2013b). While this term had referred originally to the investigation of the anatomical network structure, the rationale has been applied to the functional level as well by now (Horn et al., 2014). Functional connectomics consequently aim at going beyond the focus on anatomical wiring. Instead, this field pursues the disclosure of complementing insights into principles of functional communication that enable the emergence of coherent percepts, higher cognition, and the rise of conscious experience. Both structural and functional connectomes can be defined on multiple spatial scales, ranging from explorations on the cell level to descriptions of macroscopic interrelations using neuroimaging tools. In addition, multiple techniques and metrics are available that can be applied to explore divergent aspects of connectivity. Despite or perhaps also due to this wide range of methodological and conceptual approaches, the understanding of functional and structural connectomes in the human brain is still limited. This applies in particular to higher controlling systems of functional integration patterns, the interdependency between functional and structural connectivity, and mechanisms that lead to and result from modifications on the functional or the structural level.

The overall aim of this doctoral project was therefore to reveal new insights into organizational principles of large-scale functional networks in the human brain by examining pathological alterations in spontaneously arising functional connectivity patterns. In the following chapter, an introductory overview will be provided covering theoretical, conceptual, and experimental aspects relevant for the understanding and the investigations of functional connectivity in the healthy and the diseased brain. This includes an outline of the history of functional connectivity in resting state, a brief summary of the current understanding of the relationship between the structural and the functional connectome, an overview covering methodological aspects, and an introduction into the usage of this approach for clinical research questions. Due to the focus of this doctoral thesis on task- and stimuli-free data, methodological specificities of the acquisition or analysis of functional connectivity during task conditions are not covered. In addition, considering the main modality of the doctoral project, priority is given to functional connectivity based on functional magnetic resonance imaging (fcMRI). Details of other modalities, such as electroencephalography (EEG), magnetoencephalography (MEG), and positron emission tomography (PET), will not be discussed.

Following the terminology used in the literature on fcMRI, the terms functional connectivity, functional integration (e.g. Gamboa et al., 2014), functional interaction (e.g. Sharp et al., 2011), and functional communication (e.g. van den Heuvel and Hulshoff Pol, 2010), are used synonymously in this work to describe functional connectivity on the macroscopic level as defined by Friston (1994).

## 1.1. History of fcMRI in the absence of task and stimulation

To understand how the brain can efficiently process most diverse information, two main principles have been proposed: segregation and integration (Friston, 2011). Segregation refers to the specialization of regions for the processing of certain information or distinct processing steps. It is typically investigated with task- or stimulation-designs and based on the assumption that regions, whose activity or energy

consumption is time-locked to the temporal features of the experiment, are involved in the processing of the task, respectively the applied stimulus (e.g. Riccelli et al., 2016). A more classical approach in this context are lesion studies, where the functional relevance of an areal is derived upon reversion by the association between the structural damage and the observed behavioral deficit (e.g. D’Esposito et al., 2006). Integration, on the other hand, means the functional embedding of such distributed processing. Empirically, it can be characterized using two concepts, namely *functional* and *effective connectivity*. The first is defined as the statistical dependency between spatially remote neurophysiological events, whereas the second describes the influence of one brain region or neuron over another, thus causal relationships within a network (Friston, 1994, 2011). The neurophysiological rationale behind the concept of functional connectivity is based on the well-established assumption that some sort of binding mechanism is necessary for the brain to transfer information and to give rise to conscious perception (Engel et al., 1999; Engel and Singer, 2001; Fingelkurts et al., 2005). More than two decades ago, Singer and Gray (1995) postulated that the representations of different features of a perceptual object are integrated by synchronized oscillations of spatially remote neuronal populations, in line with the earlier formulated theory-based binding-by-synchrony hypothesis (von der Malsburg and Schneider, 1986). This theory was later refined by Fries (2015) in his communication-through-coherence theory, which proposes that pre- and post-synaptic neurons need in addition to synchronize in a way that ensures that the incoming input arrives in a temporally coordinated manner relative to the post-synaptic receiver.

Whereas both concepts of functional segregation and integration have a long history, a shift could be noticed over the last two decades from an emphasis on segregation towards integration (e.g. Fingelkurts et al., 2005; Friston, 2011). A highly relevant milestone for this shift, especially for the functional magnetic resonance imaging (fMRI) community, was the observation of a widespread correlation pattern between the activity of a so-called seed region and the remaining brain in resting state by Biswal et al. (1995), the first explicit description of *resting state functional connectivity*. Until that time, this task- and stimuli-free baseline condition had been considered to represent uninformative noise, and even Biswal was actually interested in characterizing noise sources in the brain when he discovered a dominant low-frequency ( $< 0.1$  Hz) noise source that seemed to exhibit extended spatial patterns (Biswal, 2012). The demonstration of such structured organization of functional interaction in the absence of external input or cognitive engagement introduced a whole new perspective on brain functioning, a unifying framework, and in addition a unique empirical approach. This historical and at the time highly controversial finding was followed a couple of years later by an influential paper by Raichle et al. (2001) on a concept called the default mode of the brain. The authors described a characteristic pattern of brain regions that deactivated during cognitively demanding tasks but increased its activity during rest, which is known today as the default mode network (DMN). This finding of a PET study was later replicated with fMRI (Greicius et al., 2003) and shown to be the most robust coherent pattern of functional connectivity among a series of so-called resting state networks (RSNs) (Damoiseaux et al., 2006; Fox and Greicius, 2010). Soon after, the clinical neuroscience community discovered the advantages of the resting state paradigm. Since then, the field of resting state functional connectivity has become a rapidly growing and developing neuroscientific research area, and the approach itself one of the most influential concepts and empirical paradigms in recent history of neuroimaging (Biswal, 2012). To illustrate this, between the years 2001 and 2015 nearly 3000 papers were published on the DMN alone (Raichle, 2015).

And the field is still constantly advancing. For instance, a growing number of articles could have been noticed in the last few years that have focused on dynamic features of functional connectivity. This dynamic dimension seems to be a promising expansion of the currently predominant static perspective on functional integration and might gain further relevance for both basic research and clinical applications in the near future (Calhoun et al., 2014; Kopell et al., 2014). Another relevant, but yet not fully answered research question addresses mechanisms and principles of higher-level whole-brain

spatiotemporal organization. Two concepts that are often mentioned in this context, are *multistability*, which describes the existence of multiple stable states and the shift between them triggered by external input and *metastability*, hence the spontaneous shift between transient attractor states (Tognoli and Kelso, 2014; Váša et al., 2015; Deco and Kringelbach, 2016). The thorough evaluation of derivable hypotheses of these concepts in empirical data is a matter of ongoing research. In addition, there is a recent trend towards commonly shared standards of data acquisition and analytical strategies to improve the reliability and reproducibility of methods capturing functional connectivity (e.g. Fox and Greicius, 2010; Yan et al., 2013b). Another direction of progress is the development of a more comprehensive theoretical groundwork to enhance the basic understanding of functional connectivity in resting state. This is still lacking but badly needed, considering the continuing controversy on this paradigm (Morcom and Fletcher, 2007). To this end, it will become even more important to integrate findings from divergent approaches and modalities, but this is still challenging. First, because data from different modalities vary distinctly in their temporal and spatial resolution, and second, because functional interactions are estimated based on fundamentally different neurophysiological events, such as neuronal activation, synchronized activity of neuronal assemblies, or reflections of hemodynamic responses (Horwitz, 2003). Despite relevant insights into the neuronal basis of the fMRI signal and the description of links between modalities on the micro- to macroscopic level (Logothetis et al., 2001; Fornito and Bullmore, 2010; Brookes et al., 2011; Hall et al., 2013; Garcés et al., 2016), many aspects of these relationships remain poorly understood. A future goal will therefore be to bridge these gaps. The endeavor towards a better understanding of resting state functional connectivity includes also the attempt to identify distinct sub-states, defined for example by behavioral or psychological variables (e.g. Tagliazucchi and Laufs, 2014; Pipinis et al., 2016). Finally, the term *resting state* has often been criticized because of its misleading implications and practical as well as philosophical complexity. A study by Gaab et al. (2008) revealed for example an effect of the magnetic resonance imaging (MRI) scanner noise on the activation of the DMN. This highlights that the recording set-up itself can be interpreted as some kind of experimental modulation in a broad sense. Such findings give reason to doubt the adequacy of the term *rest* in the present context. Some alternative expressions that have been suggested are *spontaneously arising fcMRI* (e.g. Rosazza and Minati, 2011), functional integration in the *absence of task and stimulation* (e.g. Vatansever et al., 2015), *intrinsic coupling* (Engel et al., 2013), and *endogenous activity* (Sporns, 2013a). Whether one of these or another term can actually replace the original one, and whether this would resolve some of controversy, remains unclear.

## 1.2. Structure to function to behavior and back

Functional communication depends on anatomical connections, considering that electrical signals are transmitted along neuronal axons. Anyway, neither does the the relationship between the structural and the functional connectome seem to be straightforward, nor has its investigation been trivial so far. In the present context, the term *structural connectome* will refer to the estimation of white matter (WM) tracts from diffusion tensor imaging (DTI) data. This approach constitutes a description of brain structure on a macroscopic level (Sporns, 2011), derived from the diffusion of water molecules in the brain, which is determined by the myelinated neuronal fibers. More fine-grained representations, which can be obtained with techniques such as tract-tracing in the macaque brain or molecular biological methods, and structural connectivity based on gray matter (GM) features (e.g. Bassett et al., 2008) are not subject of the present overview.

The dichotomy between structural and functional connectome does not reflect the current neuroanatomical understanding of the brain, but rather represents a technical terminology that emphasizes divergent methodological approaches (Fingelkurts et al., 2005). Structure shapes function, meaning that it determines the dimensionality of the functional state space, in which "neural dynamics remain fluid, variable, and sensitive to dynamic perturbations" (Sporns, 2011, p. 119). However, biological

structure is not invariant, but changes over time, triggered and influenced by a number of factors. Most obvious modifications of structural connectivity emerge until young adulthood, but reorganizations occur across the entire life span (Collin and van den Heuvel, 2013; Araneda et al., 2016). These structural changes are determined by genes, developmental factors, but also learning and experience, hence the functional repertoire that is called upon and used (Sporns, 2011). Minerbi et al. (2009) demonstrated for instance that structural modifications of synaptic connections are driven strongly by neuronal activity, while spontaneous synaptic plasticity was found to occur only to a minor extent. In addition, numerous investigations were able to reveal remarkable reorganization processes in sensory cortices of blind people, triggered by the afferent input of other sensory modalities (e.g. Araneda et al., 2016). Supekar et al. (2010) could demonstrate that rest fMRI of the DMN was comparable between children and adults despite significantly weaker structural connectivity in the younger group. Considering the strong structure-function relationship of the DMN later in life (Horn et al., 2014), it seems as if the functional relevance would precede the structural pattern. Changes of anatomical connectivity were shown even in healthy adults, for instance in response to targeted training (Scholz et al., 2009). These findings highlight the potential plasticity of brain structure. Beyond that, they disclose the reciprocal interdependency between structure and function, between temporary and permanent changes, and between alterations on divergent time scales. The structure-function interplay is therefore an integral component of the robustness and flexibility of the brain as a biological system, and the basis for its adaptability throughout life (Sporns, 2011).

The functional connectome that unfolds from the anatomical structure exhibits a distinct organization into consistent functional interaction patterns both at rest and during the performance of task (Damoiseaux et al., 2006; Smith et al., 2009). This functional organization has been found to share similarities with underlying anatomy (Sporns, 2011). Functional metrics were shown to correlate positively with DTI measures (e.g. van den Heuvel et al., 2008) and several functional networks were linked to WM tractography (e.g. van den Heuvel et al., 2009). An important insight that emerged from the investigation of similarities between the functional and the structural connectome, is that functional interaction on the macroscopic level cannot be explained by direct structural connectivity alone. Instead, indirect structural connectivity has a substantial impact as well (Koch et al., 2002; He et al., 2007; Honey et al., 2009; Sporns, 2011). From that it follows, that functional interactions occur along parallel paths in ramified networks. Two observations are in line with this finding. First, local perturbations have been demonstrated to exhibit widespread effects beyond directly connected nodes (Alstott et al., 2009; Termenon et al., 2016a). Second, the brain appears to hold a great potential to compensate at least to some extent for even severe structural disturbance (Silasi and Murphy, 2014). The impact of direct and indirect structural connectivity seems to vary across the functional connectome. By correlating voxel-specific estimates of functional and structural connectivity, Horn et al. (2014) found highest agreement for regions of the DMN, indicating that this network has the most directly connected anatomical base compared to other RSNs. The authors relate their outcome to findings by Hagmann et al. (2008) that indicate that parietal parts of the DMN represent a *structural core* of the human brain, and to the central role of the DMN in the topological organization of functional and structural connectivity. Principles of the distribution and interplay between direct and indirect structural connectivity might therefore constitute an important factor for the understanding of the organization of the functional connectome, its robustness, and its vulnerability.

The nature of the structure-function relationship can be addressed with divergent approaches as illustrated in this section, including investigations on development (e.g. Supekar et al., 2010), genetic influences (e.g. Glahn et al., 2010), or the impact of and recovery from structural impairment (e.g. Sharp et al., 2011; van Meer et al., 2012). Further insights into concrete mechanisms that mediate structural and functional connectivity have been drawn from computational modeling approaches (Sporns, 2011; Deco et al., 2015). Briefly, models of structural networks are constructed based on certain assumptions, which are tested by comparing resulting simulated functional interaction patterns

to empirical data. For instance, Cabral et al. (2012) inquired into the impact of various structural disconnections on organizational features of functional connectivity. They found similar effects on local, global, axonal, and synaptic disturbance, namely a decrease in both small-worldness and clustering, together with an increase in hierarchy, robustness, and efficiency. The authors concluded that all of those divergent types of disconnection can have dramatic impact on functional connectivity with similar qualitative effects on network topology. The comprehensive evaluation of this hypothesis based on empirical data is still outstanding though.

### 1.3. Methodological aspects of fcMRI in rest and beyond

Despite the seeming simplicity of resting state measurements, a surprisingly large variability of concrete implementations exists, not to mention the wide range of analytical approaches and metrics. Much attention is for instance still paid to the question whether participants are supposed to have their eyes open or closed, and if open, whether they should be instructed to fixate specific targets or measured without further briefing. Differential effects between those variants were identified on the strength of functional interactions as well as the consistency between sessions. For instance, Patriat et al. (2013) found in a longitudinal study slightly higher scores for the reliability of the DMN, and the attention, and auditory networks in a fixation condition when compared with closed and open eyes. The visual network, on the other hand, seemed to be more reliable when participants were instructed to have their eyes open without fixation. Moreover, with their extensive analysis of 1,147 resting state data sets from 26 scanning centers, Tagliazucchi and Laufs (2014) uncovered that subjects in eyes-closed conditions were more likely to fall asleep while fixation supported the maintenance of wakefulness during resting state measurements. Even the instruction itself was shown to influence estimates of functional connectivity (e.g. Benjamin et al., 2010), perhaps related to unintentionally triggered alterations in awareness or tension, causing differential patterns of movement or cognitive engagement. In the following, a short overview is provided, covering the most common empirical approaches and analytical options in the field of resting state fcMRI.

#### 1.3.1. Pathological disturbance and experimental manipulation

The analysis of functional connectivity in resting state is a common approach in basic and clinical research nowadays. Different applications offer the opportunity to study diverse research questions without confounding effects of cognitive or motor performance. By implication, though, these applications provide likewise the opportunity to explore functional integration from various perspectives.

Studies on structural disturbances and natural development are highly relevant for the understanding of self-regulation mechanisms determining functional reorganization in response to perturbation as well as principles underlying structure-function dependencies (see also section 1.2). In particular the exploration of differential effects of either distinct lesion locations or different types of structural impairment can be a useful approach. For instance, Irimia and Van Horn (2014) conclude from their results that the lesion location in the WM is indicative for the resulting disturbance of global functional network integrity. According to their interpretation, impairment in areas belonging to a *core scaffold of WM* lead to major alterations in functional connectivity, whereas lesions in other regions are supposed to cause much less dramatic changes. Similar findings were published for the GM, indicating that damage to cortical or subcortical hub regions is more likely related to wide-spread network alterations than disturbance in less central regions (van den Heuvel and Sporns, 2013; Gratton et al., 2012). Another example for the relevance of pathological alterations for basic work on resting state fcMRI is the commonly observed phenomenon of counterintuitive increase of fcMRI in response to structural impairment (Hillary et al., 2015). This finding is often interpreted as a sign of additional recruitment and compensation (e.g. Roosendaal et al., 2010b). The validity of such conclusions is

questionable, considering the weak theoretical foundation of the neuroscientific concept of functional compensation and the diversity of empirical findings.

While psychiatric diseases have also been linked to structural alterations, their main pathological correlate is to be found in imbalanced neurotransmitter systems and on the functional level. Investigations on changes in functional connectivity in divergent psychiatric illnesses therefore enable the exploration of influences from different neurotransmitter systems, especially in combination with pharmacological interventions. Shin et al. (2014) described for instance longitudinal changes in topological properties of functional connectivity that occurred during 16 weeks of treatment with selective serotonin reuptake inhibitors in obsessive-compulsive disorder patients. While patients exhibited significantly lower small-world efficiency at baseline, those metrics elevated considerably over time. Moreover, these changes were accompanied by distinct clinical improvement. As shown with the last example, an important advantage of resting state studies in clinical populations is the possibility to associate alterations in functional integration with clinical manifestations on the behavioral level. This matters especially for the development of theoretical concepts of the functional relevance of structured functional connectivity in task- and stimuli-free measurements, a highly complex question both philosophically and scientifically.

Anyway, considering that neither the condition *resting state* nor the observation of natural history constitute experimental manipulations in a proper sense, it is particularly helpful to take advantage of other methodological approaches in order to get the full picture of operational and organizational principles of spontaneously arising functional integration in the human brain. A promising option in this context is the application of neurostimulation techniques together with the observation of either concurrent or subsequent modifications of integration patterns. Previous findings indicate that functional interaction metrics can be modulated by transcranial magnetic stimulation (TMS) (Fox et al., 2012), transcranial alternating current stimulation (tACS) (Helfrich et al., 2014), and transcranial direct current stimulation (tDCS) (Kunze et al., 2016). Further investigations that use such tools are therefore desirable. Another legitimate mean for the manipulation of functional connectivity in healthy subjects are pharmacological interventions. For instance, van de Ven et al. (2013) found inhibiting effects of selective serotonin reuptake inhibitors on local functional interactions within the DMN, but not on its global architecture. Decreasing fcMRI in this study was moreover associated with lower self-reported alertness. This finding was interpreted as evidence for a central role of the DMN in ongoing monitoring and internal representations. It furthermore motivated the conclusion that the impact of the serotonin-system on the DMN might be relevant for the decrease of fcMRI during engagement in a task and therefore for the understanding of the DMN's contribution to cognition. Tagliazucchi et al. (2014) revealed increased temporal variability of fcMRI together with a wider repertoire of connectivity states after the intake of psilocybin, a psychoactive compound that is probably serotonin-mediated and known to cause states of unconstrained, hyper-associative cognition. The authors conclude that psilocybin causes an enhanced repertoire of metastable states that might underly the behavioral perception of an expanded state of consciousness. With this finding, they reveal relevant insights into the relationship between spontaneously arising functional interaction patterns and states of consciousness, complementing investigations on correlates of divergent pathological or physiological alterations of consciousness (e.g. Kotchoubey et al., 2013; Tagliazucchi et al., 2013). Above that, they provide further evidence for a specific functional role of temporal fluctuations of fcMRI. Two further approaches that can be applied to gain insights into the dynamic evolution of fcMRI over time are the exploration of task-induced after-effects on subsequent task-free conditions (e.g. Breckel et al., 2013), and the observation of functional integration within different states of arousal and during the transition from one into the other (Tagliazucchi and Laufs, 2014; Yang et al., 2014). These concepts reveal insights in how functional networks build up and disintegrate over time, or how the interplay between networks is orchestrated over time. Finally, advanced whole-brain computational modeling approaches are not only a powerful tool for the exploration of structure-function

relationships, but can be applied to address several other research questions in the context of spontaneous functional integration, including pathological mechanisms (Teufel and Fletcher, 2016), impact of neurotransmitter systems, or developmental aspects.

### 1.3.2. Of methods and metrics

A variety of analytical approaches and metrics for the description of functional connectivity were either developed or borrowed from other disciplines. Analytical strategies can be characterized with the help of three main categories or rather basic decisions to make while constructing such an analysis.

The term connectivity is inherently relational, describing something that is determined by characteristics of at least two elements. The first decision to make is therefore on how to define the elements of the analysis, respectively how to parcellate the brain into units that can be related to each other (de Reus and van den Heuvel, 2013). How this step is facilitated depends of course on the available data, but also on the hypothesis of interest. Analyses can be conducted on the voxel-level, which is the given resolution of the data, on the level of regions of interests (ROIs), or by computing functional interaction between whole networks. ROIs and networks can be determined with data-driven approaches, such as independent component analysis (ICA), or defined theory-based with the help of brain atlases. These atlases can be based on anatomical criteria (e.g. Tzourio-Mazoyer et al., 2002) or on functional ones (e.g. Shirer et al., 2012). In addition, it is possible to use sparse parcellation schemes (e.g. Power et al., 2011), or atlases that fully cover the brain (e.g. Tzourio-Mazoyer et al., 2002). So far, the impact of divergent parcellation strategies is not fully understood. Marrelec and Fransson (2011) found a quantitative but not qualitative influence of different parcellation schemes on the strength of fcMRI in the DMN during task. In addition, Sohn et al. (2015) point out that the application of such general templates does not account for individual differences and suggest to use subject-specific ROIs instead. In summary, it can be concluded that direct quantitative comparisons of results from differently parcellated networks is not recommended and that the choice of parcellation should be in line in with the hypothesis of interest.

Second, miscellaneous measures can be applied to quantify the statistical dependency between the activity of brain regions across time, this means the similarity in their signal fluctuations in terms of frequency, phase and amplitude (Friston, 2011; Bowyer, 2016). In resting state fcMRI research, the Pearson's correlation coefficient is by far the most widely used metric (Hindriks et al., 2016). Alternative metrics are transfer entropy, partial correlation, mutual information, or wavelet transform coherence to give a few examples.

Having decided on how to define regions of interest and their relationship, fcMRI maps can be constructed. Analytical strategies vary widely in how they proceed from this point. Functional connections can be determined pairwise between all nodes, which would result in fully connected fcMRI maps. Alternatively, functional connectivity metrics can be computed between the time-series of a single region, the seed, and the rest of the brain to capture the fcMRI profile of just one region of interest. In both cases, the constructed functional connectivity maps may or may not be thresholded. Certain conclusions can be drawn directly from such thresholded maps, for instance regarding the spread of functional interaction patterns as demonstrated in the original paper by Biswal et al. (1995). It should be noted that negative correlations are usually discarded in seed-based analyses or when thresholds are implemented, irrespective of their strengths. Whereas high positive correlations are considered to be indicative for functional communication and information transfer, the interpretation of negative correlations is still controversial, especially since it has been shown that they can result from preprocessing procedures (e.g. Murphy et al., 2009). Anyway, instead of interpreting merely the spread of a network, a far more often applied strategy is to test for differences in the strength of functional connectivity, either between groups, conditions, or time-points. Moreover, several metrics can be extracted from the fcMRI maps that describe properties of individual functional connectivity

patterns on a higher abstraction level. Especially prominent are graph theoretical metrics that capture topological features of functional integration. Graph theoretical approaches were developed in the interdisciplinary research field of *network science*. They can be applied to all kinds of *complex systems*, including social interactions, economic interrelations, and the brain. The underlying rationale results from the observation that functional and structural interactions between the elements of a system shape its overall behavior (Bullmore and Sporns, 2009). Interrelations between single elements of a network are therefore analyzed in order to learn about the macroscopic behavior of their whole. In this context, a network is defined by a definite number of nodes and their connecting edges. A variety of topological properties of such a graph can be described analytically, for example, the centrality or hierarchy of a node in its network. The application of such metrics in the context of substantially different complex systems disclosed certain similarities in some of their macroscopic organizational features, for instance the small-world property of social systems and brain networks (Bullmore and Sporns, 2009). The discovery of this field for the investigation of brain networks has led to a high number of investigations in healthy subjects and a range of different disorders with influential findings (Guye et al., 2010; van den Heuvel and Hulshoff Pol, 2010; Sporns and Betzel, 2016). Nonetheless, the application in the neuroscientific field is not without critique. Two critical steps in graph theoretical analyses are the construction of the graph and the definition of a threshold for the individual adjacency matrices, from which metrics are extracted (Bullmore and Sporns, 2009; Power et al., 2011). Results of graph theoretical analyses have been shown to be influenced substantially by the definition of nodes and the edge-threshold (e.g. van Wijk et al., 2010; Zalesky et al., 2010; de Reus and van den Heuvel, 2013). This is particularly relevant when two groups are compared that differ systematically in variables that influence the resulting adjacency matrices. For instance, when a group of patients has a globally reduced level of fcMRI in contrast to a control group, a fixed correlation threshold might lead to a lower average sparsity of edges in patients that in turn could cause significant differences in topological metrics. The informative value of such differences is of course limited. Graph theoretical analyses should be carried out and interpreted with caution for this reason.

Another option for the analysis of fcMRI maps is to apply advanced data-driven decomposition or clustering algorithms, such as ICA, principal component analysis (PCA), hierarchical clustering, spectral clustering, or self-organizing maps in order to learn about independent or otherwise definable functional connectivity clusters. Multivariate pattern recognition algorithms are another advanced method that can be used to first identify informative patterns of features in a training set and later apply this information to predict individual characteristics or the belonging to diagnostic categories (e.g. Klöppel et al., 2012).

In short, analytical approaches can be roughly categorized based on their spatial resolution, the operationalization of functional connectivity, and their focus and abstraction level. In the interest of completeness, it should be emphasized that analyses of functional connectivity in resting state can obviously also use signals recorded with other techniques, including EEG, MEG, or PET. Each modality has considerable advantages and disadvantages in comparison to all other options. While some of the above mentioned methodological and conceptual challenges of resting state analyses are independent of the modality, many specificities have to be considered for functional connectivity analysis using other modalities.

## 1.4. Translational neuroscience and clinical applications

The application of resting state fcMRI in clinical populations started very soon after the first description of this phenomenon, and has become extremely popular since (Fox and Greicius, 2010; Biswal, 2012). Already from the very first discovery of an association between clinical metrics and fcMRI, it was concluded that resting state fcMRI could serve as a marker for clinical diagnostics (Li et al., 2002; Biswal, 2012). This and subsequent findings paved the way for a whole field on the clinical application



of the resting state paradigm in combination with functional connectivity approaches. Until 2012, resting state fcMRI had been applied in 30 different pathological disorders (Biswal, 2012). Examples range from Alzheimer’s disease (e.g. Greicius et al., 2004), ADHD (e.g. Mostert et al., 2016), tinnitus (e.g. Hinkley et al., 2015), major depressive disorder (e.g. Demirtas et al., 2016), multiple sclerosis (MS) (e.g. Hawellek et al., 2011), to disorders of consciousness (e.g. Kotchoubey et al., 2013).

Fox and Greicius (2010) listed the following reasons for the success of the rest paradigm in the clinical field. First, task-related alterations in neuronal metabolism are considerably small in comparison to the energy consumption of ongoing activity (Raichle and Mintun, 2006). Task-free observations of brain functioning, in contrast, reflect processes that consume the majority of metabolic resources of the brain and might therefore provide a broader window into physiology and pathology. Second, considering the size of task-induced blood oxygen level-dependent (BOLD) modulation, clear evidence for task or stimulation effects emerges only when a large number of trials is averaged. Fox and Greicius (2010) argue that rest measurements could have a more advantageous signal-to-noise ratio, which is why scan duration can be reduced significantly in comparison to task fMRI. Third, task-designs target usually one specific functional domain. In contrast, functional integration patterns at rest are considered to represent the intrinsic architecture of functional organization and therefore the functional repertoire (Cole et al., 2014), as indicated by a remarkable similarity between functional networks identified at rest and during task (e.g. Smith et al., 2009). A single resting state fMRI session is thought to enable the inquiry into the whole range of functional systems for this reason. Fourth, the performance of a task requires in general the integration and integrity of several abilities. To give an example, an accurate group comparison of brain activation during a simple n-back task necessitates that both groups are able to understand the instructions, that the cognitive and physical endurance is at an equal level, and that both groups are physically capable of pressing the response button in the same manner. As a matter of fact, these confounds occur likely in clinical populations. Resting state measurements circumvent this issue. Fifth, taking argument two to four together explains easily why the application of resting state enables the examination of individual patients or even patient populations that had been unsuitable for fMRI measurements until then. One of the best examples to illustrate this, are patients suffering from disorders of consciousness, who are naturally incapable of following commands or perform any form of task.

The examination of alterations in spontaneously arising fcMRI in clinical samples offers a unique perspective on pathology, which can be used to disclose insights into etiology, reveal disease mechanisms, or to refine diagnostic categories. For instance the application of unsupervised classification algorithms on resting state fcMRI patterns is considered to be a powerful tool to identify subgroups of patients in disorders with a heterogeneous clinical picture, such as schizophrenia or dementia (e.g. Zhou et al., 2010; Fox and Greicius, 2010). Beyond its role for the investigation of pathology, however, different clinical applications of resting state fcMRI are discussed and inquired into. The most prominent one is the search for biomarkers that can support categorical diagnostic decisions. Such application of neuroimaging-based diagnostic tools is thought to be particularly promising when a differentiation based on other means, for example behavioral or laboratory tests, is difficult. So far, however, the empirical evidence for characteristics in resting state functional connectivity is inconsistent for most investigated disorders (Fox and Greicius, 2010). In addition, most studies focus on the contrast between healthy subjects and neurological or psychiatric patients only. Although determined specificity and sensitivity estimates are encouraging for some of these analyses (e.g. sensitivity of 85% and specificity of 77% for the distinction between Alzheimer patients and elderly subjects in Greicius et al., 2004), such contrasts are not sufficient to conclude that markers of spontaneous fcMRI can be actually used to differentiate between disorders, which is of course the real clinical challenge. It is striking, for example, that the DMN is clearly overrepresented in the literature in comparison to all other RSNs. And even more, that it has been found to be disturbed in a suspiciously large number of pathological populations (for reviews see Buckner et al., 2008; Fox and Greicius, 2010).

Trying to identify biomarkers based on group comparisons is challenging also conceptually for a simple reason: Neuroimaging findings on group differences depend on the behavioral diagnostics, whose unreliability is the main argument for neuroimaging-based biomarkers. The practical translation of such neuroscientific findings into tools for categorical diagnostics is still far from a serious realization for these reasons. Less often investigated explicitly, is the potential usage of rest fMRI in dimensional diagnostics. fMRI has already been applied to quantify cognitive or motor impairment in diverse studies (e.g. Gamboa et al., 2014), and might therefore be used to assess the chance of recovery, or to evaluate treatment effects and remission in the future. Such biomarkers could either be specific for single syndromes, disorders, or pathological families, or preferably unspecific in analogy to typical neuropsychological tests. A shift of the focus from categorical to dimensional resting state fMRI biomarkers is desirable. Last, resting state fMRI metrics could be shown to support pre-surgical mapping of functions in order to prevent unintended damage especially to brain areas involved in language and movement (Zhang et al., 2009; Fox and Greicius, 2010). In addition, the combination with EEG seems to be a promising tool to localize foci of epileptic activity (e.g. Stufflebeam et al., 2011), superior to the use of EEG alone due to the higher spatial resolution (Lee et al., 2013).

To my knowledge, none of these approaches have been integrated in clinical routines in Germany to the present day. Sufficient replication studies and comprehensive clinical trials remain to be conducted in order to pave the way for this scientific endeavor into the clinical realm.

## 2. Multiple sclerosis

MS is a multifaceted inflammatory and demyelinating disorder of the central nervous system with a pronounced neurodegenerative component. First historical descriptions, which indicate the manifestation of MS from the vantage point of the present, date back to the 14th century. Documented systematic explorations started a few centuries later with earliest scientific reports in the years 1838 and 1842 (Butler and Bennett, 2003). Despite the extensive research in the last centuries, many aspects of MS remain poorly understood to the present day.

Unlike other neurological disorders with traumatic or non-traumatic etiology, the primary structural correlate of MS are circumscribed lesions in the WM. For that reason, MS represents a unique pathological model to inquire into effects of local WM impairment on large-scale functional connectivity, and therefore to study organizational principles underlying functional connectivity in the human brain.

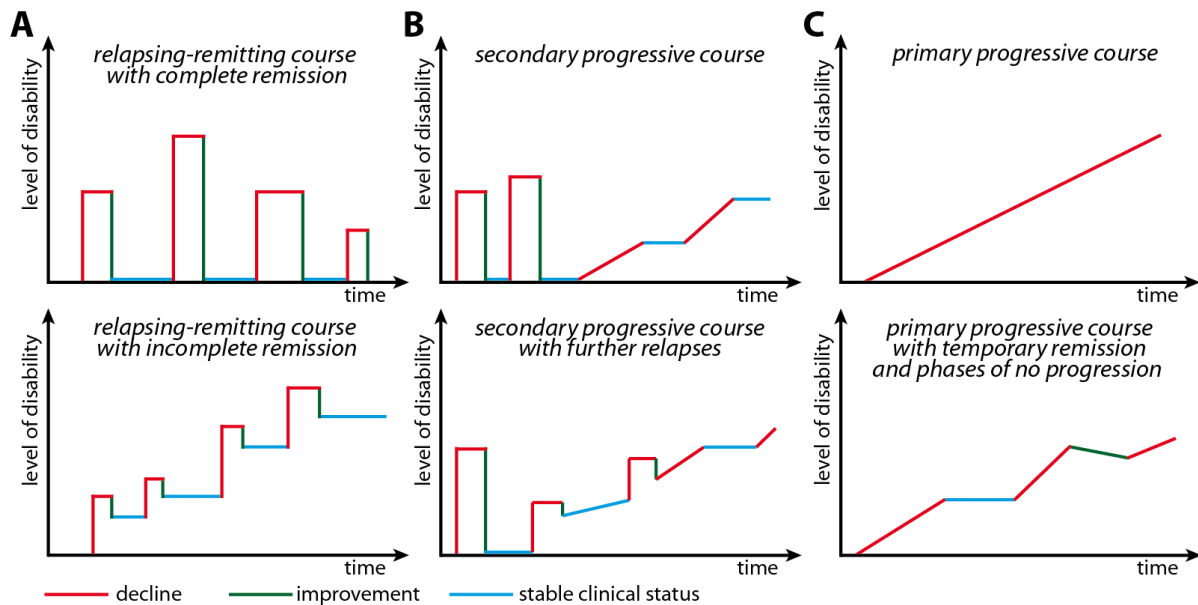
The following chapter gives an introduction into the clinical picture, the underlying (neuro-) pathology, and major diagnostic as well as therapeutic approaches. A comprehensive overview specifically over the literature on MS-related alterations in functional connectivity metrics is provided in part II. The current introductory chapter does not cover specifics of pediatric MS, which is a form of MS that manifests before the age of sixteen, observed in 0.4 to 10.5% of all MS patients (Renoux et al., 2007). In addition, it is beyond the scope of this work to go into details with respect to the wide range of differential diagnoses, especially neuromyelitis optica, which is an autoimmune disorder that shares several characteristics with MS, structural lesions in the spinal cord, animal models for MS.

### 2.1. Clinical picture

The clinical picture of MS is exceptionally diverse in terms of affected functional systems, symptom severity, and individual progression. Most frequent symptoms are found for disturbances of the motor system, damage on cranial nerves, disruption in the afferent part of the nervous system, dysfunction of the autonomic nervous system, and cognitive impairment (Köhler, 2015). More specifically, widely occurring symptoms that indicate impact on the motor system include pyramidal signs, tremor, ataxia, pareses, cerebellar signs, and spasticity. Cranial nerve damage is particularly frequent in the optical nerve and the oculomotor nerve, causing symptoms such as diplopia, nystagmus, or otherwise impaired vision. Disturbances of the afferent system often manifest as alterations of pain perception, including dysesthesia or allodynia, but also as general paresthesia. Automatic dysfunction usually appears at later stages of disease progression and can affect sexual functions and the ability to control micturition or defecation. Reported lifetime prevalence rates of psychological symptoms for depression and anxiety range between 24 and 50% (Köhler, 2015; Siegert and Abernethy, 2005; Fiest et al., 2016), and neuropsychological deficits are even found in up to 70% of all patients at some point of disease progression (Chiaravalloti and DeLuca, 2008). Most common cognitive deficits are thereby impairments of memory, attention, information processing speed, conceptual reasoning, visuospatial skills, and executive functions (Calabrese and Penner, 2007; Amato et al., 2008). Finally, a large portion of all patients is known to be affected by fatigue (65-91%; Krupp et al., 1988; Fisk et al., 1994), a distinct syndrome of perceived exhaustion and limited energy both on the physical and the mental level. While these psychological and cognitive impairments might appear as minor issues when contrasted with deficits of the motor system or dysfunction of the autonomic nervous system, they

constitute in fact a major limitation for the participation in social and professional life beyond the physical disability (Benedict et al., 2005; Amato et al., 2006). They are therefore often perceived as especially burdening and found to be one of the main determinants for quality of life (Nagaraj et al., 2013). No single symptom is specific for MS though, and inter-individual differences as well as intra-individual variability over time can be large.

The individual course of MS is hardly predictable and can range from a single neurological event with complete remission to fast progression of severe disability (Flachenecker and Zettl, 2015a). Three main subtypes are distinguished based on overall characteristics of their course (for an overview see Fig. 1).



**Figure 1. – Subtypes of multiple sclerosis.** A Relapsing-remitting subtype with and without complete remission. B Secondary progressive course after initial relapsing-remitting clinical picture and with further relapses. C Primary progressive multiple sclerosis with steady and variable course. Adapted from Flachenecker and Zettl (2015a), p. 64

In the great majority of patients (approx. 90%, Zettl et al., 2012), symptoms appear suddenly within a few days before they ameliorate substantially or even completely over the period of several weeks to months at the onset of MS. Patients may or may not experience relapses after a certain time in remission, that means abrupt reoccurrence or distinct worsening of symptoms, or the manifestation of further clinical signs. The periods between relapses as well the frequency of subsequent relapses are highly variable. If further progression of symptoms or the emergence of new deficits occur in the remission phase and without additional evidence for an acute relapse, secondary progressive MS (SPMS) is diagnosed and relapsing remitting MS (RRMS) otherwise (Fig. 1, A and B). 1 out of 10 patients suffer from the third subtype of MS, primary progressive MS (PPMS), which is characterized by a steady progressive decline that can include temporary improvement of single symptoms or the overall disability, but no complete remission (Fig. 1, C). Additional circumscribed neurological attacks may or may not occur. Two further diagnoses were added to this classification in the 2013 consensus conference of the US National Multiple Sclerosis Society Advisory Committee on Clinical Trials (Lublin et al., 2014): the clinically isolated syndrome (CIS), which is described as the first clinical manifestation of neurological signs with evidence for inflammatory demyelination, and the radiologically isolated syndrome (RIS), which is given in case of MRI findings that suggest inflammatory activity in the WM before the occurrence of clinical symptoms. Both forms can transition into all subtypes of full MS.

## 2.2. Course and prognosis

Generally speaking, the individual clinical expression of MS changes over time, usually implicating a profound worsening with respect to symptom severity, affected functional systems, and especially the neurodegenerative component of the illness. The most frequent symptoms at the very onset of MS, upon which patients consult medical help, are mild sensory disturbances, pareses, and vision impairment due to acute optic neuritis (Köhler, 2015). In the course of the disease, severe spasticity, permanent visual impairment due to atrophy of the optic nerve, cerebellar symptoms, dysfunction of the autonomic nervous system, serious alterations of the afferent system, and the interplay of the impairment of multiple functions gain in importance. The overall disability based upon the Expanded Disability Status Scale (EDSS) has been shown to be relatively stable over longer time periods. Pittock et al. (2004b) found in a retrospective longitudinal study that only about 20% of 162 patients in their sample had worsened more than two points on the EDSS scale over the observation period of ten years, with an average change of merely one point. In a second investigation, the authors revealed a median time period between initial diagnosis and a state of moderate disability (EDSS = 3) of seventeen years. A cane was necessary (EDSS = 6) after 24 years on average (Pittock et al., 2004a). A diagnosis of RRMS was found to be associated with a slower progression to moderate disability when contrasted with SPMS, as well as a lower initial EDSS score. The time period until moderate disability was not predictive for the individual course beyond that, in line with findings by Sayao et al. (2007). In their study on the longterm development of MS in patients with an EDSS score below 3 at ten years after their diagnosis, a substantial proportion of patients had an EDSS score of 6 at the twenty year follow-up or had transitioned to SPMS (together 44.3% of the 169 patients), despite their initial benign course. Pittock et al. (2004a) concluded that once a level of moderate disability was reached, further worsening was found to be more likely. This finding is reflected in the distribution of EDSS scores across the MS population. Two maxima of EDSS scores become apparent both cross-sectionally and longitudinally. Most patients have an EDSS score of either 1-1.5 or 6-7 while the average stay in those two score ranges exceeds the time in all other ones (Flachenecker and Zettl, 2015a). This indicates long stable periods in states of very low and pronounced disability and periods of more rapid deterioration in between. Tremlett et al. (2009a) report findings that indicate a relationship between the relapse frequency within 5 years after the first onset of MS and faster disease progression in terms of subtype transition and EDSS scoring in the same time frame, but no impact on long-term progression (> 10 years). Relapses that occurred later in time were also found to have a stronger short-term effect, but had in general less pronounced impact on the individual progression. Severity and duration of relapses, and location of underlying pathological processes were not taken into account for this analysis. The individual relapse frequency is variable with an average rate of about 0.5 per year (Flachenecker and Zettl, 2015a). It appears to be influenced by the age at onset in line with findings that suggest decreased inflammatory activity in higher age (Filippi et al., 2001). With respect to MS subtype, 30-50% of patients with RRMS are found to progress to the SPMS subtype ten to fifteen years after the initial diagnosis. In patients with a disease duration of over twenty years, even up to 90% show signs of neurodegenerative progression independent from acute relapses (Trojano et al., 2003; Flachenecker and Zettl, 2015a). The life expectancy is slightly reduced across the entire population of MS patients (Pittock et al., 2004b; Flachenecker and Zettl, 2015a). Anyway, MS almost never constitutes the primary cause of death. Instead, patients die from comorbidities or secondarily arising complications from severe disability, such as infection, dehydration, or pulmonary embolism. In addition, MS patients are known to have a significantly increased risk to attempt and complete suicide (Brenner et al., 2016), which should also be considered.

Making predictions for the individual course is still challenging, even when a range of clinical and demographic information is taken into account. In summary, the following variables were shown to be beneficial for a less progressive disease course: Younger age at disease onset was associated with longer

time periods until severe disability status, although the age when this phase was reached was then lower than in patient with later onset (Vukusic and Confavreux, 2007). Next, the clinical course was found to be milder in female patients (Confavreux et al., 2003; Sayao et al., 2007), and the portion of male patients among PPMS to be higher than among other subtypes (Tremlett et al., 2009b). As illustrated above, the relapse frequency within the first five years is an important determinant for further progression. This applies to RRMS only, while relapses do not seem to influence the continuous progression in the progressive subtypes (Confavreux et al., 2000). Finally, the subtype at onset is of course highly relevant for individual prognosis, with RRMS being related to a milder progression when compared to PPMS (Koch et al., 2009). Findings regarding the impact on initial symptoms are controversial. In general, no clear association seem to exist. Merely the concurrent emergence of multiple disturbances at the onset of MS seems to be related to a worse course (Flachenecker and Zettl, 2015a).

### **2.3. Epidemiology**

MS is the leading non-traumatic cause of disability among young adults and therefore an important disorder with respect to the financial burden for the general society, challenges for public health, and clinical care (Kingwell et al., 2013; Browne et al., 2014). The disorder manifests usually between age 20 and 40, with slightly earlier onset in women (Flachenecker and Zettl, 2015b). The ratio between female and male patients ranges between 2:1 and 3:1 (Alonso and Hernán, 2008), which is considered to be related to differential effects of female and male sex hormones on the immune system (Nicot, 2009). The global average point prevalence of MS is 90/100,000 (Hirtz et al., 2007) but it has been repeatedly shown to be unequally distributed across the globe. Prevalence rates were found to be lower in countries close to the equator and high in western nations, including Europe, North America, and Australia. Also within those regions, a gradient seems to emerge from highest prevalences in countries further to the north, respectively the south for the southern earth hemisphere (Koch-Henriksen and Sørensen, 2010). For instance, for Scandinavian countries, prevalence rates of up to 200/100,000 were reported together with incidence estimates between 9.2 and 11.6/100,000. In contrast, the prevalence for the Iberian peninsula was shown to range between 15 and 77/100,000, and annual incidence rates between 2.2 to 5.3/100,000 (Kingwell et al., 2013). In Germany, estimates for the prevalence range between 51 and 170/100,000 and incidence rates from 7.7 to 8/100,000 (Fasbender and Kolmel, 2008; Kingwell et al., 2013), with regional differences between north and south, but also eastern and western parts of Germany (Petersen et al., 2014). Whereas the unequal global distribution might be attributed to divergent systems of medical care, cultural differences in the interpretation of diagnostic criteria, or the availability of systematic scientific reports, the regional differences within Germany can be hardly explained with such factors. Instead, true variations in prevalence of MS seem to exist. Possible reasons for such unequal distribution are summarized in the following section. It should be noted that estimates of global and regional prevalence and incidence of MS were found to increase over the last decades (Hirtz et al., 2007). This probably reflects increased survival rates, improving clinical care, and enhanced case documentation (Browne et al., 2014), and should be taken into account when epidemiological studies from divergent decades are compared.

## 2.4. Etiology

The etiology of MS is still hardly disclosed despite a long history of research on its epidemiology, pathology, and divergent influencing variables. From what is known nowadays, a multifactorial causation must be assumed with both substantial impact from heritable as well as environmental factors. In addition, MS has a strong autoimmune component, which might be the reason for an often found simplified conceptualization as an autoimmune disorder especially in popular scientific literature. Anyway, it remains unclear to the present day whether the disturbance of the immune system constitutes the primary cause of MS or a secondary effect of unknown primary etiology, so that the above mentioned conclusion is untenable based on current scientific knowledge on MS (Winkelmann et al., 2011).

In general, current scientific approaches can be roughly assigned to three main theoretical models (Winkelmann et al., 2011):

- the autoimmune or immune dysregulation hypothesis
- the infection hypothesis
- the neurodegenerative hypothesis

Considering the variety of clinical expressions of MS, it is also plausible that there is no homogenous etiology but divergent pathological causations (Winkelmann et al., 2011; Zettl et al., 2012). Especially primary neurodegenerative pathology could be indicative for a disease with distinctly different etiology in comparison to other MS patients.

Epidemiological studies are a powerful approach for the exploration of etiological factors and the disentanglement of genetic and environmental impact. Interesting findings of this field are the unequal global distribution and the increased, respectively decreased prevalence among certain ethnic groups. The geographical distribution was related to known environmental factors such as the infection with the Epstein-Barr virus (Lünemann et al., 2007; Pender and Burrows, 2014), or the blood level of vitamin D, a secosteroid with immunomodulatory effect, whose demand is satisfied primarily by endogenous synthesis that in turn depends heavily on dermal exposure to UV radiation (e.g. van der Mei et al., 2003; Islam et al., 2007; Lucas et al., 2011). Further environmental factors, which are discussed in relation to increased prevalences in industrial nations, are smoking and fine dust pollution (Hoffmann, F in Hardt, 2015, p. 28). They are considered to activate the immune system (e.g. Öckinger et al., 2016) and to be associated with the risk for MS (Salzer et al., 2013) as well as its course (Hernán et al., 2005). Investigations into prevalences in different ethnic groups, on one hand, and migration studies, on the other hand, moreover suggest relevant interaction effects of these environmental factors with genetic predispositions. For example, prevalences among native americans but also Australian aborigines were found to be significantly lower in contrast to the caucasian population even when both groups lived in the same region (Flachenecker and Zettl, 2015b). Explorations on the effect of migration to low- or high-risk regions at different ages found a benefit from moving to low-risk regions before the age of fifteen but not later (e.g. Alter et al., 1966). This led to the assumption that the time before puberty is a critical age for a later manifestation of MS, especially for predisposed individuals (Poser, 2006).

Findings from genetic analyses in MS indicate a complex polygenic inheritance, including the involvement of divergent genes and alleles, diminished penetrance, and variable expression of genetic predisposition (Hardt, 2015). An extensive multi-side study in over fifteen countries confirmed almost all of twenty previously found risk loci for MS and identified 29 new susceptibility ones (The International Multiple Sclerosis Genetics Consortium et al., 2012). Overall, the human leukocyte antigen system, a group of genes in the human genome that is critical for the immune system, and more specifically its class I and II alleles, appear to play a central role for MS with risk-increasing but also protective effects (Link et al., 2012; The International Multiple Sclerosis Genetics Consortium et al., 2012; Hardt, 2015).

## 2.5. Pathology

The main characteristic of the pathology in MS is a selective primary demyelination in the central nervous system (Stadelmann-Nessler and Brück, 2015). It results from local inflammatory processes and manifest itself as circumscribed WM lesions on the macroscopic level. The anatomical location of such lesions can be highly variable, although they were found to occur more likely at locations with high venous density and in so-called watershed areas (Ge, 2006; Haider et al., 2016). The pathological progression of such lesions can be described both cross-sectionally as well as longitudinally due to characteristic markers for different phases of the central nervous inflammation. Acute MS lesions are generally defined by an increased activity of macrophages, detection of degradation products of myelin proteins, reactive gliosis, and infiltration of T-cells together with few B-cells (Barnett et al., 2006; Stadelmann-Nessler and Brück, 2015). Lucchinetti et al. (2000) identified four different patterns of these markers in acute lesions. Two of them (I and II) indicated autoimmune reactions while lesions with subtype III and IV were suggestive of rather infection- or toxin-related pathological causation based on the primary impairment of oligodendrocytes. The lesion type was found to be characteristic for individual patients. The emergence of inflammatory processes in the WM in MS is in general related to observable clinical relapses. However, there is neither a one-to-one relationship between single lesions and clinical symptoms, nor does every lesion trigger a manifestation on the behavioral level. Instead, a great portion of lesions is in fact clinically silent (Ge, 2006).

Already during the acute inflammation, markers for the remyelination of affected axons can be detected (Stadelmann-Nessler and Brück, 2015). The initiation of such early repair mechanisms is probably related to the response of the immune system itself, for instance to the release of beneficial growth factors (Brück, 2005). Remyelination was shown to be associated with lower clinical disability in the following remission period (Bodini et al., 2016). Permanent clinical disability, on the other hand, seems to be associated with secondary occurring axonal damage (Brück, 2005). An important function of the myelin sheath is the protection and nourishment of nerve cells. The destruction of myelin has therefore inevitably an impact on the axons they are wrapped around. Axonal impairment is no phenomenon of later disease stages only, but can be detected already during initial acute inflammation, perhaps because axons are particular prone to inflammatory mediators of the immune system after loss of their myelin sheath (Brück, 2005; Stadelmann-Nessler and Brück, 2015). This acute axonal damage was shown to have most impact at the beginning of the disease, which is one reason for the importance of early treatment in MS (Kuhlmann et al., 2002). At later stages of the disease, usually both GM and WM atrophy can be observed (e.g. Ge, 2006). Recent findings by Steenwijk et al. (2016) suggest that the GM atrophy in MS is distributed according to specific anatomical patterns and that those non-random alterations in GM are associated with the neurodegenerative component of MS, especially the cognitive decline.

Findings from an extensive body of literature on the integrity of brain functioning in MS indicate distinct alterations also on the functional level, that is in brain activation and functional integration (e.g. Kollndorfer et al., 2013; Rocca et al., 2014). In addition, meaningful associations were detected between such functional alterations and both structural impairment and behavioral symptoms (e.g. Rocca et al., 2012). To draw conclusions based on such findings is challenging due to the variety of experimental approaches and controversy results. On one hand, there is evidence for enhanced regional activation (e.g. Rocca et al., 2014) as well as increased functional integration between regions (e.g. Dogonowski et al., 2013c) that could reflect beneficial compensatory mechanisms, or maladaptive overshoot. This is opposed by findings of decreased activation and hampered functional connectivity, which might result from disturbance of underlying structure. In short, no clear picture of the functional disturbance in MS has emerged yet. For a detailed introduction into MS-related alterations in functional interaction metrics specifically during this task- and stimuli-free condition see part II.



## 2.6. Diagnostics

As stated before, the clinical expression of MS varies remarkably with no specific pathognomonic symptom or marker. Consequently, a wide range of diagnostic tools have to be taken into account to rule out multiple differential diagnoses that share characteristics with this multifaceted disease. And even if the clinical picture is suggestive of MS and no clear signs for other potential illnesses found, additional evidence has to be provided for the dissemination of pathological manifestations in space (*DIS*) and time (*DIT*) before a definite diagnosis of MS can be given (Polman et al., 2011). Historically, this criteria had to be satisfied clinically, thus with the observation of multiple neurological symptoms that occurred at distinct points in time and indicated disturbance in divergent functional systems. With technical advances and increasing scientific insight, diagnostic criteria for MS are continuously adapted (McDonald et al., 2001; Polman et al., 2005, 2011). Laboratory and neuroscientific approaches have therefore gained significantly in importance for the diagnostic process in MS over the last decades. Currently applied diagnostic criteria depend particularly on complementing and supporting evidence based on MRI assessments (see Tab. 1).

**Table 1. – 2010 McDonald criteria for the diagnosis of multiple sclerosis.**

*Adapted from Polman et al. (2011)*

clinical presentation	additional data needed for definite diagnosis
$\geq 2$ attacks; objective clinical evidence of $\geq 2$ lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack*	none**
$\geq 2$ attacks; objective clinical evidence of 1 lesion	DIS, demonstrated by: $\geq 1$ T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)***; or await a further clinical attack implicating a different CNS site
1 attack; objective clinical evidence of $\geq 2$ lesions	DIT, demonstrated by: simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time; or a new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or await a second clinical attack
1 attack; objective clinical evidence of 1 lesion (clinically isolated syndrome)	DIS and DIT, demonstrated by: for DIS: $\geq 1$ T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)***; or await a second clinical attack implicating a different CNS site; for DIT: simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time; or a new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria***: 1. evidence for DIS in the brain based on $\geq 1$ T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. evidence for DIS in the spinal cord based on $\geq 2$ T2 lesions in the cord 3. positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

Continued on next page

Table 1 – continued from previous page

- *If there is no better explanation for the clinical presentation and the criteria are met completely, the diagnosis is MS, and possible MS otherwise.*
- *Attack/relapse: patient-reported/objectively observed clinical event typical of an acute inflammatory demyelination in the CNS; current or historical; duration  $\geq 24$  hours; absence of fever/infection; documented by contemporaneous neurological examination, although undocumented historical events can provide reasonable evidence of a prior demyelinating event; reports of paroxysmal symptoms should consist of multiple episodes occurring  $\geq 24$  hours; before a definite diagnosis of MS, at least 1 attack must be corroborated by findings on neurological examination/visual evoked potential in case of prior visual disturbance/MRI consistent with demyelination in the area of the CNS*
- *\*Clinical diagnosis based on objective clinical findings for 2 attacks is most secure. Reasonable historical evidence for 1 past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristics for a prior inflammatory demyelinating event; at least 1 attack, however, must be supported by objective findings.*
- *\*\*No additional tests are required. However, it is desirable that any diagnosis of MS be made with access to imaging based on these criteria. If imaging or other tests (for instance, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS, and alternative diagnoses must be considered. There must be no better explanation for the clinical presentation, and objective evidence must be present to support a diagnosis of MS.*
- *\*\*\*Gadolinium-enhancing lesions are not required; symptomatic lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes.*

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*MS = multiple sclerosis, CNS = central nervous system, MRI = magnetic resonance imaging, PPMS = primary progressive MS, CSF = cerebrospinal fluid, DIS = dissemination in space, DIT = dissemination in time, IgG = immunoglobulin G*

A comprehensive diagnostic investigation of a potential MS case will include in most cases at least following clinical and para-clinical examinations. First, the general neurological examination is still the central element of the diagnostic process and will be applied initially to objectify and quantify subjectively perceived symptoms. It should encompass the assessment of disturbances of the cranial nerves, the exploration of motor functions, an examination of coordinative skills, equilibrioception, sensory perception, and peripheral reflexes, and finally a rating of the current mental status. Specifically for MS, it is furthermore common to quantify the overall disability for monitoring and comparative purposes using the EDSS (see Tab. 2).

Second, findings from laboratory tests on blood and liquor samples are necessary to elaborate the general health status of a patient, to exclude other illnesses that could also explain the clinical findings, to increase the evidence for inflammatory activity and responses of the immune system in the central nervous system, and to determine the phase of specific pathological processes. There is no specific marker for MS, albeit certain patterns of findings are considered to be typical for this disease, including the evidence for oligoclonal bands, increased portion of activated B-cells, and markers for immunoglobulin G in the liquor (Tumani and Rieckmann, 2015). The relevance of liquor- or blood-based biomarkers for the prediction of the progression of MS is only limited (Flachenecker and Zettl, 2015a), with the exception of few findings that were found to be indicative for later transition from CIS or RIS to definite MS, such as the presence of oligoclonal bands (Ignacio et al., 2010).

**Table 2. – Expanded Disability Status Scale. Adapted from Kurtzke (1983)**

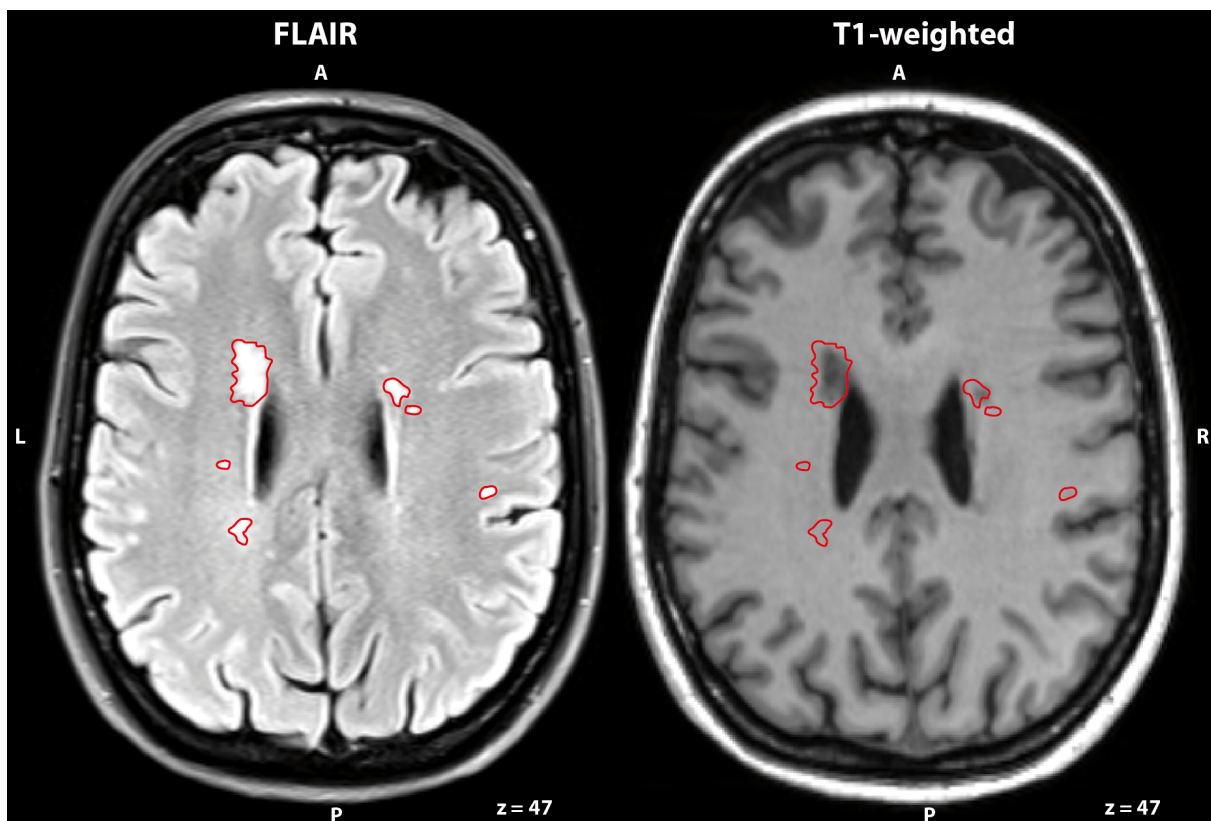
score	clinical description of level of disability
0.0	normal neurological exam (all grade 0 in all FS; cerebral grade 1 acceptable)
1.0	no disability, minimal signs in 1 FS (i.e., grade 1 excluding cerebral grade 1)
1.5	no disability, minimal signs in > 1 FS (> 1 FS grade 1 excluding cerebral grade 1)
2.0	minimal disability in 1 FS (1 FS grade 2, others 0 or 1)
2.5	minimal disability in 2 FS (2 FS grade 2, others 0 or 1)
3.0	moderate disability in 1 FS (1 FS grade 3, others 0 or 1), or mild disability in 3 or 4 FS (3/4 FS grade 2, others 0 or 1), though fully ambulatory
3.5	fully ambulatory but with moderate disability in 1 FS (grade 3) and 1 or 2 FS grade 2; or 2 FS grade 3 (others 0 or 1) or 5 grade 2 (others 0 or 1)
4.0	fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of 1 FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 meters
4.5	fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability usually consisting of 1 FS grade 4 (others or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters
5.0	ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions); (usual FS equivalents are 1 grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0)
5.5	ambulatory without aid for about 100 meters; disability severe enough to preclude full daily activities; (usual FS equivalents are 1 grade 5 alone, others 0 or 1; or combination of lesser grades usually exceeding those for step 4.0)
6.0	intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting; (usual FS equivalents are combinations with > 2 FS grade 3+)
6.5	constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting; (usual FS equivalents are combinations with > 2 FS grade 3+)
7.0	unable to walk beyond approximately 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day; (usual FS equivalents are combinations with > 1 FS grade 4+; very rarely pyramidal grade 5 alone)
7.5	unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; May require motorized wheelchair; (usual FS equivalents are combinations with > 1 FS grade 4+)
8.0	essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms; (usual FS equivalents are combinations, generally grade 4+ in several systems)
8.5	essentially restricted to bed much of day; has some effective use of arm(s); retains some self-care functions; (usual FS equivalents are combinations, generally 4+ in several systems)
9.0	helpless bed patient; can communicate and eat; (usual FS equivalents are combinations, mostly grade 4+)
9.5	totally helpless bed patient; unable to communicate effectively or eat/swallow; (usual FS equivalents are combinations, almost all grade 4+)
10.0	death due to MS

*FS = functional systems: pyramidal functions, cerebellar functions, brain stem functions, sensory functions, bowel and bladder functions, visual functions, mental functions, other*

Third, neuroimaging measurements became particularly relevant for the diagnosis of neurological diseases because they provide direct and objective evidence for structural impairment in the central nervous system, and in the case of MS, specifically for circumscribed lesions in the WM. The information that is derived this way can support, complement, and even replace clinical observations partially (see also Tab. 1). Usually, divergent MRI sequences are implemented in parallel, with each of them having advantages for the identification and description of either specific locations or phases of inflammatory activity. The application of the gadolinium in combination with a T1-weighted MRI sequence, to give an example, enables the differentiation of WM lesions in diverse phases of the inflammatory process. The paramagnetic contrast agent will accumulate only in sites with acute inflammatory

activity due to an increased permeability of the blood-cerebral barrier, so that acute lesions will be delineated brightly on the resulting T1-weighted images while lesion sites with remitting inflammation will appear dark. The finding of two lesions at divergent sites and in divergent phases would satisfy the dissemination in space and time criteria. Another useful sequence that is usually implemented in MS diagnostic is the fluid-attenuated inversion recovery (FLAIR) one, a T2-weighted sequence with high sensitivity especially for periventricular lesions due to an additional inversion pulse that suppresses the signal of the liquor. An example for a T1- and T2-weighted image of WM lesions from the present study is provided in Fig. 2. The prognostic value of conventional MRI findings is also limited, despite their relevance in initial diagnostics. Advanced structural metrics (e.g. atrophy markers, DTI-based metrics) seem to be more promising tools for prognostic purposes (Filippi et al., 2013) and therefore might get integrated in standard clinical routines in the future. The potential application of functional measurements in clinical diagnostics of MS, based on fMRI or EEG, is a matter of ongoing debate.

Finally, electrophysiological measurements are an adequate and easily applicable approach to reveal useful information, and are still widely used in everyday clinical practice, despite their subordinate relevance for current diagnostic criteria. Visually, auditory, motor, or somatosensory evoked potentials provide information independent from MRI measurements. They allow the detailed examination of the signal transmission from the receptor cells along the subsequent stages of information processing. Detected delays or even blocks in the recordings are therefore indicative for impairment of the pathways. For this reason, evoked potentials are still implemented to quantify the extend of the demyelination, to objectify clinical observations, or to identify silent lesions (Gronseth and Ashman, 2000; Reinshagen, 2015).



**Figure 2.** – Examples for MRI sequences typically implemented in MS diagnostics. **Left** Fluid-attenuated inversion recovery (FLAIR) image of MS patient 41 in native space. **Right** T1-weighted image of MS patient 41 in native space. White matter (WM) lesions, defined as hyperintensities in WM tissue based upon the FLAIR image are encircled in red. Lesion identification was done manually for illustrative purposes only. *L = left, R = right, A = anterior, P = posterior*

## **2.7. Treatment**

To the present day, there is no cure for MS. Available therapeutic approaches aim instead at maintaining or regaining the best quality of life, the highest independency, and the most participation in social and occupational life possible under the individual circumstances (Hoffmann, 2002). Roughly, they can be categorized as follows (Gemmel et al., 2015): First, short-termed interventions during acute inflammatory events that consist of high-dosed intravenously administered methylprednisolone and in some cases additional plasmapheresis. Goal of these interventions are the fast reduction of inflammatory activity and subsequently a quick recovery from the relapse. Second, long-termed drug-based treatment that modulates the immune system and influences the progression and the course of the disease. Typical options are beta interferon, glatiramer acetate, or dimethyl fumarate in moderate cases, and agents like fingolimod or natalizumab in severe ones. Third, therapeutic interventions that focus on managing symptoms, limiting physical restrictions, and prevention of secondary complications and side effects of long-term treatment. The individual treatment depends on the expression of the disease, symptom severity, the course and frequency of relapses, and of course the underlying subtype of MS.



## **Part II.**

# **Systematic Literature Review**





# 1. Introduction

The aim of this introductory part was to provide an overview over the literature on alterations in functional connectivity in MS to serve as a base for the evaluation of the representativity, novelty, and significance of the presented work. The review was supposed to evaluate the overall study quality and the appropriateness of the methodological and statistical approaches with respect to the reported study aims and the interpretation of findings. The aim was furthermore to summarize current insights in MS-specific characteristics of functional integration. All studies that matched the selection criteria and that were available at the beginning of February 2016 via the electronic library system of the University of Hamburg were therefore described with respect to their characteristics in study design, the applied methodology, their findings, and their interpretation of the results.

## 2. Material and methods

### 2.1. Search strategy and selection criteria

An extensive literature research was carried out by combining a systematic search via PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>) and the manual identification of essential publications in the reference lists of all review articles found in the first step. The electronic literature search was conducted on February, 2nd 2016 based on following search terms: *MEG* OR *magnetoencephalography* OR *EEG* OR *electroencephalography* OR *fMRI* OR *functional magnetic resonance imaging* [all fields] AND *multiple sclerosis* [Title/Abstract] AND *connectivity* OR *functional connectivity* OR *coherence* OR *functional integration* OR *effective connectivity* [all fields]. The defined endpoint of the time-period of interest was January, 31st 2016. Publications in any language other than English were excluded. Publications were selected for full-text evaluation when the analysis of functional connectivity in resting state and in adult MS patients was part of the described study or review, based on the abstract. In case of unclarity, studies were also included.

### 2.2. Description of publications

The description covered methodological aspects, an overview over the results related to the analysis of functional connectivity, and details regarding the main interpretation. All a priori-defined categories and items are listed in Tab. 3. The MS sample size was defined as the total number of MS patients and can differ from the number of patients included in statistical testing. Whether all groups contained at least 20 participants in statistical testing between MS patients and healthy controls or between MS subgroups was therefore noted separately. CIS and RIS patients were defined as a different pathological population when treated as a separate group in statistical testing. Multiple answers were possible, if more than one functional connectivity analysis was computed.

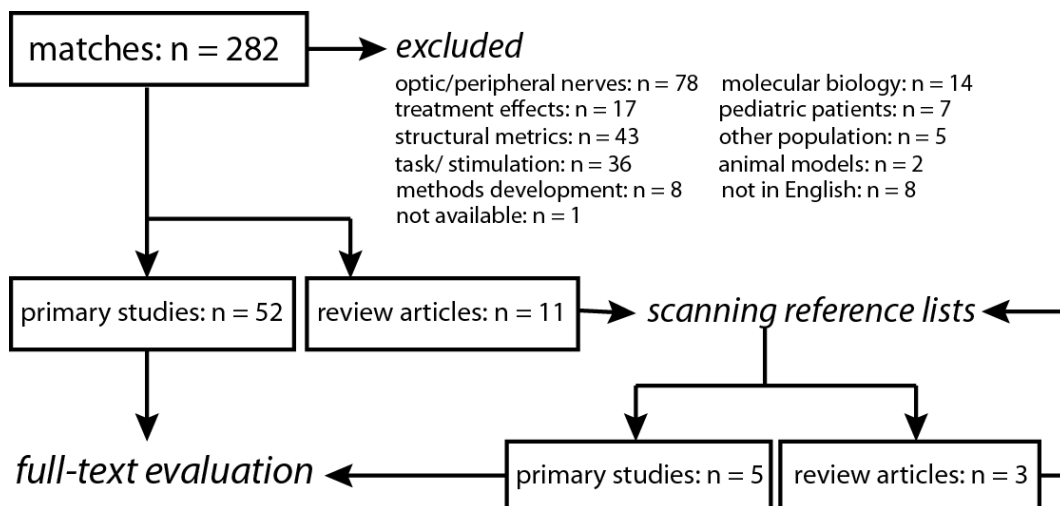
**Table 3. – Review categories for the evaluation of publications.**

Category	Subcategory	Items
Methods	MS sample	MS sample size, mean age, sex [ <i>female, male</i> ], psychiatric-behavioral comorbidities reported (depression, anxiety, or fatigue) [ <i>yes, no</i> ], MS subtype [ <i>RRMS, PPMS, SPMS, benign, not reported</i> ]
	Control group(s)	control group included [ <i>yes*</i> , <i>no</i> ], *population [ <i>healthy, MS patients, other patients</i> ], individual matching [ <i>yes, no**</i> ], **absolute group difference in sample size, significant difference in age [ <i>yes, no</i> ], significant difference in sex ratio [ <i>yes, no</i> ]
	Modality	[ <i>fMRI, EEG, MEG</i> ]
	FC analysis	<i>seed-to-voxel, seed-to-ROI, seed-to-sensor, pairwise voxel-based, pairwise ROI-based, pairwise sensor-level, graph theoretical on pairwise voxel-based, graph theoretical on pairwise ROI-based, graph theoretical on pairwise sensor-level, inter-hemispheric voxel-based, inter-hemispheric ROI-based, inter-hemispheric sensor-level, ICA for identification of network for further analysis, ICA voxel-wise statistics, ICA network-wise statistics</i>
	Statistical approach	All groups for statistical testing $n \geq 20$ [ <i>yes, no</i> ], analysis level [ <i>1st, 2nd, correlation/regression*, 2nd + correlation/ regression*</i> ], *[ <i>behavior/clinical score, structural metric</i> ]
Results	Group difference in FC	significant difference [ <i>yes*</i> , <i>no</i> ], *multiple comparison correction [ <i>yes, no</i> ], effect size reported [ <i>yes, no</i> ], confidence interval or similar reported [ <i>yes, no</i> ], direction of alteration [ <i>increase, decrease</i> ], anatomical localization
	Correlation with structural metric (if conducted)	significant [ <i>yes*, no</i> ], *multiple comparison correction [ <i>yes, no</i> ], effect size reported [ <i>yes, no</i> ], confidence interval or similar reported [ <i>yes, no</i> ], increase of functional connectivity correlated with [ <i>lower damage, higher damage</i> ], anatomical localization, statistical comparison with relationship in control group [ <i>yes, no</i> ]
	Correlation with behavioral or clinical score (if conducted)	significant [ <i>yes*, no</i> ], *multiple comparison correction [ <i>yes, no</i> ], effect size reported [ <i>yes, no</i> ], confidence interval or similar reported [ <i>yes, no</i> ], increase of functional connectivity correlated with [ <i>worse performance, better performance</i> ], anatomical localization, statistical comparison with relationship in control group [ <i>yes, no</i> ]
Discussion	Compensation	finding interpreted as compensation [ <i>yes*, no</i> ], *suggestion of mechanism, reference to theory or model [ <i>yes, no</i> ], correlation / regression analysis with behavioral / clinical conducted [ <i>yes**, no</i> ], **confirming results [ <i>yes, no</i> ]
	Biomarker	results are evidence for biomarker [ <i>yes*, no</i> ], *discussion of validity or reliability [ <i>yes, no</i> ], discussion of cost vs. usefulness in comparison to available tools [ <i>yes, no</i> ], validated in independent sample [ <i>yes, no</i> ], validated in other patient group [ <i>yes, no</i> ], compared to available tools [ <i>yes, no</i> ]

*FC = functional connectivity; asterisks indicate subsequent categories that were dependent on the selection; [x,y] = selection choices*

### 3. Results

The PubMed-based search for literature yielded 282 matches, of which 52 primary studies were selected for full-text evaluation (Fig. 3). Excluded publications covered alterations in the optic nerve or the peripheral nervous system (n = 78), treatment effects (n = 17), alterations in structural metrics (n = 43), brain functioning during task or stimulation (n = 36), molecular biological research questions (n = 14), methods development (n = 8), or focused on pediatric patients (n = 7), other pathological populations (n = 5), and animal models (n = 2). Eight study reports were not in English. By scanning the reference lists of the identified twelve reviews, five additional primary studies were found. Finally, full-text evaluation was carried out for 57 primary studies (a list of all selected publications can be found in appendix section A.1)

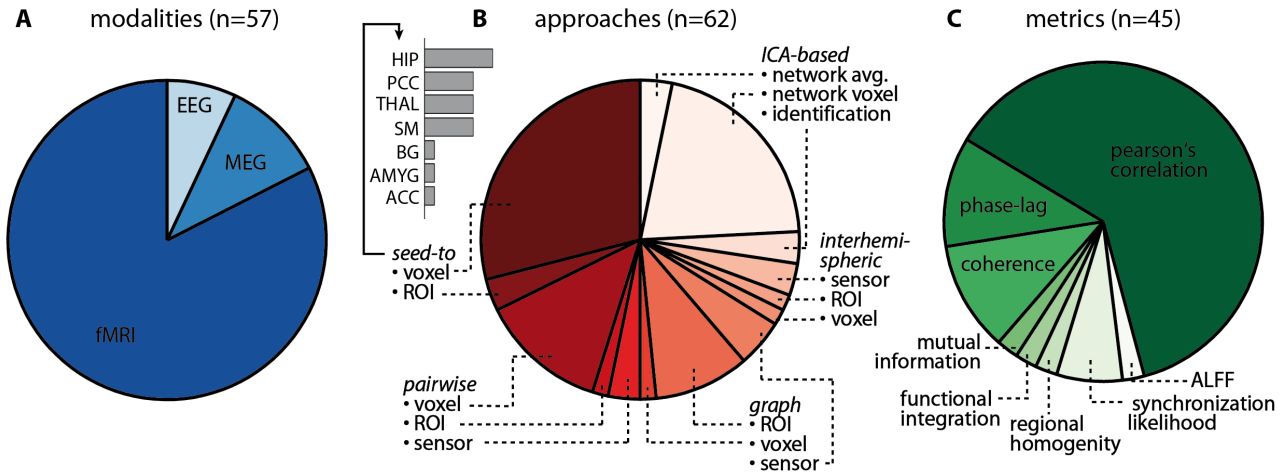


**Figure 3. – Flow chart of selection of publications for the review.** Of 282 initially found study reports, 52 primary studies and eleven review articles were included in the first step. Further primary studies (n = 5) and review articles (n = 3) were identified by scanning the reference lists of the review articles, resulting in a total number of 57 primary studies that were taken into account for the systematic review.

Taken together, 2186 data sets of MS patients were analyzed in the 57 primary studies at hand. Partial overlap between samples was reported in less than ten studies without further details. The total number contained 1391 female and 764 male patients. Sex was not reported in one study, leaving 31 patients with unknown sex. The mean MS sample size was 38.85 (median = 30, standard deviation (SD) = 36.52, range = 1-246). Patients were on average 40.29 years old (SD = 5.12) and belonged predominantly to the RRMS subtype (number of studies with a certain subtype: RRMS = 50, SPMS = 16, PPMS = 6, benign MS = 1, not reported = 6). Depression, anxiety, or fatigue scores were reported in 33 studies. A control group was included in all but one study (healthy = 54, other MS patients = 6, other patients = 3), but only five control groups were matched pairwise. The remaining control groups had 16.36 participants on average, and eleven, respectively thirteen differed significantly in age and sex.

Most studies applied fMRI (n = 47), followed by MEG (n = 6), and EEG (n = 4), while only one research team combined fMRI and MEG data (Fig. 4). Across all studies, 62 functional connectivity analyses were conducted using eight different functional connectivity metrics. Among the fifteen cat-

egories defined a priori, seed-to-voxel analyses were the most common approach, using seven different anatomical seed regions. 21 studies included whole-brain exploration at some point in their analysis, and 30 focused on a specific RSN, while the main interest in 10 studies was a single region. A priori defined foci were not sufficiently outlined or elusive in 3 publications.



**Figure 4. – Overview over applied modalities, methodological approaches, and computed metrics.** Most studies that were evaluated for this review had applied functional magnetic resonance imaging (fMRI, **A**) in combination with seed-to-voxel analyses (**B**). Functional connectivity was estimated with Pearson's correlations in most cases. (**C**). *EEG* = electroencephalography, *MEG* = magnetoencephalography, *ROI* = region of interest, *graph* = graph theoretical metrics, *HIP* = hippocampus, *PCC* = posterior cingulate cortex, *THAL* = thalamus, *SM* = sensorimotor cortex, *BG* = basal ganglia, *AMYG* = amygdala, *ACC* = anterior cingulate cortex, *ALFF* = amplitude of low-frequency fluctuations

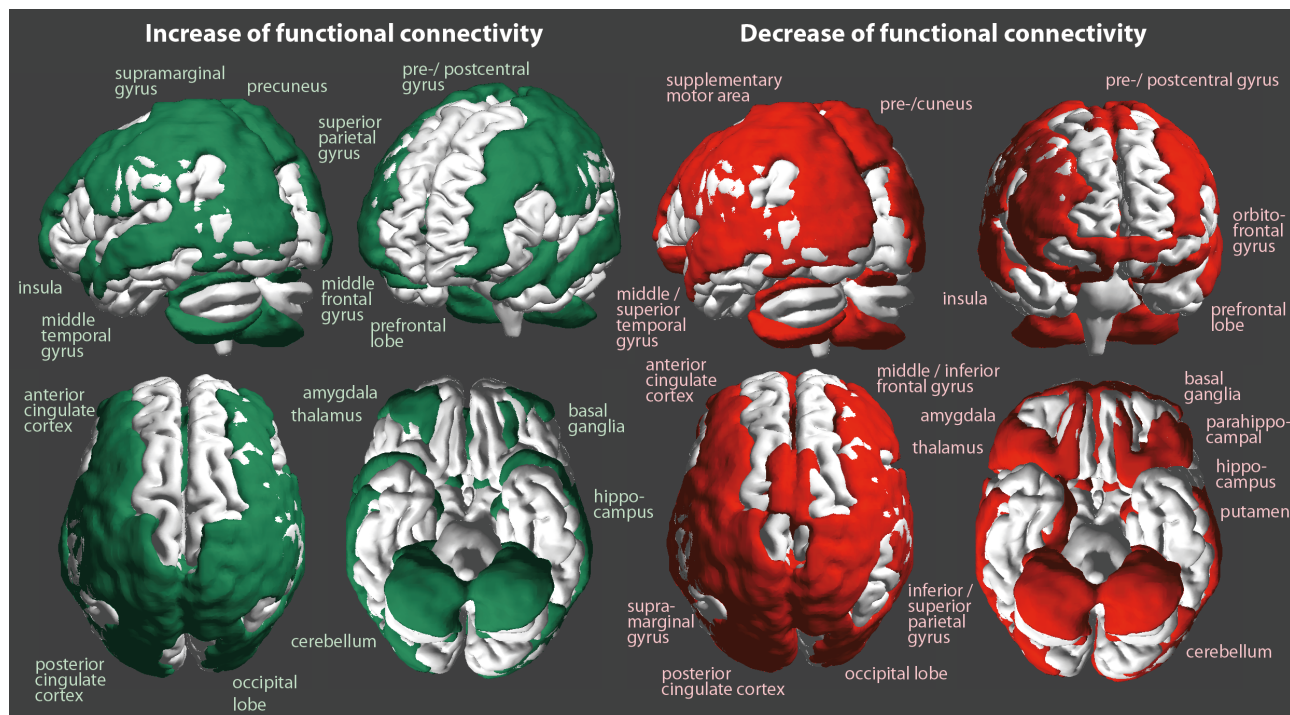
In only three studies, analyses aimed at extracting information on the individual level, for example by implementing classifiers. All other investigations focused on group differences in combination ( $n = 17$ ), or complemented by correlation or regression analyses ( $n = 32$ ) with either structural or behavioral variables ( $n = 26$ ), or both ( $n = 23$ ). The size of at least one group of comparison fell short of  $n = 20$  in 46% of the statistical analyses.

No significant group differences in functional connectivity were found in six of all 55 statistical second level analyses. 63% of the significant results were corrected for multiple comparisons at least across all voxels, ROIs, or networks included. A proper effect size or parameter of uncertainty, such as the standard deviation or a confidence interval, were reported for two, respectively four results. In total, 61 significant group differences were identified, 41% of them indicating an increase and 59% a decrease of functional connectivity in MS (for an overview of all findings see Fig. 5).

55% of correlation or regression analyses with structural metrics and 82% of those relating functional connectivity with behavioral or clinical variables yielded significant relations in MS. Proper multiple comparison correction (MCC) was reported for 37% of those results, and correlation coefficients for 74%. No study reported confidence intervals of correlations. Correlation coefficients of MS patients were compared statistically with those of a control group in five analyses only. The decrease of functional connectivity was related to worse structural, behavioral, or clinical scores in 59% of the significant results.

Authors of 31 studies interpreted increased functional connectivity as a sign for beneficial recruitment or other compensational processes, and decreased values as a lack of such mechanisms or adverse alteration. However, only 20 of these studies had actually included a correlation or regression analyses with behavioral or clinical variables. In 19 studies, group differences were interpreted as evidence for potential functional connectivity-based biomarkers. Authors of four of those studies complemented

their conclusion with a discussion on the validity and reliability of the method applied, and on possible advantages or disadvantages in comparison to available diagnostic tools.



**Figure 5. – Overview over detected increase and decrease of functional connectivity in multiple sclerosis.** Regions of the automated anatomical labeling atlas (AAL) that were found to exhibit altered functional connectivity in the publications selected for full-text evaluation. Findings that describe alterations of functional integration on the global level, in sensor space, or for which sufficient anatomical details were lacking were not taken into account for this overview.

## 4. Discussion

The systematic review of the available literature on functional connectivity in task-free conditions in MS uncovered first and foremost twofold: considerable inconsistency among findings and serious deficiencies in overall study quality.

To begin with, only a minority of all evaluated studies conducted whole-brain analyses of some sort, while most studies limited their foci a priori based on literature on functional connectivity or regional activity during task, findings in other populations, or previous investigations on the matter of interest. A priori foci encompassed several functional networks, such as the DMN, somatosensory network, or networks incorporating subcortical regions, and seven different seed-regions in only eighteen studies with seed-based approach. Overall, a wide range of networks and regions were found to be affected in MS that way, covering all lobes as well as the cerebellum. Evidence seemed to converge, if at all, for the involvement of the basal ganglia and the thalamus, cingulate regions, and middle temporal areas. The number of group contrasts that suggest either enhancement or disturbance of functional integration was almost balanced with a slightly higher number of results showing the latter. No group differences were found at all in 10% of all studies that conducted proper second level analyses,

which is astonishing, considering the overall publication bias towards significant findings. Studies, which related functional interaction with behavior, disclosed contradicting relationships with slightly more results indicating an association between functional decrease and behavioral decline, respectively increase and improvement.

Demographic characteristics of patients reflected the young age, at which the disease manifests, and the known gender ratio. The prevalence of comorbidities is high in MS and should therefore either be ruled out or reported properly to increase the internal validity of the results, that is the significance of findings for MS independent from influences from psychiatric disorders and other pathology. However, comorbidities were not reported in a substantial portion of all studies, challenging the interpretation of their outcomes. The appropriateness of the control groups can be questioned in some cases due to small sample sizes, considerable gaps between the sample sizes of patients and controls, and significant differences in demographic characteristics.

In almost half of all statistical tests, at least one group included less than twenty participants. Whether the statistical power was always sufficient for the analyses conducted, might be questioned for this reason. It should be considered that decreased statistical power due to insufficient sample sizes does not only hamper the detection of true effects, but can also facilitate false-positive findings (Button et al., 2013). Almost none of the studies reported effect sizes or parameters of uncertainty, such as confidence intervals, for their detected effects, hindering the qualitative comparison of effects from different studies and their quantitative integration in meta-analyses.

Last, one third of all investigations concluded that their results disclosed a potential biomarker for MS. However, merely three studies compared MS patients to other clinical populations, and only five investigations on relationships between functional connectivity and behavior or structure incorporated an appropriate contrast of associations found in MS and in the control group. Discussions on validity, reliability, and advantages, respectively disadvantages of functional connectivity as a potential clinical application in contrast to available diagnostic tools were lacking in almost all papers. Moreover, some studies interpreted their results as being indicative for compensational processes despite failing to uncover such a relationship or without even testing properly possible links between functional interaction and symptom severity.

Taken together, the evidence for MS-specific alterations in functional communication patterns, or relationships of such, is weak. Based on the evaluated publications, no clear pathological correlate of MS in the functional architecture at rest has emerged so far. Instead, findings are highly inconsistent and disclose that large portions of the brain are affected. Any strong conclusions regarding the clinical application of functional connectivity methods in MS does not seem to be justified based on the available literature. Instead, an open-minded and clinically-oriented evaluation of this endeavor is necessary.

**Part III.**

# **Empirical Work**





# 1. General methods

## 1.1. Study design

All analyses, which are presented in this doctoral thesis, were developed within the framework of a multimodal cross-sectional and longitudinal project on pathological alterations of structure, function, and behavior in MS called NeuConn. This exploratory study had two main objectives: First, to develop novel biomarkers for cognitive, physical and fatigue symptoms based on fMRI, DTI, MRI, MEG, or their combinations. Second, to prospectively monitor physiological and pathological changes in network integration and underlying structure over the period of twelve months.

Data of all modalities was collected within two to four consecutive days at four points in time, namely at baseline, after two weeks, after six months and finally after twelve months. Healthy controls were not examined at the six months' follow-up and no fMRI or DTI data was recorded at the two-weeks measurement. An explicit a priori power analysis to estimate the appropriate sample size was not possible due to the exploratory nature of the study and the variety of modalities. Instead, the sample size was set based on general recommendations for quantitative statistical tests and to ensure the feasibility of the project. With an intended sample size of 80 participants, the total number of planned appointments came to 800.

The present doctoral thesis focuses on cross-sectional and longitudinal analyses of MRI, fMRI, DTI, neuropsychological and clinical data. Analyses and results of MEG data or multimodal analyses are not included.

## 1.2. Participants

MS patients were recruited by the MS day clinic of the University Medical Center Hamburg-Eppendorf, Germany. Inclusion criteria were age 20-60 years, an up-to-date diagnosis of RRMS according to the revised McDonald's criteria (Polman et al., 2011), current remission, minimal disease duration of three months, and an EDSS (Kurtzke, 1983) score less or equal 3.5. Patients with a highly active disease, uncertain stability under current immunotherapy, psychiatric comorbidity, or severe cognitive deficits were excluded. Since the clinical picture of MS is known to be particularly diverse, no rules for exclusion were established based on structural, functional, or other behavioral measures. Healthy controls were recruited via advertisement in the internal hospital newsletter and among healthy relatives of patients. Exclusion criteria were previous or present neurological and psychiatric illness. Further exclusion criteria for both groups were the intake of psychoactive substances, previous neurosurgical interventions, drug abuse, and common contraindications against fMRI measurements (e.g. cardiac pacemaker, artificial magnetic heart valves, or claustrophobia). Recruitment of MS patients and healthy controls was accomplished block-wise and in alternating order to facilitate pairwise matching by sex, age, and education. At two time-points in the study course, MS patients were included before identifying matching healthy controls from a larger pool of potential healthy participants.

Written informed consent was obtained from all participants. Participants received financial compensation for time and effort, and were provided with free transportation to and from the hospital. The study was approved by the ethics review committee of the Chamber of Physicians (Aerztekammer), City of Hamburg, and was conducted in accordance with guidelines of the ethical committee of the

University of Hamburg, the declaration of Helsinki (World Health Organisation, 2013) and national legal regulations.

### 1.3. Software

All preprocessing procedures and analyses were carried out in the MATLAB and Statistics toolbox Release 2013a, The MathWorks, Inc., Natick, Massachusetts, United States (MATLAB 2013a) using in-house code, built-in functions, and commonly available software, unless reported differently. All plots were generated in MATLAB 2013a and compiled as figures using Adobe Illustrator CS5. Brain plots were visualized with the BrainNet Viewer (<http://www.nitrc.org/projects/bnv>) also running on MATLAB 2013a.

### 1.4. Neuropsychological and clinical data

Details of all neuropsychological and clinical scores that were analyzed for this doctoral thesis are summarized in Tab 4. Scores of the Hospital Anxiety and Depression Scale (HADS), Fatigue Scale for Motor and Cognitive Functions (FSMC), and the Edinburgh Handedness Inventory were computed from the raw data according to the corresponding manuals.

**Table 4. – Clinical, neuropsychological, and other assessment tools.**

Clinical tool	Subscales	Construct and scoring
<i>EDSS</i> - Extended Disability Status Scale (Kurtzke, 1983; Hobart et al., 2000)	-	level of overall disability (0-10)
<i>HADS</i> - Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983; Herrmann-Lingen et al., 1991)	HADS-A	anxiety level in patients with physical impairment (0-21), diagnostic classification (no clinical signs [ $\leq 7$ ], moderate [8-10], clinically relevant manifestation [ $\geq 11$ ])
	HADS-D	depression level in patients with physical impairment (0-21), diagnostic classification (no clinical signs [ $\leq 7$ ], moderate [8-10], clinically relevant manifestation [ $\geq 11$ ])
<i>FSMC</i> - Fatigue Scale for Motor and Cognitive Functions (Penner et al., 2009)	motor	physical fatigue level (10-50), diagnostic classification (no fatigue [ $< 22$ ], mild [22-26], moderate [27-31], severe [ $\geq 32$ ])
	cognition	cognitive fatigue level (10-50), diagnostic classification (no fatigue [ $< 22$ ], mild [22-27], moderate [28-33], severe [ $\geq 34$ ])
	sum scores	overall fatigue level (20-100), diagnostic classification (no fatigue [ $< 43$ ], mild [43-52], moderate [53-62], severe [ $\geq 63$ ])
Neuropsychological tool	Subscales	Construct
<i>VLMT</i> - Verbaler Lern- und Merkfähigkeitstest (Helmstaedter et al., 2001)	supraspan	immediate word span recall
	1-5	learning
	5-7	words forgotten over the inference trial; consolidation in longterm memory
<i>SDMT</i> - Symbol Digit Modality Test (Van Schependom et al., 2014)	-	psychomotor speed, attention, working memory
<i>PASAT</i> - Paced Auditory Serial Addition Test (Gronwall, 1977)	-	working memory, attention, information processing
<i>TAP</i> - Testbatterie zur Aufmerksamkeitsprüfung (Zimmermann and Fimm, 2007)	alertness with signal	phasic arousal

Continued on next page

Table 4 – continued from previous page

Neuropsychological tool	Subscales	Construct and scoring
<i>Block - Tapping - Test</i> (Wechsler, 1997)	alertness without signal	intrinsic alertness
	incompatibility	executive functions
	covert shift of attention	ability to focus visual attention to surroundings without changing the direction of gaze
	forward	storage capacity of visual-spatial short-term memory
	backward	learning in visual-spatial working memory
<i>RWT - Regenburger Wortfluesigkeitstest</i> (Aschenbrenner et al., 2000)	categories	semantic-categorical word fluency
	category Switching	semantic-categorical word fluency with alternating categories
	letter K	formal-lexical word fluency
Other tools	Subscale	Construct and scoring
<i>Edinburgh Handedness Inventory</i> (Oldfield, 1971)	-	measure of hand laterality: dominant hand (left [ $\leq -41$ ], both [-40-40], right [ $\geq 41$ ])

## 1.5. Neuroimaging data

### 1.5.1. MRI, fMRI, and DTI data acquisition

MRI data acquisition was performed on a 3 Tesla Magnetom Skyra whole-body system using a standard 32-channel head coil (Siemens, Erlangen, Germany). Anatomical MR images were acquired with a T1-weighted MPRAGE sequence (repetition time (TR) = 2500ms, echo time (TE) = 2.12ms, field of view = 240mm, flip angle = 9°, 256 slices covering the whole brain, voxel size 0.8x0.8x0.9mm) and a FLAIR sequence (TR = 9000ms, TE = 90ms, 43 slices, 10% gap, 320x field of view = 230mm, flip angle = 150°, voxel size 0.7x0.7x3.0mm). DTI images were acquired with a single-shot echo-planar imaging (EPI) sequence (30 directions, TR = 7200ms, TE = 87.0ms, 50 slices, no gap, 128x128 matrix, field of view = 240mm, b-value = 1000s/mm<sup>2</sup>, voxel size 1.9x1.9x2.0mm). Functional data were recorded using a T2\*-weighted gradient EPI sequence (TR = 2500ms, TE = 25ms, field of view = 250mm, flip angle = 90°, 40 slices, no gap, 94x94 matrix, voxel size 2.7x2.7x 3.0mm, duration = 625sec), while participants fixated a black cross during a so-called resting state measurement. Participants were instructed to lie still, stay awake, relax, and maintain the fixation. Head and body motion was restricted using foam and pillows. Respiration and pulse signal were only recorded for a small portion of the sample due to technical difficulties and could therefore not be taken into account for preprocessing or analyses.

### 1.5.2. Preprocessing of MRI and fMRI data

MRI data was preprocessed using the Statistical Parametric Mapping software package, version 8, Wellcome Trust Centre of Neuroimaging, London, Great Britain (SPM8) running on MATLAB 2013a. Before preprocessing, images of all participants were checked visually for rough alignment with default orientation, and manually reoriented if necessary.

WM hypointensities in T1-weighted images and WM hyperintensities in T2-weighted images indicate focal pathological modifications that occur due to acute inflammation, or permanent deterioration of myelin. WM alterations of this sort are primary symptoms in MS, but can occur to a minor extent in other neurological diseases or in the course of healthy aging as well. Extensive WM hypointensities can lead to inaccurate estimations of tissue compartments during the segmentation process (Chard et al., 2010, for an example from the present study see appendix section B.1). This in turn would

introduce systematic differences between MS patients and healthy controls when individual tissue masks are used for the analysis of fMRI data. To avoid this, individual WM lesions were identified and filled with the help of the Lesion Segmentation Toolbox version 2.0.12 (LST) for SPM8 before further preprocessing of MRI data (Schmidt et al., 2012, [www.statistical-modelling.de/lst.html](http://www.statistical-modelling.de/lst.html)). The lesion growth algorithm was applied to compute individual lesion probability maps based upon T1-weighted and FLAIR images. Using those lesion probability maps, corresponding hypointensities in T1-weighted structural images were replaced by simulated normal appearing WM. Both the lesion maps and the filled images were controlled visually for obvious failure of the algorithm. Manually marked lesion maps by an experienced radiologist were not available for a systematic validation. The total lesion volume in ml was determined automatically for each participant based on the WM lesion maps.

Further preprocessing of MRI data included co-registration with the mean functional image and segmentation into WM, GM, and cerebro-spinal fluid (CSF) compartments using the New Segment algorithm (bias regularisation = 0.0001, bias full width at half maximum (FWHM) = 60mm cutoff; based on Ashburner and Friston, 2005). Functional images were slice-time-corrected to the middle slice, realigned to the first image (2th degree B-spline interpolation) and unwarped (4th degree B-spline interpolation) to reduce susceptibility-by-movement interactions after removal of the first four volumes. Excessive volume-to-volume head movement (translation > 2mm, rotation > 2.5°) led to detailed visual inspection of all functional images as well as the realignment parameters. Volumes with movement artifacts in the course of sharp and excessive movement were discarded. Participants with less than 50% volumes left were excluded. Finally, functional and structural images were normalized to Montreal Neurological Institute (MNI) space and smoothed (8-mm FWHM gaussian kernel) using the Diffeomorphic Anatomical Registration Exponentiated Lie Algebra algorithm (DARTEL) (Ashburner, 2007). The conventional normalization procedure implemented in SPM8 warps individual data into MNI space based on a standard structural template. This approach holds the risk of introducing artificial groups differences if one group differs stronger from the standard brain than the other, for example due to structural damage. In contrast, DARTEL creates and applies a sample-specific structural template to reduce this systematic influence of normalization procedures. Because the cross-sectional analyses were conducted before the completion of the follow-up data acquisition, DARTEL was rerun including the follow-up data for the longitudinal part of this thesis. The total intensity was preserved through normalization (*no modulation*) as recommended for fMRI data. Preprocessed data was controlled visually for failure of applied algorithms.

### 1.5.3. Preprocessing of DTI data

DTI data was preprocessed with the FMRIB Software Library version 5.0.8 (FSL) ([www.fsl.fmrib.ox.ac.uk/fsl](http://www.fsl.fmrib.ox.ac.uk/fsl)). The preprocessing included the correction of head movements and eddy current distortions (reference volume = 0) using the FMRIB's Diffusion Toolbox, brain extraction using the Brain Extraction Tool (BET2,  $f = 0.2$ ; Popescu et al., 2012), and fitting of a diffusion tensor model using DTIFIT. The resulting fractional anisotropy (FA) images were spatially normalized afterwards by applying a nonlinear transformation to the implemented standard-space template and an affine transformation to MNI space using the Tract-Based Spatial Statistics (TBSS) toolbox. The FA value is derived from the eigenvalues of the diffusion tensor and represents the directionality of the diffusion in a voxel. Values range from zero to one, whereby higher values indicate greater directionality and therefore higher structural integrity of WM tracts.

## 1.6. General statistics

A few basic statistic tests were applied in more than one analysis. To reduce redundancy in the following analyses sections, their basic characteristics and test statistics are summarized below.

The t-test is a parametric test that can be applied to test a difference in a variable on interval scale. It gives the t-value ( $t$ ), which is a measure for the relation of the observed difference (for example between two groups) to the t-distribution. Results that are unlikely under the t-distribution have high t-values and low p-values. The sign of t-value reflects the group contrast. T-tests can be calculated for paired and unpaired samples. Wilcoxon signed rank tests are a non-parametric alternative to test differences between paired samples in variables on interval scale that are not normally distributed. Its test statistic is given by the Z-value ( $Z$ ) in the present work. The interpretation of its size and sign is similar to  $t$ , but its computation takes into account the ranking of differences between pairs. Differences in variables measured on nominal scale were tested using the McNemar test (binary classifications) or Chi square test ( $n_{\text{classes}} > 2$ ) in the present study. In both cases, the test statistic is given by  $\chi$ . Basically, those tests compare the observed frequencies of cases in each class with a distribution across classes that would result by chance or as predicted by a specific hypothesis.



## 2. Analysis 1: Group differences in function, structure, and behavior at baseline

### 2.1. Introduction

The aim of this analysis was to provide a comprehensive overview over structural, functional, and behavioral characteristics of both study groups. The overview was supposed to allow for an estimation of the representativity of the recruited sample and, in consequence, to facilitate the interpretation of all presented analyses of this doctoral thesis in relation to results reported by earlier neuroimaging studies on pathological mechanisms in MS.

Studies on functional characteristics in pathological populations usually aim for conclusions about abnormalities on the group level. Studies that focus specifically on individual features of fcMRI in MS or other neurological disorders, on the other hand, are rare. Nonetheless, conclusions are drawn about the potential application of those group-based results as biomarkers, implying that they possess diagnostic relevance on the individual level (e.g. Valsasina et al., 2011; Cruz Gomez et al., 2014). In a post-hoc test, individual deviations of fcMRI were therefore related to results on the group level to illustrate the variability among individuals and therefore the complexity of deriving significance for the individual case from group-based findings.

### 2.2. Material and methods

#### 2.2.1. Participants

Detailed information on recruitment criteria for MS patients and healthy controls can be found in section 1.2, part III. After the initial clinical examination, five participants were excluded from the study due to persistent technical artifacts in their MEG data caused by tooth fillings and contraceptive coils. Two patients and one healthy control had to be excluded after the baseline measurement because they did not meet the medical criteria anymore, and two more patients left the study on their own initiative. The final patient sample contained 40 MS patients (25 female, 24-60 years). From a total number of 70 eligible healthy subjects, 40 (25 female, age 29-57 years) were matched pairwise with patients by sex, age, and years of education.

#### 2.2.2. Neuropsychological and clinical data

Statistical testing of group differences in neuropsychological and clinical data was carried out in MATLAB 2013a. Statistical tests for paired samples were chosen depending on the scale of measurement and the distribution of data, which was controlled visually with histograms and analytically with Kolmogorov-Smirnov-tests (MATLAB 2013a function *kstest*, Massey, 1951). For variables measured on interval scale, paired t-tests (MATLAB 2013a function *ttest*) were conducted for normally distributed variables and Wilcoxon signed rank tests (MATLAB 2013a function *signrank*, Gibbons, 1974) otherwise. Corresponding effect size estimates were Cohen's d for t-tests, which is the mean pair difference divided by the standard deviation of pair differences (Cohen, 1988, 1992, interpretation: 0.2 = small, 0.5 = medium, 0.8 = large), and for the Wilcoxon signed rank test an approximation of the correlation coefficient, computed by dividing the Z-value by the square root of observations (esW;

Pallant, 2001, pp. 224-225; interpretation: 0.1 = small, 0.3 = medium, 0.5 = large). For variables measured on nominal scale, McNemar tests (MATLAB 2013a function *mcnemar*) were computed for binary classifications and Chi square tests (MATLAB 2013a function *crosstab*) otherwise. For neuropsychological tests, the  $\alpha$ -level was 0.05 (two-sided, false discovery rate (FDR)-corrected with  $q = 0.05$ , Benjamini and Hochberg, 1995). Demographic and clinical group differences were defined as significant at  $p < 0.05$ , uncorrected.

### 2.2.3. Structural data

#### 2.2.3.1. Analysis of gray matter

Analysis of GM was conducted in SPM8 running on MATLAB 2013a. Individual GM segments were transformed from native into MNI space using DARTEL (Ashburner, 2007). This time, the amount of signal was preserved (*modulation*), meaning that the intensity of signal of a region reduces or increases during expansion or compression when the image is transformed into standard space. This allowed for voxel-wise analysis of GM intensity as a proxy for GM atrophy. Using the Voxel Based Morphometry toolbox, Version 8 (VBM8) (Ashburner and Friston, 2000), a general linear model was fit to the data using the restricted maximum likelihood method. To correct for different brain sizes, images were proportionally scaled with respect to the individual total intracranial volumes obtained by summing up GM, WM, and CSF volumes. Group differences in GM intensity were tested for significance using paired t-tests (one-sided,  $\alpha$ -level = 0.001, family wise error (FWE)-corrected) for all voxels that had GM values  $> 0.3$  in each participant. One-sided testing was based on the hypothesis that MS patients would have lower GM intensities due to the well known GM atrophy in this disorder (see section 2.5, part I).

#### 2.2.3.2. Analysis of DTI data

Voxel-based statistical analysis of group differences in FA maps was conducted with TBSS in FSL. A sample-specific mean FA image (i.e. across all participants) was created and skeletonized. The skeleton was thresholded at 0.2, after controlling visually the alignment of the skeleton with the individual major white matter tracts. Individual FA data was projected onto the skeleton with the help of beforehand computed distance maps. Group differences in FA-values were tested for significance using permutation statistics (5000 permutations) for paired samples with the help of the *Randomise* tool (Winkler et al., 2014). The Threshold-Free Cluster Enhancement method was applied for cluster-thresholding. P-values were corrected for multiple comparisons by controlling the FWE (two-sided,  $\alpha$ -level = 0.001, FWE-corrected).

### 2.2.4. Functional connectivity

#### 2.2.4.1. Computation of individual ROI-to-ROI fcMRI

Using the MATLAB-based CONN toolbox (v14b, <http://www.nitrc.org/projects/conn>), mean BOLD signal time-series were extracted from the preprocessed data for 90 anatomical ROIs (cortical and sub-cortical regions of the automatic anatomical labeling (AAL) atlas; see appendix section B.2) covered by individual GM masks. The influence of body movements was modeled based upon the realignment parameters and regressed out of the time-series after an initial despiking that was applied to remove potential outlier scans. To account for scanner drift effects, a linear-term regressor was added to the general linear model for nuisance regression. Several investigations of spontaneous fcMRI indicate that functional interactions are best reflected in a specific frequency range of the BOLD signal (Cordes et al., 2001). High frequencies were shown to be associated with respiratory and cardiac activity (Murphy et al., 2013) and very low frequencies with scanner instabilities (Smith et al., 1999). To improve the



signal-to-noise ratio, BOLD time-series were therefore band-pass filtered (0.01-0.1Hz). Further non-neuronal contributions to BOLD signal variance from CSF and WM were reduced by applying the *CompCor* strategy (Behzadi et al., 2007). Pearson’s correlation coefficients were computed pairwise between corrected BOLD time-series and Fisher’s z-transformed to construct individual whole-brain ROI-to-ROI fcMRI matrices.

For illustration purposes only, each ROI was assigned to at least one RSNs based on previous findings (for details see appendix section B.2). Some of the ROIs of the AAL atlas cover large parts of the brains so that a distinct assignment to just one RSN was not always possible. The assignment must therefore be understood as a rough approximation for an easier understanding of the results.

#### **2.2.4.2. Second level analysis of fcMRI group differences**

Group differences in fcMRI were tested for significance separately for each of the 4005 ROI-to-ROI connections with permutation tests for paired samples (50000 permutations, two-sided,  $\alpha$ -level = 0.05, FDR-adjusted according to Yekutieli and Benjamini, 1999). Cohen’s d values for paired samples were computed as effect size.

#### **2.2.4.3. Post-hoc exploration of inter-individual variability**

To complement the results of the group analysis and to further inquire into the variability of individual alterations of fcMRI among patients, a qualitative post-hoc analysis was conducted. Each functional connection of the individual ROI-to-ROI fcMRI maps was thereby categorized as either *increased*, or *decreased* relative to the connection-specific average fcMRI of healthy controls.

In addition, *relevant* individual deviations of fcMRI were identified for each subject with two different definitions of *relevance*. First, alterations that were lower, or respectively higher than the 2.5% and the 97.5% percentiles of the connection-specific distribution of deviations in the control group were defined as *meaningful*. Second, irrespective of their relation to deviations in the control or patient group, the 5% greatest individual deviations were taken into account (*top 5%*,  $n = 200$ ; approximate amount of discriminative links in Richiardi et al., 2012). Amount and spatial distribution of those *relevant* connections were then explored. For completeness, the analysis was conducted for both groups.

## 2.3. Results

### 2.3.1. Sample

The EDSS score of one healthy controls was missing and therefore replaced by the corresponding group mean. For two healthy controls, one of fourteen items of the HADS were missing, respectively. The scores were replaced by the individual mean of the corresponding depression or anxiety subscale. Groups differed significantly ( $p < 0.05$ ) in all MS-specific clinical variables, namely EDSS, dimensional and categorical scores for general, motor, and cognitive FSMC, lesion volume, and intake of MS medications. No such group differences were detected for the handedness, depression and anxiety scores, the occurrence of sleep problems, and the intake of cortisone (Tab. 5).

**Table 5. – Demographic and clinical characteristics of the study sample.**

Demographic information	HC (n = 40)	MS (n = 40)	Test parameter	p	effect size
Age (in years; M, SD)	40.85 (8.24)	41.23 (9.49)	$t_{(39)} = -0.74$	0.46	$d = -0.12$
Sex (female-male)	25-15	25-15	-	-	-
Handedness (left-both-right)	1-1-38	4-2-34	$\chi_{(2)} = 2.36$	0.31	-
Clinical characteristics	HC (n = 40)	MS (n = 40)	Test parameter	p	effect size
Years since diagnosis (M, SD)	-	6.93 (5.00)	-	-	-
EDSS (M, SD)	0.32 (0.58)	2.03 (0.93)	$Z = -5.07$	$<0.01^*$	$esW = -0.57$
FSMC Sum (M, SD)	28.38 (6.10)	52.45 (19.49)	$Z = -4.85$	$<0.01^*$	$esW = -0.54$
no-mild-moderate-severe	40-0-0-0	14-6-6-14	$\chi_{(3)} = 38.52$	$<0.01^*$	-
FSMC Motor (M, SD)	13.98 (3.42)	25.38 (9.84)	$Z = -4.81$	$<0.01^*$	$esW = -0.54$
no-mild-moderate-severe	39-1-0-0	18-2-7-13	$\chi_{(3)} = 28.07$	$<0.01^*$	-
FSMC Cognition (M, SD)	14.40 (3.36)	27.08 (10.52)	$Z = -4.79$	$<0.01^*$	$esW = -0.57$
no-mild-moderate-severe	40-0-0-0	14-7-5-14	$\chi_{(3)} = 38.51$	$<0.01^*$	-
HADS-A (M, SD)	3.28 (2.65)	4.50 (3.20)	$Z = -1.49$	0.14	$esW = -0.17$
no-moderate-clinical	38-1-1	30-9-1	$\chi_{(2)} = 7.34$	$0.03^*$	-
HADS-D (M, SD)	2.33 (2.30)	2.93 (2.90)	$Z = -1.33$	0.18	$esW = -0.15$
no-moderate-clinical	38-2-0	37-2-1	$\chi_{(2)} = 1.01$	0.60	-
Sleep problems (no-yes)	32-8	28-12	$\chi_{(1)} = 0.75$	0.39	-
Lesion volume (in ml; M, SD)	0.13 (0.24)	11.03 (14.59)	$Z = -5.51$	$0.01^*$	$esW = -0.62$
Cortisone (yes-no-unknown)	3-32-5	3-29-8	$\chi_{(2)} = 0.84$	0.66	-
MS modifying drugs** (yes-no-unknown)	0-39-1	20**-19-1	$\chi_{(2)} = 28.90$	$<0.01^*$	-

$d =$  Cohen's  $d$ ;  $Z =$  test statistic of Wilcoxon signed rank test;  $\chi =$  test statistic of Chi square or McNemar tests;  $esW =$  effect size for Wilcoxon signed rank test; HC = healthy controls; MS = multiple sclerosis;

\*significant at  $p < 0.05$ ;

\*\*glatiramer acetate ( $n = 3$ ), natalizumab ( $n = 4$ ), beta interferon ( $n = 5$ ), fingolimod ( $n = 3$ ), dimethyl fumarate ( $n = 1$ ), unknown ( $n = 4$ )

### 2.3.2. Neuropsychological data

MS patients performed significantly worse ( $p < 0.05$ , FDR-corrected) in the VLMT 1-5 subscale (learning; Cohen's  $d = 0.56$ ), and in two subscales of the RWT (semantic-categorical word fluency with and without alternating categories; Cohen's  $d = 0.46/0.49$ ). No further meaningful differences were detected. The score of one MS patient was missing for the TAP subscale Covert shift of attention and was replaced by the average value of the remaining patients. Results are summarized in Tab. 6.

**Table 6. – Group differences in neuropsychological tests.**

Neuropsychological test	HC (n = 40)	MS (n = 40)	Test parameter	p	effect size
VLMT supraspan (M, SD)	8.45 (2.23)	7.35 (2.52)	$t_{(39)} = 2.09$	0.08	d = 0.33
1-5 (M, SD)	59.32 (7.48)	52.93 (10.62)	$t_{(39)} = 3.51$	0.02*	d = 0.56
5-7 (M, SD)	0.68 (1.53)	1.20 (1.92)	Z = -1.34	0.18	esW = -0.15
SDMT (M, SD)	61.68 (12.06)	57.73 (14.15)	$t_{(39)} = 1.48$	0.21	d = 0.23
PASAT (M, SD)	50.70 (7.87)	48.78 (9.65)	Z = 0.60	0.59	esW = 0.07
TAP Alert. + signal (M, SD)	246.90 (26.44)	264.63 (54.25)	$t_{(39)} = -1.89$	0.10	d = -0.30
Alert. - signal (M, SD)	246.68 (29.15)	267.65 (49.53)	$t_{(39)} = -2.38$	0.06	d = -0.38
Incomp. (M, SD)	505.88 (69.78)	511.28 (90.71)	$t_{(39)} = -0.34$	0.74	d = -0.05
Covert shift (M, SD)	36.58 (22.81)	49.92 (30.04)	$t_{(39)} = -2.15$	0.07	d = -0.34
Block-Tapping forw.(M, SD)	9.22 (1.85)	8.52 (1.64)	Z = 2.39	0.06	esW = 0.27
backw. (M, SD)	8.63 (1.80)	7.75 (1.82)	Z = 2.23	0.06	esW = 0.25
RWT categories (M, SD)	42.10 (9.26)	35.98 (10.60)	$t_{(39)} = 3.07$	0.03*	d = 0.49
cat. switching (M, SD)	25.78 (7.93)	21.35 (6.70)	$t_{(39)} = 2.89$	0.03*	d = 0.46
letter K (M, SD)	25.22 (4.62)	26.45 (9.21)	$t_{(39)} = -0.72$	0.55	d = -0.11

*d* = Cohen's *d*; *Z* = test statistic of Wilcoxon signed rank test; *esW* = effect size for Wilcoxon signed rank test; *HC* = healthy controls; *MS* = multiple sclerosis; \*significant at  $p < 0.05$ , FDR-corrected

### 2.3.3. Structural data

#### 2.3.3.1. Gray matter

After FWE-correction, there were no voxels that differed significantly between groups. For the purpose of display, uncorrected group differences in GM are displayed in Fig. 6, A ( $p < 0.0001$ , uncorrected, threshold for cluster-extent based thresholding = 20). MS patients showed decreased GM intensities in four clusters in the cerebellum and in one cluster in the inferior parietal lobule on the left side. Anatomical and statistical details are summarized in Tab. 7.

**Table 7. – Clusters with group differences in gray matter intensities (uncorrected).**

Contrast	size	max. T	p	$P_{(FWE)}$	x	y	z	Location
HC > MS	226	5.11	<0.01	0.05	-58	-45	48	inferior parietal lobule (L)
	600	4.85	<0.01	0.10	-28	-61	-56	cerebellum (L)
	35	4.66	<0.01	0.17	4	-55	0	cerebellum (R)
	52	4.47	<0.01	0.25	-18	-67	-33	cerebellum (L)
	419	4.47	<0.01	0.25	27	-60	-56	cerebellum (R)

*HC* = healthy controls; *MS* = multiple sclerosis; *L* = left; *R* = right; *x*, *y*, *z* = coordinates of the Montreal Neurological Institute (MNI) space

#### 2.3.3.2. White matter

Due to technical reasons, DTI data of one MS patient was not available. The corresponding healthy partner was also excluded from the analysis to maintain the individual matching, resulting in a sample size of 78 for statistical testing. There were multiple significant clusters ( $p < 0.001$ , FWE-corrected), in which healthy controls had higher FA-values than MS patients (Fig. 6, B, Tab. 8), and no significant group differences in the opposite direction.

**Table 8. – Clusters with significant group differences in fractional anisotropy (FA).**

Contrast	size	max. Z	$P_{(FWE)}$	x	y	z	Location
HC > MS	42098	7.37	<0.01	17	-17	-2	corticospinal tract (R)
	370	4.36	<0.01	16	55	15	forceps minor (R)
	121	5.71	<0.01	12	9	-1	ant. thalamic radiation (R)
	105	5.18	<0.01	13	-22	14	ant. thalamic radiation (R)

Continued on next page

Table 8 – continued from previous page

Contrast	size	max. Z	P(FWE)	x	y	z	Location
	68	3.54	<0.01	-35	-31	49	sup. longitudinal fasciculus (L)
	48	4.32	<0.01	-58	-34	-7	sup. longitudinal fasciculus (L)
	44	4.28	<0.01	-9	-58	20	cingulum (L)
	39	3.14	<0.01	-51	-15	6	sup. longitudinal fasciculus (L)
	6	3.18	<0.01	-42	-57	10	sup. longitudinal fasciculus (L)
	3	2.51	<0.01	-13	58	6	forceps minor (L), ant. thal. radiation (L)
	2	3.13	<0.01	42	-51	44	sup. longitudinal fasciculus (R)
	1	2.42	<0.01	-44	-59	12	sup. longitudinal fasciculus (L)
MS > HC	-	-	-	-	-	-	-

HC = healthy controls; MS = multiple sclerosis; L = left; R = right; x, y, z = coordinates of the Montreal Neurological Institute (MNI) space

### 2.3.3.3. White matter lesion distribution in MS patients

The distribution of WM lesions in MS patients as derived from the automatic lesion segmentation is illustrated in Fig. 6, C. The values indicate the percentage of patients with a lesion probability of at least 10% in the given voxel based on their individual unsmoothed and normalized lesion probability maps. The probability threshold has no statistical meaning, but was applied for illustration purposes only. It should be considered that subtle WM alterations that do not manifest as circumscribed lesions on the macroscopic level (so-called *normal appearing WM*) are not captured by the algorithm. White matter lesions spread widely and were found across the entire WM. The highest probability was detected periventricular in the posterior part of WM.

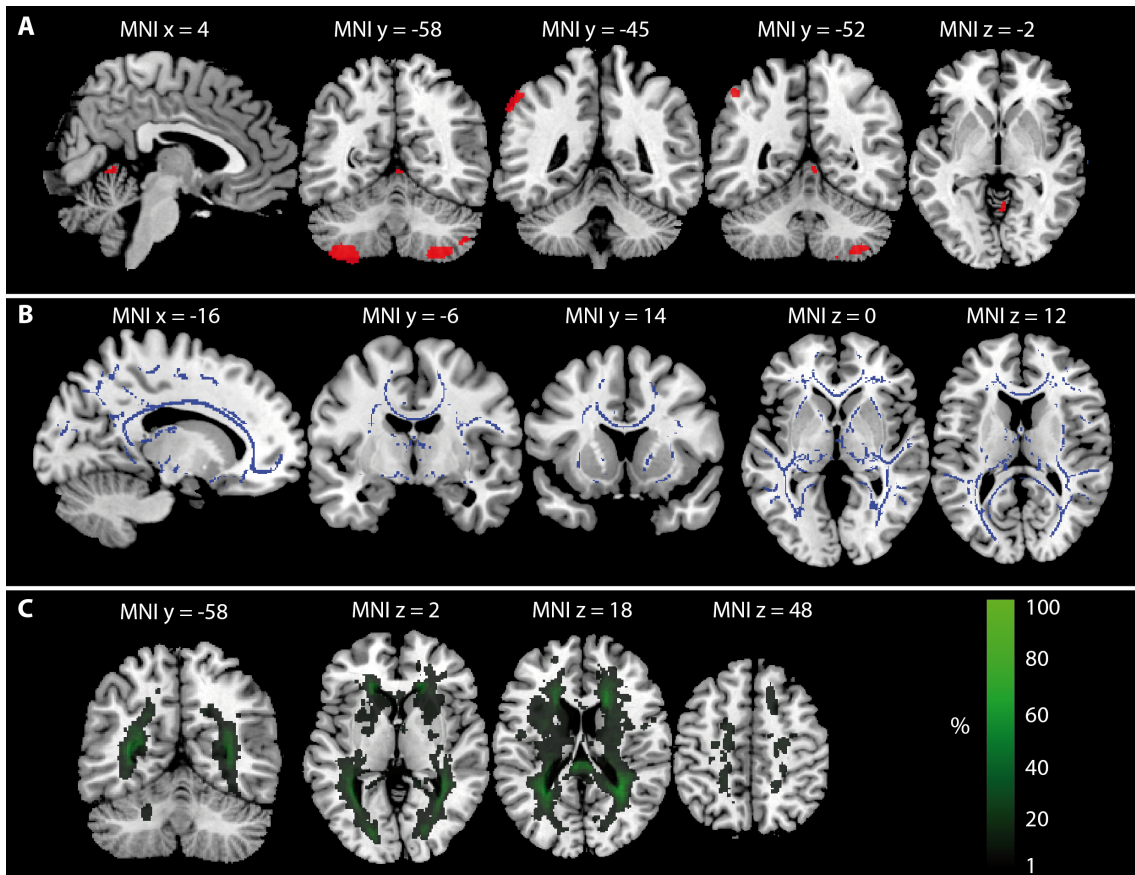


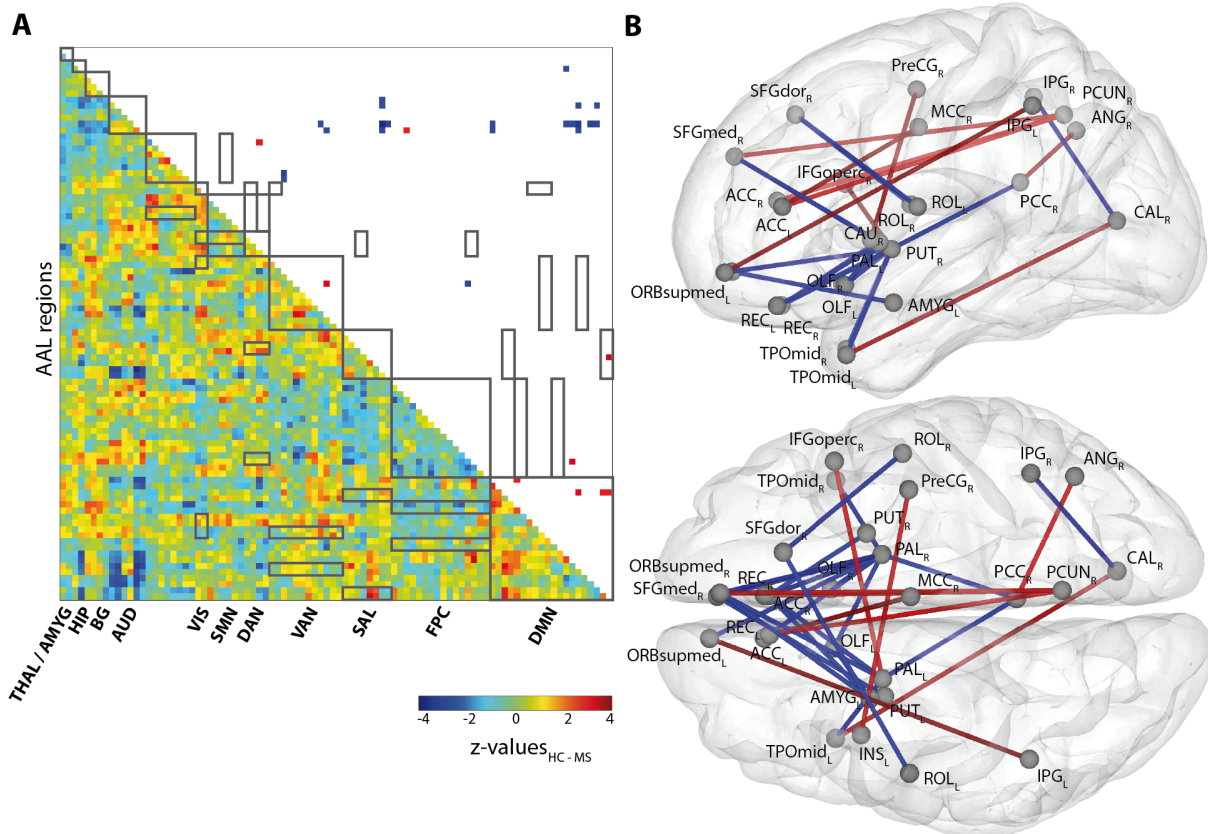
Figure 6. – Group differences in structural data. **A** Clusters with significant group differences in gray matter (red;  $p < 0.0001$ , uncorrected, cluster extend threshold = 20). **B** Significant group differences in fractional anisotropy (blue;  $p < 0.001$ , FWE-corrected). **C** Distribution of white matter lesions in MS patients. Illustrated is the percentage of patients with a probability of at least 10% for a lesion in a certain voxel.

MNI = Montreal Neurological Institute standard brain; images are displayed in neurological orientation

### 2.3.4. Functional connectivity

#### 2.3.4.1. Group differences

Volume-to-volume movement exceeded the criteria (translation > 2mm, rotation > 2.5°) in two healthy controls but no patients. Detailed inspection of both functional images and realignment parameters gave reason to discard 104 of 246 functional images in one participant (HC11) while no volumes had to be removed in the second one (HC34). Overall, study groups did not differ significantly in their translation or rotation parameters, so that systematic effects of movement on fcMRI estimates must not be assumed. There were no significant group differences in ROI-to-ROI fcMRI when multiple comparison correction was applied. Without correction, 30 group differences were identified at an  $\alpha$ -level of 0.005 (Fig. 7, Tab. 9). Controls had higher fcMRI in nine long-range connections mainly between parietal, cingulate, and frontal regions ( $|\text{Cohen's } d| = 0.50\text{-}0.62$ ). MS patients exhibited relatively increased fcMRI in 21 functional links predominantly among frontal areas and between frontal ROIs and the basal ganglia ( $|\text{Cohen's } d| = 0.49\text{-}0.73$ ). Effect sizes for the identified group differences were medium up to large.



**Figure 7. – Group differences in functional connectivity.** **A** z-values for each of the 4005 functional connections of the automatic anatomical labeling (AAL) atlas are illustrated in the lower triangle of the matrix, indicating the statistical group difference. Group contrasts with  $p < 0.005$ , uncorrected, are highlighted in the upper triangle. Warm colors indicate higher fcMRI in healthy controls (HC), while cold colors represent a relative increase of fcMRI in multiple sclerosis (MS). **B** Group differences plotted on the brain. *networks*: DMN = default mode, FPC = fronto-parietal control, SAL = salience, V/DAN = ventral/dorsal attention, SMN = somatosensory, VIS = visual, AUD = auditory, BG = basal ganglia, HIP = hippocampus, THAL = thalamus, AMYG = amygdala; L = left; R = right; for information on regions of interest see appendix section B.2

**Table 9. – Group differences in functional connectivity ( $p < 0.005$ , uncorrected).**

ROI 1	ROI 2	RSN	z	p	P(FDR)	d
cingulum ant (ACC <sub>L</sub> )	precuneus (PCUN <sub>R</sub> )	DMN	2.92	0.003	0.633	0.52
cingulum ant (ACC <sub>R</sub> )	precuneus (PCUN <sub>R</sub> )	DMN	2.92	0.003	0.632	0.52
frontal sup med (SFGmed <sub>R</sub> )	precuneus (PCUN <sub>R</sub> )	DMN	3.15	0.002	0.655	0.57
angular (ANG <sub>R</sub> )	cingulum post (PCC <sub>R</sub> )	DMN	2.85	0.004	0.632	0.50
frontal med orb (ORBsupmed <sub>L</sub> )	parietal inf (IPG <sub>L</sub> )	-	3.35	<0.001	0.55	0.62
cingulum ant (ACC <sub>L</sub> )	cingulum mid (MCC <sub>R</sub> )	SAL	3.33	<0.001	0.55	0.61
temporal pole mid (TPOmid <sub>L</sub> )	calcarine (CAL <sub>R</sub> )	VAN	3.13	0.002	0.55	0.56
precentral (PreCG <sub>R</sub> )	insula (INS <sub>L</sub> )	-	2.87	0.004	0.632	0.50
frontal inf oper (IFGoper <sub>R</sub> )	pallidum (PAL <sub>L</sub> )	-	2.94	0.003	0.632	0.52
parietal inf (IPG <sub>R</sub> )	calcarine (CAL <sub>R</sub> )	-	-3.18	0.001	0.55	-0.57
frontal sup (SFGdor <sub>R</sub> )	rolandic oper (ROL <sub>L</sub> )	-	-2.81	0.004	0.632	-0.49
frontal sup (SFGdor <sub>R</sub> )	rolandic oper (ROL <sub>R</sub> )	-	-3.25	0.001	0.554	-0.60
frontal sup med (SFGmed <sub>R</sub> )	pallidum (PAL <sub>L</sub> )	-	-3.14	0.002	0.554	-0.57
cingulum post (PCC <sub>R</sub> )	pallidum (PAL <sub>L</sub> )	-	-2.92	0.003	0.632	-0.51
rectus (REC <sub>R</sub> )	pallidum (PAL <sub>L</sub> )	-	-2.99	0.003	0.632	-0.53
temporal pole mid (TPOmid <sub>L</sub> )	pallidum (PAL <sub>L</sub> )	-	-2.99	0.003	0.632	-0.53
olfactory (OLF <sub>L</sub> )	pallidum (PAL <sub>R</sub> )	-	-3.24	0.001	0.554	-0.58
olfactory (OLF <sub>R</sub> )	pallidum (PAL <sub>R</sub> )	-	-3.36	<0.001	0.554	-0.61
frontal sup med (SFGmed <sub>R</sub> )	pallidum (PAL <sub>R</sub> )	-	-3.60	<0.001	0.554	-0.68
frontal med orb (ORBsupmed <sub>L</sub> )	pallidum (PAL <sub>R</sub> )	-	-3.12	0.002	0.554	-0.56
frontal med orb (ORBsupmed <sub>R</sub> )	pallidum (PAL <sub>R</sub> )	-	-3.05	0.002	0.632	-0.54
cingulum post (PCC <sub>R</sub> )	pallidum (PAL <sub>R</sub> )	-	-2.88	0.004	0.632	-0.50
rectus (REC <sub>L</sub> )	pallidum (PAL <sub>R</sub> )	-	-3.14	0.002	0.554	-0.56
rectus (REC <sub>R</sub> )	pallidum (PAL <sub>R</sub> )	-	-3.77	<0.001	0.554	-0.73
temporal pole mid (TPOmid <sub>R</sub> )	pallidum (PAL <sub>R</sub> )	-	-2.90	0.004	0.632	-0.51
olfactory (OLF <sub>L</sub> )	caudate (CAU <sub>L</sub> )	-	-2.82	0.004	0.632	-0.49
frontal sup medl (SFGmed <sub>R</sub> )	putamen (PUT <sub>L</sub> )	-	-3.00	0.003	0.632	-0.53
rectus (REC <sub>R</sub> )	putamen (PUT <sub>L</sub> )	-	-2.85	0.004	0.632	-0.50
rectus (REC <sub>R</sub> )	putamen (PUT <sub>R</sub> )	-	-2.92	0.004	0.632	-0.51
frontal med orb (ORBsupmed <sub>R</sub> )	amygdala (AMYG <sub>L</sub> )	-	-2.86	0.004	0.632	-0.50

*d* = Cohen's *d*; ROI = region of interest; RSN = resting state network; L = left; R = right; *d*, *z* > 0: healthy control > multiple sclerosis; *d*, *z* < 0: healthy controls < multiple sclerosis

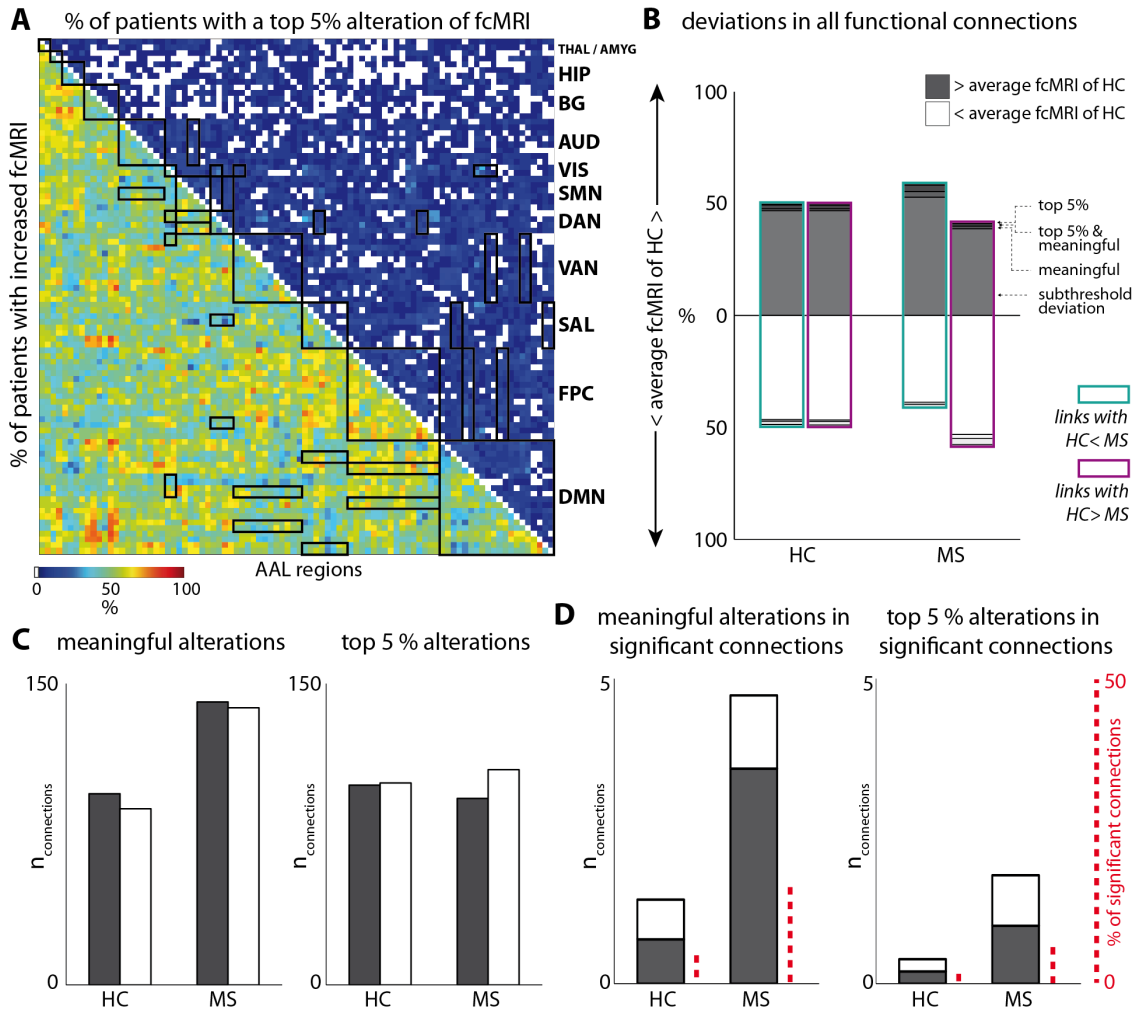
### 2.3.4.2. Post-hoc exploration of inter-individual variability in fcMRI

The post-hoc analysis revealed that the individual increase of fcMRI, that means higher fcMRI relative to the connection-specific mean value of healthy controls, corresponded best among patients in the fronto-parietal network (increase in > 75% of patients), and in functional connections connecting the basal ganglia with the DMN and the fronto-parietal with the salience network (Fig 8, A, lower triangle). There were only few connections, in which less than 25% of all patients exhibited an increase of fcMRI, indicating, by implication, that patients corresponded only weakly with respect to their individual patterns of decreased fcMRI. There was also only little matching between patients in their individual *top 5%* alterations, which were found to be distributed widely across the entire brain (Fig 8, A, upper triangle).

On average, approximately 60% of all 4005 functional connections deviated in the direction of the group contrasts in the patient group (increase when MS > HC and vice versa; Fig 8, B). *Relevant* alterations in accordance with the direction of the difference, however, were detected in only a small portion of all connections (3.17-5.98%).

In patients, on average 278.93 *meaningful* alterations were identified and still 182.88 in healthy controls (Fig 8, C). The number of *meaningful* alterations was not related to the overall lesion volume ( $r_{HC} = -0.11$ ,  $p_{HC} = 0.50$ ;  $r_{MS} = 0.03$ ,  $p_{MS} = 0.86$ ). The mean deviation from the group mean in such connections was  $|r| = 0.38$  in both groups. The *top 5%* alterations deviated slightly stronger with a mean of  $|r| = 0.42$  in patients and controls.

The ratio between average increase and decrease of fcMRI was almost balanced for both *meaningful* and *top 5%* deviations (Fig 8, C). In functional connections with significant group contrast, on the other hand, the majority of relevant deviations were positive, reflecting of course the group-based findings (Fig 8, D). Patients had on average less than five *relevant* deviations in functional connections that had differed between groups ( $n = 30$ ; Fig 8, D). In other words, in each of those functional connections with group effect, *relevant* alterations were describable on the individual level in only 2.9 of the 40 patients on average (range 0-13). The highest portion of patients with *meaningful* alterations of fcMRI were found in the connections within the DMN.



**Figure 8. – Exploration of individual deviations of functional connectivity.** **A** Lower triangle: percentage of multiple sclerosis (MS) patients with an increase of functional connectivity (fcMRI) relative to controls. The percentage of patients with a decrease is always  $100 - \%_{\text{increase}}$ . Upper triangle: percentage of patients with a top 5% alterations in each functional connection. **B** Average amount of positive (dark) and negative (light) deviations of fcMRI from the group mean in healthy controls (HC) in both groups, separate for functional connections, in which HC showed higher fcMRI and the other way around. The portion of *meaningful*, *top 5%* deviations, their overlap, and subthreshold deviations is highlighted in different shadings. **C** Number of *meaningful* and *top 5%* positive and negative alterations for both groups. **D** Number of relevant deviations in functional connections with significant group difference in fcMRI. The average percentage of significant connections, in which relevant deviations were identified is illustrated in red. *dark* = positive deviation of fcMRI in relation to healthy controls, *light* = negative deviations; networks: DMN = default mode, FPC = fronto-parietal control, SAL = salience, V/DAN = ventral/dorsal attention, SMN = somatosensory, VIS = visual, AUD = auditory, BG = basal ganglia, HIP = hippocampus, THAL = thalamus, AMYG = amygdala; L = left; R = right

## **2.4. Discussion**

### **2.4.1. Representativity of the sample**

Size and demographic characteristics of the present patient sample are comparable to those of other studies on fMRI in MS (see section 3, part II). The overall clinical picture of MS was also similar to other studies in the field. It should be noted that the overall disability was particular low as well as the levels of anxiety and depression. Cognitive deficits were furthermore limited to memory and verbal domains, while attention and information processing were shown to be preserved in disagreement with patterns of cognitive deficits typically found in MS (Calabrese and Penner, 2007). Merely the prevalence of fatigue (65% ) was in a range that was representative for the clinical population (e.g Krupp et al., 1988). Together, these characteristics do not represent the spectrum of clinical manifestations of MS but are in line with other studies in the field. The participation in such an extensive study and especially the MRI and MEG measurements are demanding and strenuous for patients. The inclusion of severely affected patients is therefore not possible or desirable both from an ethical and a scientific point of view.

### **2.4.2. White and gray matter impairment**

As expected, there was extensive WM damage in MS patients, affecting a great portion of WM tracts. The inter-individual variability of individual lesion locations and their distribution across the brain were enormous as reported by other authors (e.g. Vellinga et al., 2009). Also in correspondence, areas with highest lesion load probability were typical lesion sites in MS, namely periventricular areas and the corpus callosum (Ge, 2006; Haider et al., 2016).

Although WM lesions are still the primary symptom for the diagnosis of MS, GM alterations are attributed increasing importance for the manifestation and the development of the disease nowadays (Steenwijk et al., 2016; Chard and Miller, 2016). In the present sample, only weak signs for MS-related GM alterations were found, but consistently in the cerebellum. GM atrophy has been shown before to affect diverse areas of the brain with minor to extensive impact, including the cerebellum (Cruz Gómez et al., 2013; Grothe et al., 2016). Causal interrelation with WM pathology and the unique contribution to the clinical manifestation of MS are matters of current research.

### **2.4.3. Alterations of functional integration in MS**

In line with the present findings, previous studies have revealed both increase and decrease of functional connectivity in MS (see section 3, part II). Disturbance of functional integration became evident in this analysis between anterior and posterior parts of the DMN, with the precuneus as a key player. Functional and structural alterations in the DMN have been described before in MS (e.g. Rocca et al., 2010; Bonavita et al., 2011; Zhou et al., 2014). On the other hand, disturbances of the DMN were shown for several other psychiatric and neurological diseases as well (Buckner et al., 2008). Whether those findings reflect specific mechanisms for MS, or general pathological processes related to the hubness and centrality of major DMN components (e.g. the precuneus; van den Heuvel and Sporns, 2011) is not answered yet.

The opposite, which is the enhancement of functional integration, was found between basal ganglia nuclei and frontal regions. A specific role of the basal ganglia for MS is a subject of current discussion. Previous findings have indicated a relationship with fatigue (Chaudhuri and Behan, 2000; DeLuca et al., 2008), motor deficits (Dogonowski et al., 2013c), and cognitive impairment (DeLuca et al., 2015; Wu et al., 2016) in MS.

Hyperconnectivity of frontal regions has been shown before in divergent disorders (for a review see Hillary et al., 2015) and is thought to indicate reinforced engagement of brain regions involved in cogni-



tion, decision making and executive functioning. An in-depth exploration of the relationship between individual fcMRI expression and the clinical manifestation of cognitive disturbances is presented in the following analysis section (3, part III) and will therefore not be discussed any further at this point. Taken together, the sample characteristics indicate higher internal validity and only limited external validity in line with other studies and general recommendations for explorative investigations. Findings of functional, structural, and behavioral features are in line with previous studies, confirming that the present sample does not differ systematically from other studies in the field.

#### **2.4.4. From group to individual level, from scientific finding to clinical application**

The exploration of individual deviations of fcMRI illustrated that group-based findings were reflected only weakly on the individual level, as expected, considering the p-values of group differences in fcMRI. Strongest deviations from the group mean of healthy controls did not converge among patients and, most importantly, merely a small portion of them were found in functional connections, for which a significant group effect had been detected before. For the interpretation of this outcome, it certainly must be taken into account that the statistical evidence for the group effects was not satisfying. Findings should therefore not be overrated. Nevertheless, this exploration illustrates following major issues for the translation of neuroscientific group-based findings into clinical applications for dimensional, but also categorical diagnostics. First, while the identification of effects on the group level is of course necessary for the understanding of general pathological mechanisms, their significance for diagnostic purposes is still unclear. Typically applied statistical tests aim for the identification of converging patterns among individuals. A crucial challenge for clinical diagnostics in MS is however, on the very contrary, to improve the characterization of individual features, hence to capture differences, rather than similarities between patients, to facilitate personalized treatment or detailed prognoses. Moreover, the focus on inter-individual differences in functional integration patterns might also be useful to disclose the underlying pathophysiology of the immense inter-individual variability of clinical symptoms in MS. In addition, recent findings by Langs et al. (2016) suggest considerable inter-individual variability in the exact anatomical location of functional systems, which is a critical limitation of group-based findings both for basic research questions and clinical applications. The development of validated and reliable methodological approaches and standards for the characterization of functional interactions on the individual level is therefore desirable (Airan et al., 2016). Second, findings of fcMRI alterations are usually characterized relationally, that is in contrast to another group. As a consequence, characterization of functional variables on the individual level also require benchmarks to relate individual and group-based outcomes. In the current post-hoc analysis, the average fcMRI of healthy controls was taken as a benchmark and defined separately for each functional connection. The validity of this benchmark is certainly not sufficient for a profound statistical analysis due to the dependence between second level and post-hoc analysis, the sample size, and the overall representativity of the sample, but was applied nonetheless due to the lack of publicly available applicable functional benchmarks. It would be desirable if authors of future studies in the field of translational neuroscience consider this rather practical issue when drawing conclusions regarding the potential application of their group-based findings as biomarkers. Third, taking into account the entire body of information, for example with multivariate pattern recognition approaches (Klöppel et al., 2012), is certainly one option for individual diagnostics. Focusing on specific alterations, however, is another conceivable approach for the extraction of valuable information about individual characteristics. Results by Richiardi et al. (2012), for example, indicate that only a small amount of all functional connections actually discriminate between MS patients and healthy controls (4% of a 90x90 ROI-to-ROI matrix). However, the identification of meaningful alterations is not trivial. Relevant abnormalities of fcMRI on the individual level were defined in a very simple way in the present study. More sophisticated

methods might have come to a different conclusion. Investigations on appropriate clinical cut-offs that separate random alterations from meaningful ones on the individual level are necessary to promote the ambitious endeavor of translating neuroscientific findings into clinical tools.

#### **2.4.5. Limitations**

FcMRI was computed between pairs of ROIs based upon the AAL atlas to reduce the dimensionality of the fcMRI analysis. It should be noted that this approach holds the risk to underestimated relevant effect that might have been detected with higher spatial resolution. On the other hand, group effects can also be overestimated, especially when groups differ systematically in their GM volume and in consequence the matching between brain tissue and ROI templates. In the present analysis, only weak differences were detected in the GM and almost all of them in the cerebellum, which was not included in the fcMRI analysis. Still, a limitation due to the applied ROI-to-ROI approach should be considered for the interpretation of the present and the following analyses of this doctoral thesis.

# 3. Analysis 2: Compensation revised - On the relationship between white matter integrity, functional connectivity, and cognitive deficits in multiple sclerosis

## 3.1. Introduction

Cognitive deteriorations are frequent in MS with prevalence estimates between 40% and 70% (Chiariavalloti and DeLuca, 2008). These impairments limit the participation in social and professional life beyond physical disability and therefore are often perceived as especially burdening (Benedict et al., 2005; Amato et al., 2006; DeLuca et al., 2015). Different potential structural brain correlates of cognitive impairment in MS have been suggested (e.g. Sanfilippo et al., 2006; Calabrese et al., 2009). Recently however, Daams et al. (2016) could show with a stepwise linear regression that only few of the most commonly mentioned structural markers have unique predictive value for the cognition. Their final regression model consisted only of GM volume of subcortical regions and the severity of diffuse WM damage based on FA values, and explained less than 50% of the variance of cognition in their large sample of long-standing MS patients. Predicting individual cognitive disturbance from structural information is therefore still difficult and hardly reliable.

Both structural and behavioral symptoms have been linked to modifications of spontaneous functional integration patterns in order to characterize the role of the functional connectome in reflecting and mediating structural disturbance and behavioral manifestation in MS (e.g. Hawellek et al., 2011; Zito et al., 2014; Hulst et al., 2015; Tewarie et al., 2015). The findings of these studies are highly inconsistent. Evidence for both enhanced (Wojtowicz et al., 2014) and decreased (Gamboa et al., 2014) functional integration was found in MS patients, and increased functional connectivity was shown to be associated with preserved function (Leavitt et al., 2014) but also with greater impairment (Schoonheim et al., 2015). The actual functional impact of alterations in metrics that describe functional connectivity as well as the underlying mechanisms remain therefore unclear.

The aim of the present analysis was to go beyond the investigation of fcMRI itself and instead inquire into connection-specific interdependencies between fcMRI and both structural alterations and behavioral symptoms. FcMRI was associated with WM integrity on one side, and cognitive performance on the other, and functional connections classified based on their qualitative relation to structure and behavior. Similarities and differences between patients and controls in the resulting whole-brain patterns were examined. In addition, organizational and other characteristics of connections with similar association patterns were explored post-hoc.

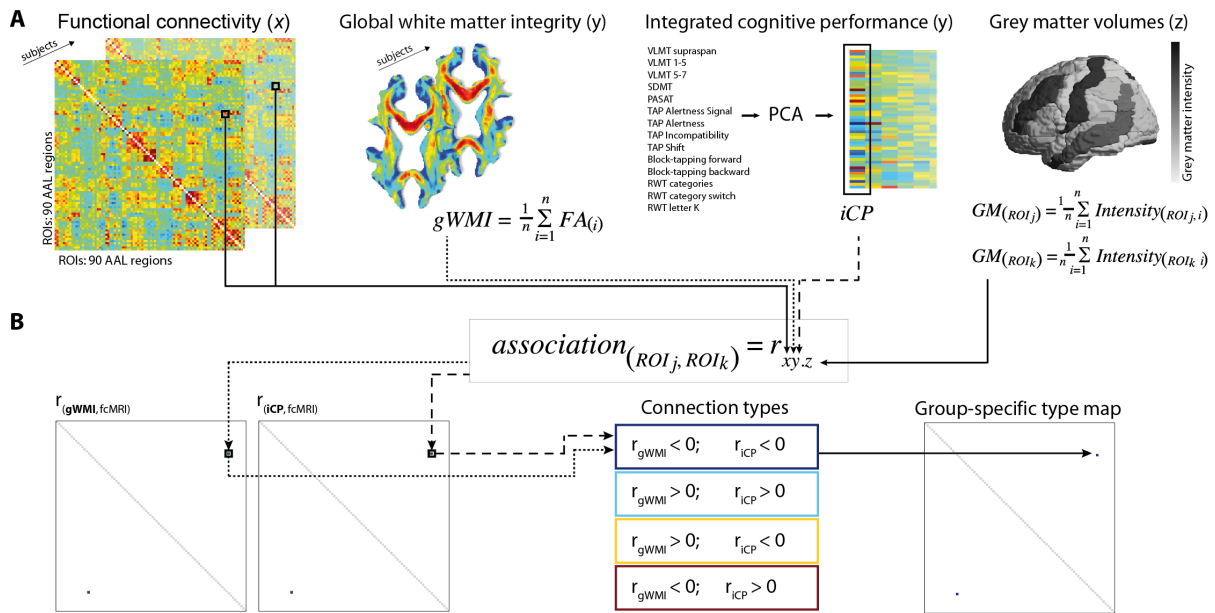
## 3.2. Material and methods

### 3.2.1. Participants

Detailed information on recruitment criteria for MS patients and healthy controls can be found in section 1.2 and section 2.2.1, part III. The sample contained 40 MS patients (25 female, 24-60 years) and 40 individually matched healthy controls (25 female, age 29-57 years; matched by sex, age, and years of education).

### 3.2.2. Computation of integrated cognitive performance score

To integrate the overall performance in the neuropsychological assessment, a PCA was computed on the z-transformed scores of all participants in fourteen neuropsychological tests (for details see section 1.4, part III) using SPSS 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Components were determined without additional rotation of the principal axes (e.g. varimax). Individual component scores, which constitute the representation of the input in principal component space, were calculated using a linear regression. The individual scores of the first component, which explains most of the variance among all components, were later used the main analysis as a measure for the integrated cognitive performance (iCP) (Fig. 9, A). The Kaiser-Meyer-Olkin measure (Kaiser, 1970) and the Bartlett's test of sphericity (Bartlett, 1937) were calculated to check the prerequisites for the PCA.



**Figure 9. – Computation of associations and type classifications.** **A** Input data. Pearson's correlations coefficients were calculated between 90 regions of interest (ROI) from the automated anatomical labeling (AAL) atlas as an estimate for functional connectivity. The global white matter integrity (gWMI) was defined as the individual average fractional anisotropy. The individual scores of the first component of a principal component analysis (PCA) on the total neuropsychological performance were used as measure for the integrated cognitive performance (iCP). Gray matter (GM) volumes of all 90 regions of interest were computed by averaging the intensities of all voxels of the structural MRI covered by an individual mask. **B** Computation of associations. Partial correlations with gray matter volumes as confounding variable were calculated to estimate the association between Fisher's z-transformed functional connectivity and either gWMI or iCP. Connections were grouped into four connection-types by their directions of associations, resulting in two group-specific type maps.  $r =$  partial correlation coefficient

### 3.2.3. Computation of global white matter integrity

Diffusion imaging measures were shown to be particularly sensitive to white matter disturbances even before lesions can be detected with conventional MRI methods (Ontaneda et al., 2014), and they are among the best predictors for cognitive disturbances (Daams et al., 2016). Furthermore, it is known that macroscopic functional interaction reflects a mixture of direct and indirect structural connectivity (He et al., 2007; Damoiseaux and Greicius, 2009; Honey et al., 2009). In order to capture the influence of WM impairment in both direct and indirect structural connections, a global metric for the integrity of the WM was preferred for this explorative analysis. The global white matter integrity (gWMI) was defined as the individual average FA value across the entire WM tissue.

DTI data was processed with the FMRIB Software Library (FSL, <http://fsl.fmrib.ox.ac.uk/fsl>) version 5.0.7. The preprocessing included the correction of head movements and eddy current distortions (reference volume = 0), brain extraction using BET2, and fitting of a diffusion tensor model using DTIFIT. The resulting FA images were spatially normalized to MNI space and averaged on the individual level across all voxels that were covered by the individual coregistered and spatially normalized WM mask (Fig. 9, A).

### 3.2.4. Computation of individual ROI-to-ROI fcMRI

Mean BOLD time-series were extracted and corrected for 90 cortical and subcortical ROIs of the AAL atlas (see appendix section B.2) with the help of the MATLAB-based CONN toolbox (v14b, <http://www.nitrc.org/projects/conn>). For details on the clean-up of time-series see section 2.2.4.1, part III. Pearson's correlation coefficients were computed pairwise between corrected BOLD time-series to construct individual whole-brain ROI-to-ROI fcMRI matrices. The correlation coefficients were Fisher's z-transformed before further use in the main analysis.

### 3.2.5. Computing and relating associations

For each functional ROI-to-ROI connection, two partial rank correlation coefficients were calculated to estimate connection-specific associations between the Fisher's z-transformed fcMRI and gWMI on one side, and iCP on the other. GM volumes of the corresponding ROIs were included as confounding variables to partial out the influence of cortical or subcortical atrophy (Fig. 9, B). Correlation coefficients were computed separately for controls and patients.

The global relationships of the resulting association maps were determined *within* and *between* groups, as well as *across* variables and groups by computing Pearson's correlations between the Fisher's z-transformed partial correlation coefficients. For the *within*-group relationship, gWMI-associations were correlated with iCP-associations of the same group. For the *between*-group relationship, gWMI-associations of the healthy group were correlated with the gWMI-associations of MS patients and the same for the iCP-associations. The global relationship *across* variables and groups was determined by relating gWMI-associations of the healthy group with iCP-associations of MS and vice versa.

To describe local correspondences, each functional connection was assigned to one of the following four connection-types depending on the directions of their two partial correlation coefficients (Fig. 9, B): connections that were negatively correlated with both gWMI and iCP (gWMI<sub>neg</sub>-iCP<sub>neg</sub>), connections with two positive partial correlation coefficients (gWMI<sub>pos</sub>-iCP<sub>pos</sub>), connections with a positive association with gWMI, but a negative one with iCP (gWMI<sub>pos</sub>-iCP<sub>neg</sub>), and the other way around (gWMI<sub>neg</sub>-iCP<sub>pos</sub>). The group difference in the frequencies of the four connection-types was tested for significance with a Chi square test (MATLAB 2013a function *crosstab*). The two groups were furthermore contrasted qualitatively to identify functional connections with identical types as well as prominent mismatch of the classification.

### 3.2.6. Exploration of reliability of associations

The classification of connection-types relied on the direction of the estimated associations only. An exploration of the reliability and relevance of those directions was therefore considered to be essential. In the present context, the direction of an association was defined as *reliable*, when the 99% asymptotic normal confidence interval of the partial correlation coefficient did not include zero (based on MATLAB 2013a function *corrcoef*). An association was defined as *relevant*, when its partial correlation was larger or equal  $r = 0.5$  ( $0.1 =$  small,  $0.3 =$  medium,  $0.5 =$  large, according to Cohen, 1988, 1992). Numbers and types of identified links that were reliably associated with gWMI, iCP, or both were determined for each group. Group differences in the distribution across connection-types were again tested using Chi square tests.

### 3.2.7. Post-hoc evaluation of characteristics of connection-types

Finally, following aspects of the connection-types were explored: First, the average absolute association strength with structure and function across all functional connections belonging to a type, and second the mean effect size with respect to the group difference in fcMRI. To test whether equally directed ( $\text{gWMI}_{\text{pos-iCP}_{\text{pos}}}$ ,  $\text{gWMI}_{\text{neg-iCP}_{\text{neg}}}$ ) and mixed types ( $\text{gWMI}_{\text{pos-iCP}_{\text{neg}}}$ ,  $\text{gWMI}_{\text{neg-iCP}_{\text{pos}}}$ ) have differential relevance for the association with either structural integrity or behavioral performance, or for the differentiation between groups, differences in association strength and effect size were tested for significance with unpaired t-test ( $\alpha = 0.01$ , uncorrected). Third, basic topological characteristics of each type of connections were determined with the help of the Brain Connectivity Toolbox (<http://www.brain-connectivity-toolbox.net/>), separately for patient and controls. As summarized in section 1.3.2, part I, graph theoretical metrics are a useful tool to describe the topological organization of a system. The aim of this sub-analysis was therefore to explore whether types would differ in such topological features, in particular metrics of integration and segregation. In the present application, each connection-type was treated as an independent system, hence equivalent to an individual person in a conventional graph theoretical analysis. The adjacency matrix of a type was a binary matrix, in which all other types were set to zero, resulting in four adjacency matrices per group. The extracted metrics were the characteristic path length, which is the average shortest path length in the network, the mean node degree, which is the average number of connected links to each node, and the maximized modularity score, which quantifies the degree, to which a network can be subdivided. Type and group differences were tested for significance separately for each metric using permutation tests ( $\alpha = 0.05$ , uncorrected, 2000 permutations). To assess the significance of type differences, the modalities were assigned randomly to each other. For the group contrast, only the group membership was permuted, whereas the individual matching between fcMRI, gWMI, and iCP was preserved.

## 3.3. Results

### 3.3.1. Sample

Detailed demographic, clinical, and neuropsychological characteristics can be found in section 2.3.1 and 2.3.2, part III.

### 3.3.2. Integrated cognitive performance

The first component of the PCA had an eigenvalue of 5.08 and explained 36.31% of the variance of the neuropsychological performance. Variables with high loadings on this component reflected memory domains and tests on information processing. Attention-related variables had the lowest loadings. The remaining thirteen components had eigenvalues between 1.89 and 0.09 and explained between 13.51%

and 0.67% of the variance. The prerequisites for a PCA were satisfied (Kaiser-Meyer-Olkin measure = 0.76, significant Bartlett's test of sphericity).

### 3.3.3. Global white matter integrity

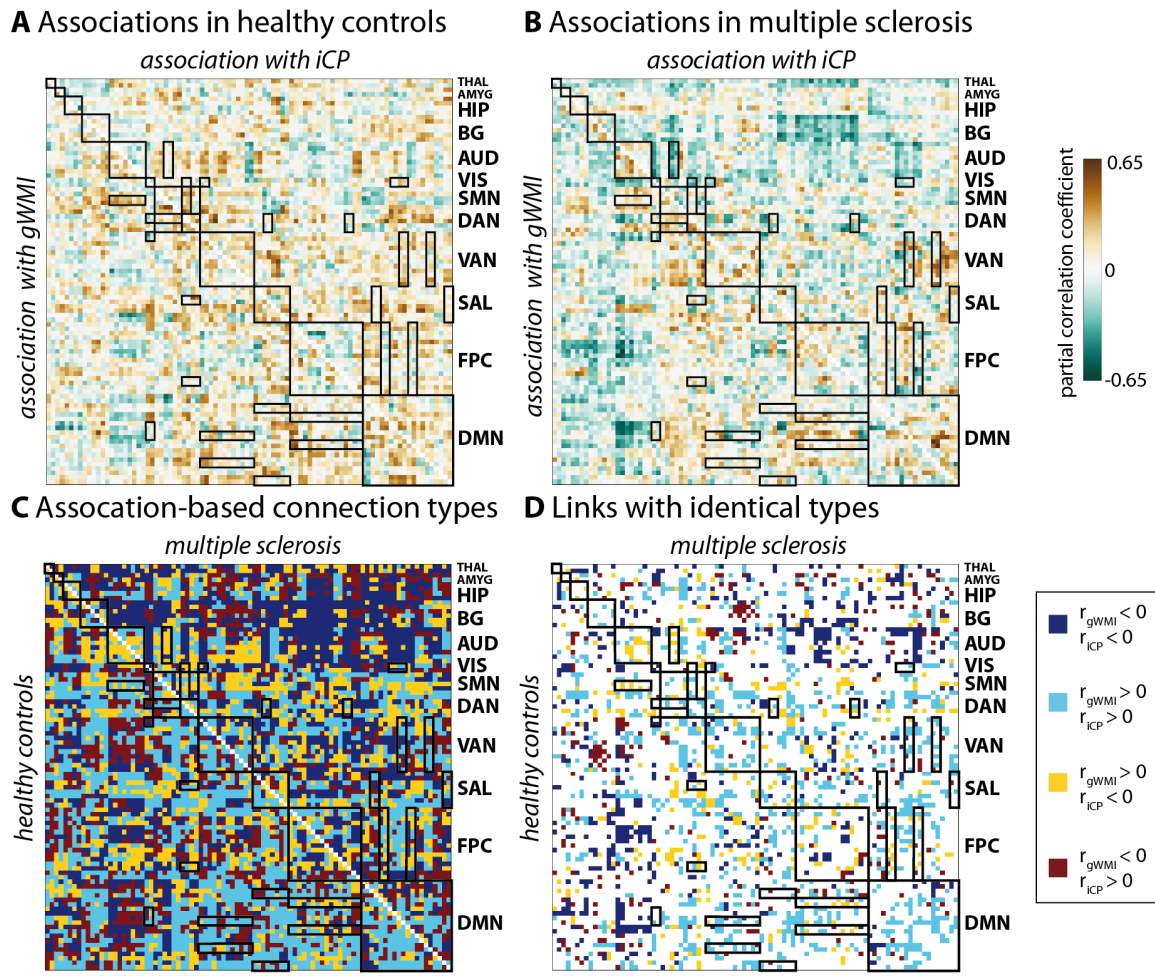
DTI data of one MS patient was missing due to technical reasons and was therefore replaced by the corresponding group mean. The mean gWMI was 0.41 (SD = 0.02) in healthy controls and 0.39 (SD = 0.03) in MS patients ( $t(39) = 2.92$ ,  $p < 0.001$ ). Lesion volume and gWMI correlated moderately ( $r = -0.44$ ,  $p < 0.01$ ) in healthy controls, and strongly in MS patients ( $r = -0.84$ ,  $p < 0.001$ ). The correlation between gWMI and iCP was weak in both controls ( $r = 0.16$ , n.s.) and MS ( $r = 0.29$ , n.s.).

### 3.3.4. Associations maps and their qualitative relationship

To account for movement artifacts, 104 of 246 functional volumes had to be discarded before the extraction of time-series for one healthy control. The resulting fcMRI matrix did not differ from others with respect to the range or distribution of fcMRI.

Partial correlation coefficients, which captured the relationship between fcMRI and gWMI, ranged between  $r = -0.50$  and  $r = 0.53$  in healthy controls and between  $r = -0.62$  and  $r = 0.58$  in MS. Associations with iCP were between  $r = -0.46$  and  $r = 0.57$ , and  $r = -0.54$  and  $r = 0.65$ , respectively. The whole-brain association maps of both groups are illustrated in Fig. 10, A and B. The global relationship between the two association maps was low in healthy controls ( $r = 0.28$ ) and slightly higher in MS ( $r = 0.37$ ). The cross-group-correlations between associations of the same kind ( $iCP_{HC}$ ,  $iCP_{MS}$ ;  $gWMI_{HC}$ ,  $gWMI_{MS}$ ), or the other association variable ( $iCP_{HC}$ ,  $gWMI_{MS}$ ;  $gWMI_{HC}$ ,  $iCP_{MS}$ ) were significantly weaker than both global interrelations within groups (all  $r < 0.2$ ,  $p_{\text{difference}} < 0.001$ ).

The classification of functional connections into four types of association patterns revealed that healthy controls and MS patients differed significantly in the distribution of the 4005 connections across the types ( $\chi_{(3)} = 414.67$   $p < 0.001$ ). In controls, 41.57% ( $n = 1665$ ) of all functional connections belonged to the  $gWMI_{\text{pos}}-iCP_{\text{pos}}$  type, followed by 21.45% ( $n = 859$ )  $gWMI_{\text{neg}}-iCP_{\text{pos}}$  connections, 19.10% ( $n = 765$ )  $gWMI_{\text{pos}}-iCP_{\text{neg}}$ , and finally 17.88% ( $n = 716$ )  $gWMI_{\text{neg}}-iCP_{\text{neg}}$  ones. In MS, on the other hand, the most frequent type was the  $gWMI_{\text{neg}}-iCP_{\text{neg}}$  one (35.71%,  $n = 1430$ ). The second most frequent type was the  $gWMI_{\text{pos}}-iCP_{\text{pos}}$  type (26.14%,  $n = 1047$ ), followed by  $gWMI_{\text{pos}}-iCP_{\text{neg}}$  (21.90%,  $n = 877$ ), and  $gWMI_{\text{neg}}-iCP_{\text{pos}}$  (16.25%,  $n = 651$ ). The group difference was most prominent in connections between subcortical and cortical regions and between the auditory and all other RSNs (Fig. 10, C). The classification matched between groups in 28.59% ( $n = 1145$ ) of all connections, with almost half of them ( $n = 518$ ) belonging to the  $gWMI_{\text{pos}}-iCP_{\text{pos}}$  type (Fig. 10, D).



**Figure 10. – Association and type matrices.** Associations between functional connectivity and the global white matter integrity (gWMI, lower triangle) as well as the integrated cognitive performance (iCP, upper triangle) in healthy controls (A) and MS patients (B). C Outcome of the classification into four connection-types based on the directions of association with gWMI and iCP for both groups. D Functional connections with identical classification outcome in controls and patients. *networks: DMN = default mode, FPC = fronto-parietal control, SAL = salience, V/DAN = ventral/dorsal attention, SMN = somatosensory, VIS = visual, AUD = auditory, BG = basal ganglia, HIP = hippocampus, THAL = thalamus, AMYG = amygdala;  $r$  = partial correlation coefficient*

### 3.3.5. Reliability of associations and resulting connection-types

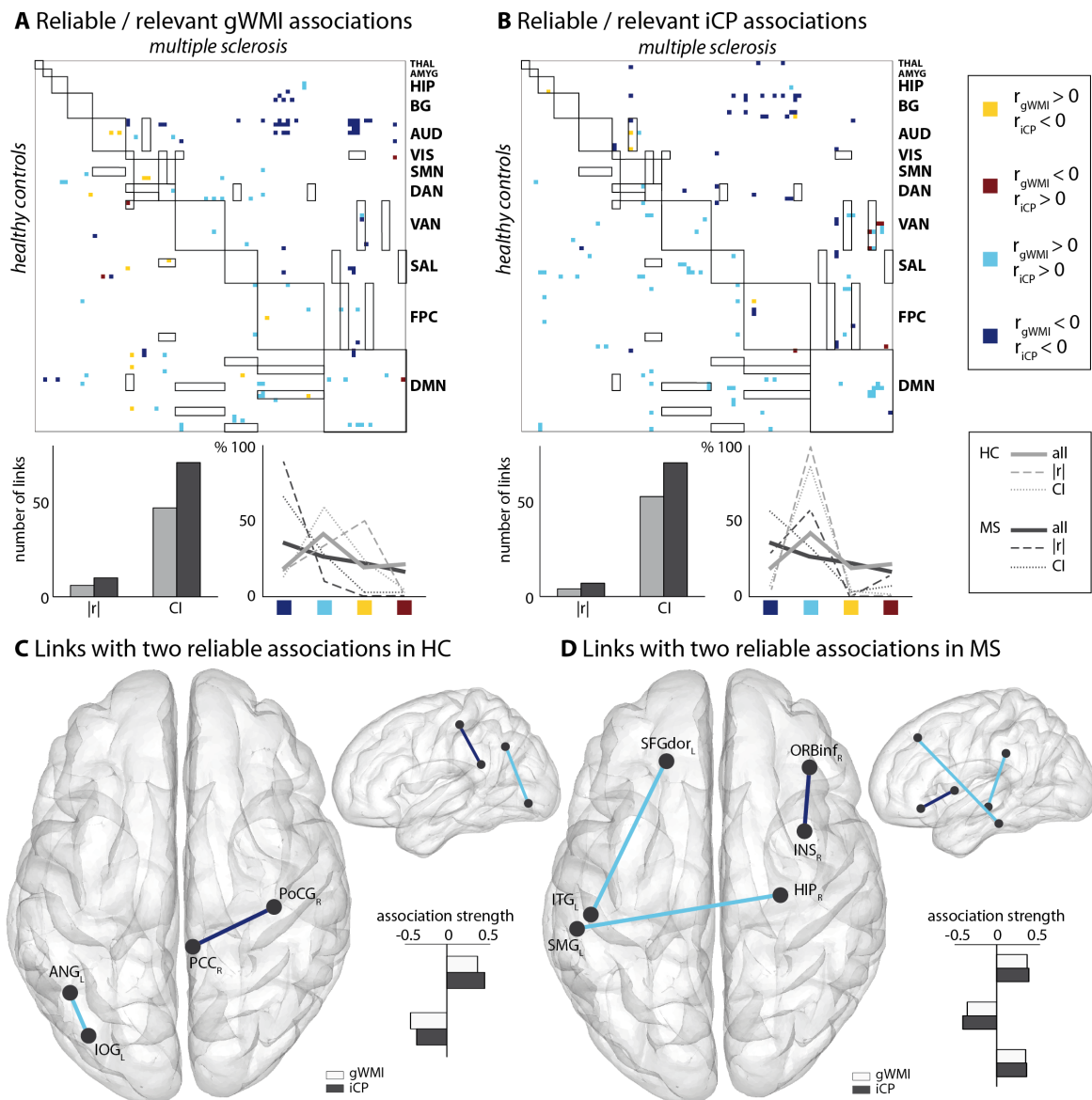
The evaluation of the reliability of the associations identified 47 functional connections in controls, which satisfied the *reliability* or *relevance* criteria for the association with gWMI (Fig. 11, A). In MS, the analysis yielded 71 links in total, with only ten of them being strongly related to gWMI ( $|r| > 0.5$ ). The distribution of the identified connections across types differed significantly between group ( $\chi_{(3)} = 35.88$   $p < 0.001$ ), with  $gWMI_{pos}$ - $iCP_{pos}$  connections being the prominent type in controls and  $gWMI_{neg}$ - $iCP_{neg}$  in MS.

53 *reliable* or *relevant* associations with iCP were identified in healthy controls and again 71 in MS (Fig. 11, B). Only four, respectively seven of those connections fulfilled the effect size criteria. 86.79% of the identified connections were  $gWMI_{pos}$ - $iCP_{pos}$  in controls, while the most frequent type in MS was again the  $gWMI_{neg}$ - $iCP_{neg}$  one ( $\chi_{(3)} = 38.18$   $p < 0.001$ ).

The majority of all functional connections with meaningful associations were located between classical RSNs in both groups. In MS, especially functional connections the basal ganglia, the auditory network, and both the fronto-parietal and the DMN were found to fit the criteria (Fig. 11, A, B). In healthy controls, no clear pattern emerged as judged by eyeballing.



Only two functional connections in controls, and three ones in MS fulfilled the reliability criterium (99% confidence interval does not include zero) for both gWMI and iCP (Fig. 11, C, D). No connection had partial correlation coefficients larger than  $|r| = 0.5$  with both metrics.



**Figure 11. – Reliable and relevant associations and their type classifications.** Location, total numbers, and type classification of functional connections that had reliable association with global white matter integrity (gWMI) (A), or the integrated cognitive performance (iCP) (B). Anatomical location and association strengths of functional connections that exhibited reliable relationships with both structural and behavioral metric in healthy controls (C), and patients (D).  $r$  = partial correlation coefficient;  $|r|$ : absolute partial correlation coefficient magnitude  $> 0.5$ ; CI: 99% confidence interval does not include  $r = 0$ ; networks: DMN = default mode, FPC = fronto-parietal control, SAL = salience, V/DAN = ventral/dorsal attention, SMN = somatosensory, VIS = visual, AUD = auditory, BG = basal ganglia, HIP = hippocampus, THAL = thalamus, AMYG = amygdala

### 3.3.6. Further post-hoc evaluation of connection-type characteristics

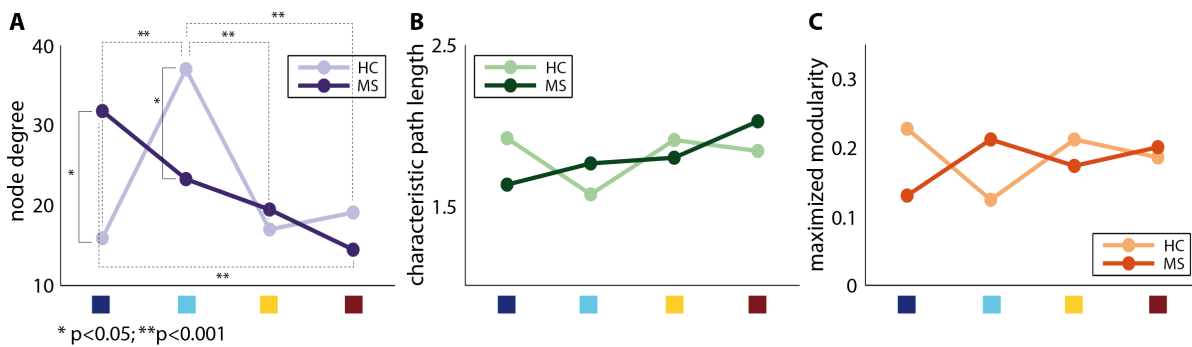
The average absolute association and Cohen’s d values were relatively small for both groups and all types (Tab. 10). Nonetheless, the difference between equally directed types and mixed ones was significant ( $p < 0.01$ ) in controls for the associations with gWMI and iCP, and for Cohen’s d, and in MS patients for both associations, but not for the effect size. In MS and controls, the associations with structure and behavior were stronger in the equally directed connection-types compared to the mixed types. Cohen’s d values in healthy controls were larger in mixed types. It must be emphasized, however, that actual differences were particularly small.

**Table 10. – Characteristics of connection-types (mean, SD of absolute values).**

	association with gWMI		association with iCP		Cohen’s d	
	HC	MS	HC	MS	HC	MS
gWMI <sub>pos</sub> -iCP <sub>pos</sub>	0.17 (0.11)	0.16 (0.11)	0.15 (0.11)	0.14 (0.11)	0.13 (0.10)	0.14 (0.10)
gWMI <sub>neg</sub> -iCP <sub>neg</sub>	0.13 (0.10)	0.16 (0.11)	0.10 (0.10)	0.16 (0.12)	0.13 (0.10)	0.13 (0.10)
gWMI <sub>pos</sub> -iCP <sub>neg</sub>	0.13 (0.10)	0.11 (0.10)	0.10 (0.10)	0.12 (0.10)	0.15 (0.11)	0.14 (0.11)
gWMI <sub>neg</sub> -iCP <sub>pos</sub>	0.10 (0.10)	0.11 (0.10)	0.12 (0.10)	0.11 (0.10)	0.14 (0.11)	0.13 (0.10)

*SD = standard deviation*

The analysis of graph theoretical features yielded significant differences between groups and types in the mean node degree only (Fig. 12, A). MS patients had a significantly higher mean density of connections in the gWMI<sub>neg</sub>-iCP<sub>neg</sub>-graph, and a lower node degree than controls for gWMI<sub>pos</sub>-iCP<sub>pos</sub> connections. The difference between the gWMI<sub>neg</sub>-iCP<sub>neg</sub> type (highest node degree) and the gWMI<sub>neg</sub>-iCP<sub>pos</sub> types (lowest node degree) in MS was significant. In controls, the high average node degree in gWMI<sub>pos</sub>-iCP<sub>pos</sub> connections differed significantly from all other connection-types. In correspondence with the pattern for the node degree, the characteristic path length (Fig. 12, B) was lowest for gWMI<sub>neg</sub>-iCP<sub>neg</sub> connections and highest for gWMI<sub>neg</sub>-iCP<sub>pos</sub> in MS, while gWMI<sub>pos</sub>-iCP<sub>pos</sub> exhibited the shortest links to each other in controls. Those differences were not significant. The pattern for the maximized modularity was similar with largest group differences in the first two types.



**Figure 12. – Graph theoretical description of connection-types.** Group and type differences in the average node degree (A), the characteristic path length (B), and the maximized modularity (C). Significant contrasts are highlighted with one asterisk for  $p < 0.05$  and two asterisks for  $p < 0.001$ . Blue: gWMI<sub>neg</sub>-iCP<sub>neg</sub>; turquoise: gWMI<sub>pos</sub>-iCP<sub>pos</sub>; yellow: gWMI<sub>pos</sub>-iCP<sub>neg</sub>; red: gWMI<sub>neg</sub>-iCP<sub>pos</sub>; HC = healthy controls; MS = multiple sclerosis patients

### 3.4. Discussion

The present analysis focused on the relationship between the expression of local fcMRI and both the underlying structural integrity and the cognitive performance in a sample of mildly affected MS patients and individually matched healthy controls. Functional connections were classified based on their associations with structure and behavior, and various features of the resulting connection-types were explored.

#### 3.4.1. Correlates of white matter impairment and cognitive disturbance in the functional connectome

To begin with, only few functional connections exhibited large associations ( $|r| > 0.5$ ) with either structure or behavior in patients or in controls. In addition, the whole-brain analysis did not yield clear evidence in favor of a protruding role of a single network for cognitive deficits, WM impairment, or the relationship between WM integrity and cognitive performance. Instead, the majority of associations that survived the reliability-test were found in inter-network connections and also group differences in associations were most prominent in connections between between subcortical and cortical networks. This finding contradicts other studies that suggest specific relationships between certain RSNs and cognitive disturbances in MS. However, many of those investigations had limited the scope of their correlational analyses a priori. These foci were usually based on previous findings in MS or other populations (e.g. Leavitt et al., 2014; Hulst et al., 2015), or derived from beforehand computed group comparisons in fcMRI (e.g. Schoonheim et al., 2014; Sbardella et al., 2015). Evidence for reliable interrelations from data-driven whole-brain explorations, in contrast, is limited (e.g. Hawellek et al., 2011). It should be considered that whole-brain analyses of ROI-based functional connectivity have to deal with a large number of statistical comparisons, not to mention voxel-wise approaches. MCC methods, such as Bonferroni or FDR, can be applied to determine p-thresholds that control for the overall  $\alpha$ -error probability so that statistically reliable conclusions can be drawn despite the large number of tests. With several hundreds to thousands comparisons, p-thresholds can become extremely low for whole-brain analyses, which holds the risk that effects are being underrated despite relevant effect sizes and meaningful ecological validity. This applies in particular to observational (patient) studies, where the amount of unexplained und uncontrollable variance is much higher than in experimental studies. As a consequence, reducing the number of contrasts can improve the chance for detecting those effects and is therefore preferable whenever possible. In the case of insufficient empirical evidence for a specific hypothesis, however, theory-driven foci can also result in misleading findings because they always display a limited perspective on the brain. Analytically derived constrains can be problematic as well. Group comparisons are most likely to become statistically significant when differences are large between groups but variability small within. Regions or functional connections with small variability within the group, in turn, might not be best suited to determine the relationship with widely distributed variables, for instance the cognitive disturbance in MS. Hypotheses on specific interrelations should be formulated carefully and underpinned with findings from unconstrained explorations of the entire functional connectome to prevent both false negative and false positive findings for this reason.

#### 3.4.2. Quantitative and qualitative similarity between association maps

The outcome of the analysis indicates that structural damage and behavioral symptoms exhibit diverging and moreover group-specific association patterns with local fcMRI. The overall relationship between whole-brain associations was small, the strongest associations did not match topographically, and no single link exhibited strong relationships with both structure and behavior ( $|r| > 0.5$ ). This divergence suggests that WM alterations and behavioral symptoms are mediated by a complex in-

terplay within the functional connectome, which cannot be explained by direct or local effects alone (Hoffmann et al., 2007).

The exploration of qualitative correspondence between local associations disclosed that almost two thirds of all functional connections correlated with both structural and behavioral metrics in the same direction, whereas one third exhibited bidirectional association patterns (that is positive correlation with gWMI and negative with iCP, or vice versa). A predominance of connections with equally directed associations was also found for functional connections with identical classifications in patients and in controls, and for links with reliable associations. In addition, equally directed connection-types were on average slightly stronger associated with structure and behavior compared to mixed types. These findings give reason to assume that the uniform translation from structure to function and onto behavior constitutes the main impact of WM alterations in both healthy subjects and MS. Anyway, a small portion of links with identical and reliable associations had still a mixed type, which suggests that bidirectional association patterns should not be ascribed solely to random variations and statistical uncertainty in the data. Instead, it can be speculated whether these association patterns reflect an unspecific effect with modulatory impact on the unidirectional translation.

While the ratio between uni- and bidirectional association patterns was comparable between patients and controls, the distribution across the four connection-types differed significantly. Generally speaking, a bias towards negative relationships with both structural and behavioral metrics was found in MS patients, whereas most functional connections in healthy subjects co-variated positively with the two variables. This pattern was reflected in characteristics describing topological features of the intra-type organization. In MS patients, functional connections that correlated negatively with both structure and behavior were integrated best and segregated the least in contrast to all other connection-types. In controls, the same applied to functional connections that were positively associated with those two variables. It must be noted, though, that the adjacency matrices obviously differed in their number of edges. Considering the dependence of graph theoretical metrics on the number of nodes and edges (van Wijk et al., 2010), these results must be understood as a different perspective on the distribution of functional connections across types, rather than an independent outcome.

### **3.4.3. Increasing functional connectivity in response to structural impairment and the concept of functional compensation**

The presented approach disclosed that fcMRI mainly decreased with lower structural integrity in healthy subjects, as one would expect intuitively, but decreased with more structural impairment in MS. Despite a growing number of studies providing evidence for enhanced fcMRI in MS and also other neurological diseases (Hillary et al., 2015), there is a lack of sufficient mechanistic models that explain how strengthening of functional integration can result from structural damage. The interpretation of such increase is unclear for this reason.

A possible explanation for the current study might be related to the divergent nature of WM alterations in those two groups. The relationship between gWMI and volume of WM lesions was low in healthy participants, supporting the assumption that WM alterations in healthy controls reflected mainly diffuse and mild degenerative processes (Bartzokis et al., 2003). In MS, on the other side, this correlation was found to be high, confirming that the dramatic decrease of global structural integrity is largely caused by focal damage. In the context of neurological patients, the term *disconnection* is often used, which suggests a complete breakdown of communication, even though such dramatic impact is rare. Instead, information flow in functional connections is rather decelerated and altered in its temporal and economic characteristics in response to dramatic structural disturbance (Hillary et al., 2015). It is reasonable to assume, that these alterations modulate the communication behavior of linked functional connections, assemblies, or even entire networks, resulting in a spread of the pathological effect beyond the structurally disturbed area (Fornito et al., 2015). On different time

scales, various forms of such secondary effects of primary structural pathology are conceivable, for example the increase of integration due to acute disinhibition (Gillebert and Mantini, 2013) or the initiation of plasticity processes that result in longstanding restructuring of interaction patterns (Fornito et al., 2015). Subtle WM alterations in the course of normal aging, on the other hand, might have less traumatic impact both primarily and secondarily. Previously described observations of enhanced fcMRI in the aging literature (e.g. Reuter-Lorenz and Cappell, 2008) might instead result from the accumulated impact of both GM and WM modifications.

Beyond that, it is of course also possible that dissimilar WM tracts are affected in MS and healthy aging. WM lesions in MS might be simply more likely to cause re-organization of large-scale functional architecture of the brain because they disturb the communication between hubs (Fornito et al., 2015), the so-called rich club organization (van den Heuvel and Sporns, 2013), central *connectors* (Gratton et al., 2012), or a central WM core (Irimia and Van Horn, 2014). Such re-organization probably has multifaceted effects, including increased functional interaction in primarily unaffected parts of the functional connectome.

Anyway, with the data at hand it remains open, how the increase of fcMRI is related exactly to the site and nature of the WM damage in MS. Subsequent analyses on this matter should integrate region-specific information on WM disturbance for in-depth explorations of possible primary and secondary effect mechanisms.

Cognitive performance was found to be related positively with fcMRI, which indicates a beneficial effect of higher fcMRI, as well as negatively, which leads to the opposite interpretation, in almost the same portion across the functional connectome. According to previous studies, most of the brain seems to be affected in MS (for an overview see Fig. 5, part II), and many functional links were found to be increased in one study and decreased in the other. Interpretations of enhanced functional integration range from compensatory mechanisms (Cruz Gómez et al., 2013; Wojtowicz et al., 2014) to maladaptive reorganization (Hulst et al., 2015; Finke et al., 2015; Schoonheim et al., 2015). Decrease of fcMRI was conceptualized as pathological correlate (Rocca et al., 2010, 2016), and the lack of increased fcMRI as a functional reserve of compensatory effects (Giorgio et al., 2015). Whereas at least some of the inconsistencies among those findings can be explained by divergent methodological approaches and metrics, the interpretative diversity clearly reflects a lack of a precise definition of the concept of compensation in MS, but also beyond. One reason for this shortcoming is the still limited understanding of the functional relevance of the observed alterations in spontaneous functional connectivity. The majority of the above mentioned studies have related task performance to features of functional interactions in resting state. In fact, the same approach has been chosen for the present investigation as well. The rationale for this relationship is provided by the observation of the resemblances between functional integration patterns in rest and in response to task and stimulation (Smith et al., 2009), which gave rise to the hypothesis that functional interactions at rest reflect at least to some extent ongoing rehearsal of the functional repertoire (Sporns, 2011). This is supported by findings of meaningful relationships between such functional interaction patterns in rest and behavioral variables in patients and healthy participants. But still, the concrete functional significance of specific alterations of functional communication in the absence of external input remains unresolved. Decrease, on one hand, has been suggested to reflect successful disengagement from previous action (Breckel et al., 2013), decreased arousal (Picchioni et al., 2013) but also structurally disturbed communication (Rocca et al., 2015). Strictly speaking, it could of course also indicate amplified anti-correlation. The validity of anti-correlations in fcMRI is still controversial, however (Murphy et al., 2009). Higher functional interaction levels, on the other side, have been related to decreased deactivation (Eryilmaz et al., 2014) as well as efficient preparation for the execution of upcoming tasks (Schultz and Cole, 2016). Going beyond that static perspective, one might take into account that the ability to (re-)act flexibly by switching between states (e.g. from rest to task) might also be influenced by specific levels of functional communication at different points in time (Schultz and Cole, 2016). Beyond that,

specific alterations of fcMRI have most likely differential indications for different behavioral variables. To give an example, enhanced functional integration might be beneficial for cognitive processing, but requires a higher demand of resources (Hillary et al., 2015), which in turn was shown to be related to higher severity of fatigue (Pravata et al., 2016). Drawing conclusions about compensational effects based on the alteration of functional connectivity alone, is therefore clearly insufficient.

A comprehensive concept of functional compensation should instead take into account the embedding of the altered link into the system, and further characteristics, such as anatomical locations, involvement in specific functional networks, and both state- and trait-related variables.

Taken together, the presented analytical approach combines information about structure, function, and behavior for each functional connection in a whole-brain framework. The findings indicate a distinction between primary or direct, and secondary or indirect effects and a complex spatially extended interplay of their adverse and modulatory impacts (Hoffmann et al., 2007). This conceptualization explains and integrates previous contradicting findings of fcMRI alterations in MS, and might also have explanatory power for the weak relationship between structural and behavioral characteristics in MS and the enormous clinical inter-individual variability. The validation of the derived assumptions on the micro-, meso-, and macro level is necessary. Furthermore, several difficulties for the interpretation of altered fcMRI from so-called resting state investigations are pointed out that challenge the conclusion of previous studies on compensational mechanisms in MS and beyond.

#### 3.4.4. Limitations

This analytical approach is not without limitations. Instead of incorporating tract-specific FA values, an estimation of the global structural integrity was used. This was done to reduce the complexity of the explorative multi-modal approach and to capture the impact of WM lesions on both direct and indirect structural connectivity (He et al., 2007; Damoiseaux and Greicius, 2009; Honey et al., 2009). A comparison with complementary analyses would be interesting to shed light on the involvement of direct and indirect structural connections in compensational mechanisms in MS.

The metric for iCP explained less than 40% of the variance of the overall performance. Other variables, such as the intelligence quotient, might have been better suited to capture the overall cognitive capability.

FcMRI was computed between ROIs instead of voxel-wise to facilitate the comparison of association maps and to reduce the computational demand. It must be noted that relevant functional interrelations with subregions of AAL ROIs might have been obscured for this reason. Moreover, RSNs were defined theory-based instead of analytically. The explanatory power for classical RSNs is therefore limited.

The overall analytical approach did not aim specifically for the identification of significant correlates of cognitive functioning within each group, but for a truly unconstrained exploration of whole-brain patterns of interrelations. No explicit MCC was applied, but it should be noted that with minimal p-values of  $p_{\text{HC}} = 0.0007$  and  $p_{\text{MS}} = 0.00003$  for reliable associations with gWMI, and  $p_{\text{HC}} = 0.0008$  and  $p_{\text{MS}} = 0.003$  for reliable associations with iCP it is unlikely that any association would have survived proper MCC due to the large number of statistical tests (4005 per group and variable, 16020 in total). Subsequent analyses should test hypotheses that can be derived from the presented findings in independent samples to evaluate their significance for this research question. Furthermore, the application of multivariate analytical methods would be desirable to identify interdependencies within the patterns.

Finally, the simple classification of connections was purely qualitative and should be validated with data-driven quantitative methods.

## 4. Analysis 3: Alterations in metrics capturing dynamics of functional connectivity

### 4.1. Introduction

Conventional analyses of fcMRI estimate statistical dependencies between time series across scanning periods of typically about five to fifteen minutes. This approach provides a static perspective on brain functioning only (Hutchison et al., 2013; Hindriks et al., 2016). Nevertheless, the analysis of *static functional connectivity* has been shown to be most useful across a wide range of research questions, although its limitations became quickly apparent as well (Kopell et al., 2014). Real life functioning, including perception, interpretation, integration, and reaction, is obviously a dynamic process, which requires flexible switching between various functional constellations in the brain. Alterations in cognition, emotions, and behavior are therefore likely reflected in temporal characteristics of functional integration. To complement findings on spatial and organizational levels with investigations on dynamic features of functional connectivity has become an uprising research question in basic and applied neuroscientific fields for this reason (Calhoun et al., 2014). To investigate moment-to-moment changes, fMRI measurements are admittedly not the modality of first choice due to their known poor temporal resolution. Nonetheless, evidence has been provided for meaningful variations of spontaneous fcMRI on much shorter time-scales (e.g. Chang and Glover, 2010; Allen et al., 2014). The functional relevance and validity of such fluctuations in resting state fcMRI is a matter of ongoing debate, however. Matter et al. (2016) summarize that dynamics of integration patterns have been linked to flexible switching between tasks (Cole et al., 2013), working memory performance (Braun et al., 2015), learning, and emotions (Betz et al., 2016). Beyond, first studies on *dynamic functional connectivity* in schizophrenia (Sakoglu et al., 2010; Du et al., 2016), depression (Demirtas et al., 2016), autism and attention deficit hyperactivity disorder (Zhang et al., 2016), or Alzheimer's disease (Jones et al., 2012) indicate a role in pathological mechanisms in both psychiatric and neurological diseases. Despite a growing body of literature on *static functional connectivity* in MS, only very little is known about the impact on dynamic features of fcMRI in this disorder and in consequence about the effect of primary WM damage on *dynamic functional connectivity*. To my knowledge, only one study has been published on dynamic features of resting state fcMRI in a sample of MS patients so far (Leonardi et al., 2013). However, the focus of this study was the methodological approach that was introduced with this paper. The explanatory power for pathological processes in MS is therefore limited.

The aim of the present analysis was therefore to extend the network perspective on MS by exploring the variability of functional connectivity based on fMRI (vfcMRI) over time within a whole-brain framework, and to relate this metric of *dynamic functional connectivity* to *static functional connectivity* and a range of clinical and neuropsychological variables.

## 4.2. Material and methods

### 4.2.1. Participants

Detailed information on recruitment criteria for MS patients and healthy controls can be found in section 1.2 and section 2.2.1, part III. The sample contained 40 MS patients (25 female, 24-60 years) and 40 individually matched healthy controls (25 female, age 29-57 years; matched by sex, age, and years of education).

### 4.2.2. Functional connectivity

#### 4.2.2.1. Computation of individual fcMRI and vfcMRI matrices

BOLD time-series for 78 ROIs of the Stanford atlas ([http://findlab.stanford.edu/functional\\_ROIs.html](http://findlab.stanford.edu/functional_ROIs.html); see appendix section B.3) were extracted using the CONN toolbox (v14b, <http://www.nitrc.org/projects/conn>). This parcellation scheme has been determined functionally in contrast to anatomically defined atlases such as the AAL atlas or the brodmann areas. It has been demonstrated to be superior in classifying states of brain functioning (Shirer et al., 2012) and might therefore be beneficial in capturing fluctuations of fcMRI, which are considered to be related to temporal dynamics of functional interaction states (Leonardi et al., 2013; Tagliazucchi et al., 2014). Time-series represented the average BOLD signal of all voxels covered by both ROI and individual GM masks over time. For details on the clean-up of time-series see section 2.2.4.1, part III. Conventional, or static fcMRI was estimated for each functional connection by computing pairwise Pearson's correlation coefficients between the time-series of all 78 ROIs (Fig. 13, A). To determine the variability of fcMRI over time (vfcMRI), a sliding-window approach was applied. Pearson's correlation coefficient are hereby computed for each of a certain number of time windows. The variability is then defined as the standard deviation across all Fisher's z-transformed window-specific fcMRI estimates (Fig. 13 B). In the present study, the window length was chosen to be 20 volumes (50sec) and the overlap to be 19 volumes (47.5sec) resulting in 226 individual windows. To reduce the influence of outlying data points at the end and the beginning of each time window, sliding windows were convolved with a Gaussian kernel ( $\sigma = 3$  volumes; tapered approach according to Allen et al., 2014). In the last step, both fcMRI and vfcMRI were averaged across 55 intra- and inter-network partitions of the functional connectome (Fig. 13, D) in order to reduce the number of group comparisons and thereby increase the statistical power.

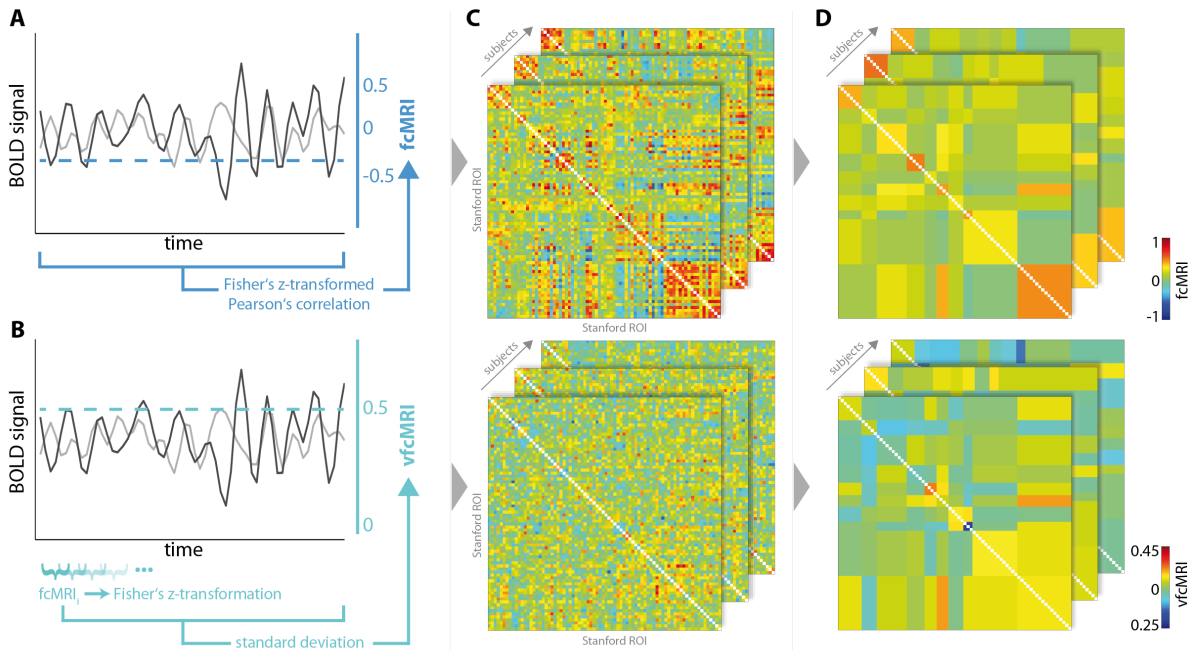
#### 4.2.2.2. Second level analysis of group differences

Group differences in fcMRI and vfcMRI were tested for significance with permutation tests for paired samples, that means that matching was preserved and only group membership assigned randomly ( $n_{\text{permutations}} = 50000$ ). Results were FDR-corrected (Yekutieli and Benjamini, 1999) separately for fcMRI and vfcMRI (significant at  $p < \text{FDR-threshold}$  with  $q = 0.15$ , two-sided). To estimate the effect size of the differences between MS patients and healthy controls, Cohen's d values for paired samples were calculated for each of the 55 intra- and inter-network partitions.

#### 4.2.3. Post-hoc analysis 1: Group differences in vfcMRI on connection level

In order to inquire further into the beforehand yielded results, group differences in vfcMRI were explored on the connection level for all partitions with significant group effect using Wilcoxon's signed rank tests (MATLAB 2013a function *signrank*, Gibbons, 1974). Group differences were significant at  $p < \text{FDR-threshold}$  with  $q = 0.15$  (one-sided for  $\text{vfcMRI}_{\text{MS}} > \text{vfcMRI}_{\text{HC}}$ , Yekutieli and Benjamini, 1999).





**Figure 13. – Computation of static and dynamic functional connectivity.** **A** Conventional static functional connectivity (fcMRI) was estimated with Pearson’s correlations, taking into account the entire time-series. **B** The variability of fcMRI over time (vfcMRI) was defined as the standard deviation across Pearson’s correlation coefficients for 226 overlapping windows. **C** Individual fcMRI and vfcMRI region of interest (ROI)-to-ROI matrices. **D** Individual fcMRI and vfcMRI mean values for 55 intra- and inter-network partitions of the functional connectome. For illustration purposes, the averaged values are plotted to match the relative size of the partitions in the original whole-brain matrix.

#### 4.2.4. Post-hoc analysis 2: Functional relevance of vfcMRI

To explore the functional relevance of vfcMRI, partial correlation coefficients were computed between the global vfcMRI, which is the individual mean vfcMRI value, and clinical as well as neuropsychological scores (for details see section 1.4, part III; Fig. 15). Age and global fcMRI were included as confounding variables to assess the relationship independent of contributions of conventional fcMRI and possible age effects on dynamics of fcMRI. The polarity of the following scores was reversed to simplify the interpretation of the partial correlation coefficients (positive: higher vfcMRI - better performance, negative: higher vfcMRI - worse performance; 0.1 = small, 0.3 = medium, 0.5 = large, according to Cohen, 1988, 1992): EDSS, FSMC motor, FSMC Cognition, HADS-A, HADS-D, VLMT 5-7, TAP Alertness without signal and with signal, TAP Covert shift of attention, and TAP Incompatibility. The partial correlation coefficients were calculated separately for patients and controls and tested for significant group differences ( $p < 0.05$ , two-sided, Equation 4.1) as

$$2 \cdot (1 - \text{normcdf}(\left| \frac{(zr_{hc} - zr_{ms})}{\sqrt{\frac{1}{n_{hc}-3} + \frac{1}{n_{ms}-3}}} \right|, 0, 1)) = p, \quad (4.1)$$

with *normcdf* being the normal cumulative distribution function and

$zr_x$  being the Fisher’s z transformed correlation coefficients.

For MS patients only, the relationship between vfcMRI of each of the partitions with significant vfcMRI group effect and the behavioral variables was computed in the same manner. Confounding variables were again the age and the average fcMRI of the corresponding partition. P-values of partial correlations were determined using a Student’s t distribution with the help of the MATLAB 2013a built-in function *partialcorr* (significant at  $p < 0.05$ ). No multiple comparison correction was applied to account for the exploratory nature of this analysis.

### 4.2.5. Post-hoc analysis 3: Validity tests

#### 4.2.5.1. Data quality

The assessment of fcMRI is sensitive for the impact of confounding factors that affect the brain globally, such as movement and technical noise. They introduce whole-brain artifacts that hold the risk of distorting the estimation of intrinsic correlation patterns. Temporal fluctuations of such noise sources are likely to influence metrics that capture dynamic features of fcMRI and group differences in these metrics can mirror distinct data quality for this reason. To rule out systematic imbalance of the data quality in the present study, group differences in movement parameters (for details see section 1.5.2, part III) and overall scanner noise were tested for significance using t-tests for paired samples (MATLAB 2013a function *ttest*; significant at  $p < 0.05$ ).

With respect to the first, differences in the maximum and average translation in three directions (left-right, front-back, up-down) and three kinds of rotation (pitch, roll, yaw) were tested.

For the latter, two volumes of interest ( $n_{\text{voxel}} = 1331$ ) were defined outside of the head, for which time-series were extracted. Group differences in the average scanner noise level were tested as well as separately for each TR (i.e. each volume). In addition, corrected time-series of all ROIs were correlated with the scanner noise time-series for each participant and group differences in the average individual relationship tested for significance.

#### 4.2.5.2. Influence of time window length and overlap

The complete data set was re-analyzed with a series of combinations of various window lengths and overlaps to evaluate the dependence of the results on these parameters (Tab. 11). For each combination, global vfcMRI and group contrasts in the partitions of the functional connectome were calculated as described above. The z-values, which resulted from the permutation tests on group differences in divergently derived vfcMRI, were compared to each other and to the average test statistic on differences between random subgroups of healthy controls ( $n_{\text{repetition}} = 1000$ ; MATLAB 2013a function *signrank*). The latter was taken as a proxy for the empirical false-positive probability, since no group differences were expected.

Next, the global relationship between divergently derived vfcMRI values was determined, as well as the correlation with the average fcMRI across time windows, and the conventional fcMRI. To this end, correlation coefficients were computed between the corresponding values of all participants and partitions, that means between two data-series with 4400 data points ( $n = 80 \text{ participants} * 55 \text{ partitions} = 4400$ ).

Last, the starting volume was shifted for the three combinations with the least overlap (v4, v5, v6; see Tab. 11) and group differences computed using Wilcoxon's signed rank tests for each partition and version. Partition-specific differences between the test statistics of the shifted and non-shifted versions were averaged to evaluate the influence of the actual segmentation of the time-series on the statistical outcome.

Table 11. – Window lengths and overlaps.

	start	length	step	overlap	$n_{tw}$
	vol	vol/sec	vol/sec	(%/vol/sec)	-
v1	1	20 / 50	1 / 2.5	95% / 19 / 47.5	226
v2	1	20 / 50	3 / 7.5	85% / 17 / 42.5	76
v3	1	20 / 50	5 / 12.5	75% / 15 / 37.5	46
v4	1	20 / 50	7 / 17.5	65% / 13 / 32.5	33
v5	1	20 / 50	9 / 22.5	55% / 11 / 27.5	26
v6	1	20 / 50	10 / 25	50% / 10 / 25	23
v7	1	30 / 75	1 / 2.5	96.7% / 29 / 72.5	216
v8	1	40 / 100	1 / 2.5	97.5% / 39 / 97.5	206
v9	1	50 / 125	1 / 2.5	98% / 49 / 122.5	196
v10	1	60 / 150	1 / 2.5	98.3% / 59 / 147.5	186
v11	1	70 / 245	1 / 2.5	98.6% / 69 / 172.5	176
evaluation of shift effect					
v4	2, 3, 4, 5, 6	20 / 50	7 / 17.5	65% / 13 / 32.5	33
v5	2, 3, 4, 5, 6	20 / 50	9 / 22.5	55% / 11 / 27.5	26
v6	2, 3, 4, 5, 6	20 / 50	10 / 25	50% / 10 / 25	23

*vol = volume; tw = time window*

## 4.3. Results

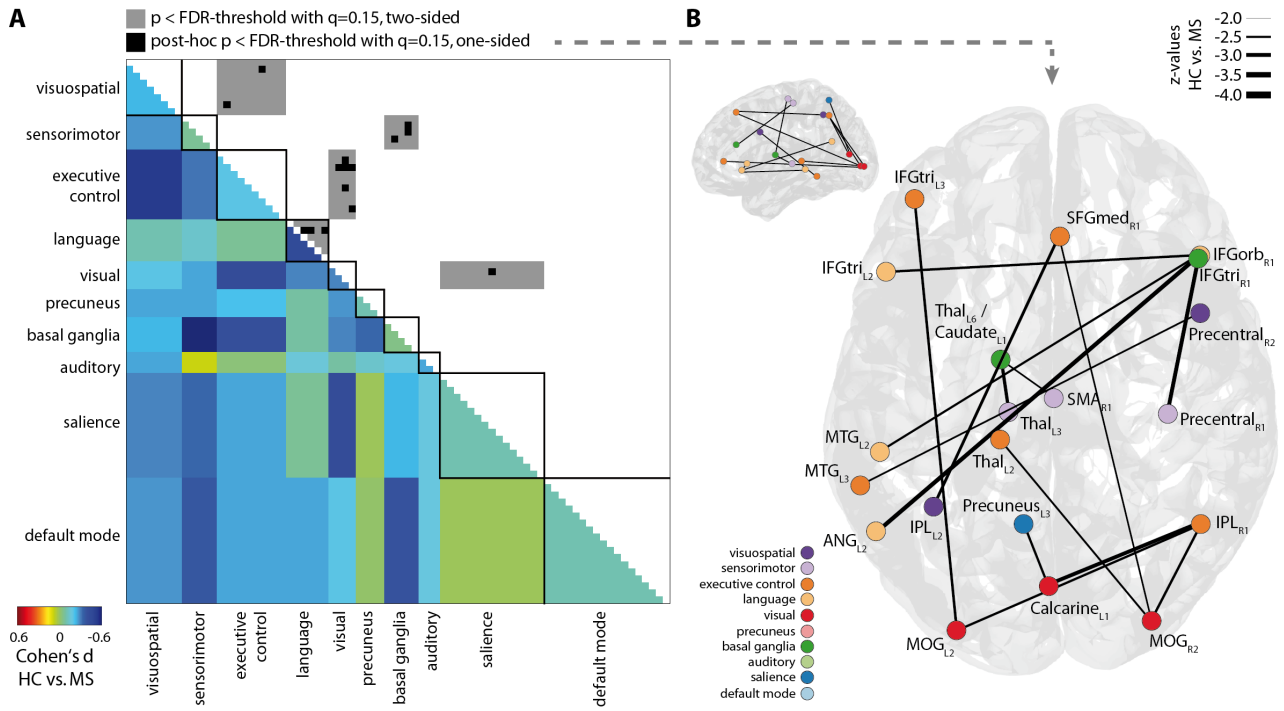
### 4.3.1. Sample

Detailed demographic, clinical, and neuropsychological characteristics can be found in section 2.3.1, and 2.3.2, part III and illustrated in Fig. 15, A.

### 4.3.2. Group differences in fcMRI and vfcMRI

Volume-to-volume movement exceeded the criteria (translation  $> 2\text{mm}$ , rotation  $> 2.5^\circ$ ) in two healthy controls and no patients. Due to excessive sharp movement, 104 of 246 functional volumes had to be discarded in one of these subjects (HC11) after careful inspection of the images and the course of the realignment parameters. The segmentation in time windows for this subject resulted in a reduced number of windows for this reason (122 in contrast to 226 for the main analysis). No volumes were excluded in the other healthy control (HC34), because the movement was uniform and caused no visual artifacts in the functional images.

The analysis disclosed significant differences ( $p < \text{FDR-threshold}$  with  $q = 0.15$ ) in the level of vfcMRI between MS patients and controls in one intra-network partition, namely the language network ( $z = -2.60$ ;  $p < 0.01$ ; Cohen's  $d = -0.45$ ; Fig. 14, A). Moreover, there were significant effects in four inter-network partitions between ROIs of the visuospatial and the executive control network ( $z = -3.03$ ;  $p < 0.01$ ; Cohen's  $d = -0.54$ ), the sensorimotor and the basal ganglia network ( $z = -3.40$ ;  $p < 0.001$ ; Cohen's  $d = -0.63$ ), the executive control and the visual network ( $z = -2.53$ ;  $p = 0.01$ ; Cohen's  $d = -0.43$ ), and the visual and the salience network ( $z = -2.46$ ;  $p = 0.01$ ; Cohen's  $d = -0.42$ ). MS patients had higher vfcMRI values than healthy controls in all five partitions. The post-hoc test revealed greater vfcMRI in the MS group in fifteen functional connections (Tab. 12, Fig. 14, B). Across all participants, the averaged vfcMRI was slightly higher in intra-networks partitions compared to inter-network ones ( $t_{(79)} = -2.43$ ,  $p = 0.02$ ). The same difference was found for MS patients alone ( $t_{(39)} = -2.43$ ,  $p = 0.02$ ), while inter- and intra-variability did not differ in healthy participant ( $t_{(39)} = -1.07$ ,  $p = 0.29$ ). No significant group differences were found with the same FDR-threshold in partition-specific fcMRI ( $p_{\min} = 0.02$ ; Cohen's  $d_{\max} = 0.35$ ).



**Figure 14. – Group differences in the variability of functional connectivity.** **A** Lower triangle: Cohen’s d for the difference between multiple sclerosis patients (MS) and controls (HC) in the variability of functional connectivity (vfcMRI) for all 55 partitions of the functional connectome. Upper triangle: significant group effects on partition (gray,  $p < \text{FDR-threshold with } q = 0.15, \text{ two-sided}$ ) and connection level (post-hoc test; black,  $p < \text{FDR-threshold with } q = 0.15, \text{ one-sided}$ ). For illustration purposes, the averaged values are plotted to match the relative size of the partitions in the original whole-brain matrix. **B** Functional connections with significantly higher vfcMRI in MS according to the post-hoc test plotted on the brain. *Line width = magnitude of z-values, node color = assignment to resting state networks, FDR = false discovery rate; for information on regions of interest see see appendix section B.3*

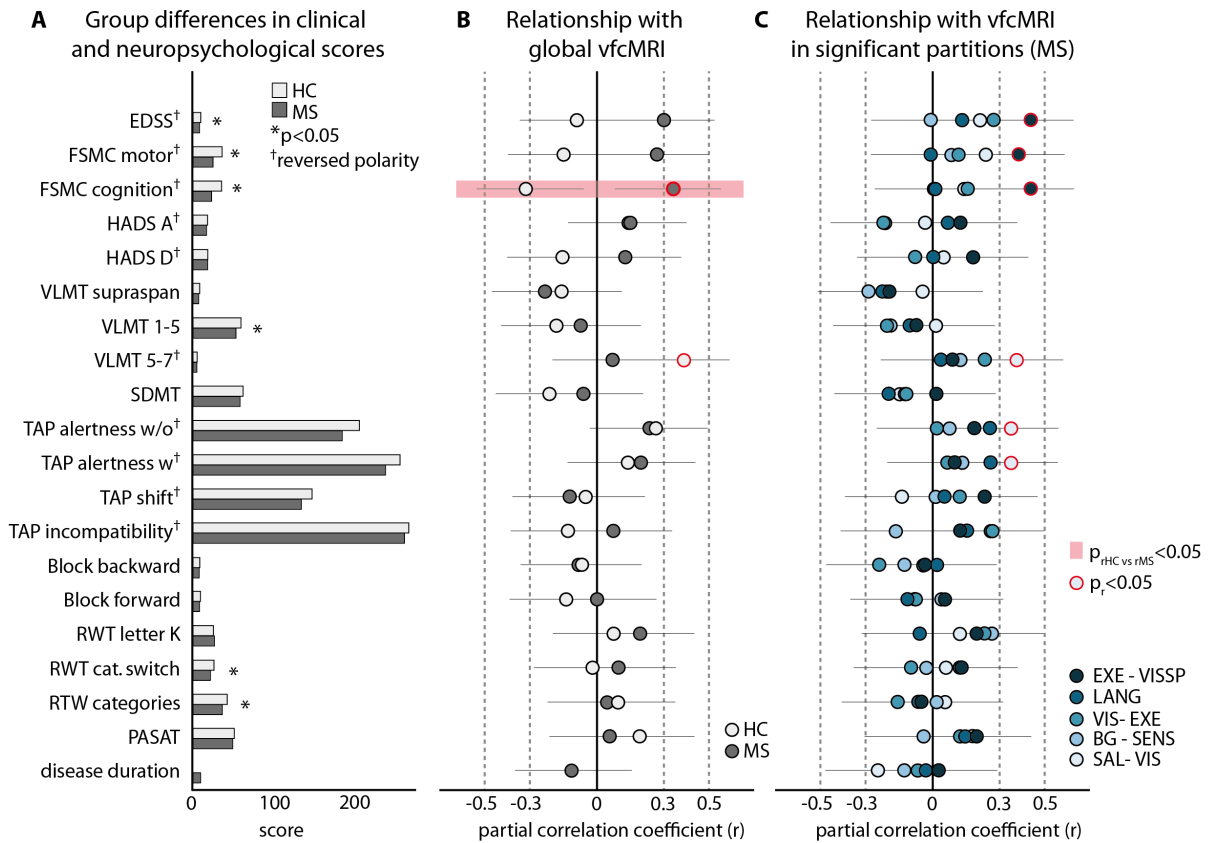
**Table 12. – Post-hoc test on group differences in the variability of functional connectivity on connection level.**

ROI 1	RSN	ROI 2	RSN	z	p	Cohen’s d
Precuneus <sub>L3</sub>	salience	Calcarine <sub>L1</sub>	visual	-2.56	0.005	-0.42
IFGtri <sub>L2</sub>	language	IFGorb <sub>R1</sub>	language	-2.58	0.005	-0.45
MTG <sub>L2</sub>	language	IFGorb <sub>R1</sub>	language	-2.51	0.006	-0.45
ANG <sub>L2</sub>	language	IFGorb <sub>R1</sub>	language	-3.14	< 0.001	-0.45
MOG <sub>L2</sub>	visual	IFGtri <sub>L3</sub>	executive	-2.63	0.004	-0.43
MOG <sub>R2</sub>	visual	Thal <sub>L2</sub>	executive	-2.45	0.007	-0.43
MOG <sub>L2</sub>	visual	IPL <sub>R1</sub>	executive	-2.56	0.005	-0.43
MOG <sub>R2</sub>	visual	IPL <sub>R1</sub>	executive	-2.59	0.005	-0.43
Calcarine <sub>L1</sub>	visual	IPL <sub>R1</sub>	executive	-3.00	0.001	-0.43
MOG <sub>R2</sub>	visual	SFGmed <sub>R1</sub>	executive	-2.37	0.009	-0.43
IFGtri <sub>R1</sub>	basal ganglia	Precentral <sub>R1</sub>	sensorimotor	-3.03	0.001	-0.63
Thal <sub>L6</sub>	basal ganglia	SMA <sub>R1</sub>	sensorimotor	-2.37	0.009	-0.63
Thal <sub>L6</sub>	basal ganglia	Thal <sub>L3</sub>	sensorimotor	-2.86	0.002	-0.63
SFGmed <sub>R1</sub>	executive	IPL <sub>L2</sub>	visuospatial	-2.74	0.003	-0.54
MTG <sub>L3</sub>	executive	Precentral <sub>R2</sub>	visuospatial	-2.41	0.008	-0.54

*ROI = region of interest; RSN = resting state network*

### 4.3.3. Post-hoc analysis 2: Relationship with neuropsychological performance and clinical status

The exploration of associations between the global vfcMRI and behavioral data revealed a significant positive relationship with FSMC cognition scores in MS ( $r = 0.34$ ,  $p = 0.04$ ). The partial correlation coefficient was significantly different ( $z = -2.92$ ,  $p < 0.01$ ; Fig. 15, B) from the one in the control group ( $r = -0.32$ ,  $p = 0.05$ ). In healthy controls, global vfcMRI correlated significantly with VLMT 5-7 scores ( $r = 0.39$ ,  $p = 0.02$ ) but not significantly stronger than in MS. The second part of the analysis disclosed significant positive associations in MS between vfcMRI in functional connections linking the executive control and the visuospatial network, and three clinical variables (Fig. 15, C): EDSS ( $r = 0.43$ ,  $p < 0.01$ ), FSMC cognition ( $r = 0.44$ ,  $p < 0.01$ ), and FSMC motor ( $r = 0.38$ ,  $p = 0.02$ ). The variability of fcMRI in connections between the salience and visual network correlated significantly with the VLMT 5-7 ( $r = 0.37$ ,  $p = 0.02$ ) and both the TAP alertness with ( $r = 0.35$ ,  $p = 0.03$ ) and without signal ( $r = 0.35$ ,  $p = 0.03$ ).



**Figure 15. – Post-hoc 2: Relationship with behavioral data.** **A** Overview over neuropsychological performance scores and clinical status of multiple sclerosis patients (MS, dark gray) and controls (HC, light gray). Significant group differences are highlighted with an asterisk. Note that the polarity of scores that are marked with a cross was reversed so that higher values always indicate better performance or status. **B** Partial correlation coefficients and 95% confidence intervals for the relationship with global variability of functional connectivity (vfcMRI) in MS (dark gray) and controls (light gray). Significant associations are emphasized with a red edge, significant group differences with a red bar. **C** Relationship with vfcMRI of the five partitions with significant group effect for MS only. *networks: EXE = executive control, VISSP = visuospatial, LANG = language, VIS = visual, BG = basal ganglia, SENS = sensorimotor, SAL = salience*

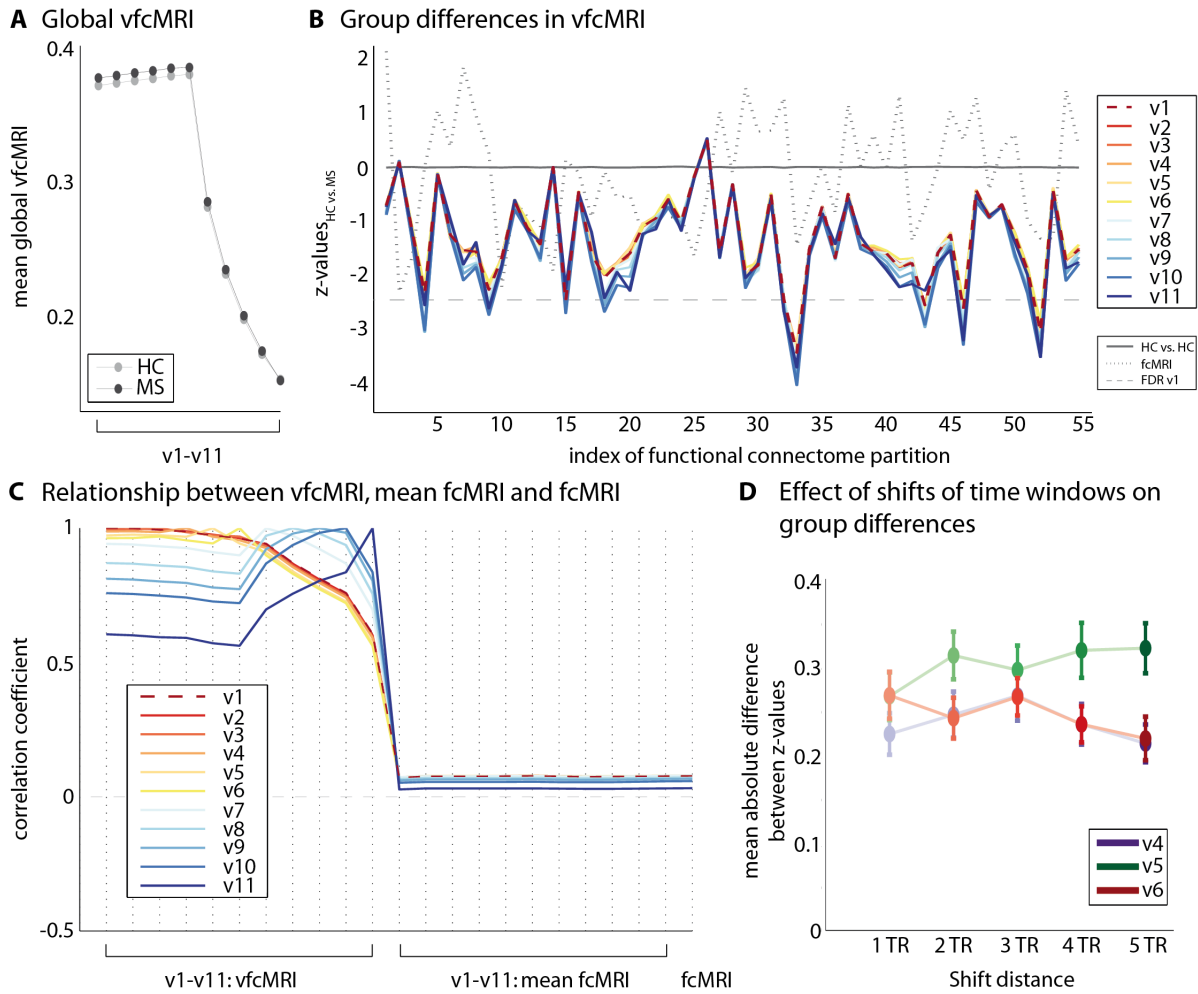
#### 4.3.4. Post-hoc analysis 3: Validity tests

##### 4.3.4.1. Data quality

There were no significant differences between MS patients and healthy controls in head movement parameters, and the overall, or time-resolved scanner noise of both volumes of interest outside of the head. Correlation coefficients that captured the individual relationship between the temporal fluctuation of the scanner noise and ROI time-series ranged between  $r = -0.29$  (volume 1 - SFG<sub>R1</sub>, healthy control) and  $r = 0.23$  (volume 1 - Thal<sub>R2</sub>, MS patient). The individual average relationships between the scanner noise time-series and all ROIs were low for both volumes of interest ( $|r_{\text{mean}}| < 0.02$ ) and did not differ between groups.

##### 4.3.4.2. Influence of time window length and overlap

The global vfcMRI level was related to the window length, with higher values for shorter time windows (Fig. 16, A). Decreasing overlap also led to increased vfcMRI, but with less striking impact (Fig. 16, A, v1-v6). MS patients had higher global vfcMRI than controls in all combinations. Despite the differences in global vfcMRI levels, z-values for group difference in the 55 partitions of the functional connectome aligned well across the various combinations. In addition, they differed considerably from z-values derived from fcMRI contrasts, and the average z-value of random subgroup comparisons in healthy controls (Fig. 16, B). Group comparisons in vfcMRI tended to yield smaller group differences when based on longer time windows ( $n_{\text{vol}} > 30$ ; v7-v11, Fig. 16, A) but seemed to have narrower distributions of differences derived from the permutation test, resulting in higher z-values, but lower effect sizes in contrast to short-window data sets ( $n_{\text{vol}} = 20$ ; v1-v6; mean absolute Cohen's  $d_{\text{long}} = 0.18$ , mean absolute Cohen's  $d_{\text{short}} = 0.22$ ). High positive relationships were found among the divergent combinations, ranging from  $r = 0.67$  to  $r = 0.99$  (Fig. 16, C). The correlation increased with similarity of the combinations, whereby window length had again the larger impact. There were no meaningful relationships between vfcMRI and the average fcMRI, or the conventional fcMRI for any combination ( $r_{\text{min}} = 0.06$ ,  $r_{\text{max}} = 0.09$ ). Last, shifting the starting volume caused alterations of z-values in a magnitude between  $z = 0.2$  and  $z = 0.35$  (Fig. 16, D; z-values in the original data set = -3.40 - 0.50).



**Figure 16. – Post-hoc 3: Validity tests on window length and overlap.** **A** Global variability of functional connectivity (vfcMRI) for controls (HC, light gray) and multiple sclerosis patients (MS, dark gray) for eleven combinations of window length and overlap. **B** z-values for group contrasts in vfcMRI of 55 partitions of the functional connectome based on the different combinations, conventional functional connectivity (fcMRI), and tests on randomly assigned subgroups of healthy controls. **C** Relationship between vfcMRI, average fcMRI, and conventional fcMRI for all combinations across all participants and functional connections. **D** Average differences between the original z-values of the 55 partitions and z-values after shifting the starting volume for 1 to 5 volumes. This post-hoc test was conducted for combinations with a window size of 20 volumes and an overlap of thirteen (v4), eleven (v5), or ten (v6) volumes. *FDR = false discovery rate, TR = repetition time*

## **4.4. Discussion**

The presented approach aimed at disclosing MS-related alterations of dynamic features of fcMRI and their relationship with clinical manifestations of neurological and neuropsychological deficits.

### **4.4.1. A new perspective on functional connectivity**

The results indicate that metrics of dynamic fcMRI provide a unique perspective on brain physiology and pathology, independent from and complementary to insights from static fcMRI analyses.

First, group differences were considerably larger in terms of statistical parameters and effect sizes for the variability of fcMRI than for the functional interaction levels estimated from the entire time series. Considering that the MS patients in the present study were affected only mildly and, with respect to the overall course of the disease, in an early phase, this gives rise to the assumption that dynamic features of fcMRI are more sensitive than conventional ones for early functional alterations. This interpretation is supported by a study on patients with chronic schizophrenia, whose authors conclude that dynamic analyses provide more insights than conventional ones and that static fcMRI alone might fail to detect relevant aspects of pathology (Sakoglu et al., 2010). An in-depth evaluation of the sensitivity and specificity of dynamic fcMRI in contrast to static fcMRI is desirable.

Second, there were no meaningful relationships between the two metrics in the present analysis, contrasting conclusions drawn by Thompson and Fransson (2015). The authors of this study also used a sliding-window approach but found a strong link between the average fcMRI level and its variance over time. It must be noted, that they did not Fisher's z-transformed the window-specific correlation coefficients, but computed the variance from raw spearman rank coefficients. The distribution of correlation coefficients is limited between -1 and 1 and, most importantly, is not equidistant, hence the distance between 0.9 and 1.0 does not equal the distance between 0.1 and 0.2. As a consequence, the variance of high correlation coefficients is always smaller than the variance across weak correlations. The detected relationship therefore necessarily emerges from the methodological approach and does not represent a statistically sound finding.

### **4.4.2. Increased variability of fcMRI in MS and its relation to behavior**

Generally speaking, the variability of functional interactions was found to be higher in patients across almost the entire functional connectome when contrasted with the control group. Significant increases were detected between several classical RSN and within the language network.

Prominent dynamics of inter-modular functional connections and integration hubs have been described before (Zalesky et al., 2014; Zhang et al., 2016). In addition, regional variability was found to be influenced by the ratio of intra- and inter-community structural connectivity (Zhang et al., 2016). Together with the present findings, this gives rise to the hypothesis that dynamic features of functional communication between classical RSNs are more than a necessary side product. Instead, they seem to have specific functional relevance for physiological functioning and might be indicative for pathological disturbances. It remains unclear, however, whether the increased variability of inter-network fcMRI constitutes an MS-specific pathological correlate, and whether this modification is related to other findings on altered static fcMRI within functional networks. The lack of studies on dynamic features of fcMRI in MS does not allow for further conclusions and also studies that focus specifically on inter-network integration in MS are rare (e.g. Rocca et al., 2012). Further investigations on inter-dependencies between static and dynamic fcMRI, regional activity, and structural connectivity, and on the electrophysiological base on dynamics of functional integration patterns (Zhang et al., 2016) are needed.

The outcome of the analysis suggests that dynamic features of functional integration are functionally relevant for both neuropsychological performance and clinical status in MS beyond the impact of static



fcMRI. Among those partitions of the functional connectome with significant group effect in vfcMRI, two exhibited relevant relationships with clinical and cognitive scores in patients. First, the variability of functional connections between executive control and visuospatial networks was associated with better clinical status in terms of overall disability and fatigue severity. Fatigue has been found to be reflected in connectivity and activity of several brain regions (e.g. Pravata et al., 2016), including frontal regions in accordance with the presented findings. To my knowledge, though, there are no studies investigating the underpinnings of fatigue with respect to dynamic fcMRI specifically for MS. In the investigation of a single healthy subject, Betzel et al. (2016) found lower fatigue severity also to be related with higher flexibility of brain functioning in motor and somatosensory regions. The concrete relationship between these two findings is unclear and probably limited due to different populations, assessment tools and methodological approaches. Further investigations are needed to draw valid conclusions from the present finding.

Second, better attention and longterm memory was related to increased variability of fcMRI between the salience and the visual network. Kucyi et al. (2016) could show just recently that the inherently dynamic nature of attention was closely linked to dynamic changes of fcMRI within and between the DMN and the salience network using a simple tapping task. Zhang et al. (2016), on the other hand, detected highest variability of interaction in brain areas that are known to be involved in integration and learning. Together, this gives rise to the assumption that higher temporal fluctuations facilitate flexible processing of the brain (Matter et al., 2016), which in turn could enhance perception, processing, storage, and probably even retrieval of information in line with the findings here.

Finally, there was a tendency for decreasing variability of fcMRI with longer disease duration, although p-levels of the correlations coefficients were far from the intended significance level. Nevertheless, it may be speculated, whether dynamics of functional interaction patterns are specifically influenced by pathological processes that are predominant in divergent phases of the disease, like functional reorganization or structural plasticity. Future studies should take this research question into account. It should be noted, that groups did not differ significantly in their performance of longterm memory or attention. On the other hand, vfcMRI of the language network was not related to the performance in verbal fluency tasks, in which MS patients performed significantly worse than healthy controls.

In summary, the results suggest that dynamic features of fcMRI, such as the variability, disclose aspects of pathology that cannot be captured with static metrics. Moreover, they indicate that increased dynamic of functional communication, especially between RSNs, have a profound beneficial effect on certain cognitive domains in MS, which seems to preserve the level of performance despite substantial WM disturbances.

#### 4.4.3. Limitations

The interpretation of the present analysis is not without limitations for a number of reasons. First, from the reported results, it cannot be derived that the findings are specific for MS or WM damage due to the lacking comparison with other pathological samples. Consequently, statements regarding the usability of dynamic features of fcMRI as biomarkers beyond the differentiation between healthy and structurally damaged brains are not valid. Second, an adequate correction for physiological noise has been found to be particular relevant for fcMRI analyses (Murphy et al., 2013). Due to technical reasons, it was not possible to record respiration and pulse signal for the majority of the participants in the present study. Although the applied *CompCor* algorithm has been shown to reduce the influence of physiological noise variance substantially (Behzadi et al., 2007), it cannot be ruled out, that the results are still influenced to some extent by physiological confounder variables. Third, the relational analysis was limited to a global score and the beforehand identified functional partitions with significant group effect. Due to that, the greatest number of possible relationships

were not even tested. A comprehensive whole-brain analysis is needed to get the full picture. Fourth, the parameters for MCC were rather liberal to account for the explorative nature of the analysis. Replications of the results in independent studies with more conservative thresholds are desirable. Fifth, the application of sliding window approaches is not without critique. Window size and overlap were chosen carefully to satisfy criteria for the analysis of dynamic fcMRI on a conceptual level as well as to meet recommendations for statistical approaches from recent literature (Hutchison et al., 2013; Wilson et al., 2015; Zalesky and Breakspear, 2015). Leonardi and Van De Ville (2015) suggest window lengths of about the longest wavelength of the BOLD signal (~100s), while Zalesky and Breakspear (2015) provide evidence for a possible detection of temporal fluctuations of fcMRI with considerably shorter time windows (~40s). Furthermore, Price et al. (2014) repeated an analysis for window sizes ranging from 20s to 240s and found regional differences for the peak performance of their classification approach depending on the window size length. To the present day, there is no methodological or practical standard for the determination of sliding window parameters, and the application of analytical solutions (e.g. Ombao and Van Belleghem, 2008) is not common yet (Lindquist et al., 2014).

Conceptually, short time windows are beneficial for the detection of dynamic changes in fcMRI, while they come with the disadvantage that estimations of co-fluctuation naturally decrease in their reliability with fewer data points (Lindquist et al., 2014). In particular, outlying data points at the very beginning or end of a data series can influence the difference between two consecutive time windows significantly and therefore overemphasize dynamic changes. Longer time windows, on the other hand, might be superior in terms of reliability but therefore less sensitive for transient fluctuations of fcMRI, thus the factor of interest. In the present study, it was attempted to balance between reliability and sensitivity by choosing a window length of 50s, maximal overlap, and tapered windows that give different weights to data points in the middle and at the extremes of each time series segment. To get further insights in this matter, the influence of window size and overlap was evaluated empirically with the data at hand. This post-hoc analysis revealed distinct impact of both window size and overlap on the general level of vfcMRI, but still good alignment of group contrasts. Longer time windows were found to be slightly more sensitive for group differences from a statistical point of view, maybe because their more reliable estimation of dynamic characteristics of fcMRI lead to a narrower distribution of values within the groups. In addition, results based on windows with incomplete overlap ( $> 1TR$ ) were shown to depend on the concrete segmentation of the time series. Although the impact did not seem to be enormous, this finding is nevertheless a reason to worry about the validity of findings based on such choice of sliding window parameter. That results of the post-hoc analysis are not corrected for differences in the absolute number of windows, although this parameter is taken into account for the computation of the variability of fcMRI defined as the standard deviation of correlation coefficients across time windows. Findings, in particular of divergent global vfcMRI levels, also reflect differences in the number of considered time windows for this reason.

To sum up, the evaluation together with the lack of systematic differences in the data quality supports the general validity of the group differences in the metric of interest. In addition, it highlights the necessity of standardized versions of dynamic features of fcMRI to allow comparisons between studies with varying parameters. In the interest of completeness, it must be mentioned that sliding window analyses are merely one approach among others that have been used to investigate temporal fluctuations of fcMRI. To the present day, however, there is no agreement on which analytical approach is best suited to disclose insights into relevant dynamics of functional communication (Hutchison et al., 2013). Further systematic evaluations of divergent methodological approaches and influencing factors, such as the cut-off frequencies for filtering, to give just one example, are still needed (Leonardi and Van De Ville, 2015). Finally, it is important to emphasize that the present findings cannot not be interpreted as proof for truly occurring fluctuations of functional interaction over time and neither can results from other studies be interpreted directly as non-stationarity of fcMRI (Hutchison et al., 2013). A major

reason for this limitation is that correlations coefficients for each time window represent only estimates of the relationship of interest. The question of whether the difference between two subsequent time windows is statistically valid and therefore indicative for true temporal fluctuation, is bound to the reliability of each correlation coefficient, thus the underlying statistical uncertainty (Hindriks et al., 2016). The reliability of metrics that describe fcMRI is influenced by a large number of factors, ranging from technical confounds, preprocessing strategies (e.g. Murphy et al., 2009, 2013), physiological and movement-related variables (e.g. Jones et al., 2008; Chang et al., 2009), to unexplained noise. Fluctuations of correlation coefficients over time can therefore be caused by the variability of each of those factors. The determination of dynamic fluctuations of fcMRI does not only require a careful monitoring of confounding variables, however, but in addition appropriate statistical testing against so-called surrogate data that correspond to the null hypothesis that functional interaction is static (Hindriks et al., 2016). The construction of such surrogate data is not trivial and different methods have been suggested over the last years (Chang and Glover, 2010; Zalesky et al., 2014; Hindriks et al., 2016). To provide further insights in the presented findings, a subsequent analysis should be conducted that incorporates statistical testing of the fluctuations themselves. Only then, it can be concluded whether the group differences and relevant interrelations with behavioral deficits in MS result from true variability of functional integrations or other sources of variance. On a conceptual level, both outcomes would certainly be highly interesting for basic and application-oriented research questions. On one side, if further analyses would indicate truly occurring dynamics of fcMRI, this would lead necessarily to the question whether such a finding reflects systematic differences in what MS patients and controls do when they are asked to do nothing. Both cognitive content as well as temporal features of switching between mental states could vary between patients and controls as a manifestation of the pathology or simply resulting from diverging circumstances of life. This rather general critique against so-called resting state measurements might be particular relevant for studies that focus on dynamic fcMRI. On the other hand, if altered vfcMRI merely reflects divergent statistical uncertainty in the MS group due to differential impact or nature of confounding variables, this alone could disclose relevant pathological mechanisms, like (regional) alterations of the hemodynamic response function (Hubbard et al., 2015) or modulated patterns of physiological processes. Furthermore, if confounding factors cause indirectly group differences in fMRI data, one might ask whether those metrics could also serve directly as much cheaper and easier to acquire biomarkers of MS. A careful and open-minded evaluation of various sources of variance for dynamics of fcMRI is needed before further conclusions regarding its usefulness in clinical applications and basic research are drawn.



# 5. Analysis 4: Functional connectivity profiles of longitudinal fatigue alterations

## 5.1. Introduction

There are several cross-sectional studies that show alterations in the strength of functional communication as well as its topological organization in MS when contrasted with healthy controls (e.g. Schoonheim et al., 2014; Liu et al., 2015b). Those deviations in functional connectivity furthermore were related to both structural damage and behavioral deficits (e.g. Hawellek et al., 2011; Leavitt et al., 2014). The pathological impact on the longitudinal development of fcMRI, however, is largely unknown to the present day. The same applies to the extent to which functional and behavioral variables co-fluctuate over time in a meaningful manner. Recently, a study by Faivre et al. (2016) provided the first longitudinal evidence for a relationship between three graph theoretical metrics and the progression of the overall disability in a sample of 38 MS patients. Building upon these findings, the aim of this study was to explore longitudinal alterations in MS with a comprehensive multimodal approach, which included complementing analyses of GM, neuropsychological performance, clinical status, and both node and edge properties of ROI-to-ROI fcMRI. Beyond that, changes in fatigue that occurred over the period of one year were related to alterations in whole-brain fcMRI to identify parts of the functional connectome that could be involved in its progression over time. Fatigue is a symptom pattern of perceived lack of energy, general exhaustion, and limited endurance, that occurs in varying forms in several neurological and other symptomatic diseases (Krupp et al., 1988; Chaudhuri and Behan, 2004). A distinction is made between peripheral fatigue, which describes the inability to sustain or maintain sufficient muscle contraction, and central fatigue, which originates in the central nervous system. The clinical manifestation of both forms can have a cognitive and a motor dimension (Chaudhuri and Behan, 2000, 2004). In MS, central fatigue is among the most frequent symptoms, affecting between 65-91% of all patients (Krupp et al., 1988; Fisk et al., 1994). Being a crucial factor for the participation in social and work life, it constitutes one of the main determinants for quality of life (Nagaraj et al., 2013). Albeit some associations were detected with other symptoms (Lerdal et al., 2007), fatigue cannot be explained by physical, cognitive, or mental disturbances alone. It is rather treated clinically and conceptually as an independent disease component with distinct etiology, epidemiology, and treatment options. Despite its huge impact on subjective and objective well-being, however, fatigue is still underrepresented and underrated in clinical neuroscience (Braley and Chervin, 2010). The underlying pathophysiology in MS, but also in general, is still poorly understood for this reason. Discussed mechanisms include alterations in neurotransmitter and neuroendocrine systems, disturbed (auto)immune responses, and dysfunction in brain activation and integration of several cortical and subcortical brain regions (Schwid et al., 2002; Krupp, 2003). Prospective longitudinal analyses on covariations with changes in parameters of structural or functional integrity of the brain might therefore reveal new perspectives on the available findings. A recent study on the relationship between global atrophy and the development of fatigue over the course of one year has found only weak associations with structural metrics though (Nourbakhsh et al., 2016). Relating longitudinal alterations of fatigue to functional parameters, as presented in the following, is the next subsequent step to shed light on the origins of fatigue in MS for this reason.

## 5.2. Material and methods

### 5.2.1. Participants

Detailed information on recruitment criteria for MS patients and healthy controls can be found in section 1.2, part III. From the original cross-sectional sample, two patients were excluded over the one year-study period due to worsening of their psychological and physiological conditions. The corresponding control subjects were also excluded to maintain the individual matching. The final sample for the longitudinal comparison contained 38 MS patients (23 female, 24-60 years) and 38 matched healthy control (23 female, age 29-57 years).

### 5.2.2. Analysis of longitudinal alterations

#### 5.2.2.1. Statistics

Testing time and interaction effects on behavioral, structural, and functional variables was carried out in MATLAB 2013a. Normal distribution was checked with Kolmogorov-Smirnov-tests (MATLAB 2013a function *kstest*, Massey, 1951) separately for each subtest of a modality (e.g. normal distribution of GM intensities of a particular ROI). Non-parametric statistical tests for paired samples were used for all subtests of a modality when more than 10% of the contrasts did not satisfy the normal distribution requirement ( $p < 0.05$ ). Parametric alternatives were applied otherwise. Time-dependent differences within each group as well as group contrasts for a given time-point were tested for significance with paired t-tests for normally distributed variables (MATLAB 2013a function *ttest*) or alternatively with Wilcoxon signed rank tests (MATLAB 2013a function *signrank*, Gibbons, 1974), two-sided respectively. Interaction effects between groups and time-points were examined either using analyses of variance (ANOVA) with two repeated measurement factors (test statistic: F-value (F)) or by testing the group contrast on individual differences between time-points with Wilcoxon signed rank tests when both the variable and the individual differences were not normally distributed. Again t-tests were applied when only the variable but not the differences did yield significant Kolmogorov-Smirnov-tests. Effect sizes, that means Cohen's d (Cohen, 1988, 1992, interpretation: 0.20 = small, 0.5 = medium, 0.8 = large), partial  $\eta^2$  (Levine and Hullett, 2002, interpretation: partial  $\eta^2 * 100$  = explained variance), or an approximation of the correlation coefficient for the non-parametric test (Pallant, 2001, pp. 224-225, interpretation: 0.10 = small, 0.3 = medium, 0.5 = large) were computed for paired samples. The particular  $\alpha$ -levels and multiple comparison strategies are reported in the corresponding methods sections below.

#### 5.2.2.2. Behavioral variables

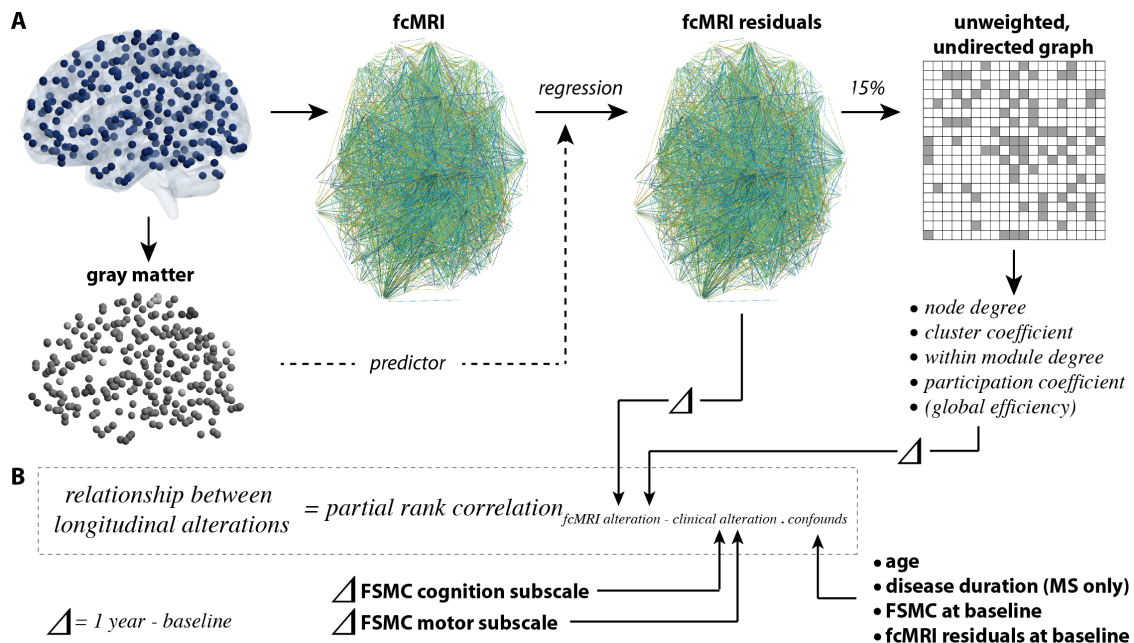
Details of all behavioral data that were collected and analyzed for this study can be found in section 1.4, part III. The polarity of following neuropsychological scores was reversed so that higher values would indicate better performance: VLMT 5-7, TAP Alertness with and without signal, TAP Covert shift of attention, and TAP Incompatibility. A PCA was computed on the z-transformed neuropsychological tests scores of healthy controls at baseline in SPSS 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). After Varimax rotation, which was applied for easier interpretation of the resulting components, component score coefficients were extracted for components with an eigenvalue larger 1. Individual factor scores for all four, respectively three time-points (baseline, two weeks, six months, 12 months) were then calculated as the sum of the neuropsychological test scores weighted by the component score coefficients. The  $\alpha$ -level for statistical testing of longitudinal alterations and interaction effects on the resulting cognitive factors and clinical variables was 0.05, FDR-corrected at  $q = 0.05$  (Yekutieli and Benjamini, 1999). Data from the two-weeks or six-months follow-ups were not subject of statistical testing. Additionally, Pearson's correlation were computed

to evaluate the interrelations between cognitive performance and longitudinal alterations in FSMC subscales ( $\alpha = 0.01$ ).

### 5.2.2.3. Structural data

Individual gray matter masks from the segmentation step (for details see section 1.5.2, part III) were normalized using the DARTEL algorithm implemented in SPM8. The amount of signal was thereby preserved (*modulation*) to allow for the evaluation of GM intensities as a proxy for cortical and subcortical atrophy. A binary group GM mask was generated that included all voxels for which the segmentation algorithm yielded a GM probability of 30% or higher in all data sets. A mask for the Power atlas (Power et al., 2011) was co-registered to that group-based GM mask while preserving the original intensities (*nearest neighbor*). Individual mean GM intensities of all voxels covered by the group GM mask were then derived for each of its 264 ROIs (Fig 17, A). Statistical testing was carried out separately for each ROI. FDR-correction for multiple comparison (Yekutieli and Benjamini, 1999) was applied across all within-factor tests ( $n_{\text{comparisons}} = 1056$ ) and across all statistical tests on interaction effects ( $n_{\text{comparisons}} = 264$ ). The  $\alpha$ -level was 0.01, FDR-corrected with  $q = 0.05$  in both cases.

The lesion volume in ml was derived during the automatic lesion segmentation step (for details see section 1.5.2, part III). The  $\alpha$ -level for statistical testing was 0.01, no multiple comparison correction was applied.



**Figure 17. – Analytical strategy.** **A** Individual average gray matter intensities for 264 regions of interest from the Power atlas were computed as well as pairwise functional connectivity (fcMRI) between them. The influence of gray matter on fcMRI was regressed out before further analytical steps to reduce the impact of group differences in cortical or subcortical atrophy on the results. Binary unweighted and undirected individual adjacency matrices were constructed based on the fifteen percent strongest functional connections ( $n = 5208$ ), from which five graph theoretical metrics were derived for each participant and time-point. Statistical analyses focused on time and interaction effects on gray matter, fcMRI, and graph theoretical metrics. **B** Relationships were assessed between either fcMRI alterations of each functional connection, or node properties of each region of interest and the longitudinal changes in two fatigue subscales of the Fatigue Scale for Motor and Cognitive Functions (FSMC). *MS* = multiple sclerosis

#### 5.2.2.4. Functional connectivity

fcMRI was estimated pairwise between 264 cortical, subcortical, and cerebellar functional ROIs (Power et al., 2011, see appendix section B.4). ROIs were modeled as 10mm diameter spheres and most of them assigned to one of ten RSNs (Cole et al., 2013). The average time-series of all GM voxels covered by both ROI and individual GM masks were extracted using the CONN toolbox (v14b, <http://www.nitrc.org/projects/conn>). For details on the clean-up of time-series see section 2.2.4.1, part III. Pearson's correlation was calculated pairwise between corrected BOLD time-series and then Fisher's z-transformed to construct individual whole-brain ROI-to-ROI fcMRI matrices. In the final step, a linear regression was computed separately for each functional connection with the fcMRI of all participants and time-points as criterion and the corresponding mean GM intensities as predictors. All further analytical steps were carried out on the residuals of this regression analysis to reduce the influence of GM differences on time or group contrasts in fcMRI (Fig 17, A).

Time and interaction effects were tested for significance ( $\alpha_{\text{uncorrected}} = 0.0001$ ) for each link in the functional connectome separately, leading to a high number of comparisons ( $n = 34716$  per contrast). Because conventional strategies for MCC were considered too conservative for this explorative whole-brain analysis, a qualitative clustering scheme was applied to identify subnetworks with coherent meaningful interaction effects. The clustering was based on the assumption that significant effects in functional links, which are not embedded in a network exhibiting a similar effect, are less reliable than functional connections that participate in such an equally behaving subnetwork. To be specific, it was checked whether detected significant interaction effects were linked to at least two other functional connections with the same directions of change of fcMRI in MS and controls, and a similar magnitude of the statistical parameter (F-values, t-values, or z-values depending on the applied test). Similarity was defined relative to the distribution of statistical parameters across all corresponding comparisons. Links with parameters within two standard deviations of the value of the reference connection (which would be the connection with significant interaction effect) were considered as similar. Please note that such a clustering is no valid substitution for a profound MCC, and that clustering criteria were chosen based on general assumptions on the minimal size of a network and similarity.

The algorithm was not applied to the within-group comparisons, nor were any other corrections for multiple comparisons applied.

#### 5.2.2.5. Graph theoretical metrics

Individual undirected and unweighted adjacency matrices were constructed by including the 15% highest functional connections of the graph ( $n = 5208$ ; Fig 17, A), which is the sparsity of links recommended by Achard et al. (2006) (see also Faivre et al., 2016). The number of nodes and edges were therefore identical across participants and time-points, ensuring that possible effects are not caused by systematic differences in these variables (van Wijk et al., 2010; Zalesky et al., 2010). It should be noted that the applied functional atlas has been developed specifically for graph theoretical analyses (Power et al., 2011) and that it has been shown to be more reliable than the AAL atlas, which is one of the most commonly used atlases in graph theoretical studies (Cao et al., 2014). The following graph theoretical metrics were extracted from the adjacency matrices with the help of the Brain Connectivity Toolbox (<http://www.brain-connectivity-toolbox.net/>). First, the node degree, which is the sum of edges connected to a node and a measure for the centrality of a node. Second, the clustering coefficient, which captures the number of triangles, in which a node is involved, relative to the possible number of triangles for that given node. It is a measure for the clustering or community characteristic of a network. In addition, two metrics were computed that describe the centrality of nodes with respect to the module structure of the network. Namely the within module degree, which indicates the centrality of a node within a module, and the participation coefficient, the intra-module version of node centrality. Finally, the global efficiency was determined, that means the average inverse



shortest path, which constitutes a measure for the overall distance of a network. Time-point differences and interaction effects were tested separately for each graph theoretical metric with an  $\alpha$ -level of 0.05, FDR-corrected with  $q = 0.05$  (Yekutieli and Benjamini, 1999).

Graphs can be thresholded in divergent ways to get individual binary adjacency matrices. The most common approach is to apply a fixed threshold at a certain functional connectivity strength, for instance  $r = 0.3$ . The strategy, which was applied in the present study, was therefore compared to divergently thresholded adjacency matrices ( $r > 0.1, 0.2, 0.3, 0.4$ ) in a post-hoc test to explore the influence of different strategies on the statistical outcome. Results on this post-hoc analysis can be found in section B.5, appendix.

### 5.2.3. FcMRI profiles of longitudinal clinical alterations

The aim of the last part of this analysis was to characterize whole-brain fcMRI profiles of longitudinal alterations of fatigue by determining connection-specific relationships between behavioral and functional changes (Fig. 17, B). First, the polarity of the FSMC subscales for cognitive and motor fatigue was reversed for easier interpretation (higher values = better clinical status). Second, individual behavioral and functional alterations were computed by subtracting baseline from one-year values ( $> 0$ : improvement, increase of fcMRI;  $< 0$ : deterioration, decrease of fcMRI). Next, partial rank correlation coefficients were computed to determine the relation between behavioral and functional changes (0.1 = small, 0.3 = medium, 0.5 = large, according to Cohen, 1988, 1992). Baseline values of following variables were taken into account as confounders in this step: age, disease duration (MS only), FSMC scores of both subscales, and the fcMRI level. Cognitive performance was not included as confounder based on the beforehand computed analysis of behavioral data, which had not yield any meaningful relationships between the cognitive domains and FSMC scores. Finally, correlation coefficients were compared between groups to identify functional connections, in which the variables were significantly stronger related in MS in contrast to controls (one-sided for  $|r_{MS}| > |r_{HC}|$ , significant at  $p < 0.001$ , uncorrected; Equation 5.1) with

$$1 - normcdf\left(\left|\frac{|zr_{ms}| - |zr_{hc}|}{\sqrt{\frac{1}{n_{ms}-3} + \frac{1}{n_{hc}-3}}}\right|, 0, 1\right) = p, \quad (5.1)$$

with *normcdf* being the normal cumulative distribution function and  $zr_x$  being the Fisher's z transformed correlation coefficients.

The beforehand mentioned clustering algorithm was applied to identify significant relationships that were embedded in a cluster of connections with similar effect. The starting points were the functional connections, which were significantly stronger correlated with the behavioral variable in MS. Similarity was evaluated based on the partial rank correlation coefficients themselves rather than the group difference, however. Coefficients that differed less than  $|r| = 0.05$  were defined as similar. The relationship between alterations in node-specific graph theoretical metrics and FSMC subscales was determined in the same manner (one-sided for  $|r_{MS}| > |r_{HC}|$ ,  $p < 0.001$ , uncorrected).

The exploration of cross-sectional associations between the FSMC subscales and functional interactions was beyond the focus of this analysis. But in the interest of completeness, whole-brain association maps for both groups can be found in the appendix section B.6.

### 5.2.4. Post-hoc analysis on fcMRI predictability

The general re-test reliability of fcMRI in stimuli-free conditions is largely unknown to the present day. To estimate the empirical re-test reliability in the present study, the predictability of fcMRI and the intraclass correlation coefficient (ICC) for three different time periods were determined for 35 MS patients. Two-week data for three MS patients were missing. Using linear regression analyses, both

two-week and one-year follow-ups were predicted by the baseline (0.5 months, 12 months), and the one-year follow-up by fcMRI at two weeks (11.5 months). For controls, only the prediction of the one-year fcMRI could be conducted, because two-week data was not available for this group.

Preprocessing, time-series extraction, clean-up, and computation of fcMRI of the two-weeks follow-up was done in the same way as for the other time-points. Note, that the post-hoc analysis was computed on the raw fcMRI estimates, hence without regression of GM values.

In the first step of the post-hoc analysis, linear regressions were computed separately for each participant across all functional connections, resulting in individual global estimations of how well the fcMRI of one time-point can predict fcMRI of another. In a second step, the individual predictability was estimated separately for each inter- and intra-network partition of the functional connectome and averaged across patients. This was repeated after permutation of the data sets, so that fcMRI of one person was predicted by the fcMRI of another randomly assigned one. Finally, the ICC (see case 3, McGraw and Wong, 1996) was determined for each functional connection and all three time periods, and then averaged across partitions. The ICC is a statistical parameter that quantifies the re-test, respectively inter-rater reliability in designs with repeated measurements. Scores can range between -1 and 1, whereby ICC values below zero equate to zero, hence no reliability. Basically, the variances within and between subjects are related with each other, so that low variability between time-points or measurements together with large variability between subjects result in high reliability estimates.

### 5.3. Results

#### 5.3.1. Sample

See Tab. 13 for full clinical and demographic information at baseline. For two healthy controls, one of fourteen item of the HADS were missing, respectively. The scores were replaced by the individual mean of the corresponding depression or anxiety subscales. None of the patients experienced an acute relapse during the study. The average inter-measurement period was 370.39 days (337-415 days) in healthy controls and 363.89 days (342-386 days) in MS ( $t_{(37)} = 2.42$ ,  $p = 0.02$ ).

**Table 13. – Demographic and clinical characteristics of the sample at baseline.**

Demographic information	HC (n = 38)	MS (n = 38)	Difference
Age (in years; mean, SD)	40.45 (8.27)	40.76 (9.51)	$t_{(37)} = -0.59$ , $p = 0.55$ , $d = 0.10$
Sex (female : male)	23 : 15	23 : 15	-
Handedness (left : both : right)	1 : 1: 36	4 : 2 : 32	$\chi_{(2)} = 2.36$ , $p = 0.31$
Clinical characteristics	HC (n = 38)	MS (n = 38)	Difference
Years since diagnosis (mean, SD)	7.05 (5.00)	-	-
EDSS (mean, SD)	0.28 (0.53)	2 (0.94)	$Z = -5.00$ , $p < 0.01^*$ , $esW = -0.57$
FSMC Sum no/mild/mod./sev.	38/0/0/0	14/6/6/12	$\chi_{(3)} = 35.08$ , $p < 0.01^*$
FSMC Mot. no/mild/mod./sev.	37/1/0/0	18/2/6/12	$\chi_{(3)} = 24.90$ , $p < 0.01^*$
FSMC Cog. no/mild/mod./sev.	38/0/0/0	14/7/4/13	$\chi_{(3)} = 35.08$ , $p < 0.01^*$
HADS-A no signs/mod./clinical	37/1/0	28/9/1	$\chi_{(2)} = 8,65$ , $p = 0.01^*$
HADS-D no signs/mod./clinical	36/2/0	36/1/1	$\chi_{(2)} = 1.33$ , $p = 0.51$
Sleep problems (no : yes)	30 : 8	26 : 12	$\chi_{(1)} = 0.75$ , $p = 0.39$
Cortisone (yes : no : unknown)	3 : 31 : 4	3 : 27 : 8	$\chi_{(2)} = 1.61$ , $p = 0.44$
MS modifying drugs (yes : no : unknown)	0 : 37 : 1	20** : 17 : 1	$\chi_{(2)} = 27,41$ , $p < 0.01^*$

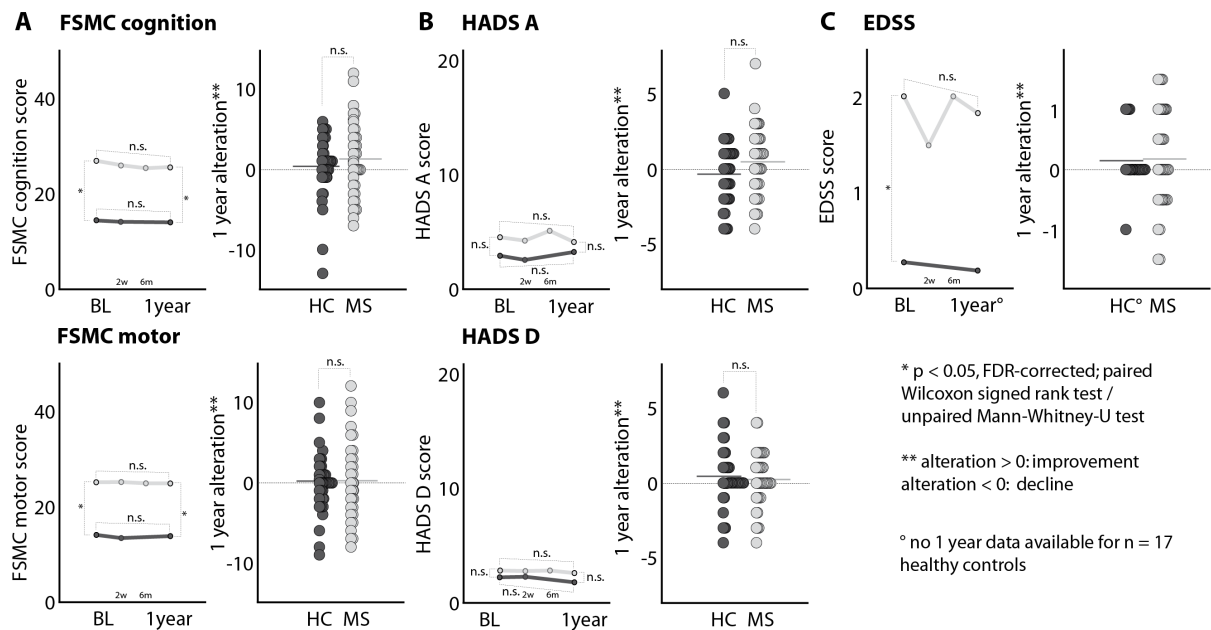
*SD = standard deviation; d = Cohen's d; Z = test statistic of Wilcoxon signed rank test;  $\chi$  = test statistic of Chi square or McNemar tests; esW = effect size for Wilcoxon signed rank test; Mot. = Motor; Cog. = Cognition; mod. = moderate; sev. = severe; \*significant at  $p < 0.05$ ;*

*\*\*Glatiramer Acetate (3), Natalizumab (4), Interferon (5), Fingolimod (3), Dimethyl Fumarate (1), unknown (2);*

*HC = healthy controls, MS = multiple sclerosis*

### 5.3.2. Longitudinal alterations of behavioral variables

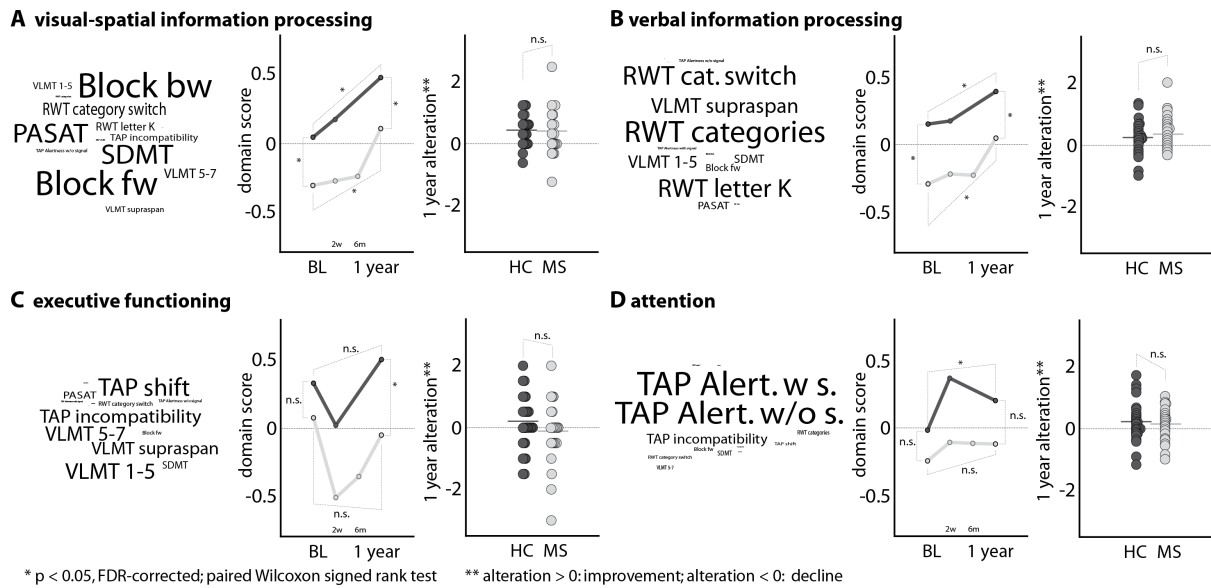
Based on the Kolmogorov-Smirnov-tests, non-parametrical tests were preferred for all clinical variables. EDSS scores for seventeen healthy controls were missing for the one-year follow-up. Group and time differences were therefore tested with a Mann-Whitney-U test for unpaired samples. Taken together, there were no significant time or interaction effects in any clinical variable on the group level. However, there were substantial changes over time in both directions on the individual level, revealing great variability of the clinical course over the period of one year (Fig. 18, A, B, and C).



**Figure 18. – Longitudinal alterations in clinical scores.** Group mean values at baseline, after two weeks, six months, and one year, complemented by individual alterations for the Fatigue Scale for Motor and Cognitive Functions (FSMC; **A**), the Hospital Anxiety and Depression Scale (HADS; **B**), and the Extended Disability Status Scale (EDSS; **C**). \* $p < 0.05$ , FDR-corrected; BL = baseline, HC = healthy controls, MS = multiple sclerosis

The PCA yielded four components that can be interpreted in the following manner based on the component scores of the neuropsychological tests: visual-spatial information processing, verbal information processing, executive functioning, and attention (Fig. 19, A, B, C, and D). Both groups improved on all for components over the course of the study. This improvement was significant in healthy controls in all but the executive functioning component and in MS patients in the first two ones. There were no significant interaction effects.

The alterations of motor and cognitive fatigue were correlated with each other (MS:  $r = 0.46$ ,  $p < 0.01$ ; HC:  $r = 0.64$ ,  $p < 0.01$ ), but not with changes in the cognitive performance (strongest correlation MS:  $r_{\text{visual-FSMC kog}} = 0.16$ ,  $p = 0.35$ ; HC:  $r_{\text{verbal-FSMC kog}} = -0.22$ ,  $p = 0.19$ ). In both groups, alterations in the visual-spatial information processing were positively correlated with changes of verbal processing (MS:  $r = 0.61$ ,  $p < 0.01$ ; HC:  $r = 0.56$ ,  $p < 0.01$ ), but not with the other two factors. There were no significant correlations between baseline scores of the four cognitive factors and the longitudinal change of both FSMC subscales (all  $|r| < 0.35$ , all  $p > 0.03$ ). In the MS group, baseline levels of visual-spatial information processing were significantly correlated with the alterations in the same domain, and the alterations in the verbal one (both  $r = -0.43$ ,  $p < 0.01$ ). In addition, executive functioning at baseline was related to its own change ( $r = -0.46$ ). In the control group, baseline scores of visual-spatial, verbal, and executive processing were significantly correlated with alterations of themselves ( $r_{\text{visual}} = -0.49$ ;  $r_{\text{verbal}} = -0.52$ ;  $r_{\text{exec.}} = -0.62$ ). Baseline levels of fatigue were correlated significantly with alterations in fatigue severity in healthy controls only.



**Figure 19. – Longitudinal alterations in cognitive performance.** A-D Group mean values at baseline, after two weeks, six months, and one year, complemented by individual alterations of performance in four cognitive domains. The word clouds represent the component scores or loadings of the fourteen neuropsychological tests on the four components that were yielded with the principal component analysis. \* $p < 0.05$ , FDR-corrected; BL = baseline, HC = healthy controls, MS = multiple sclerosis

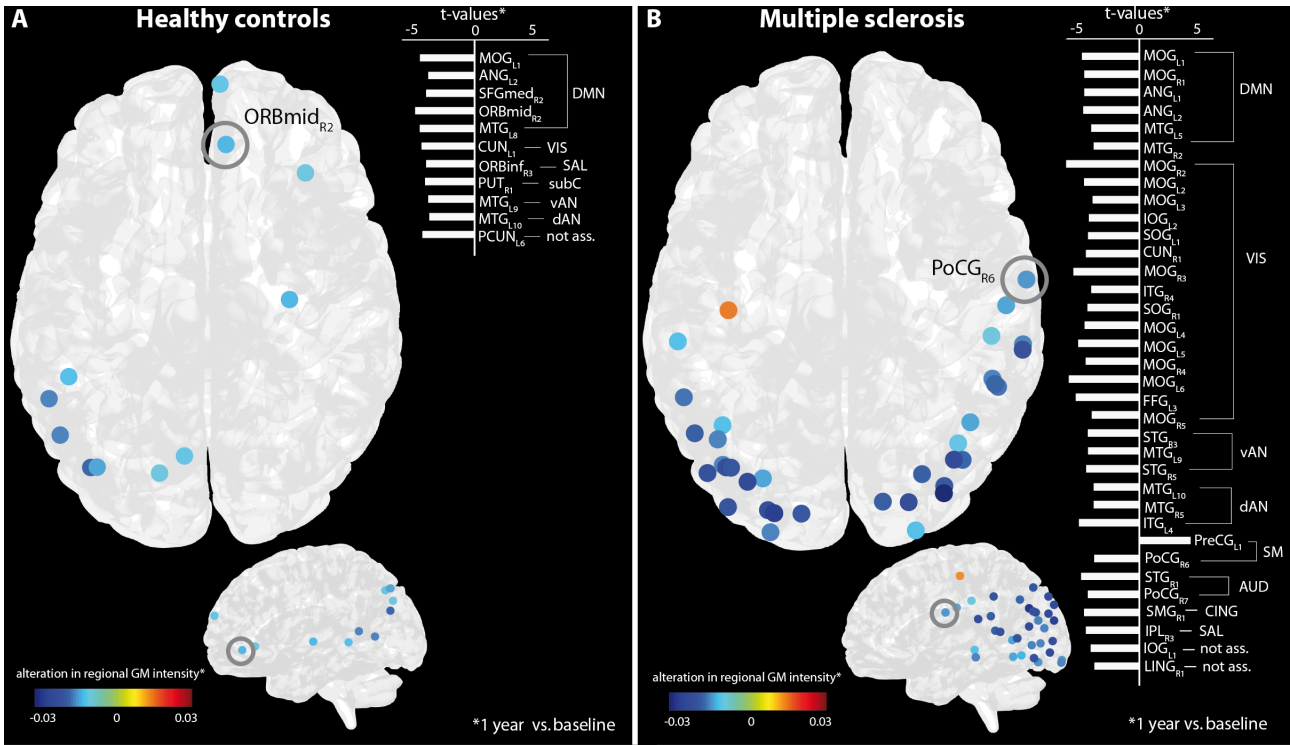
### 5.3.3. Longitudinal alterations of brain structure

Only 10 out of 264 (3.79%) Kolmogorov-Smirnov-tests on the normal distribution of GM intensities reached significance ( $p_{\min} = 0.02$ ), so that parametric testing was applied for statistical testing of group differences in all ROIs.

In healthy controls, significant decrease of GM intensity over time was found in eleven ROIs ( $p_{\text{FDR corrected}} < 0.01$ ; mean |Cohen's d| = 0.55; Fig. 20). In patients, significant alterations were identified in 35 regions ( $p_{\text{FDR corrected}} < 0.01$ ; mean |Cohen's d| = 0.62).

There were no significant interaction effects after FDR-correction. Without correction, meaningful interactions were found in the posterior central gyrus (PoCG<sub>R6</sub>;  $F_{(1)} = 9.47$ , partial  $\eta^2 = 0.002$ , explained variance = 0.23%,  $p_{\text{uncorrected}} = 0.0039$ ) and the middle orbital frontal gyrus (ORBmid<sub>R2</sub>;  $F_{(1)} = 9.67$ , partial  $\eta^2 < 0.01$ , explained variance = 0.07%,  $p_{\text{uncorrected}} = 0.0036$ ) on the right side.

There were no significant time, or interaction effects on the lesion volume according to the applied non-parametrical statistical test. In controls, the lesion volume increased on average slightly from 0.14 ml (SD = 0.24 ml; range = 0-1.14 ml) to 0.16 ml (SD = 0.28 ml; range = 0-1.40 ml;  $z = -1.33$ ,  $p = 0.18$ ). In patients, there was an average increase of the lesion volume by 13.79% from 10.95 ml at baseline (SD = 14.80 ml; range = 0.09-70.95 ml) to 11.45 ml after one year (SD = 14.86 ml; range = 0.09-66.30 ml;  $z = -1.91$ ,  $p = 0.06$ ;  $t_{\text{interaction}} = -1.69$ ,  $p = 0.30$ ).



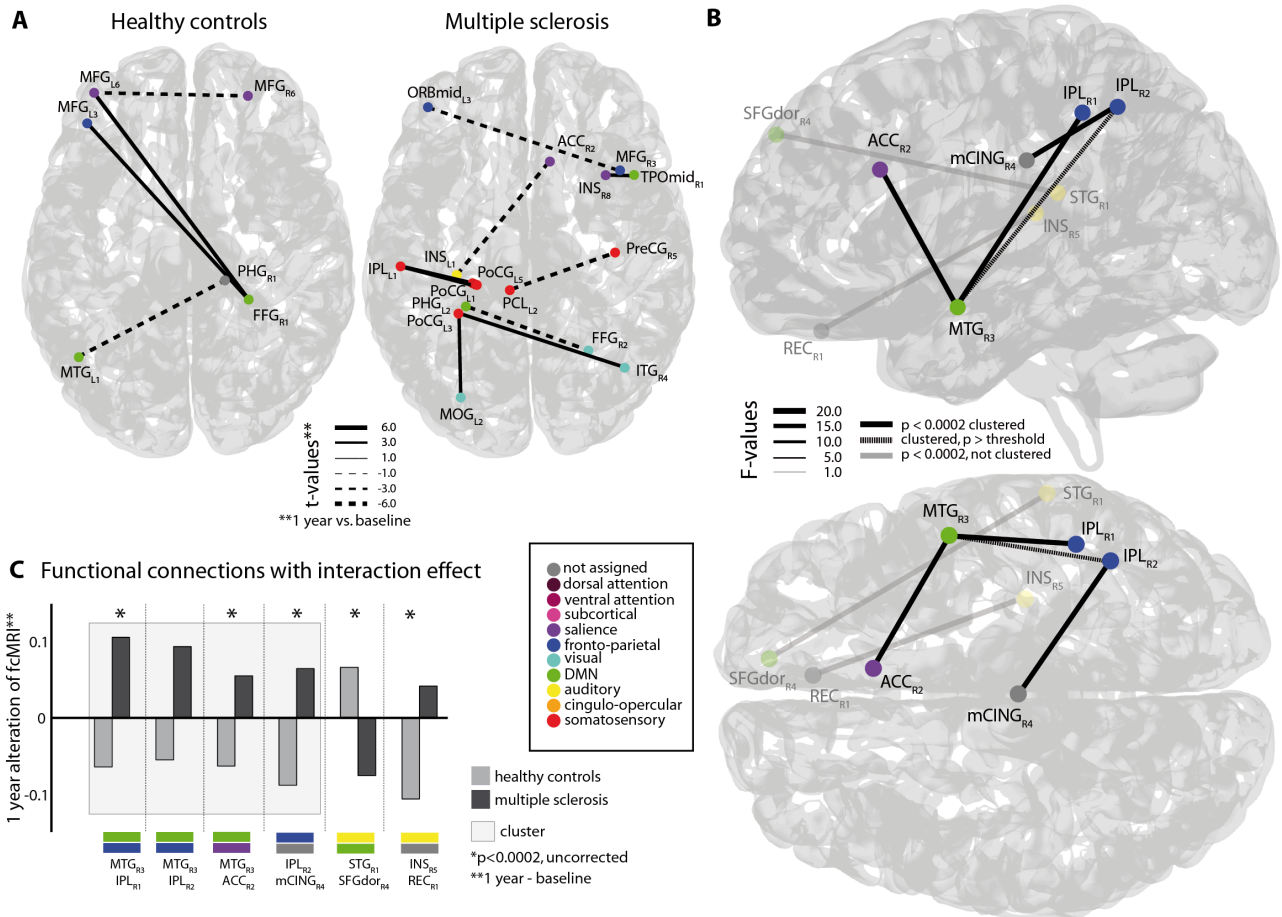
**Figure 20. – Longitudinal alterations in gray matter.** Change in gray matter intensity and t-values for regions of interest with significant time effect ( $p < 0.01$ , FDR-corrected) in healthy controls (A) and multiple sclerosis patients (B). Interaction effects ( $p < 0.01$ ) are highlighted with gray circles. *networks*: DMN = default mode, VIS = visual, SAL = salience, subC = subcortical, v/dAN = ventral/dorsal attention, SM = somatosensory, AUD = auditory, CING = cingulo-opercular, not ass. = not assigned; for information on regions of interest see appendix section B.4

### 5.3.4. Longitudinal alterations in fcMRI

After careful inspection of artifacts due to volume-to-volume movement, 104 of 246 functional volumes were discarded in one healthy controls at baseline. No further functional images had to be discarded because of movement in any other participant or time-point. Kolmogorov-Smirnov-tests yielded significant results for 16 functional connections (0.05%,  $p_{\min} = 0.01$ ), justifying parametric testing.

At the uncorrected  $\alpha$ -level of 0.0001, both increase and decrease of fcMRI over time were detected in controls and patients. In healthy participants, time effects were found in links between middle frontal brain regions (decrease), between frontal and occipito-temporal ROIs (increase), and between the middle temporal and the parahippocampal gyri (decrease; mean |Cohen’s d| = 0.66; Fig. 21, A). In MS patients meaningful longitudinal increase of fcMRI was found in inter- and intra-network connectivity of the somatosensory network, and between the right insula and the right middle temporal pole. Decrease of functional interaction was identified also for links within the somatosensory and fronto-parietal networks, as well as between cingulate, visual, postcentral, and occipito-temporal ROIs (mean |Cohen’s d| = 0.69; Fig. 21, A). Qualitatively, a pattern emerged in healthy controls consisting of decreasing fcMRI in the DMN and the salience network, and increasing inter-network interaction particularly between the DMN, the auditory, cingulo-opercular, and somatosensory networks. (for whole-brain matrices of time contrasts see appendix section B.7). No such pattern became apparent for the MS group. Along the functional connections of the somatosensory network exhibited relatively uniformly a decrease of fcMRI together with enhanced interaction with the visual and the attention networks.

There were no significant interaction effects at the pre-defined  $\alpha$ -level. Using a more liberal p-threshold ( $\alpha = 0.0002$ ) enabled the detection of five connections with notable interaction. The application of the clustering algorithm on the F-values and the direction of longitudinal changes disclosed that three of them together with one further functional connection formed a subnetwork, in which the fcMRI increased over time in MS patient and decreased in healthy controls (Fig. 21, B and C). The functional connections of the identified cluster were located in parietal, temporal, and cingulate areas of the brain and belonged to the fronto-parietal, the salience network, and the DMN. The interaction effect explained between 4.46% and 6.04% of the variance in those connections.



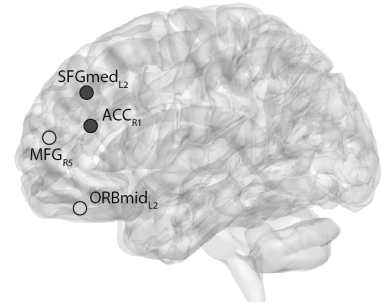
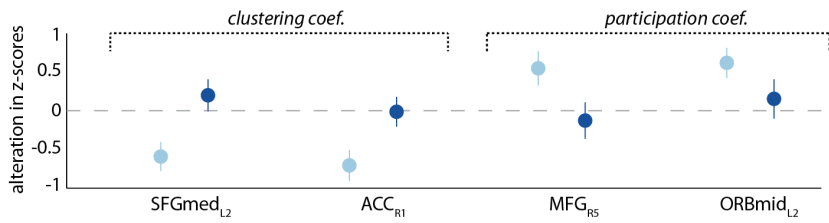
**Figure 21. – Longitudinal alterations in functional connectivity.** **A** Time effects on functional connectivity (fcMRI) in healthy controls and multiple sclerosis patients ( $p < 0.0001$ , uncorrected). Solid lines indicate longitudinal increase of fcMRI, while dashed lines reflect decreasing fcMRI. **B** Identified cluster of functional connections with meaningful interaction effect ( $p < 0.0002$ , uncorrected) or similar effect with  $p \geq 0.0002$  (dotted lines), and unclustered functional connections with meaningful interaction effect (transparent). **C** Changes in fcMRI in connections with meaningful interaction. *DMN = default mode network; for information on regions of interest see appendix section B.4; line width corresponds to magnitude of statistical parameter (t-, F-values)*

### 5.3.5. Longitudinal alterations in graph theoretical metrics

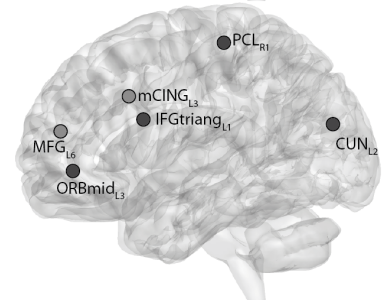
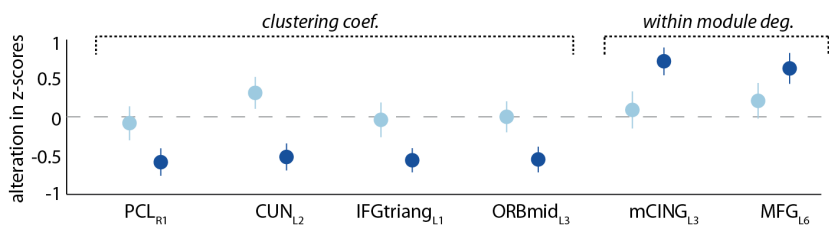
Scores of the participation coefficient and the global efficiency did not satisfy the normal distribution criterion. Non-parametrical tests were therefore applied for these two metrics and parametrical tests otherwise. With the pre-defined FDR-corrected  $\alpha$ -level, no significant effects were detected at all. Further unconstrained explorations of the results revealed that strongest time and interaction effects were detectable not before raising the p-level to 0.01, uncorrected. At this p-level, clustering coefficients

decreased over time in controls in regions of the DMN, namely the middle superior frontal gyrus and the anterior cingulate cortex (Fig. 22, A). Beyond that, the participation coefficient increased in frontal regions belonging to the salience network or unassigned. In MS patients, decreasing clustering coefficients were found in frontal areas (fronto-parietal network) as well, but also in occipital (visual network) and parietal nodes (somatosensory network) (Fig. 22, B). Interaction effects were identified for all four metrics at this p-level (Fig. 22, C). The affected nodes were located in frontal regions, but also in the insula, the basal ganglia, and the cuneus. In frontal nodes, MS patients exhibited increased node degree, clustering coefficient, and within module degree, while those metrics were decreased in the basal ganglia and the occipital node. There were no relevant effects on the global efficiency.

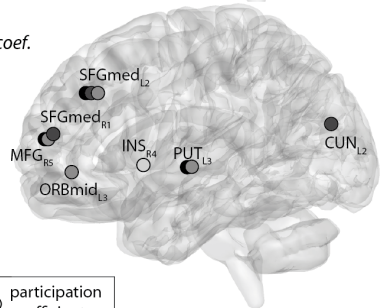
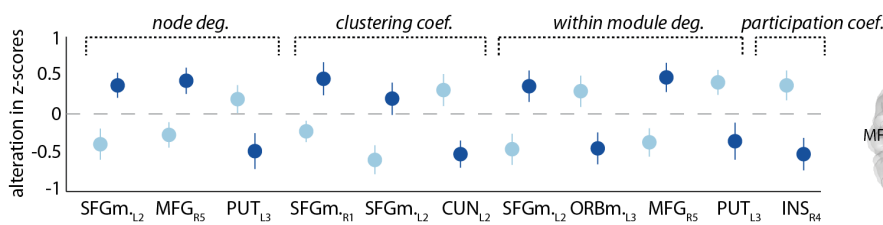
**A Longitudinal alterations in healthy controls**



**B Longitudinal alterations in MS patients**



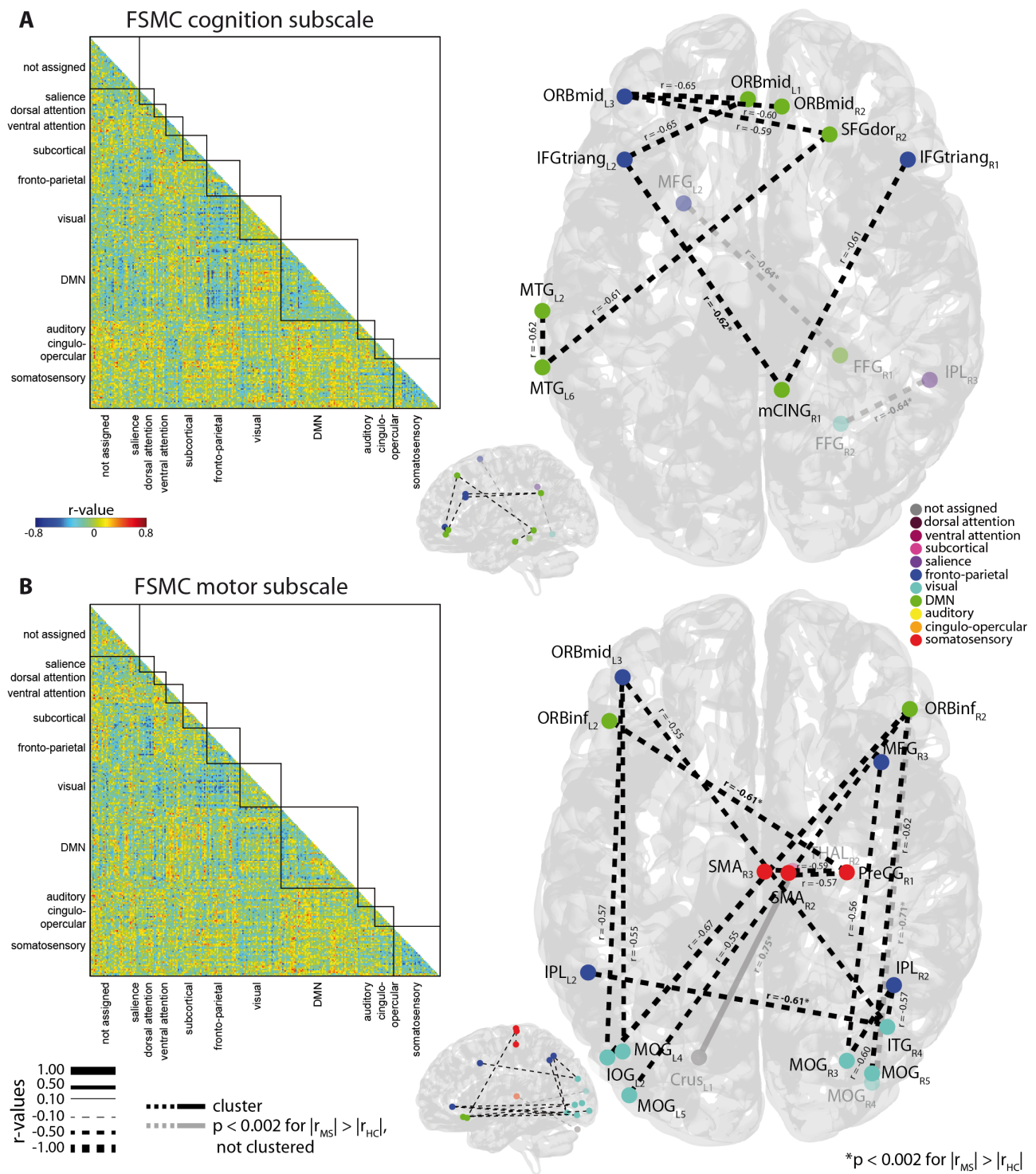
**C Interaction effects**



**Figure 22. – Longitudinal alterations in graph theoretical metrics.** Longitudinal alterations of graph theoretical metrics describing node properties ( $p < 0.01$ , uncorrected) and the anatomical location of affected nodes for healthy controls (HC, **A**), multiple sclerosis patients (MS, **B**), and for nodes with with interaction effect (**C**). For illustration purposes, alterations of all graph theoretical metrics were normalized; for information on regions of interest see appendix section B.4

**5.3.6. FcMRI profiles of longitudinal clinical alterations - fcMRI strength**

The comparison of partial rank correlation coefficients of controls and patients did not yield significant differences at the intended  $\alpha$ -level ( $p < 0.001$ , uncorrected). At a slightly higher p-level ( $p < 0.002$ , uncorrected), three functional connections were detected, whose longitudinal alterations were correlated stronger with changes of cognitive fatigue in MS than in healthy controls (Fig. 23, A).



**Figure 23.** – Relationship between longitudinal alterations in functional connectivity and fatigue severity. Left: Whole-brain functional connectivity profile of longitudinal alterations in cognitive (A) and motor (B) fatigue measured with the Fatigue Scale for Motor and Cognitive Functions (FSMC) for multiple sclerosis patients (MS). Right: Functional connections that correlated stronger in MS when contrasted with healthy controls (HC;  $p < 0.002$ , one-sided, uncorrected; marked with an asterisk) and their involvement in clusters of similar effect. Similarity threshold was an absolute difference in correlation coefficients of  $|r| = 0.05$  for the cognition subscale, resulting in one cluster, and  $|r| = 0.1$  for the relationship with motor fatigue, detecting two clusters.  $r$  = partial rank correlation coefficient; DMN = default mode network; for information on regions of interest see appendix section B.4; line width indicates the magnitude of partial rank correlation coefficients, dashed lines reflect negative correlation values



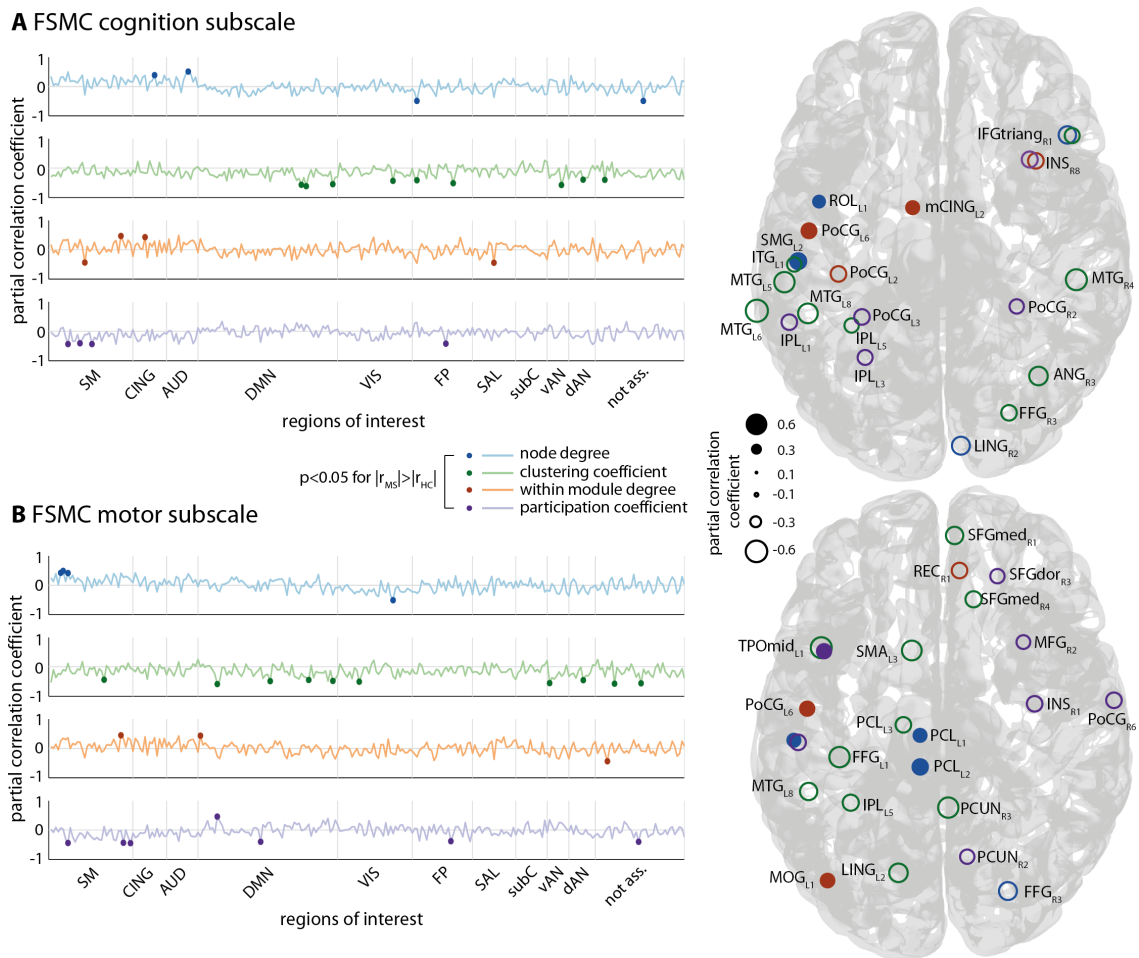
These connections were located between cingulate and temporal parts of the DMN, frontal areas of the fronto-parietal network, and between parietal ROIs. Absolute partial correlation coefficients for the relationship with the FSMC motor subscale were larger in MS in four long-range functional connections between visual areas and parietal, frontal, as well as subcortical regions (Fig. 23, B). All but one of the functional connections, whose longitudinal change were stronger associated with fatigue in MS had negative correlation coefficients. Functional connections, that correlated stronger with either motor or cognitive fatigue in healthy controls ( $p < 0.002$ , one-sided for  $|r_{\text{HC}}| > |r_{\text{MS}}|$ , uncorrected) are illustrated in Fig. 30, appendix section B.8.

Using those group differences as starting points for the cluster algorithm disclosed one cluster with similar effect ( $|r_{\text{difference}}| < 0.05$ ) for the FSMC cognition subscale (Fig. 23, A) and two clusters for the motor subscale ( $|r_{\text{difference}}| < 0.1$ , no cluster at the intended similarity threshold; Fig. 23, B). For the FSMC cognition subscale, the cluster encompassed only one functional link with meaningful group effect that connected the middle cingulate region and the inferior frontal gyrus ( $r_{\text{HC}} = -0.04$ ,  $r_{\text{MS}} = -0.62$ ,  $p = 0.002$ ). This connection was embedded in a cluster of seven functional connections between regions of the DMN and the fronto-parietal network with similar effects ( $r = -0.59$  to  $r = -0.65$  in the MS group). The first cluster of functional connections that were correlated with changes on the FSMC motor subscale included ten links, which were located between the visual network and both the fronto-parietal network and the DMN. Again only one connection exhibited a significant group effect ( $r_{\text{HC}} = -0.02$ ,  $r_{\text{MS}} = -0.53$ ,  $p = 0.002$ ). The other correlation coefficients ranged between  $r = -0.55$  and  $r = -0.67$ . The second cluster incorporated one significant connection between the somatosensory network and the DMN ( $r_{\text{HC}} = -0.004$ ,  $r_{\text{MS}} = -0.58$ ,  $p = 0.002$ ), and two further links within the somatosensory network ( $r = -0.57$  to  $r = -0.59$ ).

Cohens' d values for a time effect on fMRI in MS were relatively low in all identified clustered or unclustered connections that correlated strongly with either motor or cognitive fatigue (mean |Cohen's d| = 0.18, range |Cohen's d| = 0.02-0.47).

### 5.3.7. fMRI profiles of longitudinal clinical alterations - fMRI topology

No significant group differences in the relationship between graph theoretical metrics and FSMC subscales were found. For explorative purposes, group effects with  $p < 0.05$  are displayed in Fig. 24. Both FSMC subscales were correlated negatively with a decrease of the clustering coefficient in several nodes, but especially in ROIs belonging to the DMN. For the other three graph theoretical metrics no such pattern became apparent. Across all four metrics, most meaningful group differences were found in the fronto-parietal and the somatosensory networks for the cognition subscale, and in the somatosensory network and the DMN for the motor subscale. Partial correlation coefficients ranged between  $|r| = 0.4$  and  $|r| = 0.6$ .

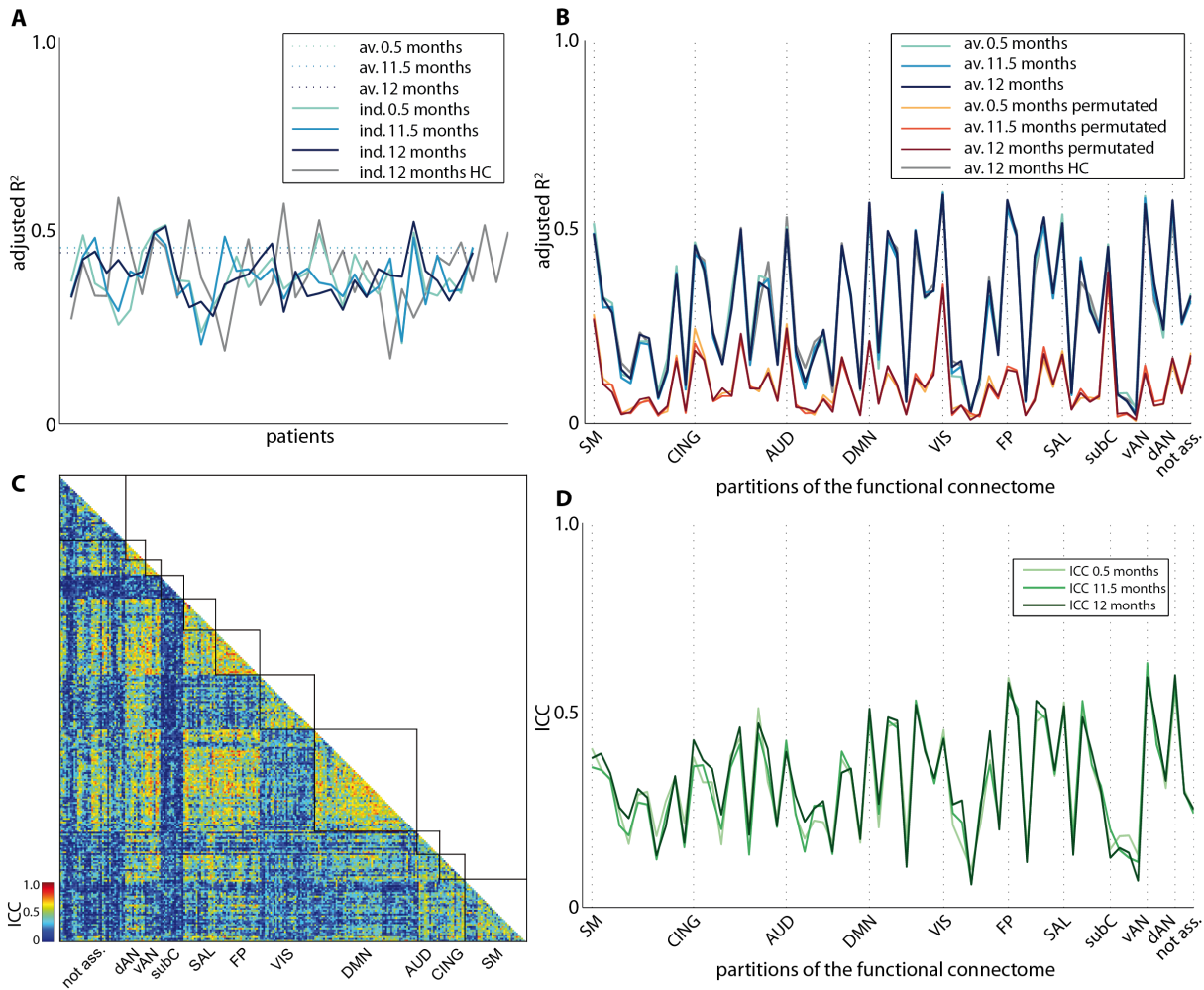


**Figure 24. – Relationship between longitudinal alterations in node properties and fatigue severity.** Left: Relationship between four graph theoretical metrics and both cognitive (A) and motor (B) fatigue as measured with the Fatigue Scale for Motor and Cognitive Functions (FSMC). Regions of interest are sorted with respect to their assignment to resting state networks. Right: Identified group differences between multiple sclerosis (MS) and controls (HC;  $p < 0.05$ , uncorrected).  $r$  = partial rank correlation coefficient; networks: SM = somatosensory, CING = cingulo-opercular, AUD = auditory, DMN = default mode, VIS = visual, FP = fronto-parietal, SAL = salience, subC = subcortical, v/dAN = ventral/dorsal attention; for information on regions of interest see appendix section B.4; node size reflects correlation magnitude, filled nodes display positive correlations

### 5.3.8. Post-hoc evaluations on fMRI predictability

The individual adjusted  $R^2$  for the linear regressions ranged between 0.21 and 0.52, indicating that between 21 and 52% of the variance of whole-brain fMRI could be explained by earlier fMRI measurements (Fig. 25, A). Prediction periods did not differ with respect to the amount of explained variance as judged by eyeballing. The exploration of the average adjusted  $R^2$ -values for separate partitions revealed that the predictability across all MS patients was drastically lower for inter-network partitions of the functional connectome than for intra-network ones (min  $R^2 = 0.04$  for partition between visual and subcortical network, max  $R^2 = 0.60$  for visual network; Fig. 25, B). This was also true for permuted data sets, indicating that within-network fMRI can be predicted better than between-network interactions even from fMRI of a randomly assigned other person. The computation of the ICC confirmed higher re-test reliability for intra-network functional connectivity (Fig. 25, C, D). Scores ranged between 0.06 (partition between visual and subcortical network) and 0.64 (ventral attention network). Estimations of the reliability of fMRI in inter-network partitions between two networks with high ICC scores, for instance between the DMN and the fronto-parietal network, yielded also higher values. Again, the re-test reliability for shorter time periods was not superior in compari-

son to longer ones. Substantially lower re-test reliability was revealed for the subcortical network and its interaction with all other networks.



**Figure 25. – Post-hoc test on the re-test reliability of functional connectivity in the present study.** **A** Individual estimates of the global predictability of functional connectivity (fcMRI) by a former fcMRI assessment for time differences of 0.5 months (patients only), 11.5 months (patients only), and 12 months. **B** Average predictability for each of 66 inter- and intra-network partitions of the functional connectome for multiple sclerosis patients, permutated patients, and healthy controls (HC). **C** Whole-brain matrix of connection-specific intraclass correlation coefficients (ICC), indicating the re-test reliability between the baseline and the 12 months follow-up. Warm colors depict higher reliability. **D** Averaged connection-specific ICC scores for all inter- and intra-network partitions of the functional connectome. Different shades of green indicate divergent time periods from the repeated measurements, ranging from 0.5 months (light green) to 12 months (dark green). *networks: SM = somatosensory, CING = cingulo-opercular, AUD = auditory, DMN = default mode, VIS = visual, FP = fronto-parietal, SAL = salience, subC = subcortical, v/dAN = ventral/dorsal attention; HC = healthy controls; adjusted  $R^2 * 100$  = portion of variance of the criterium that be explained with the applied regression model*

## 5.4. Discussion

In the present part of this doctoral thesis, alterations in behavior, brain structure, and brain function that occurred over the period of one year were explored in a sample of MS patients and healthy controls, and related to each other. Meaningful correlations between changes in fatigue severity and both edge and node properties of fcMRI were determined in order to characterize whole-brain profiles of clinical progression in longitudinal resting state fcMRI. Last, global and local re-test reliability of fcMRI was evaluated.

### 5.4.1. Behavioral alterations over time

With respect to behavioral variables, changes in cognitive abilities, severity of overall disability, fatigue, and the manifestation of depression and anxiety symptoms were evaluated.

Neuropsychological performance improved in both controls and patients over time. It must be highlighted that MS patients had completed eight neuropsychological assessments over the study course (four general appointments, four MEG measurements with on- and offline neuropsychological testing) and healthy controls still six. Based on the results, a training effect is therefore reasonable, challenging the in-depth interpretation of the neuropsychological data. The lack of significant interaction effects between groups and time-points could indicate that controls and MS patients "benefited" equally from the study participation, which might be interpreted as evidence against a general learning deficit in the present MS cohort. However, this conclusion must be taken with caution, since observed alterations probably do not reflect natural history as intended, nor a systematic training effect. Changes in cognitive and motor fatigue were not related to neuropsychological performance, so that a similar study effect on those variables must not be assumed.

The longitudinal observation of the clinical status revealed considerable individual alterations in both directions in a large portion of the sample in the overall disability, fatigue, or scores for psychological comorbidities. The substantial inter-individual variability of longitudinal alterations was reflected in the within-group contrasts, where no overall trend towards clinical improvement or deterioration over time became evident in neither MS patients nor healthy controls.

MS has a highly unpredictable and to the present day still poorly understood pathology, and the major clinical challenge is to anticipate the individual course of the disease. While traumatic and sudden impact on functionality due to acute white matter inflammations is a prominent characteristic especially in early phases, the progressive degenerative aspect of MS is nowadays known to gain importance with longer disease duration (Trojano et al., 2003). Persistent cognitive and clinical deterioration will therefore occur almost certainly at some point in time (e.g. Amato et al., 2008). However, transitional improvement of cognitive abilities or clinical status in shorter time periods is possible for numerous reasons, even if general worsening must be expected on the long run. Short-time predictions, such as for a one-year period, are therefore hardly reliable, especially for the relapsing-remitting subtype of MS. From that it follows, that the behavioral findings of this study are in line with the general understanding of MS, reflecting the dynamic nature of this disease.

### 5.4.2. Structural alterations over time

The relationship between conventional MRI outcomes and clinical symptoms in MS is complex and usually weak in empirical studies (Barkhof, 2002). Not even the occurrence of new WM lesions, in the sense of hypo- or hyperintensities in T1-, respectively T2-weighted MR images, is clearly related to the manifestation or worsening of symptoms. On the contrary, it is known that the lesion load increases annually by about 10% in RRMS (Ge, 2006) without attributable behavioral signs or clinically relevant relapses, a phenomenon called *silent lesions*. This finding can be confirmed with the average annual change of WM lesion volume in the present MS sample.

Complementing the insights on WM damage, disturbances of GM have gained attention over the last decade in MS research (Steenwijk et al., 2016). In the current study, GM intensities were found to decrease over time in MS in occipital and parietal areas belonging to the visual network and the DMN. However, meaningful signs for atrophy were detected in healthy controls as well. Together with the lack of convincing interaction effects, it remains open, whether the detected one-year GM alterations result specifically from MS pathology, or whether they primarily reflect structural alterations that occur naturally in the course of healthy aging (Fjell et al., 2010).

### 5.4.3. Functional alterations over time

The analysis of longitudinal alterations of functional integration in MS revealed both substantial increase and decrease of fcMRI in the somatosensory network, and between regions belonging to visual, fronto-parietal, salience, and auditory networks, and the DMN. In healthy controls, less functional connections exhibited significant changes and other regions were involved. Still, the affected networks were also the fronto-parietal and salience networks, and the DMN, consistent with studies indicating a specific relevance of these three networks for cognitive deterioration in healthy aging (Archer et al., 2016; Ng et al., 2016). Nevertheless, the interpretation and explanatory power of those results is bound to the question of how reliable ROI-to-ROI fcMRI can be re-tested. Although stimuli-free fMRI measurements and especially their clinical application as biomarkers became one of the top endeavors in clinical research in the last two decades, the systematic and profound evaluation of its re-test reliability is still an open question and largely ignored in the clinical field. The post-hoc test on the re-test reliability in the present study disclosed that only about 50% of the variance of a later whole-brain fcMRI pattern could get predicted by a former one. Moreover, the prediction of the two-week data turned out to be as good as the prediction of fcMRI a year later. It remains unclear, to what extent this empirical predictability reflects technical or methodological insufficiency, or pathological, respectively physiological alterations, especially since only a part of the post-hoc analysis on reliability could be conducted for healthy controls. For instance, Pinter et al. (2016) found a tendency for lower reproducibility of RSNs after only three months in MS patients when compared to controls. It is open to question whether this finding captured truly occurring changes in fcMRI over such a short time period or whether other influencing factors decreased the reproducibility of fcMRI in the patient group, for example higher measurement-to-measurement variability of physiological confounder variables. In the interests of completeness, it should be mentioned that the re-test reliability for ROI-based fcMRI in this study was in general higher than in the present analysis (ICC = 0.88-0.99 in MS, which equals 64-98% explained variance). This difference is probably related to divergent analytical strategies, in particular their focus on ICA-derived RSNs and their overlapping fractions of both time-points. The direct indications for the interpretation of the present findings is unclear for this reason. Birn et al. (2013) found intersession intra-class correlation coefficients between 0.2 and 0.4 for fcMRI estimates from scans between six and 27 minutes, more in line with the present finding. In addition, the authors revealed increasing re-test reliability for longer scan durations and more data points. To be more specific, the gain in reliability was highest for the comparison between sessions under ten minutes and those around twelve minutes. A beneficial effect of longer scan times was also suggested by a study by Shah et al. (2016), especially for the evaluation of fcMRI on the individual level. Findings of the same study indicate, moreover, regional differences in reliability. In line with the present study, lowest re-test reliability estimates were yielded for subcortical GM nuclei and regions at the junction of GM and WM. This difference could be related to less precise fit of subcortical ROI templates or inaccurate segmentation of deep GM but also to higher physiological variability of the functional communication of subcortical nuclei.

A closer look at the partition-specific scores in the present study disclosed higher re-test reliability and predictability for intra- vs. inter-network interactions. This pattern was found even when the fcMRI was predicted by another person, although with much lower certainty. This outcome confirms a reliable principle of the organization of functional interactions in the human brain that leads to functional assemblies with strong intra-network and weaker inter-network communication. This is well known and in fact the starting point and the subject of large scale functional connectivity investigations. Interestingly, the clustered interaction effects between time and groups were found in functional connections between classical RSNs rather than within, namely between inferior parietal regions of the fronto-parietal network, a temporal ROI belonging to the DMN, and cingulate areas of the salience network. Recently, a study by Yang et al. (2016) has suggested that the underlying modular struc-

ture of functional networks is controlled largely by genetic influences. The interplay between these functional networks, on the other hand, has been shown to be stronger modulated by environmental factors. It is therefore plausible to assume that pathology, whose etiology involves environmental factors, is also reflected in such inter-network functional connectivity. This fits to the present finding of strongest effects on inter-network fcMRI together with only very small effects on node properties, hence then topological organization of the functional connectome. Together, these results suggest that the significance of inter-network interactions in terms of information content on individual pathological characteristics and with respect to diagnostic, prognostic, or monitoring purposes should become a subject of future research.

In the identified cluster with meaningful interaction effect, fcMRI increased in MS patients over time and decreased in controls. An increase of fcMRI in MS has been described in several studies (e.g. Giorgio et al., 2015; Hulst et al., 2015) and has even been discussed as a fundamental, perhaps initial response of the brain to pathological modifications (Hillary et al., 2015). With the present results, a meaningful increase of the strength of functional connectivity can be shown in a prospective longitudinal study, to my knowledge for the first time in MS. The effect sizes for the interaction effects in the present study were, however, particularly small and t-tests for the time-dependent alterations in those connections did not yield significance level. In addition, it remains open to question whether the observed increase reflects beneficial recruitment due to WM disturbances in parallel tracts, equalization of deficits, or a pathological overshoot of functional communication. Further investigation into the functional relevance of such longitudinal fcMRI alterations is needed.

#### **5.4.4. Relationship between behavioral and functional alterations**

A first step towards a deeper understanding of the meaning of those longitudinal changes was the evaluation of interrelations between changes in the severity of fatigue symptoms and fcMRI across the entire functional connectome. This highly explorative whole-brain analysis revealed that patients, whose cognitive fatigue improved over time, tended to exhibit decreasing functional integration between regions and networks involved in cognitive control (Zanto and Gazzaley, 2013) and flexible exchange of information across the brain (Buckner et al., 2008). Improvement in motor fatigue, on the other hand, was correlated with decrease of inter-hemispheric long-range connectivity between areas involved in the processing of visual and somatosensory input, and regions typically engaged in impulse control, and decision making. A link between fatigue severity and both stronger brain activity and functional connectivity has been shown before (DeLuca et al., 2008), in particular for a thalamo-striato-cortical network, encompassing basal ganglia, thalamus, sensorimotor centers, and frontal regions (Engström et al., 2013). But also decreased fcMRI was found to be related to more severe symptoms (Cruz Gómez et al., 2013; Finke et al., 2015). These findings gave rise to two contrary interpretations. First, hyperconnectivity serves as a compensatory mechanism for fatigue. Decreased functional integration would therefore cause worsening of the clinical manifestation or at least prevent improvement. Second, hyperconnectivity reflects higher cognitive demand, which in turn comes with increased energy consumption and as a consequence higher fatigue burden. This theory is supported by recent findings from Pravata et al. (2016). The authors analyzed resting state fcMRI before, immediately after and 30 minutes after a cognitively demanding neuropsychological test in MS patients with fatigue. They found transient cortico-subcortical hyperconnectivity right after the task and increased interactions in fronto-temporal-occipital connections 30 minutes after completion. Most importantly, hyperconnectivity was related to higher severity of fatigue. With the present findings, further evidence is provided for the second theory, for the first time based on a prospective longitudinal study on the natural progression in MS.

The associations between fatigue and graph theoretical features were very weak and will therefore not be discussed in detail. Improvement of both motor and cognitive fatigue was correlated with lower

clustering coefficients, which indicates more efficient functional integration and in consequence less energy consumption (Fornito et al., 2016, p. 287). This outcome is therefore also in line with the second interpretation discussed above.

Taken together, the investigation of longitudinal alterations in MS revealed decreasing structural integrity in occipito-parietal areas together with functional reorganization in the frontal lobe, and a relation between their enhanced, and therefore less efficient integration and increasing severity of fatigue.

#### **5.4.5. Limitations**

Following limitations of this study should be taken into account. The selection criteria allowed for the compilation of a relatively homogenous sample of participants in terms of disease dynamics, and symptoms severity. This approach was supposed to increase the internal validity of the results following general recommendations for explorative investigations. However, the significance of the outcomes for MS patients with comorbidities or in other phases of the disease remains unclear.

It should be noted, that the applied fatigue scales are self-report assessment-tools and therefore bound to the subjective perception of symptom severity. It is well known that the participation in a study alone can influence the perception and even the manifestation of clinical symptoms. Furthermore, self-, but also external assessments are likely to be influenced by a number of temporary confounds, such as positive or negative life events, experience, or mental fitness on the day. As a consequence, it cannot be excluded that alteration of fatigue reflects study-induced placebo effects or the impact of unrelated other circumstances of life in single patients. Anyway, on the group level, there was no evidence for a general study effect. Together with the satisfying scientific quality criteria of the FSMC (Penner et al., 2009), the available fatigue scores seem to constitute a valid variable to correlate brain function with.

The implemented fMRI sequence had a length of approximately ten minutes with 246 data points, which ranges midfield in comparison to other studies in the clinical resting state field. Longer time-series might improve the re-test reliability as discussed above. The benefit from longer scan durations in clinical studies must albeit be put into perspective with the burden of an fMRI scan for the patients. A careful evaluation of the advantages and disadvantages of divergent sequences is therefore necessary.

The acquisition of individual physiological data was technically not feasible in the present study, even though their inclusion certainly improves data quality and therefore the reliability. The implementation of physiological recordings should therefore become a required standard for clinical studies in the future.

The applied measures of structural damage cannot provide a comprehensive picture of the overall disturbance, because the WM lesion volume was extracted automatically rather than by an experienced clinician, and because estimates for GM atrophy were ROI-specific. The validity of these metrics was nevertheless considered sufficient for the purpose of the present study. Anyway, more advanced methodological approaches are desirable for further longitudinal analyses.

From a statistical point of view, the sample size is relatively small for a correlational whole-brain analysis such as the one applied here. Relevant effects might have been underestimated due to the lack of statistical power and identified effects are certainly not particularly reliable in statistical terms. Subsequent studies should follow a confirming approach with a-priori defined foci on circumscribed parts of the functional connectome to confirm or refute the results presented here with higher statistical power and appropriate strategies for MCC.

In general, effects on the group level were very small. This is also related to the observation that individual alterations pointed in both directions. Statistical analyses on the sheer magnitude of change, irrespective of its direction, might have yielded more significant effects. Such an approach is useful to identify functional connections, in which modifications are in general most likely to occur

over time. However, it is not suited to reveal insights into functional alterations that correlate with the clinical manifestation of symptoms, hence to investigate the underlying research question of the present project.

Finally, it must be stated that the results must not be interpreted as potential biomarkers for the development of MS or the prognosis of long-term alterations of fatigue symptoms in this disease. First, all patients in this study had radiologically secured, thus clinically definite MS. To investigate the development of MS in unsecured cases, someone would have to focus on longitudinal alterations in patient with RIS or CIS diagnosis at baseline. Second, MS patients were not compared to any other pathological population. Whether the detected effects are MS-specific or general responses to structural disturbances in the brain cannot be derived from the outcome of this study. Third, the observation period was particularly short for MS. Longer observations are desirable. Last, levels of the variables at baseline were regressed out before computing the relationships between their longitudinal modifications for the final part of the analysis. As a consequence, the results are not indicative for meaningful cross-sectional interrelations, nor are they influenced by them.



**Part IV.**

**General Discussion**



# Synoptic summary

The presented work provides a comprehensive multimodal characterization of a sample of patients suffering from RRMS, comprising structural, functional, and behavioral findings from both cross-sectional and longitudinal analyses. With a special focus on pathological alterations in the functional connectome, diverse aspects of fcMRI and their relationship with cognitive, clinical, and structural variables were examined within a whole-brain framework. The results of this explorative investigation were put into perspective with current literature and conceptual work on functional compensation, dynamics of functional integration, and the development of neuroimaging-based biomarkers. The overall significance of this doctoral thesis for basic research towards a general understanding of physiological organization and pathological intrusion of the human functional connectome, as well as the clinical application and practical usefulness of those insights are summarized in the following sections. Further details on specifics of methodology, findings, or underlying concepts of each analysis can be found in the corresponding discussion sections.

## 1. Organizational principles of functional connectivity

### 1.1. White matter disturbances, the functional connectome, and the concept of functional compensation

Though atrophy of GM has gained attention as an important component of MS in the past years (Steenwijk et al., 2016), inflammatory processes in the WM are still considered the primary pathological correlate, conceptually as well as clinically. The investigation of functional integration characteristics in MS therefore constitutes a unique approach for studying the impact of circumscribed WM damage on brain functioning in humans, distinguishing MS from other clinical populations with traumatic or non-traumatic structural pathology.

The present work provides further insights into the complex and non-trivial effect of WM disturbances on the functional connectome. To begin with, the unconstrained whole-brain approach disclosed substantial differences between structure-function and function-behavior interrelation patterns, suggesting that the translation of WM impairment into manifestations on the behavioral level cannot be explained by local effects alone. Instead, this finding can be interpreted as a sign for a meaningful mediating role of indirect or secondary effects in the functional connectome, facilitating the ramification of the pathological impact beyond primarily affected functional connections. Such functional disease propagation could happen in conjunction with, or complementary to topological reorganization processes within the functional architecture and structural plasticity. The functional expansion of pathological effects does not occur randomly, but emerging patterns are thought to be constrained by the underlying functional network structure (Fornito et al., 2015). Functional widespread of the effect of GM damage and TMS stimulation has been described before and in addition put into relation

with the manifestation of deficits on the behavioral level (Eldaief et al., 2011; Gillebert et al., 2011; Gillebert and Mantini, 2013). In addition, a case study by Jones et al. (2011) disclosed secondarily occurring disruption of the DMN in response to an acute MS lesion close to the thalamus. Altered afferent input was suggested as a possible mechanism for such functional propagation, in accordance with a phenomenon called *functional diaschisis* (Silasi and Murphy, 2014; Fornito et al., 2015; Eldaief et al., 2016). With the present doctoral thesis, further evidence is provided that encourages the transfer of this concept of disease propagation onto the impact of circumscribed WM lesion on the functional connectome.

The findings furthermore lead to the conclusion that WM-related deficiency of one part of the functional connectome does not necessarily have to disturb functional communication elsewhere, but might also facilitate it. This could be explained, to give an example, by reduced or altered inhibitory input, or, on a higher level of abstraction, the disturbance of the normal interplay between functional specialization and integration (Fornito et al., 2015). From this it follows, that the occurrence of meaningful secondary effects constitutes a possible mechanism for the frequently described, yet counterintuitive enhancement of functional communication in the presence of WM damage.

Increased functional connectivity is often interpreted as functional compensation, hence beneficial additional recruitment of cortical or subcortical resources for the successful accomplishment of cognitive or motor tasks. While this assumption could indeed be supported by findings of relevant associations between increasing functional integration and improved behavioral performance in MS, almost the same number of publications provide evidence for the opposite relationship (see section 3, part II). By conducting a whole-brain analysis, both the maintenance of cognitive abilities and their decline were shown to be associated with the enhancement as well as the decrease of functional connectivity in the present doctoral thesis, reflecting the diversity of previous findings in just one dataset. This finding together with the outcome of the systematic literature review draws attention to substantial shortcomings of the conceptualization of functional compensation in the presence of WM lesions. The conceptual distinction of equally relevant primary and secondary impact introduces a new perspective on potential modulatory, or compensatory effect mechanisms. A wide range of hypotheses can be derived from this concept and tested on various temporal and spatial scales, for instance by using neurostimulation techniques, or by further in-depth inquiries into interdependencies of functional modulatory processes and the structural adaptability of the brain. Moreover, it provides an integrative framework for the interpretation of seemingly contradicting and highly diverse findings from previous studies.

Finally, the contradicting results and their interpretations in the current literature on functional compensation in pathological populations disclose remarkable deficiency in the current understanding of the functional relevance of spontaneous functional integration and its relation to behavioral performance (for a detailed discussion see section 3.4, part III). Many details of the exact interrelations are yet to be determined and conceptual groundwork is needed.

## **1.2. Inter-network functional connectivity**

There is strong and often replicated evidence for a highly structured organization of functional integration in the human brain during so-called rest, in response to stimulation, and during the execution of tasks (Smith et al., 2009). Several assemblies of regions were identified that exhibit stronger functional connectivity with one another than with other brain areas. Characterizing and relating those emerging networks to behavior or pathology has been particularly fruitful for a wide range of research questions. Nevertheless, focusing on within-network connectivity only, naturally represents a limited picture of functional integration in the brain. Going beyond this restricted perspective was therefore one of the main reasons to apply consequently whole-brain analyses for the exploration of pathological modifications of fcMRI in the present doctoral thesis. And indeed, the findings provide evidence for

a meaningful functional role of static and dynamic features of functional communication that occurs between specialized networks. Especially the temporal variability of functional between-network interactions was shown to be related to cognitive performance. In line with previous publications touching on this matter, this finding might reflect a substantial role of inter-network functional connectivity for flexible switching between mental or functional states. The investigation of longitudinal alterations of fcMRI indicated moreover that inter-network functional connectivity might have higher sensitivity for physiological development and pathological progression. This interpretation is supported by studies on the differential role of inter- and intra-network interaction in aging (e.g. Ng et al., 2016; Grady et al., 2016), and on pathological alterations in neurological and psychiatric disorders (e.g. Janssen et al., 2013; Cai et al., 2016).

Results by Eldaief et al. (2016) suggest that between-network communication is influenced by certain functional hubs in the frontal part of the DMN. More specifically, they found that circumscribed GM lesions in the medial prefrontal cortex were more likely to modify the interaction of the DMN with other RSNs than the integration within the DMN itself. In addition, Grady et al. (2016) found that inter-network connectivity of the fronto-parietal control network predicted age-dependent decrease of within-network interaction of the DMN. Functional connectivity between the control network and others moreover predicted cognitive performance. The authors interpreted this outcome as supporting evidence for a regulating role of the fronto-parietal control network for flexible switching in dynamic cognitive processing. It remains speculation, whether the central role of the DMN in a wide range of pathological diseases could be explained by its relation to inter-network integration. Still, these findings certainly provide the rationale to further look into this matter and extend the focus from intra- to inter-network functional connectivity in basic and applied neuroscience. Finally, Yang et al. (2016) report findings that indicate that the interplay between functional modules of the brain is largely influenced by environmental factors, while the modular architecture was found to be determined by genetic influences. According to this result, functional inter-network connectivity might indeed be particularly prone to pathological effects and structural impairment. Further research on this question as well as adequate replication studies are necessary to confirm this interpretation.

Taken together, interaction between specialized assemblies might be involved in orchestrating whole-brain integration over time. A profound evaluation of inter-network connectivity with respect to its significance for cognition, pathology, or moment-to-moment functioning is therefore desirable to complement the current one-sided perspective on functional communication in the human brain.

### **1.3. Longitudinal alterations of functional interaction in the healthy brain**

The outcome of the longitudinal analysis revealed that meaningful alterations of functional connectivity could be observed after the relatively short period of only one year even in healthy participants. Physiological modifications of functional connectivity are known to occur naturally in the course of the ontogenetic development and have been described before in the context of adolescence (e.g. Richmond et al., 2016) as well as healthy aging (e.g. Spreng et al., 2016). At the same time, however, resting state fcMRI is thought to yield reproducible patterns across states and subjects (Damoiseaux et al., 2006; Fornito and Bullmore, 2010), and to express reliable individual characteristics (Finn et al., 2015; Tavor et al., 2016). Taken together, this gives rise to questions such as how individual characteristics develop over time, which aspects of functional connectivity are more or less stable, respectively prone to developmental influences, and how functional evolution relates to longitudinal changes in structural connectivity. For instance, the relationship between functional and structural connectivity has been shown to be age-dependent (Betz et al., 2014), and inter- and intra-network communication to change differently over time (Betz et al., 2014; Archer et al., 2016; Ng et al., 2016; Grady et al., 2016). With

respect to the latter, functional alterations in healthy aging seem to be characterized by decreasing fcMRI within functional networks together with intensified communication between networks. The statistical analysis of the present study did not yield obvious evidence supporting or contradicting this observation. The qualitative evaluation of the whole-brain matrix of alterations, on the other hand, disclosed clearly such a pattern in the control group in particular for inter- and intra-network interactions of the DMN.

Beyond that, findings of longitudinal alterations of fcMRI in healthy participants give reason to take a closer look at the most relevant prerequisite for the interpretation of such outcome, namely the re-test reliability of spontaneous fcMRI. Even though all potential clinical applications would rely critically on basic scientific quality criteria such as validity, reliability, and objectivity, not much attention has been given so far to the explicit evaluation of those metrics in the clinical neuroimaging field (Shah et al., 2016). The re-test reliability is also a limiting factor for all longitudinal studies in the area of basic research concerning resting state fcMRI or functional integration in general. Building on earlier cross-sectional work on divergent influencing variables on functional communication (for a short overview see e.g. Castellanos et al., 2013), a recent trend can be detected in the field towards a systematic investigation of re-test-reliability for this reason (e.g. Yan et al., 2013a; Cao et al., 2014; Zuo et al., 2014; Andellini et al., 2015; Shah et al., 2016; Marchitelli et al., 2016; Termenon et al., 2016b; Colclough et al., 2016).

On a general level, identical or at least similar scan conditions seem to be particular relevant for sufficient re-test reliability, considering the impact of state variables on resting state functional connectivity (Geerligts et al., 2015). Study planning should therefore include considerations of influences from circadian fluctuations (Shannon et al., 2013), seasonal effects on the brain (Mc Mahon et al., 2016), or physiological variables such as hunger and arousal (e.g. Lohmann et al., 2010). In addition, several technical and methodological factors can diminish or enhance the re-test reliability, such as the scan duration and certain preprocessing strategies (e.g. Andellini et al., 2015; Shirer et al., 2015, and discussion section 5.4.3, part III). Empirical findings are mixed, and range from low re-test reliability scores for intersession comparisons in Birn et al. (2013) to high ICC scores of around 0.75 for seed-to-ROI multiband fcMRI in Shah et al. (2016). Evaluations of reliability in other modalities paint a similar picture. Colclough et al. (2016) tested the re-test reliability of twelve methods for the characterization of functional connectivity in the alpha-band based on MEG data and found distinct differences. For instance, the consistency of simple amplitude correlations was found to be satisfactory while the re-test reliability for the imaginary part of coherency was poor. Conclusions regarding the overall re-test reliability of spontaneous functional connectivity are difficult to draw for this reason. It must be noted that reported estimates of reliability moreover refer to divergent features of functional networks, such as edge or node properties, network integrity and widespread, or global measures of functional integration. How they relate to each other, is not clear yet. Beyond that, several different terms are used equivalently irrespective of their conceptual base or use in other disciplines, including *reliability*, *reproducibility*, *repeatability*, *replicability*, *validity*, and *bias-variance tradeoff*. The inconsistent terminology is certainly not an isolated or circumscribed problem, but rather a component of a larger methodological issue concerning the replicability of neuroscientific findings (Ioannidis, 2005; Button et al., 2013). An increasing effort towards a uniform conceptual framework and standardized approaches for the assessment and comparison of reliability of resting state functional connectivity is therefore desirable. In addition, further comprehensive longitudinal studies are needed to fully disclose organizational principles underlying healthy evolution of functional integration over time, and to enlarge the body of data for profound evaluations of the reliability of longitudinal functional connectivity analyses.

## 2. Clinical and translational neuroimaging

### 2.1. Insights into neuropathological correlates of multiple sclerosis from a neuroimaging perspective

The conducted analyses revealed relevant new aspects of the neuropathology of MS that complement but also extend previous findings. First, a prominent role of a single functional network for the differentiation between MS patients and healthy controls, or the manifestation of cognitive deficits and fatigue, could not be confirmed by the results of either the cross-sectional and longitudinal study parts, or the systematic literature review. The evaluation of the available literature disclosed moreover that a substantial portion of evidence for specifically affected or involved RSNs was yielded from investigations with predefined foci on certain parts of the functional connectome, which were only partially justified by previous explorative analyses. In addition, substantial deficiency in study quality was revealed. The overall validity and reliability of the body of evidence for the functional relevance of a specific network for MS is therefore questionable. Furthermore, it should be emphasized that altered functional connectivity of a region should not be taken as evidence for the functional disturbance of the associated network per se. Strictly speaking, such conclusion would require evidence for modified interaction within the corresponding network, while the detection of altered communication with nodes of other networks would rather indicate a pathological correlate in between-network interactions. That the one does not necessarily occur concurrently with the other could be shown just recently (Eldaief et al., 2016). To conclude that within-network alterations are indicative of modifications in the between-network integration or the other way around, is not valid for this reason. The present findings strongly suggest that a differentiation between inter- and intra-network connectivity is relevant not only on the conceptual level, but might actually improve the understanding of pathological effects in MS in line with findings by Rocca et al. (2012). Group differences in functional integration features were most evident in links connecting the basal ganglia with the DMN, the salience, and attention networks, and between several cortical RSNs. In addition, the most reliable functional correlates of overall cognitive performance and WM integrity were identified in inter-network connectivity, as well. Last and most importantly, the major outcome of the longitudinal analyses was also found in functional connections between classical RSNs. This indicates that disease progression and symptom development over time is reflected particularly in cross-network functional connectivity, rather than in intra-network integration of specific functional assemblies. In sum, the findings of this doctoral thesis provide evidence for a specific role of communication patterns between functional networks in MS. The in-depth investigation of the prognostic and diagnostic value of inter-network fcMRI is suggested.

Second, no clear picture with respect to a predominant direction of MS-related fcMRI alterations emerged from the literature review or the conducted analyses. While such inconsistency is certainly related to the variability of both methods and illness, it is important to emphasize at this point that the terms *increase* or *decrease* are inherently relational, that means defined with reference to some sort of empirical benchmark. Any conclusions drawn from such empirical results are therefore highly dependent on the suitability of the analyzed contrasts. The systematic literature review disclosed that the selection of control participants was insufficient in some studies, raising the question whether at least some of the inconsistency in neuroimaging findings on MS-related fcMRI alterations might issue from this shortage. In addition, while this terminology necessarily follows the logic of statistical testing, it should be noted that its meaning for the interpretation of actual levels of functional integration

within a group or on the individual level is very limited.

Third, with this doctoral thesis I provide to my knowledge the first evidence for alterations of dynamic fcMRI in MS. The variability of fcMRI was increased in MS patients in functional connections between several RSNs and within the language network. Moreover, higher temporal variability in between-network connections was found to be associated with better cognitive performance and lower neurological symptom burden. This finding indicates that dynamic features of fcMRI have indeed physiological and pathological relevance, possibly related to flexible information processing and state-to-state switching. The outcome of the analysis on dynamics of fcMRI implies for this reason that this methodological strategy constitutes a unique approach towards fcMRI that seems to capture aspects of the pathology of MS that cannot be detected with conventional analyses of fcMRI alone. While several methodological shortcomings have to be considered and overcome in the future (for details see section 4.4, part III), analyzing dynamic features of functional integration could reveal novel insights in neuropathology in general. Developing and adjusting experimental designs for the most beneficial application of this approach is needed and could involve, for instance, continuous data acquisition across changing conditions to examine explicitly the transition from one state to another (e.g. rest to stimulation). Apart from this, insights from fMRI-based analyses should be put into relation with other modalities to disclose the precise neurophysiological and structural underpinnings of dynamic fcMRI metrics.

Fourth, the longitudinal analysis disclosed an association between increasing functional integration over time and worsening of both cognitive and motor fatigue symptoms over the one year study period. Whereas this finding does not provide direct evidence for causality, it still constitutes the first support from a prospective longitudinal study for a prominent theory that proposes a relationship between fatigue and amplified energy consumption due to hyperconnectivity. The outcome is therefore highly relevant for the investigation of the neuropathological correlate of fatigue and requires appropriate replication studies and integration with modalities capturing energy consumption, such as PET.

Finally, it must be highlighted another time that MS is a complex syndrome with a multifaceted clinical picture and multifactorial etiology. None of the studies that were evaluated in the systematic review had put their findings into perspective with insights from other disciplines, although interrelations between suggested effect mechanisms on diverse levels of observation are most likely. As a matter of fact, this critique applies in the same way to the current study. A major challenge for upcoming research on MS will therefore become to bring together insights from a range of disciplines that investigate physiology and pathology on the micro, meso, and macro level, from a theoretical, and empirical perspective, and with explanatory and confirmatory approaches.

## **2.2. In search for new biomarkers**

Extracting clinically useful information from the functional connectome has become a major goal in clinical neuroscience over the last two decades. Especially task- and stimulation-free measurements are considered to constitute a promising tool for neuroimaging-aided diagnostics due to their relatively simple application and unbiased characterization of functional organization patterns. However, the development of biomarkers based on this particular approach, but also in general, is a lengthy and complex process. Conceptual, methodological, and practical issues that came up during the course of this study, or were indicated by either the findings or the systematic literature review are summarized in the following section to provide an overview of the current status quo and challenges of this endeavor in the context of MS.

From a clinical perspective, fcMRI-based tools could aid the diagnostic process in MS with respect to at least three major challenges. First, the clinical picture of MS is extremely diverse, which is why a wide range of differential diagnoses have to be ruled out during the diagnostic process. The identification of MS-specific signatures of functional integration would have great clinical relevance for



this reason. Second, it is still challenging to predict the course of CIS, or RIS patients, hence patients with isolated symptoms that indicate MS, but no definite diagnosis. Biomarkers that disclose insights into individual pathogenesis and the expected progression are desirable (Tumani and Rieckmann, 2015), especially since targeted therapeutic interventions at this early stage could be shown to influence the manifestation of full MS significantly (Comi et al., 2009). Third, functional connectivity metrics might also support dimensional diagnostics, that is the assessment of symptom severity for both cross-sectional description and longitudinal monitoring.

The translation of neuroimaging findings into clinical tools for such purpose is indeed extremely difficult for several reasons. On a general level, acquisition and processing of neuroimaging data can vary considerably due to a large number of technical parameters, methodological alternatives, and differences in hardware, with considerable impact on functional connectivity metrics (Jovicich et al., 2016). Developing a commonly shared standard routine that can be applied as a benchmark is necessary to compare individual findings from different sites and time-points, and would be helpful to integrate group-based findings. Next, the evaluation of macroscopic network characteristics prerequisites the parcellation of the brain in some way. Parcellation approaches, however, vary widely with respect to their resolution (from voxels to lobes) and underlying rationale (e.g. anatomical, functional, network-based). In the present study, ROI-based solutions were preferred to reduce the dimensionality of the applied whole-brain analyses. Relevant effects that occur in subdivisions of ROIs might have been obscured for this reason. Further systematic evaluations of diverse anatomical and functional parcellation schemes is probably useful to find the most appropriate method for clinical applications (e.g. de Reus and van den Heuvel, 2013). As for all kind of diagnostic information, valid benchmarks are a basic requirement for the interpretation of individual information. In the present study the average fcMRI of forty healthy controls was used as a reference to identify relative increase or decrease in MS on the individual level. Better-validated age- and sex-specific benchmarks from larger samples are of course necessary for the real application of neuroimaging tools in clinical diagnostics. Last, the interpretability of individual biomarkers is obviously determined by its overall reliability (for a comprehensive discussion on the reliability of clinical diagnoses see Kraemer, 2014). The re-test reliability of whole-brain functional connectivity was found to be only mid-size in the present study, and also other studies have revealed at least partially insufficient reliability of metrics capturing functional integration based on fMRI data and other modalities (see also section 1.3, part IV). Apart from general recommendations, for instance the consideration of physiological confounds or longer measurements, there is still no consensus on how to improve the reliability of fcMRI. Without significant progress on this issue, an application of functional connectivity-based metrics in a clinical context is highly unlikely in the near future.

More specifically and with respect to the above mentioned challenges in MS, it must be emphasized that whereas several studies describe meaningful alterations of functional connectivity in this disease, none of them could provide sufficient evidence for the specificity of such findings to the present day. A main reason for this shortcoming is the one-sided comparison with only healthy controls that most studies in the field apply, including the present one. Strictly speaking, the only valid conclusion regarding potential biomarkers that can be drawn from such contrast refers to the differentiation between brain-damaged patients and healthy controls, an information that obviously does not have particular clinical relevance. To illustrate the problem, the DMN has been found to be altered in a wide range of disorders, including MS (Rocca et al., 2010; Leavitt et al., 2014), Alzheimer's disease (Greicius et al., 2004), disorders of consciousness (Fernández-Espejo et al., 2012), schizophrenia (Wang et al., 2016), and autism (Spencer et al., 2012; Washington et al., 2014). Whether these findings indeed reflect specific alterations in subdivisions of the DMN, or a general correlate of functional or structural disturbance in the brain, perhaps related to the central role of the DMN, is impossible to disentangle based on these studies. Hence, while the contrast between pathological samples and healthy controls is useful to investigate basic pathological mechanisms, it is not suited to identify

disorder-specific characteristics and therefore biomarkers for categorical diagnostics. Comparative studies across divergent pathologies are needed instead (e.g. Klöppel et al., 2012). With respect to RIS and CIS patients, prospective studies are required to identify fcMRI-based biomarkers with predictive value for the individual course. Finally, as stated before, MS is an exceptionally diverse disease. The representativity of most research samples for the overall population or the individual patient is therefore rather limited. The ethical and scientific reasons that underlie this shortcoming may be reasonable, but it must be highlighted that they also question the general applicability of functional neuroimaging measurements in the diagnostic process for MS patients.

To sum up, the realization of clinical applications of potential biomarkers based on features of the functional connectome is still at an early stage for MS. Improvement of reliability and validity of applied metrics is needed. In addition, open-minded and unconstrained evaluations of the usefulness and prognostic value of such tools in MS will become essential in the future to stay in line with principles of good scientific practice and ethical prerequisites for clinical research. This should involve particularly the careful consideration of benefits and disadvantages of biomarkers based upon functional neuroimaging, including monetary aspects, invested time, technical and methodological demand, clinical availability of different modalities, sensitivity and specificity, and significance for the individual patient against all other available options. For instance, a prominent argument for resting state measurements is its easy application in comparison to task designs. While this is certainly true, fMRI and MEG task- and stimulation-free measurements are still expensive and moreover most demanding for patients. Benefits of resting state measurements should therefore exceed not only those of task designs, but usable information should also be clearly superior when contrasted with conventional neurological, psychological, neuropsychological, or other clinical assessment tools, and in comparison to so-called confounder variables of fcMRI.

### 3. General limitations

Following general limitations of this work should be taken into account, in addition to the limitations of the applied methodological approaches that are discussed in the corresponding sections above. First, the reported findings are not independent on the statistical or the content level, considering that all four analyses were computed based on one single data set. Such an exploratory multimodal and longitudinal observation is on the other hand also a unique opportunity to relate a wide range of aspects and findings to one another. The interdependency of the presented outcomes is therefore both a weakness and a strength of the project. To draw further conclusions regarding the validity and reliability of the results, independent confirmatory studies are of course necessary. Second, the sample of MS patients of the NeuConn project is representative for the neuroscientific research field on fMRI in this disorder, but not for the general population of MS. The significance of the outcome for other patients is therefore unclear. Last, pathological processes can have multifaceted effects on the brain, for instance on its metabolic or hemodynamic characteristics. In addition, functional modifications that result from structural alterations have again an effect on the anatomy of the brain, which in turn might have an impact on the functional level due to the reciprocal relationship between structure and function (see section 1.2, part I). Conclusions concerning principles of the structural or the functional organization of the healthy brain have to be drawn with caution for this reason and confirmed using other experimental or methodological approaches.

### 4. Conclusion

With this doctoral thesis, an up-to-date comprehensive overview is provided, covering status quo, limitations, and upcoming challenges for the investigation of functional integration in MS, as well as for the development of potential clinical applications based on functional interaction metrics.

Based upon this review, it can be stated that the findings from both cross-sectional and longitudinal analyses constitute cutting-edge insights into the pathological underpinnings of MS, in particular into the mediating role of the functional connectome in the translation of structural impairment into behavioral symptoms.

Beyond that, relevant conclusions concerning the interrelationship between brain structure, brain function, and behavior were indicated. They have the potential to enhance the understanding of organizational principles of functional connectivity in the human brain, and furthermore motivate subsequent neuroscientific investigations of the manifold hypotheses that can be derived from the presented findings.



## References

- Achard, S., Salvador, R., Whitcher, B., Suckling, J., and Bullmore, E. (2006). A resilient, low-frequency, small-world human brain functional network with highly connected association cortical hubs. *The Journal of Neuroscience*, 26(1):63–72.
- Airan, R. D., Vogelstein, J. T., Pillai, J. J., Caffo, B., Pekar, J. J., and Sair, H. I. (2016). Factors affecting characterization and localization of interindividual differences in functional connectivity using MRI. *Human Brain Mapping*, 37(5):1986–1997.
- Allen, E. A., Damaraju, E., Plis, S. M., Erhardt, E. B., Eichele, T., and Calhoun, V. D. (2014). Tracking whole-brain connectivity dynamics in the resting state. *Cerebral Cortex*, 24(3):663–676.
- Alonso, A. and Hernán, M. A. (2008). Temporal trends in the incidence of multiple sclerosis: a systematic review. *Neurology*, 71(2):129–135.
- Alstott, J., Breakspear, M., Hagmann, P., Cammoun, L., and Sporns, O. (2009). Modeling the impact of lesions in the human brain. *PLoS Computational Biology*, 5(6):e1000408.
- Alter, M., Leibowitz, U., and Speer, J. (1966). Risk of multiple sclerosis related to age at immigration to israel. *Archives of Neurology*, 15(3):234–237.
- Amato, M. P., Zipoli, V., and Portaccio, E. (2006). Multiple sclerosis-related cognitive changes: a review of cross-sectional and longitudinal studies. *Journal of the Neurological Sciences*, 245(1-2):41–46.
- Amato, M. P., Zipoli, V., and Portaccio, E. (2008). Cognitive changes in multiple sclerosis. *Expert Review of Neurotherapeutics*, 8(10):1585–1596.
- Andellini, M., Cannata, V., Gazzellini, S., and Bernardi, B. (2015). Test-retest reliability of graph metrics of resting state MRI functional brain networks: A review.
- Araneda, R., Renier, L. A., Rombaux, P., Cuevas, I., and De Volder, A. G. (2016). Cortical plasticity and olfactory function in early blindness. *Frontiers in Systems Neuroscience*, 10:75.
- Archer, J. A., Lee, A., Qiu, A., and Chen, S.-H. A. (2016). A comprehensive analysis of connectivity and aging over the adult life span. *Brain Connectivity*, 6(2):169–185.
- Aschenbrenner, A., Tucha, O., and Lange, K. W. (2000). *RWT Regensburger Wortflüssigkeits-Test. Handanweisung*. Hogrefe Verlag, Göttingen.
- Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *NeuroImage*, 38(1):95–113.
- Ashburner, J. and Friston, K. J. (2000). Voxel-based morphometry - The methods. *NeuroImage*, 11(6):805–821.
- Ashburner, J. and Friston, K. J. (2005). Unified segmentation. *NeuroImage*, 26(3):839–851.
- Barkhof, F. (2002). The clinico-radiological paradox in multiple sclerosis revisited. *Current Opinion in Neurology*, 15(3):239–45.

- Barnett, M. H., Henderson, A. P. D., and Prineas, J. W. (2006). The macrophage in MS: Just a scavenger after all? Pathology and pathogenesis of the acute MS lesion. *Multiple Sclerosis Journal*, 12(2):121–132.
- Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences*, 160(901):268–282.
- Bartzokis, G., Cummings, J. L., Sultzer, D., Henderson, V. W., Nuechterlein, K. H., and Mintz, J. (2003). White matter structural integrity in healthy aging adults and patients with Alzheimer disease: a magnetic resonance imaging study. *Archives of Neurology*, 60(3):393–398.
- Basile, B., Castelli, M., Monteleone, F., Nocentini, U., Caltagirone, C., Centonze, D., Cercignani, M., and Bozzali, M. (2014). Functional connectivity changes within specific networks parallel the clinical evolution of multiple sclerosis. *Multiple Sclerosis Journal*, 20(8):1050–1057.
- Bassett, D. S., Bullmore, E., Verchinski, B. A., Mattay, V. S., Weinberger, D. R., and Meyer-Lindenberg, A. (2008). Hierarchical organization of human cortical networks in health and schizophrenia. *The Journal of Neuroscience*, 28(37):9239–9248.
- Behzadi, Y., Restom, K., Liau, J., and Liu, T. T. (2007). A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *NeuroImage*, 37(1):90–101.
- Benedict, R. H., Wahlig, E., Bakshi, R., Fishman, I., Munschauer, F., Zivadinov, R., and Weinstock-Guttman, B. (2005). Predicting quality of life in multiple sclerosis: accounting for physical disability, fatigue, cognition, mood disorder, personality, and behavior change. *Journal of the Neurological Sciences*, 231(1-2):29–34.
- Benjamin, C., Lieberman, D. A., Chang, M., Ofen, N., Whitfield-Gabrieli, S., Gabrieli, J. D. E., and Gaab, N. (2010). The influence of rest period instructions on the default mode network. *Frontiers in Human Neuroscience*, 4:218.
- Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B*, 57(1):289–300.
- Betzal, R. F., Byrge, L., He, Y., Goñi, J., Zuo, X.-N., and Sporns, O. (2014). Changes in structural and functional connectivity among resting-state networks across the human lifespan. *NeuroImage*, 102:345–357.
- Betzal, R. F., Satterthwaite, T. D., Gold, J. I., and Bassett, D. S. (2016). A positive mood, a flexible brain. *arXiv preprint 2016; arXiv:1601.07881*.
- Birn, R. M., Molloy, E. K., Patriat, R., Parker, T., Meier, T. B., Kirk, G. R., Nair, V. A., Meyerand, M. E., and Prabhakaran, V. (2013). The effect of scan length on the reliability of resting-state fMRI connectivity estimates. *NeuroImage*, 83:550–558.
- Biswal, B. B. (2012). Resting state fMRI: A personal history. *NeuroImage*, 62(2):938–944.
- Biswal, B. B., Zerrin Yetkin, F., Haughton, V. M., and Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine*, 34(4):537–541.
- Bodini, B., Veronese, M., García-Lorenzo, D., Battaglini, M., Poirion, E., Chardain, A., Freeman, L., Louapre, C., Tchikviladze, M., Papeix, C., Dollé, F., Zalc, B., Lubetzki, C., Bottlaender, M., Turkheimer, F., and Stankoff, B. (2016). Dynamic imaging of individual remyelination profiles in multiple sclerosis. *Annals of Neurology*, 79(5):726–738.

- Bonavita, S., Gallo, A., Sacco, R., Corte, M. D., Biseco, A., Docimo, R., Lavorgna, L., Corbo, D., Costanzo, A. D., Tortora, F., Cirillo, M., Esposito, F., and Tedeschi, G. (2011). Distributed changes in default-mode resting-state connectivity in multiple sclerosis. *Multiple Sclerosis Journal*, 17(4):411–422.
- Bowyer, S. M. (2016). Coherence a measure of the brain networks: past and present. *Neuropsychiatric Electrophysiology*, 2(1).
- Braley, T. J. and Chervin, R. D. (2010). Fatigue in multiple sclerosis: mechanisms, evaluation, and treatment. *Sleep*, 33(8):1061–1067.
- Braun, U., Schäfer, A., Walter, H., Erk, S., Romanczuk-Seiferth, N., Haddad, L., Schweiger, J. I., Grimm, O., Heinz, A., Tost, H., Meyer-Lindenberg, A., and Bassett, D. S. (2015). Dynamic re-configuration of frontal brain networks during executive cognition in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 112(37):11678–11683.
- Breckel, T. P. K., Thiel, C. M., Bullmore, E. T., Zalesky, A., Patel, A. X., and Giessing, C. (2013). Long-term effects of attentional performance on functional brain network topology. *PLoS One*, 8(9):e74125.
- Brenner, P., Burkill, S., Jokinen, J., Hillert, J., Bahmanyar, S., and Montgomery, S. (2016). Multiple sclerosis and risk of attempted and completed suicide - a cohort study. *European Journal of Neurology*, 23(8):1329–1336.
- Brookes, M. J., Woolrich, M., Luckhoo, H., Price, D., Hale, J. R., Stephenson, M. C., Barnes, G. R., Smith, S. M., and Morris, P. G. (2011). Investigating the electrophysiological basis of resting state networks using magnetoencephalography. *Proceedings of the National Academy of Sciences of the United States of America*, 108(40):16783–16788.
- Browne, P., Chandraratna, D., Angood, C., Tremlett, H., Baker, C., Taylor, B. V., and Thompson, A. J. (2014). Atlas of multiple sclerosis 2013: A growing global problem with widespread inequity. *Neurology*, 83(11):1022–1024.
- Brück, W. (2005). The pathology of multiple sclerosis is the result of focal inflammatory demyelination with axonal damage. *Journal of Neurology*, 252(Suppl 5):v3–9.
- Buckner, R. L., Andrews-Hanna, J. R., and Schacter, D. L. (2008). The brain’s default network: Anatomy, function, and relevance to disease. *Annals of the New York Academy of Sciences*, 1124:1–38.
- Bullmore, E. and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature Reviews. Neuroscience*, 10(3):186–198.
- Butler, M. A. and Bennett, T. L. (2003). In search of a conceptualization of multiple sclerosis: A historical perspective. *Neuropsychology Review*, 13(2):93–112.
- Button, K. S., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., and Munafò, M. R. (2013). Power failure: Why small sample size undermines the reliability of neuroscience. *Nature Reviews. Neuroscience*, 14(5):365–376.
- Cabral, J., Hugues, E., Kringelbach, M. L., and Deco, G. (2012). Modeling the outcome of structural disconnection on resting-state functional connectivity. *NeuroImage*, 62(3):1342–1353.

- Cai, S., Chong, T., Peng, Y., Shen, W., Li, J., von Deneen, K. M., Huang, L., and Alzheimer's Disease Neuroimaging Initiative (2016). Altered functional brain networks in amnesic mild cognitive impairment: A resting-state fMRI study. *Brain Imaging and Behavior*, pages 1–13.
- Calabrese, M., Agosta, F., Rinaldi, F., Mattisi, I., Grossi, P., Favaretto, A., Atzori, M., Bernardi, V., Barachino, L., Rinaldi, L., Perini, P., Gallo, P., and Filippi, M. (2009). Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. *Archives of Neurology*, 66(9):1144–1150.
- Calabrese, P. and Penner, I. K. (2007). Cognitive dysfunctions in multiple sclerosis - A "multiple disconnection syndrome"? *Journal of Neurology*, 254(Suppl 2):II/18–II/21.
- Calhoun, V. D. D., Miller, R., Pearlson, G., and Adali, T. (2014). The chronnectome: Time-varying connectivity networks as the next frontier in fMRI data discovery. *Neuron*, 84(2):262–274.
- Cao, H., Plichta, M. M., Schäfer, A., Haddad, L., Grimm, O., Schneider, M., Esslinger, C., Kirsch, P., Meyer-Lindenberg, A., and Tost, H. (2014). Test-retest reliability of fMRI-based graph theoretical properties during working memory, emotion processing, and resting state. *NeuroImage*, 84:888–900.
- Castellanos, F. X., Di Martino, A., Craddock, R. C., Mehta, A. D., and Milham, M. P. (2013). Clinical applications of the functional connectome. *NeuroImage*, 80:527–540.
- Chang, C., Cunningham, J. P., and Glover, G. H. (2009). Influence of heart rate on the BOLD signal: the cardiac response function. *NeuroImage*, 44(3):857–869.
- Chang, C. and Glover, G. H. (2010). Time-frequency dynamics of resting-state brain connectivity measured with fMRI. *NeuroImage*, 50(1):81–98.
- Chard, D. T., Jackson, J. S., Miller, D. H., and Wheeler-Kingshott, C. A. (2010). Reducing the impact of white matter lesions on automated measures of brain gray and white matter volumes. *Journal of Magnetic Resonance Imaging*, 32(1):223–228.
- Chard, D. T. and Miller, D. H. (2016). What lies beneath grey matter atrophy in multiple sclerosis? *Brain*, 139(Pt 1):7–10.
- Chaudhuri, A. and Behan, P. O. (2000). Fatigue and basal ganglia. *Journal of the Neurological Sciences*, 179(1-2):34–42.
- Chaudhuri, A. and Behan, P. O. (2004). Fatigue in neurological disorders. *The Lancet*, 363(9413):978–988.
- Chiaravalloti, N. D. and DeLuca, J. (2008). Cognitive impairment in multiple sclerosis. *The Lancet Neurology*, 7(12):1139–1151.
- Cogliati Dezza, I., Zito, G., Tomasevic, L., Filippi, M. M., Ghazaryan, A., Porcaro, C., Squitti, R., Ventriglia, M., Lupoi, D., and Tecchio, F. (2015). Functional and structural balances of homologous sensorimotor regions in multiple sclerosis fatigue. *Journal of Neurology*, 262(3):614–622.
- Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. L. Erlbaum Associates, Hillsdale N.J., 2 edition.
- Cohen, J. (1992). A power primer. *Psychological Bulletin*, 112(1):155–159.
- Colasanti, A., Guo, Q., Giannetti, P., Wall, M. B., Newbould, R. D., Bishop, C., Onega, M., Nicholas, R., Ciccarelli, O., Muraro, P. A., Malik, O., Owen, D. R., Young, A. H., Gunn, R. N., Piccini, P., Matthews, P. M., and Rabiner, E. A. (2015). Hippocampal neuroinflammation, functional connectivity, and depressive symptoms in multiple sclerosis. *Biological Psychiatry*, 80(1):62–72.



- Colclough, G. L., Woolrich, M. W., Tewarie, P. K., Brookes, M. J., Quinn, A. J., and Smith, S. M. (2016). How reliable are MEG resting-state connectivity metrics? *NeuroImage*, 138:284–293.
- Cole, M. W., Bassett, D. S., Power, J. D., Braver, T. S., and Petersen, S. E. (2014). Intrinsic and task-evoked network architectures of the human brain. *Neuron*, 83(1):238–251.
- Cole, M. W., Reynolds, J. R., Power, J. D., Repovs, G., Anticevic, A., and Braver, T. S. (2013). Multi-task connectivity reveals flexible hubs for adaptive task control. *Nature Neuroscience*, 16(9):1348–1355.
- Collin, G. and van den Heuvel, M. P. (2013). The ontogeny of the human connectome: development and dynamic changes of brain connectivity across the life span. *The Neuroscientist*, 19(6):616–628.
- Comi, G., Martinelli, V., Rodegher, M., Moiola, L., Bajenaru, O., Carra, A., Elovaara, I., Fazekas, F., Hartung, H. P., Hillert, J., King, J., Komoly, S., Lubetzki, C., Montalban, X., Myhr, K. M., Ravnborg, M., Rieckmann, P., Wynn, D., Young, C., and Filippi, M. (2009). Effect of glatiramer acetate on conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome (PreCISe study): A randomised, double-blind, placebo-controlled trial. *The Lancet*, 374(9700):1503–1511.
- Confavreux, C., Vukusic, S., and Adeleine, P. (2003). Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. *Brain*, 126(Pt 4):770–782.
- Confavreux, C., Vukusic, S., Moreau, T., and Adeleine, P. (2000). Relapses and progression of disability in multiple sclerosis. *New England Journal of Medicine*, 343(20):1430–1438.
- Cordes, D., Haughton, V. M., Arfanakis, K., Carew, J. D., Turski, P. A., Moritz, C. H., Quigley, M. A., and Meyerand, M. E. (2001). Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. *AJNR. American Journal of Neuroradiology*, 22(7):1326–1333.
- Cover, K. S., Vrenken, H., Geurts, J. J. G., van Oosten, B. W., Jelles, B., Polman, C. H., Stam, C. J., and van Dijk, B. W. (2006). Multiple sclerosis patients show a highly significant decrease in alpha band interhemispheric synchronization measured using MEG. *NeuroImage*, 29(3):783–788.
- Cruz Gómez, Á. J., Ventura Campos, N., Belenguer, A., Ávila, C., and Forn, C. (2013). Regional brain atrophy and functional connectivity changes related to fatigue in multiple sclerosis. *PLoS One*, 8(10):e77914.
- Cruz Gomez, Á. J., Ventura-Campos, N., Belenguer, A., Ávila, C., and Forn, C. (2014). The link between resting-state functional connectivity and cognition in MS patients. *Multiple Sclerosis Journal*, 20(3):338–348.
- Daams, M., Steenwijk, M. D., Schoonheim, M. M., Wattjes, M. P., Balk, L. J., Tewarie, P. K., Killestein, J., Uitdehaag, B. M., Geurts, J. J., and Barkhof, F. (2016). Multi-parametric structural magnetic resonance imaging in relation to cognitive dysfunction in long-standing multiple sclerosis. *Multiple Sclerosis Journal*, 22(5):608–619.
- Damoiseaux, J. S. and Greicius, M. D. (2009). Greater than the sum of its parts: A review of studies combining structural connectivity and resting-state functional connectivity. *Brain Structure & Function*, 213(6):525–533.
- Damoiseaux, J. S., Rombouts, S. A. R. B., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., and Beckmann, C. F. (2006). Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences of the United States of America*, 103(37):13848–13853.

- de Reus, M. A. and van den Heuvel, M. P. (2013). The parcellation-based connectome: Limitations and extensions. *NeuroImage*, 80:397–404.
- Deco, G. and Kringelbach, M. L. (2016). Metastability and coherence: Extending the communication through coherence hypothesis using a whole-brain computational perspective. *Trends in Neurosciences*, 39(3):125–135.
- Deco, G., Tononi, G., Boly, M., and Kringelbach, M. L. (2015). Rethinking segregation and integration: contributions of whole-brain modelling. *Nature Reviews. Neuroscience*, 16(7):430–439.
- DeLuca, G. C., Yates, R. L., Beale, H., and Morrow, S. A. (2015). Cognitive impairment in multiple sclerosis: Clinical, radiologic and pathologic insights. *Brain Pathology*, 25(1):79–98.
- DeLuca, J., Genova, H. M., Hillary, F. G., and Wylie, G. (2008). Neural correlates of cognitive fatigue in multiple sclerosis using functional MRI. *Journal of the Neurological Sciences*, 270(1-2):28–39.
- Demirtas, M., Tornador, C., Falcón, C., López-Solà, M., Hernández-Ribas, R., Pujol, J., Menchón, J. M., Ritter, P., Cardoner, N., Soriano-Mas, C., and Deco, G. (2016). Dynamic functional connectivity reveals altered variability in functional connectivity among patients with major depressive disorder. *Human Brain Mapping*, 37(8):2918–2930.
- D’Esposito, M., Cooney, J. W., Gazzaley, A., Gibbs, S. E. B., and Postle, B. R. (2006). Is the prefrontal cortex necessary for delay task performance? Evidence from lesion and fMRI data. *Journal of the International Neuropsychological Society*, 12(2):248–260.
- Dogonowski, A.-M., Andersen, K. W., Madsen, K. H., Sørensen, P. S., Paulson, O. B., Blinkenberg, M., and Siebner, H. R. (2013a). Multiple sclerosis impairs regional functional connectivity in the cerebellum. *NeuroImage. Clinical*, 4:130–138.
- Dogonowski, A. M., Siebner, H. R., Soelberg Sørensen, P., Paulson, O. B., Dyrby, T. B., Blinkenberg, M., and Madsen, K. H. (2013b). Resting-state connectivity of pre-motor cortex reflects disability in multiple sclerosis. *Acta Neurologica Scandinavica*, 128(5):328–35.
- Dogonowski, A.-M., Siebner, H. R., Soelberg Sørensen, P., Wu, X., Biswal, B., Paulson, O. B., Dyrby, T. B., Skimminge, A., Blinkenberg, M., and Madsen, K. H. (2013c). Expanded functional coupling of subcortical nuclei with the motor resting-1 Dogonowski A-M, Siebner HR, Sørensen PS, et al. Expanded functional coupling of subcortical nuclei with the motor resting-state network in multiple sclerosis. *Mult Scler J* 2013; 1. *Multiple Sclerosis Journal*, 19(5):559–566.
- Droby, A., Yuen, K. S. L., Muthuraman, M., Reitz, S.-C., Fleischer, V., Klein, J., Gracien, R.-M., Ziemann, U., Deichmann, R., Zipp, F., and Groppa, S. (2015). Changes in brain functional connectivity patterns are driven by an individual lesion in MS: A resting-state fMRI study. *Brain Imaging and Behavior*, page [Epub ahead of print].
- Du, Y., Pearlson, G. D., Yu, Q., He, H., Lin, D., Sui, J., Wu, L., and Calhoun, V. D. (2016). Interaction among subsystems within default mode network diminished in schizophrenia patients: A dynamic connectivity approach. *Schizophrenia Research*, 170(1):55–65.
- Eldaief, M. C., Halko, M. A., Buckner, R. L., and Pascual-Leone, A. (2011). Transcranial magnetic stimulation modulates the brain’s intrinsic activity in a frequency-dependent manner. *Proceedings of the National Academy of Sciences of the United States of America*, 108(52):21229–21234.
- Eldaief, M. C., McMains, S., Hutchison, R. M., Halko, M. A., and Pascual-Leone, A. (2016). Reconfiguration of intrinsic functional coupling patterns following circumscribed network lesions. *Cerebral Cortex*, page [Epub ahead of print].

- Engel, A. K., Fries, P., König, P., Brecht, M., and Singer, W. (1999). Temporal binding, binocular rivalry, and consciousness. *Consciousness and Cognition*, 8(2):128–151.
- Engel, A. K., Gerloff, C., Hilgetag, C. C., and Nolte, G. (2013). Intrinsic coupling modes: Multiscale interactions in ongoing brain activity. *Neuron*, 80(4):867–886.
- Engel, A. K. and Singer, W. (2001). Temporal binding and the neural correlates of sensory awareness. *Trends in Cognitive Sciences*, 5(1):16–25.
- Engström, M., Flensner, G., Landtblom, A. M., Ek, A. C., and Karlsson, T. (2013). Thalamo-striato-cortical determinants to fatigue in multiple sclerosis. *Brain and Behavior*, 3(6):715–728.
- Eryilmaz, H., Van De Ville, D., Schwartz, S., and Vuilleumier, P. (2014). Lasting impact of regret and gratification on resting brain activity and its relation to depressive traits. *The Journal of Neuroscience*, 34(23):7825–7835.
- Eshaghi, A., Riyahi-Alam, S., Saeedi, R., Roostaei, T., Nazeri, A., Aghsaei, A., Doosti, R., Ganjgahi, H., Bodini, B., Shakourirad, A., Pakravan, M., Ghana’ati, H., Firouznia, K., Zarei, M., Azimi, A. R., and Sahraian, M. A. (2015). Classification algorithms with multi-modal data fusion could accurately distinguish neuromyelitis optica from multiple sclerosis. *NeuroImage. Clinical*, 7:306–314.
- Faivre, A., Rico, A., Zaaraoui, W., Crespy, L., Reuter, F., Wybrecht, D., Soulier, E., Malikova, I., Confort-Gouny, S., Cozzone, P. J., Pelletier, J., Ranjeva, J.-P., and Audoin, B. (2012). Assessing brain connectivity at rest is clinically relevant in early multiple sclerosis. *Multiple Sclerosis Journal*, 18(9):1251–1258.
- Faivre, A., Rico, A., Zaaraoui, W., Reuter, F., Confort-Gouny, S., Guye, M., Pelletier, J., Ranjeva, J.-P., and Audoin, B. (2015). Brain functional plasticity at rest and during action in multiple sclerosis patients. *Journal of Clinical Neuroscience*, 22(9):1438–1443.
- Faivre, A., Robinet, E., Guye, M., Rousseau, C., Maarouf, A., Le Troter, A., Zaaraoui, W., Rico, A., Crespy, L., Soulier, E., Confort-Gouny, S., Pelletier, J., Achard, S., Ranjeva, J.-P., and Audoin, B. (2016). Depletion of brain functional connectivity enhancement leads to disability progression in multiple sclerosis: A longitudinal resting-state fMRI study. *Multiple Sclerosis Journal*, page [Epub ahead of print].
- Fasbender, P. and Kolmel, H. W. (2008). Incidence of multiple sclerosis in the urban area of Erfurt, Thuringia, Germany. *Neuroepidemiology*, 30(3):147–151.
- Fernández-Espejo, D., Soddu, A., Cruse, D., Palacios, E. M., Junque, C., Vanhaudenhuyse, A., Rivas, E., Newcombe, V., Menon, D. K., Pickard, J. D., Laureys, S., and Owen, A. M. (2012). A role for the default mode network in the bases of disorders of consciousness. *Annals of Neurology*, 72(3):335–343.
- Fiest, K., Walker, J., Bernstein, C., Graff, L., Zarychanski, R., Abou-Setta, A., Patten, S., Sareen, J., Bolton, J., Marriott, J., Fisk, J., Singer, A., and Marrie, R. (2016). Systematic review and meta-analysis of interventions for depression and anxiety in persons with multiple sclerosis. *Multiple Sclerosis and Related Disorders*, 5:12–26.
- Filippi, M., Absinta, M., and Rocca, M. (2013). Future MRI tools in multiple sclerosis. *Journal of the Neurological Sciences*, 331(1-2):14–18.
- Filippi, M., Wolinsky, J. S., Sormani, M. P., Comi, G., and The European/Canadian Glatiramer Acetate Study Group (2001). Enhancement frequency decreases with increasing age in relapsing-remitting multiple sclerosis. *Neurology*, 56(3):422–423.

- Fingelkurts, A. A., Fingelkurts, A. A., and Kähkönen, S. (2005). Functional connectivity in the brain-is it an elusive concept? *Neuroscience and Biobehavioral Reviews*, 28(8):827–836.
- Finke, C., Schlichting, J., Papazoglou, S., Scheel, M., Freing, A., Soemmer, C., Pech, L. L., Pajkert, A., Pfüller, C., Wuerfel, J., Ploner, C., Paul, F., and Brandt, A. U. (2015). Altered basal ganglia functional connectivity in multiple sclerosis patients with fatigue. *Multiple Sclerosis Journal*, 21(7):925–934.
- Finn, E. S., Shen, X., Scheinost, D., Rosenberg, M. D., Huang, J., Chun, M. M., Papademetris, X., and Constable, R. T. (2015). Functional connectome fingerprinting: Identifying individuals using patterns of brain connectivity. *Nature Neuroscience*, 18:1664–1671.
- Fisk, J. D., Pontefract, A., Ritvo, P. G., Archibald, C. J., and Murray, T. (1994). The impact of fatigue on patients with multiple sclerosis. *The Canadian Journal of Neurological Sciences*, 21(1):9–14.
- Fjell, A. M., Westlye, L. T., Espeseth, T., Reinvang, I., Dale, A. M., Holland, D., and Walhovd, K. B. (2010). Cortical gray matter atrophy in healthy aging cannot be explained by undetected incipient cognitive disorders: a comment on Burgmans et al. (2009). *Neuropsychology*, 24(2):258–263; discussion 264–266.
- Flachenecker, P. and Zettl, U. (2015a). Kapitel 7 - Krankheitsverlauf und Prognose. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 59–69. Elsevier, München, 6. edition.
- Flachenecker, P. and Zettl, U. K. (2015b). Kapitel 2 - Epidemiologie. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 11–17. Elsevier, München, 6. edition.
- Fornito, A. and Bullmore, E. T. (2010). What can spontaneous fluctuations of the blood oxygenation-level-dependent signal tell us about psychiatric disorders? *Current Opinion in Psychiatry*, 23(3):239–249.
- Fornito, A., Zalesky, A., and Breakspear, M. (2015). The connectomics of brain disorders. *Nature Reviews Neuroscience*, 16(3):159–172.
- Fornito, A., Zalesky, A., and Bullmore, E. (2016). *Fundamentals of Brain Network Analysis*. Elsevier, Oxford, 1. edition.
- Fox, M. D. and Greicius, M. (2010). Clinical applications of resting state functional connectivity. *Frontiers in Systems Neuroscience*, 4:19.
- Fox, M. D., Halko, M. A., Eldaief, M. C., and Pascual-Leone, A. (2012). Measuring and manipulating brain connectivity with resting state functional connectivity magnetic resonance imaging (fcMRI) and transcranial magnetic stimulation (TMS). *NeuroImage*, 62(4):2232–2243.
- Fries, P. (2015). Rhythms for cognition: Communication through coherence. *Neuron*, 88(1):220–235.
- Friston, K. J. (1994). Functional and effective connectivity in neuroimaging: A synthesis. *Human Brain Mapping*, 2(1-2):56–78.
- Friston, K. J. (2011). Functional and effective connectivity: A review. *Brain Connectivity*, 1(1):13–36.
- Gaab, N., Gabrieli, J. D., and Glover, G. H. (2008). Resting in peace or noise: Scanner background noise suppresses default-mode network. *Human Brain Mapping*, 29(7):858–867.

- Gamboa, O. L., Tagliazucchi, E., Von Wegner, F., Jurcoane, A., Wahl, M., Laufs, H., and Ziemann, U. (2014). Working memory performance of early MS patients correlates inversely with modularity increases in resting state functional connectivity networks. *NeuroImage*, 94:385–395.
- Garcés, P., Pereda, E., Hernández-Tamames, J. A., Del-Pozo, F., Maestú, F., and Pineda-Pardo, J. A. (2016). Multimodal description of whole brain connectivity: A comparison of resting state MEG, fMRI, and DWI. *Human Brain Mapping*, 37(1):20–34.
- Ge, Y. (2006). Multiple sclerosis: The role of MR imaging. *AJNR. American Journal of Neuroradiology*, 27(6):1165–1176.
- Geerligs, L., Rubinov, M., Cam-CAN, and Henson, R. N. (2015). State and trait components of functional connectivity: Individual differences vary with mental state. *The Journal of Neuroscience*, 35(41):13949–13961.
- Gemmel, C., Haas, J., Harzheim, M., Hoffmann, F. A., Hoffmann, V., Köhler, W., Martin, R., Pöhlau, D., Schippling, S., Schöttner, R., and Stangel, M. (2015). Kapitel 18 - Pathophysiologisch ansetzende Therapie. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 247–319. Elsevier, München, 6. edition.
- Gibbons, J. D. (1974). Nonparametric statistical methods. *Technometrics*, 16(3):477–478.
- Gillebert, C. R. and Mantini, D. (2013). Functional connectivity in the normal and injured brain. *The Neuroscientist*, 19(5):509–522.
- Gillebert, C. R., Mantini, D., Thijs, V., Sunaert, S., Dupont, P., and Vandenberghe, R. (2011). Lesion evidence for the critical role of the intraparietal sulcus in spatial attention. *Brain*, 134(Pt 6):1694–1709.
- Giorgio, A., Stromillo, M. L., De Leucio, A., Rossi, F., Brandes, I., Hakiki, B., Portaccio, E., Amato, M. P., and De Stefano, N. (2015). Appraisal of brain connectivity in radiologically isolated syndrome by modeling imaging measures. *The Journal of Neuroscience*, 35(2):550–558.
- Glahn, D. C., Winkler, A. M., Kochunov, P., Almasy, L., Duggirala, R., Carless, M. A., Curran, J. C., Olvera, R. L., Laird, A. R., Smith, S. M., Beckmann, C. F., Fox, P. T., and Blangero, J. (2010). Genetic control over the resting brain. *Proceedings of the National Academy of Sciences of the United States of America*, 107(3):1223–1228.
- Grady, C., Sarraf, S., Saverino, C., and Campbell, K. (2016). Age differences in the functional interactions among the default, frontoparietal control, and dorsal attention networks. *Neurobiology of Aging*, 41:159–172.
- Gratton, C., Nomura, E. M., Pérez, F., and D’Esposito, M. (2012). Focal brain lesions to critical locations cause widespread disruption of the modular organization of the brain. *Journal of Cognitive Neuroscience*, 24(6):1275–1285.
- Greicius, M. D., Krasnow, B., Reiss, A. L., and Menon, V. (2003). Functional connectivity in the resting brain: A network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 100(1):253–258.
- Greicius, M. D., Srivastava, G., Reiss, A. L., and Menon, V. (2004). Default-mode network activity distinguishes Alzheimer’s disease from healthy aging: Evidence from functional MRI. *Proceedings of the National Academy of Sciences of the United States of America*, 101(13):4637–4642.

- Gronseth, G. S. and Ashman, E. J. (2000). Practice parameter: The usefulness of evoked potentials in identifying clinically silent lesions in patients with suspected multiple sclerosis (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*, 54(9):1720–1725.
- Gronwall, D. M. A. (1977). Paced auditory serial-addition task: A measure of recovery from concussion. *Perceptual and Motor Skills*, 44(2):367–373.
- Grothe, M., Lotze, M., Langner, S., and Dressel, A. (2016). The role of global and regional gray matter volume decrease in multiple sclerosis. *Journal of Neurology*, 263(6):1137–1145.
- Guye, M., Bettus, G., Bartolomei, F., Cozzone, P. J., Guye, M., Bettus, G., Cozzone, P. J., and Bartolomei, F. (2010). Graph theoretical analysis of structural and functional connectivity MRI in normal and pathological brain networks. *MAGMA*, 23(5-6):409–421.
- Hagmann, P., Cammoun, L., Gigandet, X., Meuli, R., Honey, C. J., Wedeen, V. J., and Sporns, O. (2008). Mapping the structural core of human cerebral cortex. *PLoS Biology*, 6(7):e159.
- Haider, L., Zrzavy, T., Hametner, S., Höftberger, R., Bagnato, F., Grabner, G., Trattinig, S., Pfeifenbring, S., Brück, W., and Lassmann, H. (2016). The topography of demyelination and neurodegeneration in the multiple sclerosis brain. *Brain*, 139(Pt 3):807–815.
- Hall, E. L., Robson, S. E., Morris, P. G., and Brookes, M. J. (2013). The relationship between MEG and fMRI. *NeuroImage*, 102(Pt 1):80–91.
- Hardmeier, M., Schoonheim, M. M., Geurts, J., Hillebrand, A., Polman, C. H., Barkhof, F., and Stam, C. J. (2012). Cognitive dysfunction in early multiple sclerosis: Altered centrality derived from resting-state functional connectivity using magneto-encephalography. *PLoS One*, 7(7):e42087.
- Hardt, C. (2015). Kapitel 3 - Genetik und Umweltfaktoren. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 19–30. Elsevier, München, 6. edition.
- Hawellek, D. J., Hipp, J. F., Lewis, C. M., Corbetta, M., and Engel, A. K. (2011). Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, 108(47):19066–19071.
- He, B. J., Shulman, G. L., Snyder, A. Z., and Corbetta, M. (2007). The role of impaired neuronal communication in neurological disorders. *Current Opinion in Neurology*, 20(6):655–660.
- Helfrich, R. F., Knepper, H., Nolte, G., Strüber, D., Rach, S., Herrmann, C. S., Schneider, T. R., and Engel, A. K. (2014). Selective modulation of interhemispheric functional connectivity by HD-tACS shapes perception. *PLoS Biology*, 12(12):e1002031.
- Helmstaedter, C., Lendt, M., and Lux, S. (2001). *Verbaler Lern- und Merkfähigkeitstest: VLMT, Manual*. Beltz Test GmbH, Göttingen.
- Hernán, M. A., Jick, S. S., Logroscino, G., Olek, M. J., Ascherio, A., and Jick, H. (2005). Cigarette smoking and the progression of multiple sclerosis. *Brain*, 128(6):1461–1465.
- Herrmann-Lingen, C., Scholz, K. H., and Kreuzer, H. (1991). [Psychologic screening of patients of a cardiologic acute care clinic with the German version of the Hospital Anxiety and Depression Scale]. *Psychotherapie, Psychosomatik, medizinische Psychologie*, 41(2):83–92.
- Hillary, F. G., Roman, C. A., Venkatesan, U., Rajtmajer, S. M., Bajo, R., and Castellanos, N. D. (2015). Hyperconnectivity is a fundamental response to neurological disruption. *Neuropsychology*, 29(1):59–75.

- Hindriks, R., Adhikari, M. H., Murayama, Y., Ganzetti, M., Mantini, D., Logothetis, N. K., and Deco, G. (2016). Can sliding-window correlations reveal dynamic functional connectivity in resting-state fMRI? *NeuroImage*, 127:242–256.
- Hinkley, L. B., Mizuri, D., Hong, O., Nagarajan, S. S., and Cheung, S. W. (2015). Increased striatal functional connectivity with auditory cortex in tinnitus. *Frontiers in Human Neuroscience*, 9:568.
- Hirtz, D., Thurman, D. J., Gwinn-Hardy, K., Mohamed, M., Chaudhuri, A. R., and Zalutsky, R. (2007). How common are the "common" neurologic disorders? *Neurology*, 68(5):326–337.
- Hobart, J., Freeman, J., and Thompson, A. (2000). Kurtzke scales revisited: the application of psychometric methods to clinical intuition. *Brain*, 123(Pt 5):1027–1040.
- Hoffmann, F. (2002). Kapitel 17 - Symptomatische Therapie. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 182–236. Elsevier, München, 6. edition.
- Hoffmann, S., Tittgemeyer, M., and von Cramon, D. Y. (2007). Cognitive impairment in multiple sclerosis. *Current Opinion in Neurology*, 20(3):275–280.
- Honey, C. J., Sporns, O., Cammoun, L., Gigandet, X., Thiran, J. P., Meuli, R., and Hagmann, P. (2009). Predicting human resting-state functional connectivity from structural connectivity. *Proceedings of the National Academy of Sciences of the United States of America*, 106(6):2035–2040.
- Horn, A., Ostwald, D., Reisert, M., and Blankenburg, F. (2014). The structural-functional connectome and the default mode network of the human brain. *NeuroImage*, 102(Pt 1):142–151.
- Horwitz, B. (2003). The elusive concept of brain connectivity. *NeuroImage*, 19(2 Pt 1):466–470.
- Hubbard, N. A., Turner, M., Hutchison, J. L., Ouyang, A., Strain, J., Oasay, L., Sundaram, S., Davis, S., Remington, G., Brigante, R., Huang, H., Hart, J., Frohman, T., Frohman, E., Biswal, B. B., and Rypma, B. (2015). Multiple sclerosis-related white matter microstructural change alters the BOLD hemodynamic response. *Journal of Cerebral Blood Flow & Metabolism*, page [Epub ahead of print].
- Hulst, H. E., Schoonheim, M. M., Van Geest, Q., Uitdehaag, B. M., Barkhof, F., and Geurts, J. J. (2015). Memory impairment in multiple sclerosis: Relevance of hippocampal activation and hippocampal connectivity. *Multiple Sclerosis Journal*, 21(13):1705–1712.
- Hutchison, R. M., Womelsdorf, T., Allen, E. A., Bandettini, P. A., Calhoun, V. D., Corbetta, M., Della Penna, S., Duyn, J. H., Glover, G. H., Gonzalez-Castillo, J., Handwerker, D. a., Keilholz, S., Kiviniemi, V., Leopold, D. a., de Pasquale, F., Sporns, O., Walter, M., and Chang, C. (2013). Dynamic functional connectivity: Promise, issues, and interpretations. *NeuroImage*, 80:360–378.
- Ignacio, R. J., Liliana, P., and Edgardo, C. (2010). Oligoclonal bands and MRI in clinically isolated syndromes: Predicting conversion time to multiple sclerosis. *Journal of Neurology*, 257(7):1188–1191.
- Ioannidis, J. P. A. (2005). Why Most Published Research Findings Are False. *PLoS Medicine*, 2(8):e124.
- Irimia, A. and Van Horn, J. D. (2014). Systematic network lesioning reveals the core white matter scaffold of the human brain. *Frontiers in Human Neuroscience*, 8:51.
- Islam, T., Gauderman, W. J., Cozen, W., and Mack, T. M. (2007). Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. *Neurology*, 69(4):381–388.

- Janssen, A. L., Boster, A., Patterson, B. A., Abduljalil, A., and Prakash, R. S. (2013). Resting-state functional connectivity in multiple sclerosis: An examination of group differences and individual differences. *Neuropsychologia*, 51(13):2918–2929.
- Jones, D. T., Mateen, F. J., Lucchinetti, C. F., Jack, C. R., and Welker, K. M. (2011). Default mode network disruption secondary to a lesion in the anterior thalamus. *Archives of Neurology*, 68(2):242–247.
- Jones, D. T., Vemuri, P., Murphy, M. C., Gunter, J. L., Senjem, M. L., Machulda, M. M., Przybelski, S. A., Gregg, B. E., Kantarci, K., Knopman, D. S., Boeve, B. F., Petersen, R. C., and Jack, C. R. (2012). Non-stationarity in the "resting brain's" modular architecture. *PLoS One*, 7(6):e39731.
- Jones, T. B., Bandettini, P. a., and Birn, R. M. (2008). Integration of motion correction and physiological noise regression in fMRI. *NeuroImage*, 42(2):582–590.
- Jovicich, J., Minati, L., Marizzoni, M., Marchitelli, R., Sala-Llonch, R., Bartrés-Faz, D., Arnold, J., Benninghoff, J., Fiedler, U., Roccatagliata, L., Picco, A., Nobili, F., Blin, O., Bombois, S., Lopes, R., Bordet, R., Sein, J., Ranjeva, J.-P., Didic, M., Gros-Dagnac, H., Payoux, P., Zoccatelli, G., Alessandrini, F., Beltramello, A., Bargalló, N., Ferretti, A., Caulo, M., Aiello, M., Cavaliere, C., Soricelli, A., Parnetti, L., Tarducci, R., Floridi, P., Tzolaki, M., Constantinidis, M., Drevelegas, A., Rossini, P. M., Marra, C., Schönknecht, P., Hensch, T., Hoffmann, K.-T., Kuijjer, J. P., Visser, P. J., Barkhof, F., Frisoni, G. B., and PharmaCog Consortium (2016). Longitudinal reproducibility of default-mode network connectivity in healthy elderly participants: A multicentric resting-state fMRI study. *NeuroImage*, 124(Pt A):442–454.
- Kaiser, H. F. (1970). A second generation little jiffy. *Psychometrika*, 35(4):401–415.
- Kingwell, E., Marriott, J. J., Jetté, N., Pringsheim, T., Makhani, N., Morrow, S. A., Fisk, J. D., Evans, C., Béland, S. G., Kulaga, S., Dykeman, J., Wolfson, C., Koch, M. W., and Marrie, R. A. (2013). Incidence and prevalence of multiple sclerosis in Europe: A systematic review. *BMC Neurology*, 13:128.
- Klöppel, S., Abdulkadir, A., Jack, C. R., Koutsouleris, N., Mourão-Miranda, J., and Vemuri, P. (2012). Diagnostic neuroimaging across diseases. *NeuroImage*, 61(2):457–463.
- Koch, M., Kingwell, E., Rieckmann, P., and Tremlett, H. (2009). The natural history of primary progressive multiple sclerosis. *Neurology*, 73(23):1996–2002.
- Koch, M. A., Norris, D. G., and Hund-Georgiadis, M. (2002). An investigation of functional and anatomical connectivity using magnetic resonance imaging. *NeuroImage*, 16(1):241–250.
- Koch-Henriksen, N. and Sørensen, P. S. (2010). The changing demographic pattern of multiple sclerosis epidemiology. *The Lancet Neurology*, 9(5):520–532.
- Koenig, K. A., Lowe, M. J., Lin, J., Sakaie, K. E., Stone, L., Bermel, R. A., Beall, E. B., Rao, S. M., Trapp, B. D., and Phillips, M. D. (2013). Sex differences in resting-state functional connectivity in multiple sclerosis. *AJNR. American Journal of Neuroradiology*, 34(12):2304–2311.
- Köhler, W. (2015). Kapitel 6 - Klinik. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 55–62. Elsevier, München, 6. edition.
- Kollndorfer, K., Krajnik, J., Woitek, R., Freiherr, J., Prayer, D., and Schöpf, V. (2013). Altered likelihood of brain activation in attention and working memory networks in patients with multiple sclerosis: An ALE meta-analysis. *Neuroscience & Biobehavioral Reviews*, 37(10):2699–2708.



- Kopell, N. J., Gritton, H. J., Whittington, M. A., and Kramer, M. A. (2014). Beyond the connectome: The dynamome. *Neuron*, 83(6):1319–1328.
- Kotchoubey, B., Merz, S., Lang, S., Markl, A., Müller, F., Yu, T., and Schwarzbauer, C. (2013). Global functional connectivity reveals highly significant differences between the vegetative and the minimally conscious state. *Journal of Neurology*, 260(4):975–983.
- Kraemer, H. C. (2014). The reliability of clinical diagnoses: State of the art. *Annual Review of Clinical Psychology*, 10(1):111–130.
- Krupp, L. B. (2003). Fatigue in multiple sclerosis: Definition, pathophysiology and treatment. *CNS drugs*, 17(4):225–234.
- Krupp, L. B., Alvarez, L. A., LaRocca, N. G., Scheinberg, L. C., Freal JE, Kraft GH, C. J., TJ, M., et al Schumacher GA, Beebe G, Kibler RF, JF, K., Scott PJ, H. E., LS, R., Whitlock FA, S. M., et al Rabins PV, Brooks BR, O'Donnell P, and et al Joffe RT, Lippert GP, Gray TA (1988). Fatigue in Multiple Sclerosis. *Archives of Neurology*, 45(4):435–437.
- Kucyi, A., Hove, M. J., Esterman, M., Hutchison, R. M., and Valera, E. M. (2016). Dynamic brain network correlates of spontaneous fluctuations in attention. *Cerebral Cortex*, page [Epub ahead of print].
- Kuhlmann, T., Lingfeld, G., Bitsch, A., Schuchardt, J., and Brück, W. (2002). Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain*, 125(Pt 10):2202–2212.
- Kunze, T., Hunold, A., Haueisen, J., Jirsa, V., and Spiegler, A. (2016). Transcranial direct current stimulation changes resting state functional connectivity: A large-scale brain network modeling study. *NeuroImage*, 140:174–187.
- Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology*, 33(11):1444–1452.
- Langs, G., Wang, D., Golland, P., Mueller, S., Pan, R., Sabuncu, M. R., Sun, W., Li, K., and Liu, H. (2016). Identifying shared brain networks in individuals by decoupling functional and anatomical variability. *Cerebral Cortex*, 26(10):4004–4014.
- Leavitt, V. M., Paxton, J., and Sumowski, J. F. (2014). Default network connectivity is linked to memory status in multiple sclerosis. *Journal of the International Neuropsychological Society*, 20(9):937–944.
- Lee, M. H., Hacker, C. D., Snyder, A. Z., Corbetta, M., Zhang, D., Leuthardt, E. C., and Shimony, J. S. (2012). Clustering of resting state networks. *PloS One*, 7(7):e40370.
- Lee, M. H., Smyser, C. D., and Shimony, J. S. (2013). Resting-state fMRI: A review of methods and clinical applications. *American Journal of Neuroradiology*, 34(10):1866–1872.
- Lenne, B., Blanc, J.-L., Nandrino, J.-L., Gallois, P., Hauteceœur, P., and Pezard, L. (2013). Decrease of mutual information in brain electrical activity of patients with relapsing-remitting multiple sclerosis. *Behavioural Neurology*, 27(2):201–212.
- Leocani, L., Locatelli, T., Martinelli, V., Rovaris, M., Falautano, M., Filippi, M., Magnani, G., and Comi, G. (2000). Electroencephalographic coherence analysis in multiple sclerosis: Correlation with clinical, neuropsychological, and MRI findings. *Journal of Neurology, Neurosurgery, and Psychiatry*, 69(2):192–198.

- Leonardi, N., Richiardi, J., Gschwind, M., Simioni, S., Annoni, J. M., Schlupe, M., Vuilleumier, P., and Van De Ville, D. (2013). Principal components of functional connectivity: A new approach to study dynamic brain connectivity during rest. *NeuroImage*, 83:937–950.
- Leonardi, N. and Van De Ville, D. (2015). On spurious and real fluctuations of dynamic functional connectivity during rest. *NeuroImage*, 104:430–436.
- Lerdal, A., Gulowsen Celius, E., Krupp, L., and Dahl, A. A. (2007). A prospective study of patterns of fatigue in multiple sclerosis. *European Journal of Neurology*, 14(12):1338–1343.
- Levine, T. R. and Hullett, C. R. (2002). Eta squared, partial eta squared, and misreporting of effect size in communication research. *Human Communication Research*, 28(4):612–625.
- Li, S.-J., Li, Z., Wu, G., Zhang, M.-J., Franczak, M., and Antuono, P. G. (2002). Alzheimer disease: Evaluation of a functional MR imaging index as a marker. *Radiology*, 225(1):253–259.
- Lindquist, M. A., Xu, Y., Nebel, M. B., and Caffo, B. S. (2014). Evaluating dynamic bivariate correlations in resting-state fMRI: A comparison study and a new approach. *NeuroImage*, 1(101):531–546.
- Link, J., Kockum, I., Lorentzen, A. R., Lie, B. A., Celius, E. G., Westerlind, H., Schaffer, M., Alfredsson, L., Olsson, T., Brynedal, B., Harbo, H. F., and Hillert, J. (2012). Importance of human leukocyte antigen (HLA) class I and II alleles on the risk of multiple sclerosis. *PLoS One*, 7(5):e36779.
- Liu, Y., Dai, Z., Duan, Y., Huang, J., Ren, Z., Liu, Z., Dong, H., Shu, N., Vrenken, H., Wattjes, M. P., Barkhof, F., He, Y., and Li, K. (2016). Whole brain functional connectivity in clinically isolated syndrome without conventional brain MRI lesions. *European Radiology*, 26(9):2982–2991.
- Liu, Y., Duan, Y., Huang, J., Ren, Z., Ye, J., Dong, H., Shi, F.-D., Barkhof, F., Vrenken, H., Wattjes, M. P., Wang, J., and Li, K. (2015a). Multimodal quantitative MR imaging of the thalamus in multiple sclerosis and neuromyelitis optica. *Radiology*, 277(3):784–792.
- Liu, Y., Liang, P., Duan, Y., Huang, J., Ren, Z., Jia, X., Dong, H., Ye, J., Shi, F.-d., Butzkueven, H., and Li, K. (2015b). Altered thalamic functional connectivity in multiple sclerosis. *European Journal of Radiology*, 84(4):703–708.
- Liu, Y., Liang, P., Duan, Y., Jia, X., Yu, C., Zhang, M., Wang, F., Zhang, M., Dong, H., Ye, J., Butzkueven, H., and Li, K. (2011). Brain plasticity in relapsing-remitting multiple sclerosis: Evidence from resting-state fMRI. *Journal of the Neurological Sciences*, 304(1-2):127–131.
- Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., and Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature*, 412(6843):150–157.
- Lohmann, G., Margulies, D. S., Horstmann, A., Pleger, B., Lepsien, J., Goldhahn, D., Schloegl, H., Stumvoll, M., Villringer, A., and Turner, R. (2010). Eigenvector centrality mapping for analyzing connectivity patterns in fMRI data of the human brain. *PLoS One*, 5(4):e10232.
- Loitfelder, M., Filippi, M., Rocca, M., Valsasina, P., Ropele, S., Jehna, M., Fuchs, S., Schmidt, R., Neuper, C., Fazekas, F., and Enzinger, C. (2012). Abnormalities of resting state functional connectivity are related to sustained attention deficits in MS. *PLoS One*, 7(8):e42862.
- Louapre, C., Perlberg, V., García-Lorenzo, D., Urbanski, M., Benali, H., Assouad, R., Galanaud, D., Freeman, L., Bodini, B., Papeix, C., Tourbah, A., Lubetzki, C., Lehericy, S., and Stankoff, B. (2014). Brain networks disconnection in early multiple sclerosis cognitive deficits: An anatomofunctional study. *Human Brain Mapping*, 35(9):4706–4717.

- Lowe, M. J., Beall, E. B., Sakaie, K. E., Koenig, K. A., Stone, L., Marrie, R. A., and Phillips, M. D. (2008). Resting state sensorimotor functional connectivity in multiple sclerosis inversely correlates with transcallosal motor pathway transverse diffusivity. *Human Brain Mapping*, 29(7):818–827.
- Lowe, M. J., Koenig, K. A., Beall, E. B., Sakaie, K. A., Stone, L., Bermel, R., and Phillips, M. D. (2014). Anatomic connectivity assessed using pathway radial diffusivity is related to functional connectivity in monosynaptic pathways. *Brain Connectivity*, 4(7):558–565.
- Lowe, M. J., Phillips, M. D., Lurito, J. T., Mattson, D., Dziedzic, M., and Mathews, V. P. (2002). Multiple sclerosis: Low-frequency temporal blood oxygen level-dependent fluctuations indicate reduced functional connectivity initial results. *Radiology*, 224(1):184–192.
- Lublin, F. D., Reingold, S. C., Cohen, J. A., Cutter, G. R., Sorensen, P. S., Thompson, A. J., Wolinsky, J. S., Balcer, L. J., Banwell, B., Barkhof, F., Bebo, B., Calabresi, P. A., Clanet, M., Comi, G., Fox, R. J., Freedman, M. S., Goodman, A. D., Inglese, M., Kappos, L., Kieseier, B. C., Lincoln, J. A., Lubetzki, C., Miller, A. E., Montalban, X., O'Connor, P. W., Petkau, J., Pozzilli, C., Rudick, R. A., Sormani, M. P., Stuve, O., Waubant, E., and Polman, C. H. (2014). Defining the clinical course of multiple sclerosis: The 2013 revisions. *Neurology*, 83(3):278–286.
- Lucas, R. M., Ponsonby, A.-L., Dear, K., Valery, P. C., Pender, M. P., Taylor, B. V., Kilpatrick, T. J., Dwyer, T., Coulthard, A., Chapman, C., van der Mei, I., Williams, D., and McMichael, A. J. (2011). Sun exposure and vitamin D are independent risk factors for CNS demyelination. *Neurology*, 76(6):540–548.
- Lucchinetti, C., Brück, W., Parisi, J., Scheithauer, B., Rodriguez, M., and Lassmann, H. (2000). Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. *Annals of Neurology*, 47(6):707–717.
- Lünemann, J. D., Kamradt, T., Martin, R., and Münz, C. (2007). Epstein-barr virus: Environmental trigger of multiple sclerosis? *Journal of Virology*, 81(13):6777–6784.
- Marchitelli, R., Minati, L., Marizzoni, M., Bosch, B., Bartrés-Faz, D., Müller, B. W., Wiltfang, J., Fiedler, U., Roccatagliata, L., Picco, A., Nobili, F., Blin, O., Bombois, S., Lopes, R., Bordet, R., Sein, J., Ranjeva, J. P., Didic, M., Gros-Dagnac, H., Payoux, P., Zoccatelli, G., Alessandrini, F., Beltramello, A., Bargalló, N., Ferretti, A., Caulo, M., Aiello, M., Cavaliere, C., Soricelli, A., Parnetti, L., Tarducci, R., Floridi, P., Tsolaki, M., Constantinidis, M., Drevelegas, A., Rossini, P. M., Marra, C., Schönknecht, P., Hensch, T., Hoffmann, K. T., Kuijjer, J. P., Visser, P. J., Barkhof, F., Frisoni, G. B., and Jovicich, J. (2016). Test-retest reliability of the default mode network in a multi-centric fMRI study of healthy elderly: Effects of data-driven physiological noise correction techniques. *Human Brain Mapping*, 37(6):2114–2132.
- Marrelec, G. and Fransson, P. (2011). Assessing the influence of different ROI selection strategies on functional connectivity analyses of fMRI data acquired during steady-state conditions. *PloS One*, 6(4):e14788.
- Massey, F. J. (1951). The kolmogorov-smirnov test for goodness of fit. *Source Journal of the American Statistical Association*, 46(253):68–78.
- Matter, M. G., Betzel, R. F., and Bassett, D. S. (2016). The flexible brain. *Brain*, 139(Pt 8):2110–2112.
- Mc Mahon, B. E. M., Andersen, S. B., Madsen, M. K., Hjordt, L. V., Hageman, I., Dam, H., Svarer, C., da Cunha-Bang, S., Baaré, W., Madsen, J., Hasholdt, L., Holst, K., Frokjaer, V. G., and Knudsen, G. M. (2016). Seasonal difference in brain serotonin transporter binding predicts symptom severity in patients with seasonal affective disorder. *Brain*, 139:1605–1614.

- McDonald, W. I., Compston, A., Edan, G., Goodkin, D., Hartung, H. P., Lublin, F. D., McFarland, H. F., Paty, D. W., Polman, C. H., Reingold, S. C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., van den Noort, S., Weinshenker, B. Y., and Wolinsky, J. S. (2001). Recommended diagnostic criteria for multiple sclerosis: Guidelines from the International Panel on the diagnosis of multiple sclerosis. *Annals of Neurology*, 50(1):121–127.
- McGraw, K. O. and Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychological Methods*, 1(1):30–46.
- Minerbi, A., Kahana, R., Goldfeld, L., Kaufman, M., Marom, S., and Ziv, N. E. (2009). Long-term relationships between synaptic tenacity, synaptic remodeling, and network activity. *PLoS Biology*, 7(6):e1000136.
- Morcom, A. M. and Fletcher, P. C. (2007). Does the brain have a baseline? Why we should be resisting a rest. *NeuroImage*, 37(4):1073–1082.
- Mostert, J. C., Shumskaya, E., Mennes, M., Onnink, A. M. H., Hoogman, M., Kan, C. C., Arias Vasquez, A., Buitelaar, J., Franke, B., and Norris, D. G. (2016). Characterising resting-state functional connectivity in a large sample of adults with ADHD. *Progress in Neuropsychopharmacology & Biological Psychiatry*, 67:82–91.
- Murphy, K., Birn, R. M., and Bandettini, P. A. (2013). Resting-state fMRI confounds and cleanup. *NeuroImage*, 80:349–359.
- Murphy, K., Birn, R. M., Handwerker, D. A., Jones, T. B., and Bandettini, P. A. (2009). The impact of global signal regression on resting state correlations: Are anti-correlated networks introduced? *NeuroImage*, 44(3):893–905.
- Nagaraj, K., Taly, A. B., Gupta, A., Prasad, C., and Christopher, R. (2013). Prevalence of fatigue in patients with multiple sclerosis and its effect on the quality of life. *Journal of Neurosciences in Rural Practice*, 4(3):278–282.
- Ng, K. K., Lo, J. C., Lim, J. K., Chee, M. W., and Zhou, J. (2016). Reduced functional segregation between the default mode network and the executive control network in healthy older adults: A longitudinal study. *NeuroImage*, 133:321–330.
- Nicot, A. (2009). Gender and sex hormones in multiple sclerosis pathology and therapy. *Frontiers in Bioscience*, 14:4477–4515.
- Nourbakhsh, B., Azevedo, C., Nunan-Saah, J., Maghzi, A.-H., Spain, R., Pelletier, D., and Waubant, E. (2016). Longitudinal associations between brain structural changes and fatigue in early MS. *Multiple Sclerosis and Related Disorders*, 5:29–33.
- Öckinger, J., Hagemann-Jensen, M., Kullberg, S., Engvall, B., Eklund, A., Grunewald, J., Piehl, F., Olsson, T., and Wahlström, J. (2016). T-cell activation and HLA-regulated response to smoking in the deep airways of patients with multiple sclerosis. *Clinical Immunology*, 169:114–120.
- Oldfield, R. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*, 9:97–113.
- Ombao, H. and Van Bellegem, S. (2008). Evolutionary coherence of nonstationary signals. *IEEE Transactions on Signal Processing*, 56(6):2259–2266.

- Ontaneda, D., Sakaie, K., Lin, J., Wang, X., Lowe, M. J., Phillips, M. D., and Fox, R. J. (2014). Identifying the start of multiple sclerosis injury: A serial DTI study. *Journal of Neuroimaging*, 24(6):569–576.
- Pallant, J. (2001). *SPSS survival manual*. Open University Press, Ballmoor, Philadelphia, 10 edition.
- Patriat, R., Molloy, E. K., Meier, T. B., Kirk, G. R., Nair, V. A., Meyerand, M. E., Prabhakaran, V., and Birn, R. M. (2013). The effect of resting condition on resting-state fMRI reliability and consistency: A comparison between resting with eyes open, closed, and fixated. *NeuroImage*, 78:463–473.
- Pender, M. P. and Burrows, S. R. (2014). Epstein-barr virus and multiple sclerosis: Potential opportunities for immunotherapy. *Clinical & Translational Immunology*, 3(10):e27.
- Penner, I. K., Raselli, C., Stöcklin, M., Opwis, K., Kappos, L., and Calabrese, P. (2009). The Fatigue Scale for Motor and Cognitive Functions (FSMC): Validation of a new instrument to assess multiple sclerosis-related fatigue. *Multiple Sclerosis Journal*, 15(12):1509–1517.
- Petersen, G., Wittmann, R., Arndt, V., and Göppfarth, D. (2014). Epidemiologie der Multiplen Sklerose in Deutschland. *Nervenarzt*, 85(8):990–998.
- Petsas, N., Tomassini, V., Filippini, N., Sbardella, E., Tona, F., Piattella, M. C., Pozzilli, C., Wise, R. G., and Pantano, P. (2015). Impaired functional connectivity unmasked by simple repetitive motor task in early relapsing-remitting multiple sclerosis. *Neurorehabilitation and Neural Repair*, 29(6):557–567.
- Picchioni, D., Duyn, J. H., and Horowitz, S. G. (2013). Sleep and the functional connectome. *NeuroImage*, 80:387–396.
- Pinter, D., Beckmann, C., Koini, M., Pirker, E., Filippini, N., Pichler, A., Fuchs, S., Fazekas, F., and Enzinger, C. (2016). Reproducibility of resting state connectivity in patients with stable multiple sclerosis. *PLoS One*, 11(3):e0152158.
- Pipinis, E., Melynyte, S., Koenig, T., Jarutyte, L., Linkenkaer-Hansen, K., Ruksenas, O., and Griskova-Bulanova, I. (2016). Association between resting-state microstates and ratings on the amsterdam resting-state questionnaire. *Brain Topography*, page [Epub ahead of print].
- Pittock, S. J., Mayr, W. T., McClelland, R. L., Jorgensen, N. W., Weigand, S. D., Noseworthy, J. H., and Rodriguez, M. (2004a). Disability profile of MS did not change over 10 years in a population-based prevalence cohort. *Neurology*, 62(4):601–606.
- Pittock, S. J., Mayr, W. T., McClelland, R. L., Jorgensen, N. W., Weigand, S. D., Noseworthy, J. H., Weinshenker, B. G., and Rodriguez, M. (2004b). Change in MS-related disability in a population-based cohort: A 10-year follow-up study. *Neurology*, 62(1):51–59.
- Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F. D., Montalban, X., O’Connor, P., Sandberg-Wollheim, M., Thompson, A. J., Waubant, E., Weinshenker, B., and Wolinsky, J. S. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of Neurology*, 69(2):292–302.
- Polman, C. H., Reingold, S. C., Edan, G., Filippi, M., Hartung, H.-P., Kappos, L., Lublin, F. D., Metz, L. M., McFarland, H. F., O’Connor, P. W., Sandberg-Wollheim, M., Thompson, A. J., Weinshenker, B. G., and Wolinsky, J. S. (2005). Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Annals of Neurology*, 58(6):840–846.

- Popescu, V., Battaglini, M., Hoogstrate, W. S., Verfaillie, S. C. J., Sluimer, I. C., van Schijndel, R. a., van Dijk, B. W., Cover, K. S., Knol, D. L., Jenkinson, M., Barkhof, F., de Stefano, N., and Vrenken, H. (2012). Optimizing parameter choice for FSL-Brain Extraction Tool (BET) on 3D T1 images in multiple sclerosis. *NeuroImage*, 61(4):1484–1494.
- Poser, C. M. (2006). The multiple sclerosis trait and the development of multiple sclerosis: Genetic vulnerability and environmental effect. *Clinical Neurology and Neurosurgery*, 108(3):227–233.
- Power, J. D., Cohen, A. L., Nelson, S. S. M., Wig, G. S., Barnes, K. A., Church, J. A., Vogel, A. C., Laumann, T. O., Miezin, F. M., Schlaggar, B. L., and Petersen, S. E. (2011). Functional network organization of the human brain. *Neuron*, 72(4):665–678.
- Pravata, E., Zecca, C., Sestieri, C., Caulo, M., Riccitelli, G. C., Rocca, M. A., Filippi, M., Cianfoni, A., and Gobbi, C. (2016). Hyperconnectivity of the dorsolateral prefrontal cortex following mental effort in multiple sclerosis patients with cognitive fatigue. *Multiple Sclerosis Journal*, page [Epub ahead of print].
- Price, T., Wee, C.-Y., Gao, W., and Shen, D. (2014). Multiple-Network Classification of Childhood Autism. *Medical Image Computing and Computer-Assisted Intervention*, 17(Pt 3):177–184.
- Raichle, M. E. (2015). The brain’s default mode network. *Annual Review of Neuroscience*, 38(1):433–447.
- Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., and Shulman, G. L. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America*, 98(2):676–682.
- Raichle, M. E. and Mintun, M. A. (2006). Brain work and brain imaging. *Annual Review of Neuroscience*, 29:449–476.
- Reinshagen, A. (2015). Kapitel 14 - Neurophysiologie und MS. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 185–200. Elsevier, München, 6. edition.
- Renoux, C., Vukusic, S., Mikaeloff, Y., Edan, G., Clanet, M., Dubois, B., Debouverie, M., Brochet, B., Lebrun-Frenay, C., Pelletier, J., Moreau, T., Lubetzki, C., Vermersch, P., Roullet, E., Magy, L., Tardieu, M., Suissa, S., and Confavreux, C. (2007). Natural history of multiple sclerosis with childhood onset. *New England Journal of Medicine*, 356(25):2603–2613.
- Reuter-Lorenz, P. A. and Cappell, K. A. (2008). Neurocognitive aging and the compensation hypothesis. *Current Directions in Psychological Science*, 17(3):177–182.
- Riccelli, R., Passamonti, L., Cerasa, A., Nigro, S., Cavalli, S. M., Chiriaco, C., Valentino, P., Nistico, R., and Quattrone, A. (2016). Individual differences in depression are associated with abnormal function of the limbic system in multiple sclerosis patients. *Multiple Sclerosis Journal*, 22(8):1094–1105.
- Richiardi, J., Gschwind, M., Simioni, S., Annoni, J.-M., Greco, B., Hagmann, P., Schlupe, M., Vuilleumier, P., and Van De Ville, D. (2012). Classifying minimally disabled multiple sclerosis patients from resting state functional connectivity. *NeuroImage*, 62(3):2021–2033.
- Richmond, S., Johnson, K. A., Seal, M. L., Allen, N. B., and Whittle, S. (2016). Development of Brain Networks and Relevance of Environmental and Genetic Factors: A Systematic Review. *Neuroscience & Biobehavioral Reviews*, 71:215–239.

- Rocca, M. A., Pravatà, E., Valsasina, P., Radaelli, M., Colombo, B., Vacchi, L., Gobbi, C., Comi, G., Falini, A., and Filippi, M. (2015). Hippocampal-DMN disconnectivity in MS is related to WM lesions and depression. *Human Brain Mapping*, 36(12):5051–5063.
- Rocca, M. A., Valsasina, P., Absinta, M., Riccitelli, G., Rodegher, M. E., Misci, P., Rossi, P., Falini, A., Comi, G., and Filippi, M. (2010). Default-mode network dysfunction and cognitive impairment in progressive MS. *Neurology*, 74(16):1252–1259.
- Rocca, M. A., Valsasina, P., Hulst, H. E., Abdel-Aziz, K., Enzinger, C., Gallo, A., Pareto, D., Riccitelli, G., Muhlert, N., Ciccarelli, O., Barkhof, F., Fazekas, F., Tedeschi, G., Arévalo, M. J., Filippi, M., and The MAGNIMS fMRI Study Group (2014). Functional correlates of cognitive dysfunction in multiple sclerosis: A multicenter fMRI Study. *Human Brain Mapping*, 35(12):5799–5814.
- Rocca, M. A., Valsasina, P., Martinelli, V., Misci, P., Falini, A., Comi, G., and Filippi, M. (2012). Large-scale neuronal network dysfunction in relapsing-remitting multiple sclerosis. *Neurology*, 79(14):1449–1457.
- Rocca, M. A., Valsasina, P., Meani, A., Falini, A., Comi, G., and Filippi, M. (2016). Impaired functional integration in multiple sclerosis: A graph theory study. *Brain Structure and Function*, 221(1):115–131.
- Romascano, D., Meskaldji, D.-E., Bonnier, G., Simioni, S., Rotzinger, D., Lin, Y.-C., Menegaz, G., Roche, A., Schlupe, M., Pasquier, R. D., Richiardi, J., Van De Ville, D., Daducci, A., Sumpf, T., Fraham, J., Thiran, J.-P., Krueger, G., and Granziera, C. (2015). Multicontrast connectometry: A new tool to assess cerebellum alterations in early relapsing-remitting multiple sclerosis. *Human Brain Mapping*, 36(4):1609–19.
- Roosendaal, S. D., Hulst, H. E., Vrenken, H., Feenstra, H. E., Castelijns, J. A., Pouwels, P. J., Barkhof, F., and Geurts, J. J. (2010a). Structural and functional hippocampal changes in multiple sclerosis patients with intact memory. *Radiology*, 255(2):595–604.
- Roosendaal, S. D., Schoonheim, M. M., Hulst, H. E., Sanz-Arigita, E. J., Smith, S. M., Geurts, J. J. G., and Barkhof, F. (2010b). Resting state networks change in clinically isolated syndrome. *Brain*, 133(Pt 6):1612–1621.
- Rosazza, C. and Minati, L. (2011). Resting-state brain networks: Literature review and clinical applications. *Neurological Sciences*, 32(5):773–785.
- Sakoglu, Ü., Pearlson, G. D., Kiehl, K. A., Wang, Y. M., Michael, A. M., and Calhoun, V. D. (2010). A method for evaluating dynamic functional network connectivity and task-modulation: Application to schizophrenia. *MAGMA*, 23(5-6):351–366.
- Salzer, J., Hallmans, G., Nyström, M., Stenlund, H., Wadell, G., and Sundström, P. (2013). Smoking as a risk factor for multiple sclerosis. *Multiple Sclerosis Journal*, 19(8):1022–1027.
- Sanfilippo, M. P., Benedict, R. H. B., Weinstock-Guttman, B., and Bakshi, R. (2006). Gray and white matter brain atrophy and neuropsychological impairment in multiple sclerosis. *Neurology*, 66(5):685–692.
- Sayao, A.-L., Devonshire, V., and Tremlett, H. (2007). Longitudinal follow-up of "benign" multiple sclerosis at 20 years. *Neurology*, 68(7):496–500.
- Sbardella, E., Tona, F., Petsas, N., Upadhyay, N., Piattella, M., Filippini, N., Prosperini, L., Pozzilli, C., and Pantano, P. (2015). Functional connectivity changes and their relationship with clinical

- disability and white matter integrity in patients with relapsing-remitting multiple sclerosis. *Multiple Sclerosis Journal*, 21(13):1681–1692.
- Schmidt, P., Gaser, C., Arsic, M., Buck, D., Förchler, A., Berthele, A., Hoshi, M., Ilg, R., Schmid, V. J., Zimmer, C., Hemmer, B., and Mühlau, M. (2012). An automated tool for detection of FLAIR-hyperintense white-matter lesions in Multiple Sclerosis. *NeuroImage*, 59(4):3774–3783.
- Scholz, J., Klein, M. C., Behrens, T. E. J., and Johansen-Berg, H. (2009). Training induces changes in white-matter architecture. *Nature Neuroscience*, 12(11):1370 – 1371.
- Schoonheim, M. M., Geurts, J., Landi, D., Douw, L., van der Meer, M. L., Vrenken, H., Polman, C. H., Barkhof, F., and Stam, C. J. (2013). Functional connectivity changes in multiple sclerosis patients: A graph analytical study of MEG resting state data. *Human Brain Mapping*, 34(1):52–61.
- Schoonheim, M. M., Geurts, J., Wiebenga, O. T., De Munck, J. C., Polman, C. H., Stam, C. J., Barkhof, F., and Wink, A. M. (2014). Changes in functional network centrality underlie cognitive dysfunction and physical disability in multiple sclerosis. *Multiple Sclerosis Journal*, 20(8):1058–1065.
- Schoonheim, M. M., Hulst, H. E., Brandt, R., Strik, M., Wink, A. M., Uitehaag, B., Barkhof, F., and Geurts, J. J. (2015). Thalamus structure and function determines severity of cognitive impairment in multiple sclerosis. *Neurology*, 84(8):776–783.
- Schoonheim, M. M., Hulst, H. E., Landi, D., Ciccarelli, O., Roosendaal, S. D., Sanz-Arigita, E. J., Vrenken, H., Polman, C. H., Stam, C. J., Barkhof, F., and Geurts, J. (2012). Gender-related differences in functional connectivity in multiple sclerosis. *Multiple Sclerosis Journal*, 18(2):164–173.
- Schultz, D. H. and Cole, M. W. (2016). Higher intelligence is associated with less task-related brain network reconfiguration. *The Journal of Neuroscience*, 36(33):8551–8561.
- Schwid, S. R., Covington, M., Segal, B. M., and Goodman, A. D. (2002). Fatigue in multiple sclerosis: Current understanding and future directions. *Journal of Rehabilitation Research and Development*, 39(2):211–224.
- Shah, L. M., Cramer, J. A., Ferguson, M. A., Birn, R. M., and Anderson, J. S. (2016). Reliability and reproducibility of individual differences in functional connectivity acquired during task and resting state. *Brain and Behavior*, 6(5):e00456.
- Shannon, B. J., Dosenbach, R. A., Su, Y., Vlassenko, A. G., Larson-Prior, L. J., Nolan, T. S., Snyder, A. Z., and Raichle, M. E. (2013). Morning-evening variation in human brain metabolism and memory circuits. *Journal of Neurophysiology*, 109(5):1444–14456.
- Sharp, D. J., Beckmann, C. F., Greenwood, R., Kinnunen, K. M., Bonnelle, V., De Boissezon, X., Powell, J. H., Counsell, S. J., Patel, M. C., and Leech, R. (2011). Default mode network functional and structural connectivity after traumatic brain injury. *Brain*, 134(Pt 8):2233–2247.
- Shin, D.-J., Jung, W. H., He, Y., Wang, J., Shim, G., Byun, M. S., Jang, J. H., Kim, S. N., Lee, T. Y., Park, H. Y., and Kwon, J. S. (2014). The effects of pharmacological treatment on functional brain connectome in obsessive-compulsive disorder. *Biological Psychiatry*, 75(8):606–614.
- Shirer, W. R., Jiang, H., Price, C. M., Ng, B., and Greicius, M. D. (2015). Optimization of rs-fMRI Pre-processing for enhanced signal-noise separation, test-retest reliability, and group discrimination. *NeuroImage*, 117:67–79.



- Shirer, W. R., Ryali, S., Rykhlevskaia, E., Menon, V., and Greicius, M. D. (2012). Decoding subject-driven cognitive states with whole-brain connectivity patterns. *Cerebral Cortex*, 22(1):158–165.
- Siebert, R. J. and Abernethy, D. A. (2005). Depression in multiple sclerosis: A review. *Journal of Neurology, Neurosurgery, and Psychiatry*, 76(4):469–475.
- Silasi, G. and Murphy, T. H. (2014). Stroke and the connectome: How connectivity guides therapeutic intervention. *Neuron*, 83(6):1354–1368.
- Singer, W. and Gray, C. M. (1995). Visual feature integration and the temporal correlation hypothesis. *Annual Review of Neuroscience*, 18:555–586.
- Smith, A. M., Lewis, B. K., Ruttimann, U. E., Ye, F. Q., Sinnwell, T. M., Yang, Y., Duyn, J. H., and Frank, J. A. (1999). Investigation of low frequency drift in fMRI signal. *NeuroImage*, 9(5):526–533.
- Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., Filippini, N., Watkins, K. E., Toro, R., Laird, A. R., and Beckmann, C. F. (2009). Correspondence of the brain’s functional architecture during activation and rest. *Proceedings of the National Academy of Sciences of the United States of America*, 106(31):13040–13045.
- Sohn, W. S., Yoo, K., Lee, Y.-B., Seo, S. W., Na, D. L., and Jeong, Y. (2015). Influence of ROI selection on resting state functional connectivity: An individualized approach for resting state fMRI analysis. *Frontiers in Neuroscience*, 9:280.
- Spencer, M. D., Chura, L. R., Holt, R. J., Suckling, J., Calder, A. J., Bullmore, E. T., and Baron-Cohen, S. (2012). Failure to deactivate the default mode network indicates a possible endophenotype of autism. *Molecular Autism*, 3(1):15.
- Sporns, O. (2011). The human connectome: A complex network. *Annals of the New York Academy of Sciences*, 1224:109–125.
- Sporns, O. (2013a). Structure and function of complex brain networks. *Dialogues in Clinical Neuroscience*, 15(3):247–262.
- Sporns, O. (2013b). The human connectome: Origins and challenges. *NeuroImage*, 80:53–61.
- Sporns, O. and Betzel, R. F. (2016). Modular brain networks. *Annual Review of Psychology*, 67(1):613–640.
- Sporns, O., Tononi, G., and Kötter, R. (2005). The human connectome: A structural description of the human brain. *PLoS Computational Biology*, 1(4):e42.
- Spreng, R. N., Stevens, W. D., Viviano, J. D., and Schacter, D. L. (2016). Attenuated anticorrelation between the default and dorsal attention networks with aging: Evidence from task and rest. *Neurobiology of Aging*, 45:149–160.
- Stadelmann-Nessler, C. and Brück, W. (2015). Kapitel 4 - Pathologie und Pathophysiologie. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 31–36. Elsevier, München, 6. edition.
- Steenwijk, M. D., Geurts, J. J., Daams, M., Tijms, B. M., Wink, A. M., Balk, L. J., Tewarie, P. K., Uitdehaag, B. M., Barkhof, F., Vrenken, H., and Pouwels, P. J. (2016). Cortical atrophy patterns in multiple sclerosis are non-random and clinically relevant. *Brain*, 139(Pt 1):115–126.

- Stufflebeam, S. M., Liu, H., Sepulcre, J., Tanaka, N., Buckner, R. L., and Madsen, J. R. (2011). Localization of focal epileptic discharges using functional connectivity magnetic resonance imaging. *Journal of Neurosurgery*, 114(6):1693–1697.
- Supekar, K., Uddin, L. Q., Prater, K., Amin, H., Greicius, M. D., and Menon, V. (2010). Development of functional and structural connectivity within the default mode network in young children. *NeuroImage*, 52(1):290–301.
- Tagliazucchi, E., Behrens, M., and Laufs, H. (2013). Sleep neuroimaging and models of consciousness. *Frontiers in Psychology*, 4:256.
- Tagliazucchi, E., Carhart-Harris, R., Leech, R., Nutt, D., and Chialvo, D. R. (2014). Enhanced repertoire of brain dynamical states during the psychedelic experience. *Human Brain Mapping*, 35(11):5442–5456.
- Tagliazucchi, E. and Laufs, H. (2014). Decoding wakefulness levels from typical fMRI resting-state data reveals reliable drifts between wakefulness and sleep. *Neuron*, 82(3):695–708.
- Tavor, I., Parker Jones, O., Mars, R. B., Smith, S. M., Behrens, T. E., and Jbabdi, S. (2016). Task-free MRI predicts individual differences in brain activity during task performance. *Science*, 352(6282):216–220.
- Termenon, M., Achard, S., Jaillard, A., and Delon-Martin, C. (2016a). The "hub disruption index," a reliable index sensitive to the brain networks reorganization. A study of the contralesional hemisphere in stroke. *Frontiers in Computational Neuroscience*, 10:84.
- Termenon, M., Jaillard, A., Delon-Martin, C., and Achard, S. (2016b). Reliability of graph analysis of resting state fMRI using test-retest dataset from the Human Connectome Project. *NeuroImage*, page [Epub ahead of print].
- Teufel, C. and Fletcher, P. C. (2016). The promises and pitfalls of applying computational models to neurological and psychiatric disorders. *Brain*, 139(Pt 10):2600–2608.
- Tewarie, P., Hillebrand, A., Schoonheim, M. M., van Dijk, B. W., Geurts, J. J., Barkhof, F., Polman, C. H., and Stam, C. J. (2014a). Functional brain network analysis using minimum spanning trees in Multiple Sclerosis: An MEG source-space study. *NeuroImage*, 88:308–318.
- Tewarie, P., Schoonheim, M. M., Schouten, D. I., Polman, C. H., Balk, L. J., Uitdehaag, B. M. J., Geurts, J. J. G., Hillebrand, A., Barkhof, F., and Stam, C. J. (2015). Functional brain networks: Linking thalamic atrophy to clinical disability in multiple sclerosis, a multimodal fMRI and MEG Study. *Human Brain Mapping*, 36(2):603–618.
- Tewarie, P., Schoonheim, M. M., Stam, C. J., van der Meer, M. L., van Dijk, B. W., Barkhof, F., Polman, C. H., and Hillebrand, A. (2013). Cognitive and clinical dysfunction, Altered MEG resting-state networks and thalamic atrophy in multiple sclerosis. *PLoS One*, 8(7):e69318.
- Tewarie, P., Steenwijk, M. D., Tijms, B. M., Daams, M., Balk, L. J., Stam, C. J., Uitdehaag, B. M. J., Polman, C. H., Geurts, J. J. G., Barkhof, F., Pouwels, P. J. W., Vrenken, H., and Hillebrand, A. (2014b). Disruption of structural and functional networks in long-standing multiple sclerosis. *Human Brain Mapping*, 35(12):5946–5961.
- The International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium 2, Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C. A., Patsopoulos, N. A., Moutsianas, L., Dilthey, A., Su, Z., Freeman, C., Hunt, S. E., Edkins, S., Gray, E., Booth, D. R., Potter,

- S. C., Goris, A., Band, G., Oturai, A. B., Strange, A., Saarela, J., Bellenguez, C., Fontaine, B., Gillman, M., Hemmer, B., Gwilliam, R., Zipp, F., Jayakumar, A., Martin, R., Leslie, S., Hawkins, S., Giannoulatou, E., D'alfonso, S., Blackburn, H., Martinelli Boneschi, F., Liddle, J., Harbo, H. F., Perez, M. L., Spurkland, A., Waller, M. J., Mycko, M. P., Ricketts, M., Comabella, M., Hammond, N., Kockum, I., McCann, O. T., Ban, M., Whittaker, P., Kempainen, A., Weston, P., Hawkins, C., Widaa, S., Zajicek, J., Dronov, S., Robertson, N., Bumpstead, S. J., Barcellos, L. F., Ravindrarajah, R., Abraham, R., Alfredsson, L., Ardlie, K., Aubin, C., Baker, A., Baker, K., Baranzini, S. E., Bergamaschi, L., Bergamaschi, R., Bernstein, A., Berthele, A., Boggild, M., Bradfield, J. P., Brassat, D., Broadley, S. A., Buck, D., Butzkueven, H., Capra, R., Carroll, W. M., Cavalla, P., Celius, E. G., Cepok, S., Chiavacci, R., Clerget-Darpoux, F., Clysters, K., Comi, G., Cossburn, M., Cournu-Rebeix, I., Cox, M. B., Cozen, W., Cree, B. A. C., Cross, A. H., Cusi, D., Daly, M. J., Davis, E., de Bakker, P. I. W., Debouverie, M., D'hooghe, M. B., Dixon, K., Dobosi, R., Dubois, B., Ellinghaus, D., Elovaara, I., Esposito, F., Fontenille, C., Foote, S., Franke, A., Galimberti, D., Ghezzi, A., Glessner, J., Gomez, R., Gout, O., Graham, C., Grant, S. F. A., Guerini, F. R., Hakonarson, H., Hall, P., Hamsten, A., Hartung, H.-P., Heard, R. N., Heath, S., Hobart, J., Hoshi, M., Infante-Duarte, C., Ingram, G., Ingram, W., Islam, T., Jagodic, M., Kabesch, M., Kermode, A. G., Kilpatrick, T. J., Kim, C., Klopp, N., Koivisto, K., Larsson, M., Lathrop, M., Lechner-Scott, J. S., Leone, M. A., Leppä, V., Liljedahl, U., Bomfim, I. L., Lincoln, R. R., Link, J., Liu, J., Lorentzen, A. R., Lupoli, S., Macciardi, F., Mack, T., Marriott, M., Martinelli, V., Mason, D., McCauley, J. L., Mentch, F., Mero, I.-L., Mihalova, T., Montalban, X., Mottershead, J., Myhr, K.-M., Naldi, P., Ollier, W., Page, A., Palotie, A., Pelletier, J., Piccio, L., Pickersgill, T., Piehl, F., Pobywajlo, S., Quach, H. L., Ramsay, P. P., Reunanen, M., Reynolds, R., Rioux, J. D., Rodegher, M., Roesner, S., Rubio, J. P., Rückert, I.-M., Salvetti, M., Salvi, E., Santaniello, A., Schaefer, C. A., Schreiber, S., Schulze, C., Scott, R. J., Sellebjerg, F., Selmaj, K. W., Sexton, D., Shen, L., Simms-Acuna, B., Skidmore, S., Sleiman, P. M. A., Smestad, C., Sørensen, P. S., Søndergaard, H. B., Stankovich, J., Strange, R. C., Sulonen, A.-M., Sundqvist, E., Syvänen, A.-C., Taddeo, F., Taylor, B., Blackwell, J. M., Tienari, P., Bramon, E., Tourbah, A., Brown, M. A., Tronczynska, E., Casas, J. P., Tubridy, N., Corvin, A., Vickery, J., Jankowski, J., Villoslada, P., Markus, H. S., Wang, K., Mathew, C. G., Wason, J., Palmer, C. N. A., Wichmann, H.-E., Plomin, R., Willoughby, E., Rautanen, A., Winkelmann, J., Wittig, M., Trembath, R. C., Yaouanq, J., Viswanathan, A. C., Zhang, H., Wood, N. W., Zuvich, R., Deloukas, P., Langford, C., Duncanson, A., Oksenberg, J. R., Pericak-Vance, M. A., Haines, J. L., Olsson, T., Hillert, J., Ivinson, A. J., De Jager, P. L., Peltonen, L., Stewart, G. J., Hafler, D. A., Hauser, S. L., McVean, G., Donnelly, P., and Compston, A. (2012). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*, 476(7359):214–219.
- Thompson, W. H. and Fransson, P. (2015). The mean-variance relationship reveals two possible strategies for dynamic brain connectivity analysis in fMRI. *Frontiers in Human Neuroscience*, 9:398.
- Tognoli, E. and Kelso, J. A. S. (2014). The metastable brain. *Neuron*, 81(1):35–48.
- Tona, F., Petsas, N., Sbardella, E., Prosperini, L., Carmellini, M., Pozzilli, C., and Pantano, P. (2014). Multiple sclerosis: Altered thalamic resting-state functional connectivity and its effect on cognitive function. *Radiology*, 271(3):814–821.
- Tremlett, H., Yousefi, M., Devonshire, V., Rieckmann, P., Zhao, Y., and UBC Neurologists (2009a). Impact of multiple sclerosis relapses on progression diminishes with time. *Neurology*, 73(20):1616–1623.
- Tremlett, H., Zhao, Y., Devonshire, V., and UBC Neurologists (2009b). Natural history comparisons

- of primary and secondary progressive multiple sclerosis reveals differences and similarities. *Journal of Neurology*, 256(3):374–381.
- Trojano, M., Paolicelli, D., Bellacosa, A., and Cataldo, S. (2003). The transition from relapsing-remitting MS to irreversible disability: Clinical evaluation. *Neurological Sciences*, 24(Suppl 5):S268–270.
- Tumani, H. and Rieckmann, P. (2015). Kapitel 11 - Marker des Liquor cerebrospinalis und des Blutes im Überblick. In Schmidt, R. M., Hoffmann, F., Faiss, J. H., and Köhler, W., editors, *Multiple Sklerose*, pages 123–135. Elsevier, München, 6. edition.
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., and Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*, 15(1):273–289.
- Valsasina, P., Rocca, M. A., Absinta, M., Sormani, M. P., Mancini, L., De Stefano, N., Rovira, A., Gass, A., Enzinger, C., Barkhof, F., Wegner, C., Matthews, P. M., and Filippi, M. (2011). A multicentre study of motor functional connectivity changes in patients with multiple sclerosis. *The European Journal of Neuroscience*, 33(7):1256–1263.
- van de Ven, V., Wingen, M., Kuypers, K. P. C., Ramaekers, J. G., and Formisano, E. (2013). Escitalopram decreases cross-regional functional connectivity within the default-mode network. *PLoS One*, 8(6):e68355.
- van den Heuvel, M. P. and Hulshoff Pol, H. E. (2010). Exploring the brain network: A review on resting-state fMRI functional connectivity. *European Neuropsychopharmacology*, 20(8):519–534.
- van den Heuvel, M. P., Mandl, R., Luigjes, J., and Hulshoff Pol, H. E. (2008). Microstructural organization of the cingulum tract and the level of default mode functional connectivity. *The Journal of Neuroscience*, 28(43):10844–10851.
- van den Heuvel, M. P., Mandl, R. C., Kahn, R. S., and Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Human Brain Mapping*, 30(10):3127–3141.
- van den Heuvel, M. P. and Sporns, O. (2011). Rich-club organization of the human connectome. *The Journal of Neuroscience*, 31(44):15775–15786.
- van den Heuvel, M. P. and Sporns, O. (2013). Network hubs in the human brain. *Trends in Cognitive Sciences*, 17(12):683–696.
- van der Mei, I. A., Ponsonby, A.-L., Dwyer, T., Blizzard, L., Simmons, R., Taylor, B. V., Butzkueven, H., and Kilpatrick, T. (2003). Past exposure to sun, skin phenotype, and risk of multiple sclerosis: Case-control study. *BMJ*, 327(7410):316.
- van Meer, M. P. A., Otte, W. M., van der Marel, K., Nijboer, C. H., Kavelaars, A., van der Sprenkel, J. W., Viergever, M. A., and Dijkhuizen, R. M. (2012). Extent of bilateral neuronal network reorganization and functional recovery in relation to stroke severity. *The Journal of Neuroscience*, 32(13):4495–4507.
- Van Schependom, J., D’hooghe, M. B., Cleynhens, K., D’hooge, M., Haelewyck, M. C., De Keyser, J., and Nagels, G. (2014). The Symbol Digit Modalities Test as sentinel test for cognitive impairment in multiple sclerosis. *European Journal of Neurology*, 21(9):1219–1225, e71–72.

- van Wijk, B. C. M., Stam, C. J., and Daffertshofer, A. (2010). Comparing brain networks of different size and connectivity density using graph theory. *PLoS One*, 5(10):e13701.
- Váša, F., Shanahan, M., Hellyer, P. J., Scott, G., Cabral, J., and Leech, R. (2015). Effects of lesions on synchrony and metastability in cortical networks. *NeuroImage*, 118:456–467.
- Vatansever, D., Menon, X. D. K., Manktelow, A. E., Sahakian, B. J., and Stamatakis, E. A. (2015). Default mode dynamics for global functional integration. *The Journal of Neuroscience*, 35(46):15254–15262.
- Vellinga, M., Geurts, J., Rostrup, E., Uitdehaag, B., Polman, C., Barkhof, F., and Vrenken, H. (2009). Clinical correlations of brain lesion distribution in multiple sclerosis. *Journal of Magnetic Resonance Imaging*, 29(4):768–773.
- von der Malsburg, C. and Schneider, W. (1986). A Neural Cocktail-Party Processor. *Biological Cybernetics*, 54(1):29–40.
- Vukusic, S. and Confavreux, C. (2007). Natural history of multiple sclerosis: Risk factors and prognostic indicators. *Current Opinion in Neurology*, 20(3):269–274.
- Walker, C. (2014). The fun of science. *Science*, 344(6189):1314.
- Wang, H., Guo, W., Liu, F., Wang, G., Lyu, H., Wu, R., Chen, J., Wang, S., Li, L., and Zhao, J. (2016). Patients with first-episode, drug-naive schizophrenia and subjects at ultra-high risk of psychosis shared increased cerebellar-default mode network connectivity at rest. *Scientific Reports*, 6:26124.
- Washington, S. D., Gordon, E. M., Brar, J., Warburton, S., Sawyer, A. T., Wolfe, A., Mease-Ference, E. R., Girton, L., Hailu, A., Mbwana, J., Gaillard, W. D., Kalbfleisch, M. L., and VanMeter, J. W. (2014). Dysmaturation of the default mode network in autism. *Human Brain Mapping*, 35(4):1284–1296.
- Wechsler, D. (1997). *WMS-III administration and scoring manual*. The Psychological Corporation, Harcourt Brace Jovanovich, San Antonio.
- Wilson, R. S., Mayhew, S. D., Rollings, D. T., Goldstone, A., Przewdzik, I., Arvanitis, T. N., and Bagshaw, A. P. (2015). Influence of epoch length on measurement of dynamic functional connectivity in wakefulness and behavioural validation in sleep. *NeuroImage*, 112:169–179.
- Winkelmann, A., Löbermann, M., Reisinger, E., and Zettl, U. (2011). Multiple Sklerose und Infektionskrankheiten. *Aktuelle Neurologie*, 38:339–350.
- Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., and Nichols, T. E. (2014). Permutation inference for the general linear model. *NeuroImage*, 92:381–397.
- Wojtowicz, M., Mazerolle, E. L., Bhan, V., and Fisk, J. D. (2014). Altered functional connectivity and performance variability in relapsing-remitting multiple sclerosis. *Multiple Sclerosis Journal*, 20(11):1453–1463.
- World Health Organisation (2013). Declaration of Helsinki World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. *The Journal of the American Medical Association*, 310(20):2191–2194.
- Wu, L., Zhang, Y., Zhou, F., Gao, L., He, L., Zeng, X., and Gong, H. (2016). Altered intra- and interregional synchronization in relapsing-remitting multiple sclerosis: A resting-state fMRI study. *Neuropsychiatric Disease and Treatment*, 12:853–862.

- Yan, C.-G., Cheung, B., Kelly, C., Colcombe, S., Craddock, R. C., Di Martino, A., Li, Q., Zuo, X.-N., Castellanos, F. X., and Milham, M. P. (2013a). A comprehensive assessment of regional variation in the impact of head micromovements on functional connectomics. *NeuroImage*, 76:183–201.
- Yan, C.-G., Craddock, R. C., Zuo, X.-N., Zang, Y.-F., and Milham, M. P. (2013b). Standardizing the intrinsic brain: Towards robust measurement of inter-individual variation in 1000 functional connectomes. *NeuroImage*, 80:246–262.
- Yang, Z., Craddock, R. C., Margulies, D. S., Yan, C.-G., and Milham, M. P. (2014). Common intrinsic connectivity states among posteromedial cortex subdivisions: Insights from analysis of temporal dynamics. *NeuroImage*, 93(Pt 1):124–137.
- Yang, Z., Zuo, X.-N., McMahon, K. L., Craddock, R. C., Kelly, C., de Zubicaray, G. I., Hickie, I., Bandettini, P. A., Castellanos, F. X., Milham, M. P., and Wright, M. J. (2016). Genetic and environmental contributions to functional connectivity architecture of the human brain. *Cerebral Cortex*, 26(5):2341–2352.
- Yekutieli, D. and Benjamini, Y. (1999). Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics. *Journal of Statistical Planning and Inference*, 82(1-2):171–196.
- Zalesky, A. and Breakspear, M. (2015). Towards a statistical test for functional connectivity dynamics. *NeuroImage*, 114:466–470.
- Zalesky, A., Fornito, A., Cocchi, L., Gollo, L. L., and Breakspear, M. (2014). Time-resolved resting-state brain networks. *Proceedings of the National Academy of Sciences of the United States of America*, 111(28):10341–10346.
- Zalesky, A., Fornito, A., Harding, I. H., Cocchi, L., Yücel, M., Pantelis, C., and Bullmore, E. T. (2010). Whole-brain anatomical networks: Does the choice of nodes matter? *NeuroImage*, 50(3):970–983.
- Zanto, T. P. and Gazzaley, A. (2013). Fronto-parietal network: Flexible hub of cognitive control. *Trends in Cognitive Sciences*, 17(12):602–603.
- Zettl, U., Stüve, O., and Patejdl, R. (2012). Immune-mediated CNS diseases: A review on nosological classification and clinical features. *Autoimmunity Reviews*, 11(3):167–173.
- Zhang, D., Johnston, J. M., Fox, M. D., Leuthardt, E. C., Grubb, R. L., Chicoine, M. R., Smyth, M. D., Snyder, A. Z., Raichle, M. E., and Shimony, J. S. (2009). Preoperative sensorimotor mapping in brain tumor patients using spontaneous fluctuations in neuronal activity imaged with functional magnetic resonance imaging: Initial experience. *Neurosurgery*, 65(6 Suppl):226–236.
- Zhang, J., Cheng, W., Liu, Z., Zhang, K., Lei, X., Yao, Y., Becker, B., Liu, Y., Kendrick, K. M., Lu, G., and Feng, J. (2016). Neural, electrophysiological and anatomical basis of brain-network variability and its characteristic changes in mental disorders. *Brain*, 139(8):247–256.
- Zhou, F., Zhuang, Y., Gong, H., Wang, B., Wang, X., Chen, Q., Wu, L., and Wan, H. (2014). Altered inter-subregion connectivity of the default mode network in relapsing remitting multiple sclerosis: A functional and structural connectivity study. *PLoS One*, 9(7):e101198.
- Zhou, F., Zhuang, Y., Wang, L., Zhang, Y., Wu, L., Zeng, X., and Gong, H. (2015). Disconnection of the hippocampus and amygdala associated with lesion load in relapsing - remitting multiple sclerosis: A structural and functional connectivity study. *Neuropsychiatric Disease and Treatment*, 11:1749–1765.

- Zhou, J., Greicius, M. D., Gennatas, E. D., Growdon, M. E., Jang, J. Y., Rabinovici, G. D., Kramer, J. H., Weiner, M., Miller, B. L., and Seeley, W. W. (2010). Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. *Brain*, 133(Pt 5):1352–1367.
- Zigmond, A. S. and Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta psychiatrica Scandinavica*, 67(6):361–370.
- Zimmermann, P. and Fimm, B. (2007). *TAP Testbatterie zur Aufmerksamkeitspruefung (Version 2.1)*. Psytest, Herzogenrath.
- Zito, G., Luders, E., Tomasevic, L., Lupoi, D., Toga, A. W., Thompson, P. M., Rossini, P. M., Filippi, M. M., and Tecchio, F. (2014). Inter-hemispheric functional connectivity changes with corpus callosum morphology in multiple sclerosis. *Neuroscience*, 266:47–55.
- Zuo, X.-N., Anderson, J. S., Bellec, P., Birn, R. M., Biswal, B. B., Blautzik, J., Breitner, J. C. S., Buckner, R. L., Calhoun, V. D., Castellanos, F. X., Chen, A., Chen, B., Chen, J., Chen, X., Colcombe, S. J., Courtney, W., Craddock, R. C., Di Martino, A., Dong, H.-M., Fu, X., Gong, Q., Gorgolewski, K. J., Han, Y., He, Y., He, Y., Ho, E., Holmes, A., Hou, X.-H., Huckins, J., Jiang, T., Jiang, Y., Kelley, W., Kelly, C., King, M., LaConte, S. M., Lainhart, J. E., Lei, X., Li, H.-J., Li, K., Li, K., Lin, Q., Liu, D., Liu, J., Liu, X., Liu, Y., Lu, G., Lu, J., Luna, B., Luo, J., Lurie, D., Mao, Y., Margulies, D. S., Mayer, A. R., Meindl, T., Meyerand, M. E., Nan, W., Nielsen, J. A., O'Connor, D., Paulsen, D., Prabhakaran, V., Qi, Z., Qiu, J., Shao, C., Shehzad, Z., Tang, W., Villringer, A., Wang, H., Wang, K., Wei, D., Wei, G.-X., Weng, X.-C., Wu, X., Xu, T., Yang, N., Yang, Z., Zang, Y.-F., Zhang, L., Zhang, Q., Zhang, Z., Zhang, Z., Zhao, K., Zhen, Z., Zhou, Y., Zhu, X.-T., and Milham, M. P. (2014). An open science resource for establishing reliability and reproducibility in functional connectomics. *Scientific Data*, 1:140049.

# Abbreviations

**AAL** automatic anatomical labeling

**ANOVA** analyses of variance

**BOLD** blood oxygen level-dependent

**CIS** clinically isolated syndrome

**CSF** cerebro-spinal fluid

**DARTEL** Diffeomorphic Anatomical Registration Exponentiated Lie Algebra algorithm

**DMN** default mode network

**DTI** diffusion tensor imaging

**EDSS** Expanded Disability Status Scale

**EEG** electroencephalography

**EPI** echo-planar imaging

**F** F-value

**FA** fractional anisotropy

**fcMRI** functional connectivity based on functional magnetic resonance imaging

**FDR** false discovery rate

**FLAIR** fluid-attenuated inversion recovery

**fmRI** functional magnetic resonance imaging

**FSL** FMRIB Software Library version 5.0.8

**FSMC** Fatigue Scale for Motor and Cognitive Functions

**FWE** family wise error

**FWHM** full width at half maximum

**GM** gray matter

**gWMI** global white matter integrity

**HADS** Hospital Anxiety and Depression Scale

**ICA** independent component analysis

**ICC** intraclass correlation coefficient

**iCP** integrated cognitive performance



- LST** Lesion Segmentation Toolbox version 2.0.12
- MATLAB 2013a** MATLAB and Statistics toolbox Release 2013a, The MathWorks, Inc., Natick, Massachusetts, United States
- MCC** multiple comparison correction
- MEG** magnetoencephalography
- MNI** Montreal Neurological Institute
- MRI** magnetic resonance imaging
- MS** multiple sclerosis
- PCA** principal component analysis
- PET** positron emission tomography
- PPMS** primary progressive MS
- RIS** radiologically isolated syndrome
- ROI** regions of interest
- RRMS** relapsing remitting MS
- RSN** resting state network
- SD** standard deviation
- SPM8** Statistical Parametric Mapping software package, version 8, Wellcome Trust Centre of Neuroimaging, London, Great Britain
- SPMS** secondary progressive MS
- t** t-value
- tACS** transcranial alternating current stimulation
- TBSS** Tract-Based Spatial Statistics
- tDCS** transcranial direct current stimulation
- TE** echo time
- TMS** transcranial magnetic stimulation
- TR** repetition time
- VBM8** Voxel Based Morphometry toolbox, Version 8
- vfcMRI** variability of functional connectivity based on fMRI
- WM** white matter
- Z** Z-value

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# Contributions

## Other involved scientists

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## Supervision

Academic supervision of this doctoral project was provided by CH and AKE.

## NeuConn project

AKE, ChH, SMG, and GN are co-principal investigators of the NeuConn project. They planned the project, developed the study concept and its design, and obtained funding. JP and SMG compiled the neuropsychological and clinical assessment battery. SuS and JP were responsible for the coordination of the appointments and the acquisition of neuroimaging and neuropsychological data. I was involved in the data acquisition of the neuroimaging data. ChH is the head of the MS day clinic and was responsible for the recruitment and all clinical examinations.

## Systematic literature review

I developed the idea and the categories for the systematic literature review. I did the literature research, evaluated the publications, summarized the results, and interpreted the findings.

## Empirical work

I preprocessed all data, developed and conducted the analyses and statistical testing, created the figures, and interpreted the results. AKE and GN were involved in the development of the concept of analysis 2, in particular the graph theoretical part, and reviewed the draft of the corresponding study report. AKE reviewed the draft of the manuscript for publication of analysis 4.

**Confirmed by:** Prof. Dr. Christoph Herrmann

# Declarations

## **Statement of good scientific practice / Erklärung zur guten wissenschaftlichen Praxis** according to / gemäß §9 (2) h)

This is to confirm that I am aware of the guidelines of good scientific practice of the Carl von Ossietzky University Oldenburg and that I observed them.


Hiermit erkläre ich, dass mir die Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg bekannt sind und von mir befolgt wurden.

## **Statement of independent work / Erklärung der selbstständigen Erarbeitung** according to / gemäß §12 (2) b)

I hereby confirm that I completed the work independently and used only the indicated resources.

Ich bestätige hiermit, dass ich die Arbeit selbstständig verfasst und nur die angegebenen Hilfsmittel benutzt habe.

Sarah J. Büttof

A handwritten signature in blue ink, appearing to read 'S. Büttof', with a stylized flourish at the end.

# Curriculum Vitae and Publications

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## Education

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Since April 2014 **Carl von Ossietzky University Oldenburg,**  
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March 2013 - **University Medical Center Hamburg-Eppendorf,**  
October 2016 **Department of Neurophysiology and Pathophysiology**  
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Principal Investigator: Prof. Andreas Engel, MD  
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Ministry of Education and Research (BMBF)

October 2006 - **Albert-Ludwigs-University Freiburg,**  
October 2012 **Department of Psychology**  
*Studies of Psychis (Diploma, Final Mark: 1,1)*

Special Research Subject: Neurobiological basics  
Major Specializations: Clinical psychology  
Educational psychology, family therapy  
Minor Specialization: Psychopathology

July 2011 - **Eberhard Karls University Tübingen,**  
May 2012 **Institute for Medical Psychology and Behavioural Neurobiology**  
*Diploma Thesis (Mark: 1,0)*

Topic: Functional connectivity in severe disorders of con-  
sciousness: Resting state and emotional reactivity  
Supervision: Prof. Dr. Ulrike Halsband, Freiburg University  
Prof. Dr. Boris Kotchoubey, Tübingen University

---

October 2007 - **Albert-Ludwigs-University Freiburg,**  
January 2011 **Medical Faculty**  
*Studies of Medicine (as Second Major Subject; no degree)*

August 2009 - **University of Massachusetts Boston, USA,**  
June 2010 **Psychology Department**  
*Master / PhD-Program Clinical Psychology (no degree)*

August 1997 - **Albert-Schweitzer-UNESCO-Schule Offenbach a.M.**  
June 2006 *Abitur (Final Mark: 1,0)*

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## Publications

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Körner, M., **Bütof, S.**, Müller, C., Zimmermann, L, Becker, S, Bengel, J. (2016). Interprofessional teamwork and team interventions in chronic care: A systematic review. *Journal of interprofessional care*, 30(1): 15-28.

Kotchoubey, B., **Bütof, S.**, Sitaram, R. (2015). Flagrant misconduct of reviewers and editor: A case study. *Science and engineering ethics*, 21(4): 829-835.

**Bueto, S. J.**, Hawellek D. J., Gold, S. M., Siemonsen, S., Heesen, C., Nolte, G., Engel, A. K. (2014). Association between integrity of white matter, cognitive deficits and spontaneous functional connectivity in multiple sclerosis. *Brain Connectivity*, 4(9): A1-A169 [Abstracts Fourth Biennial Conference on Resting State Brain Connectivity September 11-13, 2014].

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## Conference posters

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**Bueto, S. J.**, Ewald, A., Peffer, T., Heesen, C., Gold, S. M., Nolte, G., Engel, A. K. (2015, June). Altered dynamics of spontaneous functional connectivity in multiple sclerosis: An fMRI and MEG study. Poster presented at the Annual meeting of the Organization of Human Brain Mapping 2015, Honolulu, USA

**Bueto, S. J.**, Hawellek, D. J., Gold, S. M., Siemonsen, S., Heesen, C., Nolte, G., Engel, A. K. (2014, September). Association between integrity of white matter, cognitive deficits and spontaneous functional connectivity in multiple sclerosis. Poster presented at the biennial Resting State Conference, Cambridge, MA, USA

**Bueto, S. J.**, Kordon, A., van Eimeren, T., Hohagen, F., Zurowski, B. (2014, June). From rest to task and back. Functional connectivity in obsessive compulsive disorder. Poster presented at the Annual meeting of the Organization of Human Brain Mapping 2014, Hamburg, Germany

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## Talks

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07-02-2014 *Functional and structural connectivity in neurological patients: Pitfalls and challenges*, Educational lecture series, Institute for Neuro- and Bioinformatics, University of Lübeck, Germany

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# Appendix



# A. Systematic literature review

## A.1. Publications

Table 14. – Publications selected for full-text evaluation.

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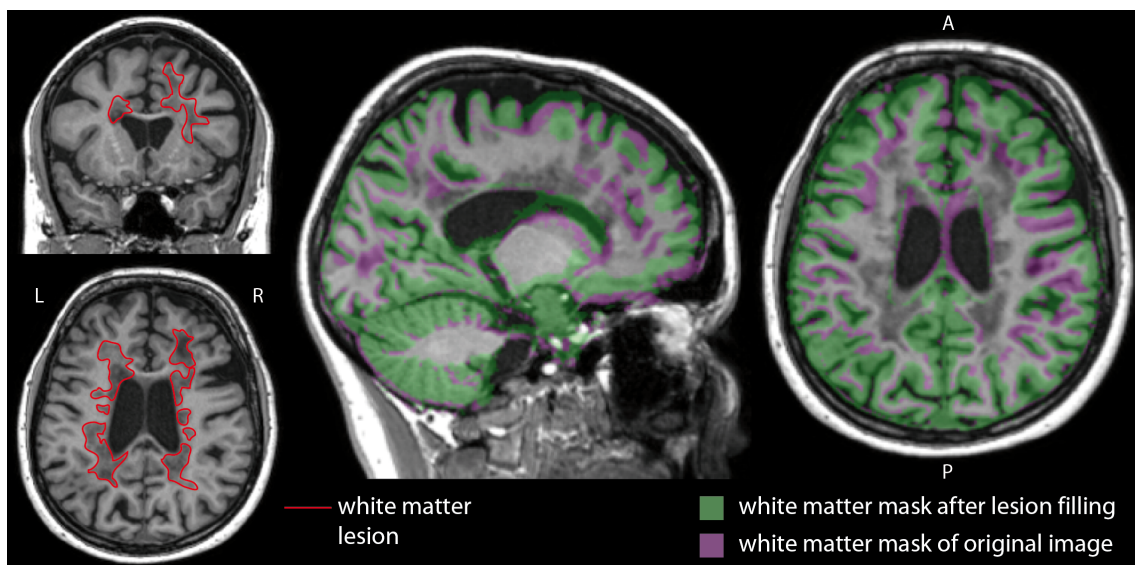
1	Colasanti et al. (2015)	2	Liu et al. (2016)	3	Droby et al. (2015)
2	Rocca et al. (2015)	5	Zhou et al. (2015)	6	Faivre et al. (2015)
7	Liu et al. (2015a)	8	Sbardella et al. (2015)	9	Hulst et al. (2015)
10	Liu et al. (2015b)	11	Eshaghi et al. (2015)	12	Giorgio et al. (2015)
13	Cogliati Dezza et al. (2015)	14	Romascano et al. (2015)	15	Petsas et al. (2015)
16	Finke et al. (2015)	17	Tewarie et al. (2015)	18	Rocca et al. (2016)
19	Leavitt et al. (2014)	20	Lowe et al. (2014)	21	Tewarie et al. (2014b)
22	Zhou et al. (2014)	23	Louapre et al. (2014)	24	Wojtowicz et al. (2014)
25	Zito et al. (2014)	26	Tona et al. (2014)	27	Janssen et al. (2013)
28	Gamboa et al. (2014)	29	Schoonheim et al. (2014)	30	Basile et al. (2014)
31	Leonardi et al. (2013)	32	Tewarie et al. (2013)	33	Tewarie et al. (2014a)
34	Cruz Gomez et al. (2014)	35	Koenig et al. (2013)	36	Rocca et al. (2010)
37	Lenne et al. (2013)	38	Liu et al. (2011)	39	Rocca et al. (2012)
40	Hardmeier et al. (2012)	41	Richiardi et al. (2012)	42	Faivre et al. (2012)
43	Hawellek et al. (2011)	44	Schoonheim et al. (2013)	45	Schoonheim et al. (2012)
46	Bonavita et al. (2011)	47	Jones et al. (2011)	48	Roosendaal et al. (2010a)
49	Lowe et al. (2008)	50	Cover et al. (2006)	51	Lowe et al. (2002)
52	Leocani et al. (2000)	53	Cruz Gómez et al. (2013)	54	Loitfelder et al. (2012)
55	Dogonowski et al. (2013a)	56	Dogonowski et al. (2013b)	57	Dogonowski et al. (2013c)

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## B. Empirical work

### B.1. General methods: White matter lesions and tissue segmentation

Simplified, tissue segmentation algorithms in SPM8 estimate the probability of belonging to a tissue class in an iterative manner by starting from a standard tissue template and the distribution of voxel intensities of each tissue class based on the standard probability map. On T1-weighted images, cortex and subcortical regions are illustrated in dark gray, while myelinated fibers are shown in light gray. Circumscribed demyelination, so-called WM lesions, are logically visualized as dark spots in bright areas. This can lead to substantial misclassification of WM in two ways (Chard et al., 2010). First, initial estimates of the distribution of voxel intensities in the WM will be biased towards lower values or darker shades. This can result in an overestimation of the WM segment, and therefore an artificially decreased GM volume. On the other hand, WM lesion can be classified as GM at some point of the segmentation algorithm, leading to an altered average intensity of the GM and in consequence to larger GM segments. Both cases will in turn introduce systematic group differences when individuals with WM pathology are compared to healthy participants in analyses, which integrate tissue masks into their pipeline. An example of the second case is illustrated in Fig. 26.



**Figure 26.** – Example of the effect of white matter lesions on the segmentation of T1-weighted images. Left: White matter lesions marked in red on the T1-weighted image of a multiple sclerosis patient. Lesions were marked manually for illustrative purposes only. Middle and right: Gray matter masks from tissue segmentation based on the original T1-weighted image (purple) or on the same image with filled lesions (green). Tissue segmentation was done with the New Segment algorithm as implemented in SPM8. *L = left; R = right; A = anterior; P = posterior*

## B.2. Analyses 1 and 2: Automated Anatomical Labeling atlas, Tzourio-Mazoyer et al. (2002)

**Table 15. – Regions of interest of the Automated Anatomical Labeling atlas (AAL).**

Name	Abbreviation	center (MNI)			RSN
		x	y	z	
anterior cingulum (L)	ACC <sub>L</sub>	-5	35	14	DMN, SAL
anterior cingulum (R)	ACC <sub>R</sub>	8	37	16	DMN, SAL
olfactory cortex (L)	OLF <sub>L</sub>	-9	15	-12	DMN
olfactory cortex (R)	OLF <sub>R</sub>	9	16	-11	DMN
medial part of the superior frontal cortex (L)	SFGmed <sub>L</sub>	-6	49	31	DMN, VAN
medial part of the superior frontal cortex (R)	SFGmed <sub>R</sub>	8	51	30	DMN, VAN
medial orbital of the superior frontal gyrus (L)	ORBsupmed <sub>L</sub>	-6	54	-7	DMN
medial orbital of the superior frontal gyrus (R)	ORBsupmed <sub>R</sub>	7	52	-7	DMN
orbital part of the inferior frontal gyrus (L)	ORBinf <sub>L</sub>	-37	31	-12	DMN
orbital part of the inferior frontal gyrus (R)	ORBinf <sub>R</sub>	40	32	-12	DMN
inferior temporal gyrus (L)	ITG <sub>L</sub>	-51	-28	-23	DMN, VAN, VIS
inferior temporal gyrus (R)	ITG <sub>R</sub>	53	-31	-22	DMN, VAN, VIS
middle occipital gyrus (L)	MOG <sub>L</sub>	-33	-81	16	DMN, VIS
middle occipital gyrus (R)	MOG <sub>R</sub>	36	-80	19	DMN, VIS
angular gyrus (L)	ANG <sub>L</sub>	-45	-61	36	DMN, FPC
angular gyrus (R)	ANG <sub>R</sub>	45	-60	39	DMN, FPC
precuneus (L)	PCUN <sub>L</sub>	-8	-56	48	DMN, SAL
precuneus (R)	PCUN <sub>R</sub>	9	-56	44	DMN, SAL
posterior cingulum (L)	PCC <sub>L</sub>	-6	-43	25	DMN
posterior cingulum (R)	PCC <sub>R</sub>	6	-42	22	DMN
lingual gyrus (L)	LING <sub>L</sub>	-16	-68	-5	FPC
lingual gyrus (R)	LING <sub>R</sub>	15	-67	-4	FPC
inferior parietal lobule, excluding supramarginal and angular gyri (L)	IPL <sub>L</sub>	-44	-46	47	FPC, DAN
inferior parietal lobule, excluding supramarginal and angular gyri (R)	IPL <sub>R</sub>	46	-46	50	FPC, DAN
supramarginal gyrus (L)	SMG <sub>L</sub>	-57	-34	30	FPC
supramarginal gyrus (R)	SMG <sub>R</sub>	57	-32	35	FPC
triangular part of the inferior frontal gyrus (L)	IFGtriang <sub>L</sub>	-47	30	14	FPC
triangular part of the inferior frontal gyrus (R)	IFGtriang <sub>R</sub>	49	30	14	FPC
orbital part of the superior frontal gyrus (L)	ORBsup <sub>L</sub>	-18	47	-13	FPC
orbital part of the superior frontal gyrus (R)	ORBsup <sub>R</sub>	18	48	-14	FPC
orbital part of the middle frontal gyrus (L)	ORBmid <sub>L</sub>	-32	50	-10	FPC
orbital part of the middle frontal gyrus (R)	ORBmid <sub>R</sub>	32	53	-11	FPC
opercular part of the inferior frontal gyrus (L)	IFGoper <sub>L</sub>	-49	13	19	FPC
opercular part of the inferior frontal gyrus (R)	IFGoper <sub>R</sub>	49	15	21	FPC
middle frontal gyrus (L)	MFG <sub>L</sub>	-34	33	36	FPC
middle frontal gyrus (R)	MFG <sub>R</sub>	37	33	34	FPC
gyrus rectus (L)	REC <sub>L</sub>	-6	37	-18	SAL
gyrus rectus (R)	REC <sub>R</sub>	7	36	-18	SAL
median cingulate region (L)	DCG <sub>L</sub>	-7	-15	42	SAL
median cingulate region (R)	DCG <sub>R</sub>	7	-9	40	SAL
superior parietal gyrus (L)	SPG <sub>L</sub>	-25	-60	59	SAL, DAN
superior parietal gyrus (R)	SPG <sub>R</sub>	25	-59	62	SAL, DAN
cuneus (L)	CUN <sub>L</sub>	-7	-80	27	SAL
cuneus (R)	CUN <sub>R</sub>	13	-79	28	SAL
temporal pole, superior temporal gyrus (L)	TPOsup <sub>L</sub>	-41	15	-20	VAN
temporal pole, superior temporal gyrus (R)	TPOsup <sub>R</sub>	47	15	-17	VAN
temporal pole, middle temporal gyrus (L)	TPOmid <sub>L</sub>	-37	15	-34	VAN
temporal pole, middle temporal gyrus (R)	TPOmid <sub>R</sub>	43	15	-32	VAN
middle temporal gyrus (L)	MTG <sub>L</sub>	-57	-34	-2	VAN

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Table 15 – continued from previous page

middle temporal gyrus (R)	MTG <sub>R</sub>	57	-37	-2	VAN
cortex surrounding the calcarine fissure (L)	CAL <sub>L</sub>	-8	-79	6	VAN
cortex surrounding the calcarine fissure (R)	CAL <sub>R</sub>	15	-73	9	VAN
dorsolateral superior frontal gyrus (L)	SFGdor <sub>L</sub>	-20	35	42	VAN
dorsolateral superior frontal gyrus (R)	SFGdor <sub>R</sub>	21	31	44	VAN
fusiform gyrus (L)	FFG <sub>L</sub>	-32	-40	-20	VAN, VIS
fusiform gyrus (R)	FFG <sub>R</sub>	33	-39	-20	VAN, VIS
precentral gyrus (L)	PreCG <sub>L</sub>	-40	-6	51	DAN, SMN
precentral gyrus (R)	PreCG <sub>R</sub>	40	-8	52	DAN, SMN
inferior occipital gyrus (L)	IOG <sub>L</sub>	-37	-78	-8	DAN, VIS
inferior occipital gyrus (R)	IOG <sub>R</sub>	37	-82	-8	DAN, VIS
supplementary motor area (L)	SMA <sub>L</sub>	-6	5	61	SMN
supplementary motor area (R)	SMA <sub>R</sub>	8	0.2	62	SMN
postcentral gyrus (L)	PoCG <sub>L</sub>	-44	-23	49	SMN, AUD
postcentral gyrus (R)	PoCG <sub>R</sub>	40	-26	53	SMN, AUD
paracentral lobule (L)	PCL <sub>L</sub>	-9	-25	70	SMN
paracentral lobule (R)	PCL <sub>R</sub>	7	-32	68	SMN
superior occipital gyrus (L)	SOG <sub>L</sub>	-18	-84	28	VIS
superior occipital gyrus (R)	SOG <sub>R</sub>	24	-81	31	VIS
rolandic operculum (L)	ROL <sub>L</sub>	-48	-9	14	AUD
rolandic operculum (R)	ROL <sub>R</sub>	52	-6	15	AUD
heschl gyrus (L)	HES <sub>L</sub>	-43	-19	10	AUD
heschl gyrus (R)	HES <sub>R</sub>	45	-17	10	AUD
superior temporal gyrus (L)	STG <sub>L</sub>	-54	-21	7	AUD
superior temporal gyrus (R)	STG <sub>R</sub>	57	-22	7	AUD
insula (L)	INS <sub>L</sub>	-36	7	3	AUD
insula (R)	INS <sub>R</sub>	38	6	2	AUD
nucleus pallidum (L)	PAL <sub>L</sub>	-19	-0.03	0.2	BG
nucleus pallidum (R)	PAL <sub>R</sub>	20	0.2	0.2	BG
nucleus caudate (L)	CAU <sub>L</sub>	-13	11	9	BG
nucleus caudate (R)	CAU <sub>R</sub>	14	12	9	BG
putamen (L)	PUT <sub>L</sub>	-25	4	2	BG
putamen (R)	PUT <sub>R</sub>	27	5	3	BG
hippocampus (L)	HIP <sub>L</sub>	-26	-21	-10	HIP
hippocampus (R)	HIP <sub>R</sub>	28	-20	-10	HIP
parahippocampal gyrus (L)	PHG <sub>L</sub>	-22	-16	-21	HIP
parahippocampal gyrus (R)	PHG <sub>R</sub>	24	-15	-21	HIP
amygdala (L)	AMYG <sub>L</sub>	-24	-1	-17	AMYG
amygdala (R)	AMYG <sub>R</sub>	26	1	-18	AMYG
thalamus (L)	THAL <sub>L</sub>	-12	-18	8	THAL
thalamus (R)	THAL <sub>R</sub>	12	-18	8	THAL

*MNI = Montreal Neurological Institute standard brain; RSN = assignment to resting state networks based on Damoiseaux et al. (2006); Smith et al. (2009); Lee et al. (2012);*

*networks: DMN = default mode, FPC = fronto-parietal, SAL = salience, VAN = ventral attention, DAN = dorsal attention, SMN = somatosensory, VIS = visual, AUD = auditory, BG = basal ganglia, HIP = hippocampus, AMYG = amygdala, THAL = thalamus*

### B.3. Analysis 3: Stanford atlas, Shirer et al. (2012)

Table 16. – Regions of interest of the Stanford atlas.

Name	Abbreviation	MNI coordinates			RSN
		x	y	z	
middle superior frontal gyrus (L1)	SFGmed <sub>L1</sub>	-5	46	20	default mode
angular gyrus (L1)	ANG <sub>L1</sub>	-47	-73	36	default mode
superior frontal gyrus (R1)	SFG <sub>R1</sub>	18	34	53	default mode
precuneus (L1)	Precuneus <sub>L1</sub>	1	-57	30	default mode
median cingulate cortex (R1)	MCC <sub>R1</sub>	2	-19	40	default mode
angular gyrus (R1)	ANG <sub>R1</sub>	50	-67	33	default mode
thalamus (L4)	Thal <sub>L4</sub>	-1	-11	8	default mode
hippocampus (L1)	Hip <sub>L1</sub>	-24	-31	-10	default mode
hippocampus (R1)	Hip <sub>R1</sub>	26	-24	-14	default mode
precuneus (L2)	Precuneus <sub>L2</sub>	-12	-61	17	default mode
middle frontal gyrus (L1)	MFG <sub>L1</sub>	-26	6	60	default mode
fusiform gyrus (L1)	Fusiform <sub>L1</sub>	-29	-40	-13	default mode
middle occipital gyrus (L1)	MOG <sub>L1</sub>	-36	-86	33	default mode
precuneus (R1)	Precuneus <sub>R1</sub>	13	-56	16	default mode
precuneus (R2)	Precuneus <sub>R2</sub>	1	62	56	default mode
superior frontal gyrus (R2)	SFG <sub>R2</sub>	24	22	50	default mode
fusiform gyrus (R1)	Fusiform <sub>R1</sub>	29	-35	16	default mode
middle occipital gyrus (R1)	MOG <sub>R1</sub>	44	-78	33	default mode
middle frontal gyrus (L2)	MFG <sub>L2</sub>	-33,	42	28	saliency
insula (L1)	Ins <sub>L1</sub>	-43	1	2	saliency
supplementary motor area (L1)	SMA <sub>L1</sub>	-1	13	52	saliency
middle frontal gyrus (R1)	MFG <sub>R1</sub>	27	43	33	saliency
opercular part of the inferior frontal gyrus (R1)	IFGoper <sub>R1</sub>	41	14	3	saliency
middle frontal gyrus (L3)	MFG <sub>L3</sub>	-41	31	36	saliency
inferior parietal gyrus (L1)	IPL <sub>L1</sub>	-58	-43	40	saliency
precuneus (L3)	Precuneus <sub>L3</sub>	-8	-57	63	saliency
paracentral lobule (R1)	PCL <sub>R1</sub>	12	-32	48	saliency
superior parietal gyrus (R1)	SPG <sub>R1</sub>	21	-53	71	saliency
supramarginal gyrus (R1)	SMG <sub>R1</sub>	59	-35	39	saliency
thalamus (L5)	Thal <sub>L5</sub>	-13	-25	8	saliency
insula (L2)	Ins <sub>L2</sub>	-37	-16	-2	saliency
thalamus (R1)	Thal <sub>R1</sub>	12	-17	12	saliency
insula (R1)	Ins <sub>R1</sub>	40	-8	-5	saliency
superior temporal gyrus (L1), heschl gyrus	STG <sub>L1</sub>	-57	-18	11	auditory
superior temporal gyrus (R1)	STG <sub>R1</sub>	55	-9	10	auditory
thalamus (R2)	Thal <sub>R2</sub>	13	-18	2	auditory
thalamus (R3), nucleus caudate (R2)	Thal <sub>R3</sub> , Caudate <sub>R2</sub>	14	-4	12	basal ganglia
thalamus (L6), nucleus caudate (L1)	Thal <sub>L6</sub> , Caudate <sub>L1</sub>	-15	-6	11	basal ganglia
triangular part of the inferior frontal gyrus (L1)	IFGtri <sub>L1</sub>	-46	18	28	basal ganglia
triangular part of the inferior frontal gyrus (R1)	IFGtri <sub>R1</sub>	47	26	23	basal ganglia
pons (L1)	Pons <sub>L1</sub>	-6	-27	-36	basal ganglia
median cingulate cortex (R2)	MCC <sub>R2</sub>	2	-31	30	precuneus
precuneus (R3)	Precuneus <sub>R3</sub>	3	-76	41	precuneus
superior parietal gyrus (L1)	SPG <sub>L1</sub>	-36	-68	47	precuneus
angular gyrus (R2)	ANG <sub>R2</sub>	39	-66	47	precuneus
middle occipital gyrus (L2)	MOG <sub>L2</sub>	-28	-91	0	visual
middle occipital gyrus (R2)	MOG <sub>R2</sub>	33	-87	0	visual
cortex surrounding the calcarine fissure (L1)	Calcarine <sub>L1</sub>	0	-77	11	visual
thalamus (L1)	Thal <sub>L1</sub>	-18	-27	-1	visual
triangular part of the inferior frontal gyrus (L2)	IFGtri <sub>L2</sub>	-51	21	1	language
middle temporal gyrus (L1)	MTG <sub>L1</sub>	-53	-4	-18	language

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Table 16 – continued from previous page

middle temporal gyrus (L2)	MTG <sub>L2</sub>	-52	-34	-3	language
angular gyrus (L2)	ANG <sub>L2</sub>	-54	-60	23	language
orbital part of the inferior frontal gyrus (R1)	IFGorb <sub>R1</sub>	48	27	-5	language
superior temporal gyrus (R2)	STG <sub>R2</sub>	55	-45	13	language
middle frontal gyrus (L4)	MFG <sub>L4</sub>	-31	18	54	executive
triangular part of the inferior frontal gyrus (L3)	IFGtri <sub>L3</sub>	-42	44	5	executive
angular gyrus (L3)	ANG <sub>L3</sub>	-41	-69	47	executive
middle temporal gyrus (L3)	MTG <sub>L3</sub>	-59	-45	-10	executive
thalamus (L2)	Thal <sub>L2</sub>	-15	-31	5	executive
middle frontal gyrus (R2)	MFG <sub>R2</sub>	37	22	48	executive
middle frontal gyrus (R3)	MFG <sub>R3</sub>	36	52	7	executive
inferior parietal gyrus (R1)	IPL <sub>R1</sub>	48	57	48	executive
middle superior frontal gyrus (R1)	SFGmed <sub>R1</sub>	4	33	51	executive
nucleus caudate (R1)	Caudate <sub>R1</sub>	12	-1	18	executive
precentral (L1)	Precentral <sub>L1</sub>	-34	-27	63	sensorimotor
precentral (R1)	Precentral <sub>R1</sub>	38	-23	60	sensorimotor
supplementary motor area (R1)	SMA <sub>R1</sub>	2	-18	64	sensorimotor
thalamus (L3)	Thal <sub>L3</sub>	-12	-22	2	sensorimotor
thalamus (R4)	Thal <sub>R4</sub>	11	-22	-1	sensorimotor
superior frontal gyrus (L1)	SFG <sub>L1</sub>	-28	-7	58	visuospatial
inferior parietal gyrus (L2)	IPL <sub>L2</sub>	-36	-52	49	visuospatial
precentral (L2)	Precentral <sub>L2</sub>	-49	8	31	visuospatial
inferior occipital gyrus L1	IOG <sub>L1</sub>	-48	-68	-5	visuospatial
superior frontal gyrus (R3)	SFG <sub>R3</sub>	27	-3	58	visuospatial
inferior parietal gyrus (R2)	IPL <sub>R2</sub>	37	-51	50	visuospatial
precentral (R2)	Precentral <sub>R2</sub>	48	9	33	visuospatial
inferior temporal gyrus (R1)	ITG <sub>R1</sub>	50	-60	-9	visuospatial

*MNI = Montreal Neurological Institute standard brain; RSN = assignment to resting state networks*

## B.4. Analysis 4: Power atlas, Power et al. (2011)

Table 17. – Regions of interest of the Stanford atlas.

Name	Abbreviation	MNI coordinates			RSN
		x	y	z	
precuneus (L1)	PCUN <sub>L1</sub>	-7	-52	61	somatosensory
median cingulate cortex (L1)	mCING <sub>L1</sub>	-14	-18	40	somatosensory
supplementary motor area (L1)	SMA <sub>L1</sub>	0	-15	47	somatosensory
supplementary motor area (R1)	SMA <sub>R1</sub>	10	-2	45	somatosensory
paracentral lobule (L1)	PCL <sub>L1</sub>	-7	-21	65	somatosensory
paracentral lobule (L2)	PCL <sub>L2</sub>	-7	-33	72	somatosensory
postcentral gyrus (R1)	PoCG <sub>R1</sub>	13	-33	75	somatosensory
inferior parietal lobule (L1)	IPL <sub>L1</sub>	-54	-23	43	somatosensory
precentral gyrus (R1)	PreCG <sub>R1</sub>	29	-17	71	somatosensory
precuneus (R5)	PCUN <sub>R5</sub>	10	-46	73	somatosensory
postcentral gyrus (L1)	PoCG <sub>L1</sub>	-23	-30	72	somatosensory
precentral gyrus (L1)	PreCG <sub>L1</sub>	-40	-19	54	somatosensory
postcentral gyrus (R2)	PoCG <sub>R2</sub>	29	-39	59	somatosensory
postcentral gyrus (R3)	PoCG <sub>R3</sub>	50	-20	42	somatosensory
postcentral gyrus (L2)	PoCG <sub>L2</sub>	-38	-27	69	somatosensory
precentral gyrus (R2)	PreCG <sub>R2</sub>	20	-29	60	somatosensory
precentral gyrus (R3)	PreCG <sub>R3</sub>	44	-8	57	somatosensory
postcentral gyrus (L3)	PoCG <sub>L3</sub>	-29	-43	61	somatosensory

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Table 17 – continued from previous page

supplementary motor area (R2)	SMA <sub>R2</sub>	10	-17	74	somatosensory
postcentral gyrus (R4)	PoCG <sub>R4</sub>	22	-42	69	somatosensory
postcentral gyrus (L4)	PoCG <sub>L4</sub>	-45	-32	47	somatosensory
postcentral gyrus (L5)	PoCG <sub>L5</sub>	-21	-31	61	somatosensory
paracentral lobule (L3)	PCL <sub>L3</sub>	-13	-17	75	somatosensory
precentral gyrus (R4)	PreCG <sub>R4</sub>	42	-20	55	somatosensory
precentral gyrus (L2)	PreCG <sub>L2</sub>	-38	-15	69	somatosensory
superior parietal gyrus (L1)	SPG <sub>L1</sub>	-16	-46	73	somatosensory
paracentral lobule (R1)	PCL <sub>R1</sub>	2	-28	60	somatosensory
supplementary motor area (R3)	SMA <sub>R3</sub>	3	-17	58	somatosensory
precentral gyrus (R5)	PreCG <sub>R5</sub>	38	-17	45	somatosensory
postcentral gyrus (L6)	PoCG <sub>L6</sub>	-49	-11	35	somatosensory
insular (R1)	INS <sub>R1</sub>	36	-9	14	somatosensory
postcentral gyrus (R5)	PoCG <sub>R5</sub>	51	-6	32	somatosensory
postcentral gyrus (L7)	PoCG <sub>L7</sub>	-53	-10	24	somatosensory
postcentral gyrus (R6)	PoCG <sub>R6</sub>	66	-8	25	somatosensory
postcentral gyrus (R8)	PoCG <sub>R8</sub>	47	-30	49	somatosensory
supplementary motor area (L2)	SMA <sub>L2</sub>	-3	2	53	cingulo-opercular
supramarginal gyrus (R1)	SMG <sub>R1</sub>	54	-28	34	cingulo-opercular
dorsolateral superior frontal gyrus (R1)	SFG <sub>dorR1</sub>	19	-8	64	cingulo-opercular
dorsolateral superior frontal gyrus (L1)	SFG <sub>dorL1</sub>	-16	-5	71	cingulo-opercular
median cingulate cortex (L2)	mCING <sub>L2</sub>	-10	-2	42	cingulo-opercular
insular (R2)	INS <sub>R2</sub>	37	1	-4	cingulo-opercular
supplementary motor area (R4)	SMA <sub>R4</sub>	13	-1	70	cingulo-opercular
supplementary motor area (R5)	SMA <sub>R5</sub>	7	8	51	cingulo-opercular
rolandic operculum (L1)	ROL <sub>L1</sub>	-45	0	9	cingulo-opercular
insular (R3)	INS <sub>R3</sub>	49	8	-1	cingulo-opercular
claustrum (L1)	Clastrum <sub>L1</sub>	-34	3	4	cingulo-opercular
temporal pole, superior temporal gyrus (L1)	TPO <sub>supL1</sub>	-51	8	-2	cingulo-opercular
median cingulate cortex (L3)	mCING <sub>L3</sub>	-5	18	34	cingulo-opercular
insular (R4)	INS <sub>R4</sub>	36	10	1	cingulo-opercular
insular (R5)	INS <sub>R5</sub>	32	-26	13	auditory
superior temporal gyrus (R1)	STG <sub>R1</sub>	65	-33	20	auditory
superior temporal gyrus (R2)	STG <sub>R2</sub>	58	-16	7	auditory
rolandic operculum (L2)	ROL <sub>L2</sub>	-38	-33	17	auditory
superior temporal gyrus (L1)	STG <sub>L1</sub>	-60	-25	14	auditory
superior temporal gyrus (L2)	STG <sub>L2</sub>	-49	-26	5	auditory
rolandic operculum (R1)	ROL <sub>R1</sub>	43	-23	20	auditory
supramarginal gyrus (L1)	SMG <sub>L1</sub>	-50	-34	26	auditory
supramarginal gyrus (L2)	SMG <sub>L2</sub>	-53	-22	23	auditory
rolandic operculum (L3)	ROL <sub>L3</sub>	-55	-9	12	auditory
rolandic operculum (R2)	ROL <sub>R2</sub>	56	-5	13	auditory
postcentral gyrus (R7)	PoCG <sub>R7</sub>	59	-17	29	auditory
insular (L1)	INS <sub>L1</sub>	-30	-27	12	auditory
default mode middle occipital gyrus (L1)	MOG <sub>L1</sub>	-41	-75	26	default mode
orbital part of the middle frontal gyrus (R1)	ORB <sub>midR1</sub>	6	67	-4	default mode
gyrus rectus (R2)	REC <sub>R2</sub>	8	48	-15	default mode
precuneus (L2)	PCUN <sub>L2</sub>	-13	-40	1	default mode
orbital part of the superior frontal gyrus (L1)	ORB <sub>supL1</sub>	-18	63	-9	default mode
middle temporal gyrus (L1)	MTG <sub>L1</sub>	-46	-61	21	default mode
middle occipital gyrus (R1)	MOG <sub>R1</sub>	43	-72	28	default mode
temporal pole, middle temporal gyrus (L1)	TPO <sub>midL1</sub>	-44	12	-34	default mode
temporal pole, middle temporal gyrus (R1)	TPO <sub>midR1</sub>	46	16	-30	default mode
middle temporal gyrus (L2)	MTG <sub>L2</sub>	-68	-23	-16	default mode
angular gyrus (L1)	ANG <sub>L1</sub>	-44	-65	35	default mode
angular gyrus (L2)	ANG <sub>L2</sub>	-39	-75	44	default mode
precuneus (L3)	PCUN <sub>L3</sub>	-7	-55	27	default mode
precuneus (R6)	PCUN <sub>R6</sub>	6	-59	35	default mode

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precuneus (L4)	PCUN <sub>L4</sub>	-11	-56	16	default mode
precuneus (L5)	PCUN <sub>L5</sub>	-3	-49	13	default mode
median cingulate cortex (R1)	mCING <sub>R1</sub>	8	-48	31	default mode
precuneus (R7)	PCUN <sub>R7</sub>	15	-63	26	default mode
median cingulate cortex (L4)	mCING <sub>L4</sub>	-2	-37	44	default mode
precuneus (R1)	PCUN <sub>R1</sub>	11	-54	17	default mode
angular gyrus (R1)	ANG <sub>R1</sub>	52	-59	36	default mode
dorsolateral superior frontal gyrus (R2)	SFG <sub>dorR2</sub>	23	33	48	default mode
medial part of the superior frontal cortex (L1)	SFG <sub>medL1</sub>	-10	39	52	default mode
dorsolateral superior frontal gyrus (L2)	SFG <sub>dorL2</sub>	-16	29	53	default mode
middle frontal gyrus (L1)	MFG <sub>L1</sub>	-35	20	51	default mode
dorsolateral superior frontal gyrus (R3)	SFG <sub>dorR3</sub>	22	39	39	default mode
dorsolateral superior frontal gyrus (R4)	SFG <sub>dorR4</sub>	13	55	38	default mode
dorsolateral superior frontal gyrus (L3)	SFG <sub>dorL3</sub>	-10	55	39	default mode
dorsolateral superior frontal gyrus (L4)	SFG <sub>dorL4</sub>	-20	45	39	default mode
medial part of the superior frontal cortex (R1)	SFG <sub>medR1</sub>	6	54	16	default mode
medial part of the superior frontal cortex (R2)	SFG <sub>medR2</sub>	6	64	22	default mode
anterior cingulate cortex (L1)	ACC <sub>L1</sub>	-7	51	-1	default mode
medial part of the superior frontal cortex (R3)	SFG <sub>medR3</sub>	9	54	3	default mode
orbital part of the middle frontal gyrus (L1)	ORB <sub>midL1</sub>	-3	44	-9	default mode
orbital part of the middle frontal gyrus (R2)	ORB <sub>midR2</sub>	8	42	-5	default mode
anterior cingulate cortex (L2)	ACC <sub>L2</sub>	-11	45	8	default mode
medial part of the superior frontal cortex (L2)	SFG <sub>medL2</sub>	-2	38	36	default mode
anterior cingulate cortex (L3)	ACC <sub>L3</sub>	-3	42	16	default mode
dorsolateral superior frontal gyrus (L5)	SFG <sub>dorL5</sub>	-20	64	19	default mode
medial part of the superior frontal cortex (L3)	SFG <sub>medL3</sub>	-8	48	23	default mode
middle temporal gyrus (R1)	MTG <sub>R1</sub>	65	-12	-19	default mode
middle temporal gyrus (L4)	MTG <sub>L4</sub>	-56	-13	-10	default mode
middle temporal gyrus (L5)	MTG <sub>L5</sub>	-58	-30	-4	default mode
middle temporal gyrus (R2)	MTG <sub>R2</sub>	65	-31	-9	default mode
middle temporal gyrus (L6)	MTG <sub>L6</sub>	-68	-41	-5	default mode
medial part of the superior frontal cortex (R4)	SFG <sub>medR4</sub>	13	30	59	default mode
anterior cingulate cortex (R1)	ACC <sub>R1</sub>	12	36	20	default mode
middle temporal gyrus (R3)	MTG <sub>R3</sub>	52	-2	-16	default mode
parahippocampal gyrus (L2)	PHG <sub>L2</sub>	-26	-40	-8	default mode
fusiform gyrus (R1)	FFG <sub>R1</sub>	27	-37	-13	default mode
fusiform gyrus (L2)	FFG <sub>L2</sub>	-34	-38	-16	default mode
crus1 (R1)	Crus1 <sub>R1</sub>	28	-77	-32	default mode
temporal pole, middle temporal gyrus (R2)	TPO <sub>midR2</sub>	52	7	-30	default mode
middle temporal gyrus (L7)	MTG <sub>L7</sub>	-53	3	-27	default mode
angular gyrus (R2)	ANG <sub>R2</sub>	47	-50	29	default mode
middle temporal gyrus (L8)	MTG <sub>L8</sub>	-49	-42	1	default mode
orbital part of the inferior frontal gyrus (L2)	ORB <sub>infL2</sub>	-46	31	-13	default mode
orbital part of the inferior frontal gyrus (R2)	ORB <sub>infR2</sub>	49	35	-12	default mode
lingual gyrus (R4)	LING <sub>R4</sub>	18	-47	-10	visual
middle occipital gyrus (R2)	MOG <sub>R2</sub>	40	-72	14	visual
cortex surrounding the calcarine fissure (R1)	CAL <sub>R1</sub>	8	-72	11	visual
cortex surrounding the calcarine fissure (L1)	CAL <sub>L1</sub>	-8	-81	7	visual
middle occipital gyrus (L2)	MOG <sub>L2</sub>	-28	-79	19	visual
lingual gyrus (R5)	LING <sub>R5</sub>	20	-66	2	visual
middle occipital gyrus (L3)	MOG <sub>L3</sub>	-24	-91	19	visual
fusiform gyrus (R2)	FFG <sub>R2</sub>	27	-59	-9	visual
lingual gyrus (L2)	LING <sub>L2</sub>	-15	-72	-8	visual
cortex surrounding the calcarine fissure (L2)	CAL <sub>L2</sub>	-18	-68	5	visual
inferior occipital gyrus (R1)	IOG <sub>R1</sub>	43	-78	-12	visual
inferior occipital gyrus (L2)	IOG <sub>L2</sub>	-47	-76	-10	visual
superior occipital gyrus (L1)	SOG <sub>L1</sub>	-14	-91	31	visual
cuneus (R1)	CUN <sub>R1</sub>	15	-87	37	visual

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middle occipital gyrus (R3)	MOG <sub>R3</sub>	29	-77	25	visual
lingual gyrus (R6)	LING <sub>R6</sub>	20	-86	-2	visual
cuneus (R2)	CUN <sub>R2</sub>	15	-77	31	visual
lingual gyrus (L3)	LING <sub>L3</sub>	-16	-52	-1	visual
inferior temporal gyrus (R4)	ITG <sub>R4</sub>	42	-66	-8	visual
superior occipital gyrus (R1)	SOG <sub>R1</sub>	24	-87	24	visual
cuneus (R3)	CUN <sub>R3</sub>	6	-72	24	visual
middle occipital gyrus (L4)	MOG <sub>L4</sub>	-42	-74	0	visual
fusiform gyrus (R3)	FFG <sub>R3</sub>	26	-79	-16	visual
cuneus (L1)	CUN <sub>L1</sub>	-16	-77	34	visual
cuneus (L2)	CUN <sub>L2</sub>	-3	-81	21	visual
middle occipital gyrus (L5)	MOG <sub>L5</sub>	-40	-88	-6	visual
middle occipital gyrus (R4)	MOG <sub>R4</sub>	37	-84	13	visual
cortex surrounding the calcarine fissure (R2)	CAL <sub>R2</sub>	6	-81	6	visual
middle occipital gyrus (L6)	MOG <sub>L6</sub>	-26	-90	3	visual
fusiform gyrus (L3)	FFG <sub>L3</sub>	-33	-79	-13	visual
middle occipital gyrus (R5)	MOG <sub>R5</sub>	37	-81	1	visual
precentral gyrus (L3)	PreCG <sub>L3</sub>	-44	2	46	fronto-parietal
triangular part of the inferior frontal gyrus (R1)	IFG <sub>triangR1</sub>	48	25	27	fronto-parietal
triangular part of the inferior frontal gyrus (L1)	IFG <sub>triangL1</sub>	-47	11	23	fronto-parietal
inferior parietal lobule (L2)	IPL <sub>L2</sub>	-53	-49	43	fronto-parietal
middle frontal gyrus (L2)	MFG <sub>L2</sub>	-23	11	64	fronto-parietal
inferior temporal gyrus (R5)	ITG <sub>R5</sub>	58	-53	-14	fronto-parietal
orbital part of the superior frontal gyrus (R2)	ORB <sub>supR2</sub>	24	45	-15	fronto-parietal
orbital part of the middle frontal gyrus (R3)	ORB <sub>midR3</sub>	34	54	-13	fronto-parietal
precentral gyrus (R6)	PreCG <sub>R6</sub>	47	10	33	fronto-parietal
precentral gyrus (L4)	PreCG <sub>L4</sub>	-41	6	33	fronto-parietal
middle frontal gyrus (L3)	MFG <sub>L3</sub>	-42	38	21	fronto-parietal
middle frontal gyrus (R1)	MFG <sub>R1</sub>	38	43	15	fronto-parietal
inferior parietal lobule (R1)	IPL <sub>R1</sub>	49	-42	45	fronto-parietal
inferior parietal lobule (L3)	IPL <sub>L3</sub>	-28	-58	48	fronto-parietal
inferior parietal lobule (R2)	IPL <sub>R2</sub>	44	-53	47	fronto-parietal
middle frontal gyrus (R2)	MFG <sub>R2</sub>	32	14	56	fronto-parietal
angular gyrus (R3)	ANG <sub>R3</sub>	37	-65	40	fronto-parietal
inferior parietal lobule (L4)	IPL <sub>L4</sub>	-42	-55	45	fronto-parietal
middle frontal gyrus (R3)	MFG <sub>R3</sub>	40	18	40	fronto-parietal
middle frontal gyrus (L4)	MFG <sub>L4</sub>	-34	55	4	fronto-parietal
orbital part of the middle frontal gyrus (L3)	ORB <sub>midL3</sub>	-42	45	-2	fronto-parietal
angular gyrus (R4)	ANG <sub>R4</sub>	33	-53	44	fronto-parietal
orbital part of the middle frontal gyrus (R4)	ORB <sub>midR4</sub>	43	49	-2	fronto-parietal
triangular part of the inferior frontal gyrus (L2)	IFG <sub>triangL2</sub>	-42	25	30	fronto-parietal
medial part of the superior frontal cortex (L4)	SFG <sub>medL4</sub>	-3	26	44	fronto-parietal
median cingulate cortex (R2)	mCING <sub>R2</sub>	11	-39	50	salience
inferior parietal lobule (R3)	IPL <sub>R3</sub>	55	-45	37	salience
precentral gyrus (R7)	PreCG <sub>R7</sub>	42	0	47	salience
middle frontal gyrus (R4)	MFG <sub>R4</sub>	31	33	26	salience
triangular part of the inferior frontal gyrus (R2)	IFG <sub>triangR2</sub>	48	22	10	salience
insular (L2)	INS <sub>L2</sub>	-35	20	0	salience
insular (R7)	INS <sub>R7</sub>	36	22	3	salience
orbital part of the inferior frontal gyrus (R3)	ORB <sub>infR3</sub>	37	32	-2	salience
insular (R8)	INS <sub>R8</sub>	34	16	-8	salience
anterior cingulate cortex (L4)	ACC <sub>L4</sub>	-11	26	25	salience
supplementary motor area (L4)	SMA <sub>L4</sub>	-1	15	44	salience
middle frontal gyrus (L5)	MFG <sub>L5</sub>	-28	52	21	salience
anterior cingulate cortex (L5)	ACC <sub>L5</sub>	0	30	27	salience
median cingulate cortex (R3)	mCING <sub>R3</sub>	5	23	37	salience
anterior cingulate cortex (R2)	ACC <sub>R2</sub>	10	22	27	salience
middle frontal gyrus (R5)	MFG <sub>R5</sub>	31	56	14	salience

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middle frontal gyrus (R6)	MFG <sub>R6</sub>	26	50	27	salience
middle frontal gyrus (L6)	MFG <sub>L6</sub>	-39	51	17	salience
thalamus (R1)	THAL <sub>R1</sub>	6	-24	0	subcortical
thalamus (L1)	THAL <sub>L1</sub>	-2	-13	12	subcortical
thalamus (L2)	THAL <sub>L2</sub>	-10	-18	7	subcortical
thalamus (R2)	THAL <sub>R2</sub>	12	-17	8	subcortical
brainstem (L1)	Brainstem <sub>L1</sub>	-5	-28	-4	subcortical
putamen (L1)	PUT <sub>L1</sub>	-22	7	-5	subcortical
putamen (L2)	PUT <sub>L2</sub>	-15	4	8	subcortical
putamen (R1)	PUT <sub>R1</sub>	31	-14	2	subcortical
putamen (R2)	PUT <sub>R2</sub>	23	10	1	subcortical
putamen (R3)	PUT <sub>R3</sub>	29	1	4	subcortical
putamen (L3)	PUT <sub>L3</sub>	-31	-11	0	subcortical
nucleus pallidum (R1)	PAL <sub>R1</sub>	15	5	7	subcortical
thalamus (R3)	THAL <sub>R3</sub>	9	-4	6	subcortical
supplementary motor area (L3)	SMA <sub>L3</sub>	-10	11	67	ventral attention
superior temporal gyrus (R3)	STG <sub>R3</sub>	54	-43	22	ventral attention
middle temporal gyrus (L9)	MTG <sub>L9</sub>	-56	-50	10	ventral attention
superior temporal gyrus (L3)	STG <sub>L3</sub>	-55	-40	14	ventral attention
superior temporal gyrus (R4)	STG <sub>R4</sub>	52	-33	8	ventral attention
middle temporal gyrus (R4)	MTG <sub>R4</sub>	51	-29	-4	ventral attention
superior temporal gyrus (R5)	STG <sub>R5</sub>	56	-46	11	ventral attention
triangular part of the inferior frontal gyrus (R3)	IFG <sub>triangR3</sub>	53	33	1	ventral attention
triangular part of the inferior frontal gyrus (L3)	IFG <sub>triangL3</sub>	-49	25	-1	ventral attention
precuneus (R4)	PCUN <sub>R4</sub>	10	-62	61	dorsal attention
middle temporal gyrus (L10)	MTG <sub>L10</sub>	-52	-63	5	dorsal attention
superior parietal gyrus (R1)	SPG <sub>R1</sub>	22	-65	48	dorsal attention
middle temporal gyrus (R5)	MTG <sub>R5</sub>	46	-59	4	dorsal attention
superior parietal gyrus (R2)	SPG <sub>R2</sub>	25	-58	60	dorsal attention
inferior parietal lobule (L5)	IPL <sub>L5</sub>	-33	-46	47	dorsal attention
middle occipital gyrus (L7)	MOG <sub>L7</sub>	-27	-71	37	dorsal attention
precentral gyrus (L5)	PreCG <sub>L5</sub>	-32	-1	54	dorsal attention
inferior temporal gyrus (L4)	ITG <sub>L4</sub>	-42	-60	-9	dorsal attention
superior parietal gyrus (L2)	SPG <sub>L2</sub>	-17	-59	64	dorsal attention
precentral gyrus (R8)	PreCG <sub>R8</sub>	29	-5	54	dorsal attention
inferior occipital gyrus (L1)	IOG <sub>L1</sub>	-25	-98	-12	not assigned
lingual gyrus (R1)	LING <sub>R1</sub>	27	-97	-13	not assigned
orbital part of the superior frontal gyrus (R1)	ORB <sub>supR1</sub>	24	32	-18	not assigned
inferior temporal gyrus (L1)	ITG <sub>L1</sub>	-56	-45	-24	not assigned
gyrus rectus (R1)	REC <sub>R1</sub>	8	41	-24	not assigned
parahippocampal gyrus (L1)	PHG <sub>L1</sub>	-21	-22	-20	not assigned
parahippocampal gyrus (R1)	PHG <sub>R1</sub>	17	-28	-17	not assigned
fusiform gyrus (L1)	FFG <sub>L1</sub>	-37	-29	-26	not assigned
inferior temporal gyrus (R1)	ITG <sub>R1</sub>	65	-24	-19	not assigned
inferior temporal gyrus (R2)	ITG <sub>R2</sub>	52	-34	-27	not assigned
inferior temporal gyrus (R3)	ITG <sub>R3</sub>	55	-31	-17	not assigned
orbital part of the inferior frontal gyrus (R1)	ORB <sub>infR1</sub>	34	38	-12	not assigned
middle temporal gyrus (L3)	MTG <sub>L3</sub>	-58	-26	-15	not assigned
insular (R6)	INS <sub>R6</sub>	27	16	-17	not assigned
orbital part of the inferior frontal gyrus (L1)	ORB <sub>infL1</sub>	-31	19	-19	not assigned
posterior cingulate cortex (L1)	PCC <sub>L1</sub>	-2	-35	31	not assigned
precuneus (L6)	PCUN <sub>L6</sub>	-7	-71	42	not assigned
precuneus (R2)	PCUN <sub>R2</sub>	11	-66	42	not assigned
precuneus (R3)	PCUN <sub>R3</sub>	4	-48	51	not assigned
lingual gyrus (R2)	LING <sub>R2</sub>	8	-91	-7	not assigned
lingual gyrus (R3)	LING <sub>R3</sub>	17	-91	-14	not assigned
lingual gyrus (L1)	LING <sub>L1</sub>	-12	-95	-13	not assigned
orbital part of the middle frontal gyrus (L2)	ORB <sub>midL2</sub>	-21	41	-20	not assigned

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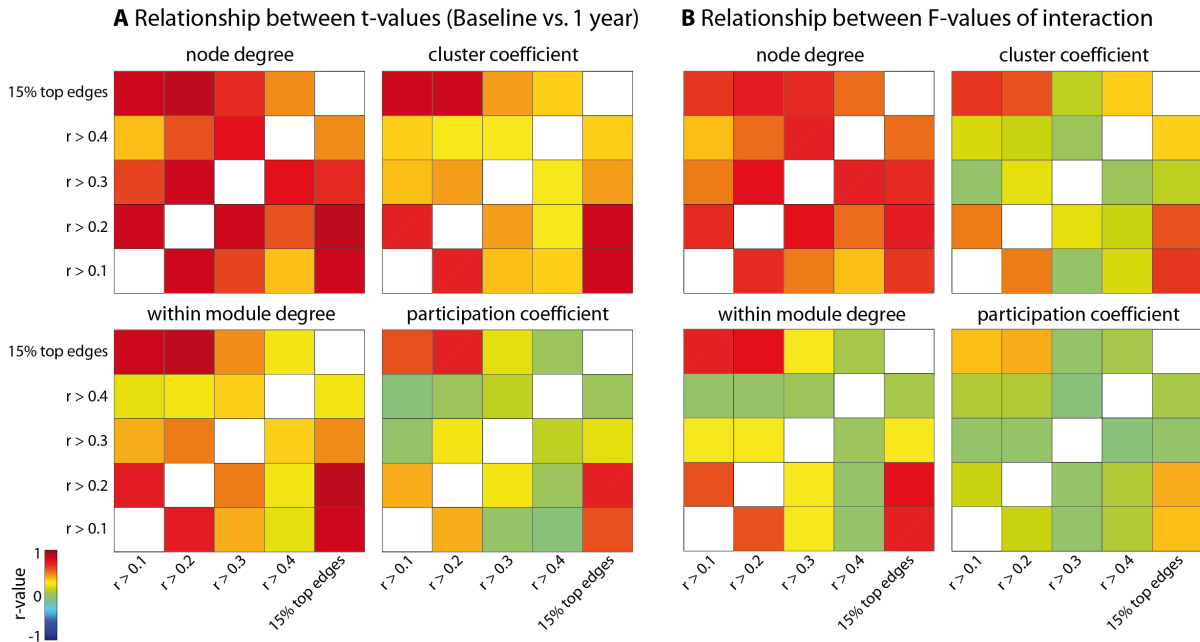
crus1 (L1)	Crus1 <sub>L1</sub>	-18	-76	-24	not assigned
crus2 (R1)	Crus2 <sub>R1</sub>	17	-80	-34	not assigned
crus1 (R2)	Crus1 <sub>R2</sub>	35	-67	-34	not assigned
median cingulate cortex (R4)	mCING <sub>R4</sub>	2	-24	30	not assigned
cerebellum (L1)	Cerebellum <sub>L1</sub>	-16	-65	-20	not assigned
cerebellum (L2)	Cerebellum <sub>L2</sub>	-32	-55	-25	not assigned
cerebellum (R1)	Cerebellum <sub>R1</sub>	22	-58	-23	not assigned
vermis (R1)	Vermis <sub>R1</sub>	1	-62	-18	not assigned
fusiform gyrus (R4)	FFG <sub>R4</sub>	33	-12	-34	not assigned
fusiform gyrus (L4)	FFG <sub>L4</sub>	-31	-10	-36	not assigned
inferior temporal gyrus (R6)	ITG <sub>R6</sub>	49	-3	-38	not assigned
inferior temporal gyrus (L2)	ITG <sub>L2</sub>	-50	-7	-39	not assigned
inferior temporal gyrus (L3)	ITG <sub>L3</sub>	-47	-51	-21	not assigned
inferior temporal gyrus (R7)	ITG <sub>R7</sub>	46	-47	-17	not assigned

*MNI = Montreal Neurological Institute standard brain; RSN = assignment to resting state networks according to Cole et al. (2013)*

## B.5. Analysis 4: Post-hoc evaluation of the influence of different adjacency matrices on statistical results

The construction strategy, which was applied in the present study, was compared to divergently thresholded graphs ( $r > 0.1, 0.2, 0.3, 0.4$ ) in a post-hoc test to explore the influence of different adjacency matrices on statistical outcome. The global similarity between statistical results based on the five divergent adjacency matrices was assessed pairwise using Pearson's correlation. For instance, F-values quantifying the statistical interaction effect on the node-specific cluster coefficient based on matrix  $x$  were correlated with the corresponding F-values from matrix  $y$ . The similarity between t-values capturing the within-group effect of time was estimated across both groups, that is for data series with two times 264 values. This resulted in eight correlation coefficient matrices, one for each metric and statistical contrast (see Fig. 27).

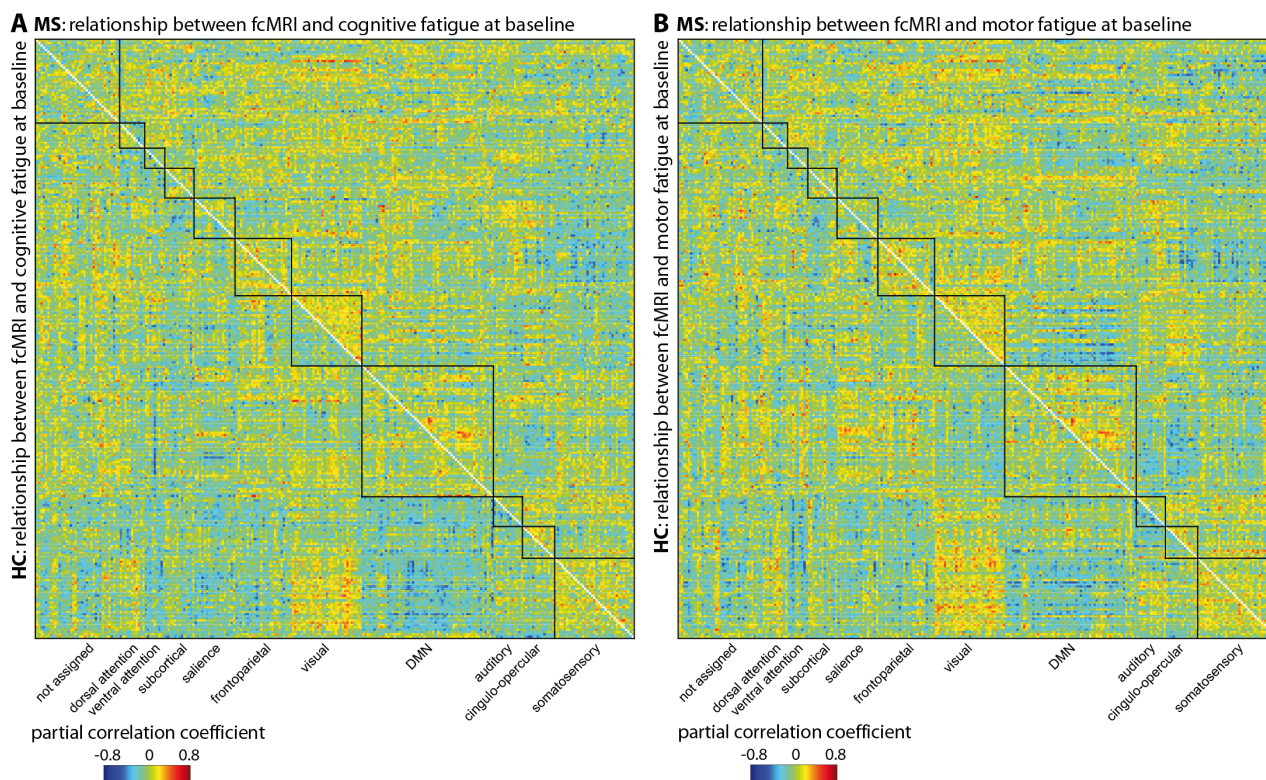
Qualitatively, divergent thresholding strategies seemed to have a greater impact on F-values than t-values, and a differential effect on the graph theoretical metrics. The node degree appeared to be the most robust metric, while differences between matrices were largest for the participation coefficient (node degree:  $r_{t, \text{mean}} = 0.71, r_{F, \text{mean}} = 0.62$ ; participation coefficient:  $r_{t, \text{mean}} = 0.24, r_{F, \text{mean}} = 0.11$ ). Results from the original adjacency matrix used in the main analysis were most similar to those based on the one that included functional connections with correlation coefficients  $> 0.2$ . Least similarity was found with the outcome of the matrix with the highest threshold ( $r > 0.4$ ). This relationship was reflected by the average number of edges resulting from each thresholding strategy, in line with previous reports on the substantial influence of the number of edges and nodes on graph theoretical results (van Wijk et al., 2010, mean  $n_{0.1} = 8901.13$ , mean  $n_{0.2} = 3496.73$ , mean  $n_{0.3} = 1084.85$ , mean  $n_{0.4} = 282.23$ ,  $n_{15\%} = 5208$ ).



**Figure 27.** – Similarity between statistical parameter on graph theoretical metrics based on divergent graphs. **A** Pearson's correlation coefficients for the relationship between graph- and node-specific t-values for the within-group effect of time on four graph theoretical metrics. **B** Pearson's correlation coefficients for the global similarity between F-values describing the interaction effect between time and group. *Warm colors indicate positive relationships*

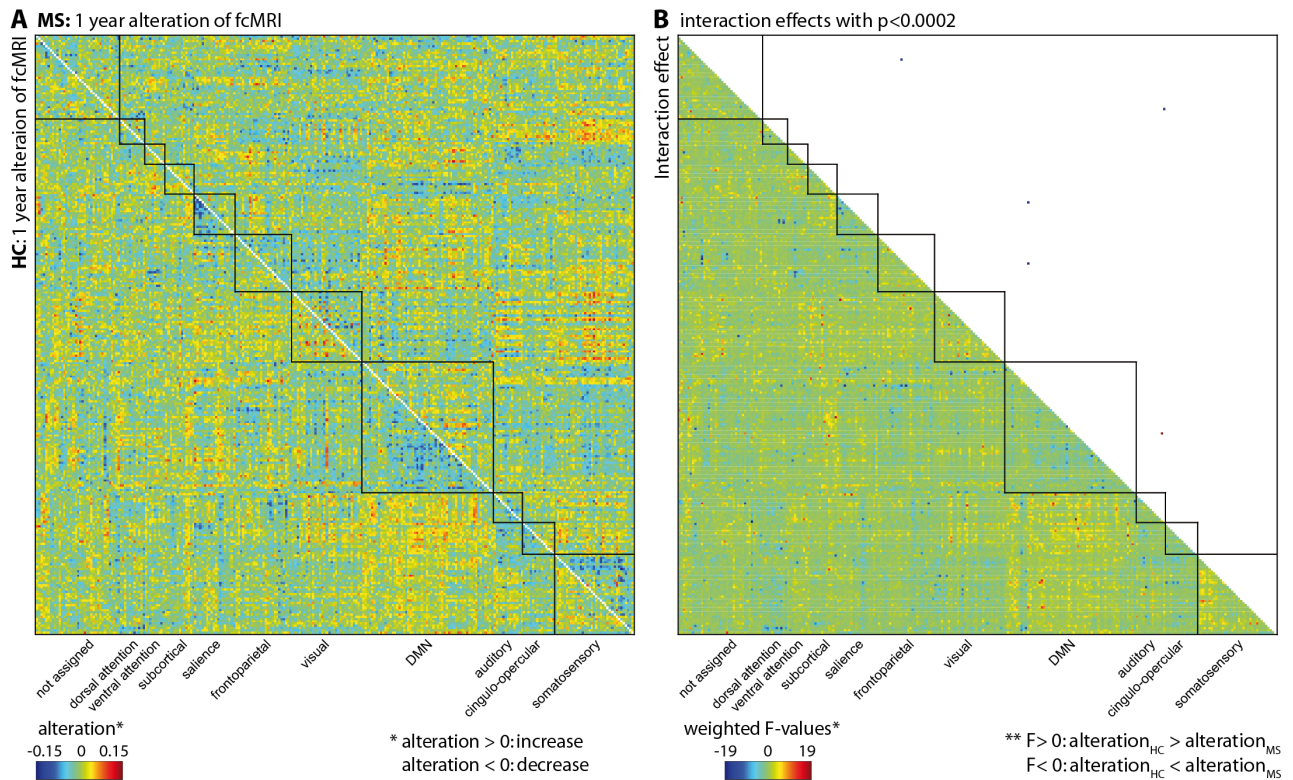
## B.6. Analysis 4: Association between FSMC and fcMRI at baseline

The relationship between the FSMC subscales and fcMRI at baseline was determined with partial rank correlations, separately for each functional connection. Confounder variables were age and disease duration (MS only). Resulting whole-brain association maps are illustrated in Fig. 28.



**Figure 28.** – Relationship between FSMC and fcMRI at baseline. **A** Connection-specific partial rank correlation coefficients for the association between functional connectivity (fcMRI) and cognitive fatigue measured with the Fatigue Scale for Motor and Cognitive Functions at baseline. **B** Whole-brain matrix of regional associations between functional connectivity (fcMRI) and motor fatigue measured with the Fatigue Scale for Motor and Cognitive Functions at baseline. Warm colors indicate positive relationships; lower triangle = healthy controls (HC); upper triangle = multiple sclerosis patients (MS); regions are assigned to ten resting state networks as displayed at the bottom of the matrices according to Cole et al. (2013)

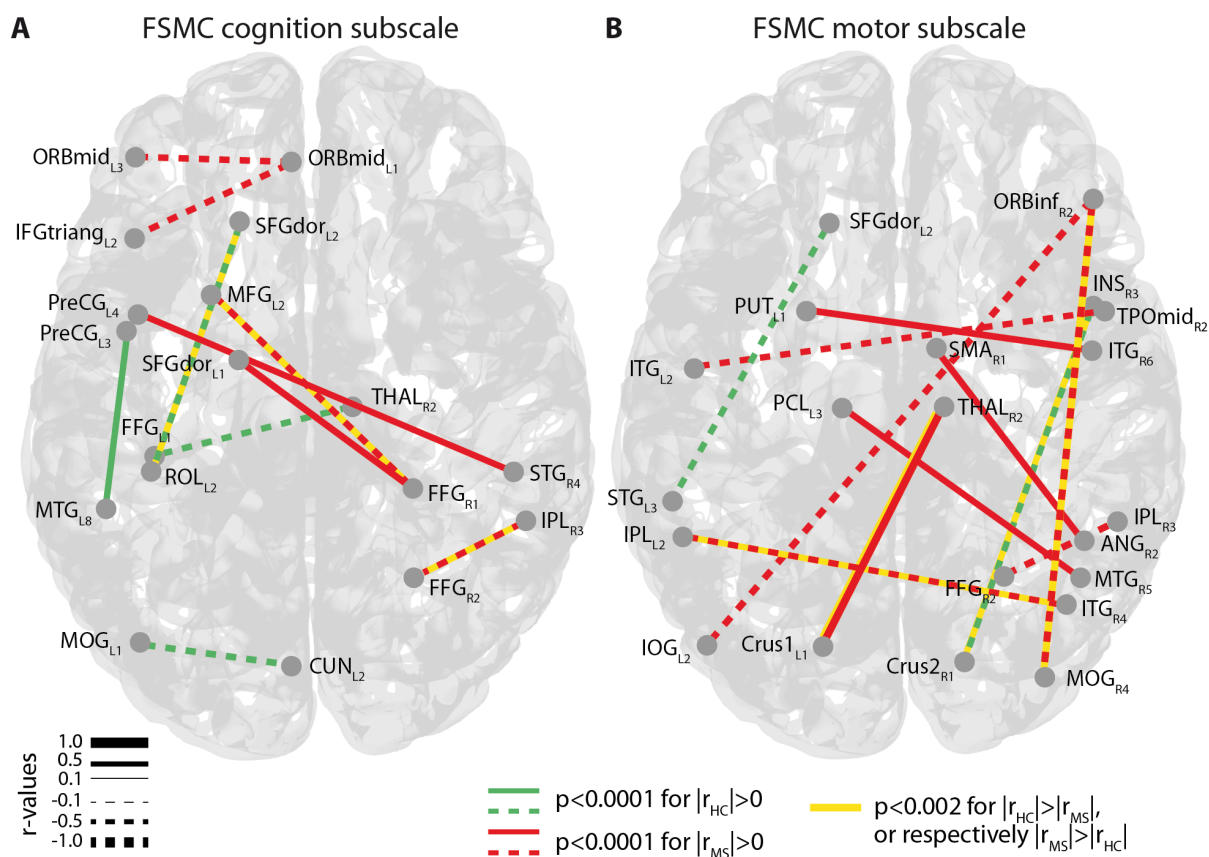
## B.7. Analysis 4: Whole-brain matrices for longitudinal fcMRI alterations



**Figure 29. – Longitudinal alterations in fcMRI.** **A** Alterations in whole-brain functional connectivity (fcMRI) computed between 264 regions of interest in healthy controls (HC) and multiple sclerosis patients (MS). The alteration is the difference between fcMRI at the 1 year follow-up and at baseline. Positive values depict increase over time, while negative values indicate a decrease of fcMRI. **B** Weighted F-values for the interaction effect between group and time-point based on analyses of variances. Computed F-values were multiplied with -1 when the value for the longitudinal alteration was larger in MS patients than controls, indicating that fcMRI increased more in MS, increased in MS and decreased in HC, or decreased less in MS. *Regions are assigned to ten resting state networks as displayed at the bottom of the matrices according to Cole et al. (2013)*



## B.8. Analysis 4: Resting state profiles of fatigue alteration over time in MS patients and controls



**Figure 30.** – Relationship between longitudinal alterations of functional connectivity and fatigue in patients and controls. Functional connections that were significantly ( $p<0.0001$ ) associated with the change in cognitive (**A**) and motor (**B**) fatigue severity over one year in multiple sclerosis (MS) are displayed in red. Significant associations in healthy controls (HC) are depicted in green. Functional connections that additionally differed between groups are highlighted in yellow.