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A neuroimaging approach to clarifying cognitive decline in multiple sclerosis: implications for rehabilitation

By

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"Il cervello: se lo coltivi funziona. Se lo lasci andare e lo metti in pensione si indebolisce.
La sua plasticità è formidabile. Per questo bisogna continuare a pensare."

“The brain: it works if you nurture it. It weakens if you neglect it and put in on retirement.
Its plasticity is impressive. This is why we must persevere with reasoning.”

Rita Levi Montalcini

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Abstract

Multiple sclerosis (MS) is a complex disease characterised by a wide range of symptoms, including cognitive impairment. In particular, patients with MS show processing speed (PS) deficits that may impact other functions. Despite considerable research in the field, the neural correlates of core MS-related cognitive manifestations have not been fully elucidated yet. Similarly, knowledge on whether non-pharmacological approaches are effective to manage these symptoms and the underlying neuroplastic mechanisms supporting any effects is still lacking.

Current understanding of cognitive deficits in MS relies on the hypothesis of a disconnection across brain networks. In this thesis, a series of studies were carried out to investigate the structural and functional connectivity correlates of PS performance in people with relapsing-remitting (RRMS) and secondary progressive MS (SPMS). These findings were subsequently used to test the effects of cognitive rehabilitation specifically designed to stimulate cross-network communication.

Performance on different tests of PS abilities was consistently found to be supported by microstructural integrity of the corpus callosum and other frontal associative white matter tracts. At the functional level three different networks emerged to support PS performance in MS, namely the left fronto-parietal, the salience and the default mode networks. Results were comparable across different MS phenotypes.

Finally, forty-five patients with RRMS volunteered to receive cognitive rehabilitation. A set of computerised multi-domain exercises aimed at facilitating integration of information across different functional networks was used in two conditions, with and without PS demands, to test whether stressing PS abilities of patients may have wider neurocognitive effects. Treatments were compared to care as usual. Only rehabilitation without PS demands induced cognitive improvements and salience network modulation compared to the other two conditions.

Non-pharmacological treatments can modulate cognition in MS, but need to consider PS abilities of patients and integrate knowledge on cerebral functional reorganisation.

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AHSCT: autologous hematopoietic stem cell transplantation; **ANOVA**: Analysis of variance; **BBB**: blood-brain barrier; **CCSVI**: chronic cerebrospinal venous insufficiency; **CIS**: clinically isolated syndrome; **CNS**: central nervous system; **CSF**: cerebrospinal fluid; **DMN**: default mode network; **DMT**: disease modifying treatment; **DSCT**: Digit Symbol Coding Test; **DTI**: diffusion tensor imaging; **EAE**: experimental autoimmune encephalitis; **EBV**: Epstein-Barr virus; **EDSS**: Expanded Disability Status Scale; **FA**: fractional anisotropy; **FC**: functional connectivity; **FLAIR**: fluid attenuated inversion recovery; **FSL**: FMRIB Software Library, **FSS**: Fatigue Severity Scale; **FWE**: Family Wise Error; **GAD-7**: 7-item Generalised Anxiety Disorder; **GM**: grey matter; **HLA**: human leukocyte antigen; **HHV-6**: human herpes virus 6; **ICA**: independent component analysis; **MFIS**: Modified Fatigue Impact Scale; **MMSE**: Mini Mental State Examination; **MNI**: Montreal Neurological Institute; **MRI**: magnetic resonance imaging; **MS**: multiple sclerosis; **MSFC**: Multiple Sclerosis Functional Composite; **MSQoL-54**: Multiple Sclerosis Quality of Life Instrument; **PASAT**: Paced Auditory Serial Addition Test; **PFC**: prefrontal cortex; **PHQ-9**: 9-item Patient Health Questionnaire; **PPMS**: primary progressive multiple sclerosis; **PS**: processing speed; **RIS**: radiologically isolated syndrome; **ROI**: region of interest; **RRMS**: relapsing-remitting multiple sclerosis; **RS-fMRI**: resting-state functional magnetic resonance imaging; **SDMT**: Symbol Digit Modalities Test; **SNP**: single nucleotide polymorphism; **SPM**: Statistical Parametric Mapping; **SPMS**: secondary progressive multiple sclerosis; **TBSS**: tract-based spatial statistics; **TFCE**: threshold-free cluster enhancement, **TIV**: Total intracranial volume; **TLV**: total lesion volume; **TMT**: Trail Making Test; **UVR**: ultraviolet radiations; **VMPFC**: ventromedial prefrontal cortex; **WM**: white matter

Chapter 1 | Multiple sclerosis

Multiple sclerosis (MS) is an immune-mediated disease characterised mainly by an abnormal inflammatory response targeting the central nervous system (CNS) (Compston and Coles, 2008). Previous research has highlighted high levels of burden for both patients affected by MS and their caregivers, especially in terms of time and economic resources (Bayen et al., 2015, Kobelt et al., 2006). Moreover, this disease is recognised as one of the major causes of disability in young adults (Milo and Kahana, 2010). Indeed MS onset can occur quite early in life, usually between 20 and 50 years of age, but with a peak commonly reported around 30 years of age (Compston et al., 2006).

The estimated worldwide prevalence for MS is about 2.3-2.5 million people (Browne et al., 2014, Compston et al., 2006), although figures are variable and show a geographical gradient (Milo and Kahana, 2010). Over the past five decades a steady rise in the number of people affected has been reported not only in prevalence (probably due to improved care opportunities and subsequent increased survival (Howard et al., 2016)), but also in incidence rate, indicating higher disease risk (Melcon et al., 2014). A recent analysis of multiple datasets on MS prevalence from all over the world has led to the proposal of a new and more detailed classification of countries/geographical areas depending on five MS risk classes (Wade, 2014).

It is worth noting that it is also well-established that there is a difference in prevalence between the sexes: females are more likely to develop MS with a women-to-men ratio of about 2.6 (Compston et al., 2006). These skewed numbers may be driven by sexual differences in hormonal and genetic influences on both the nervous and the immune systems, with the evidence being provided by studies in animal models (Ramien et al., 2016). Similarly, the women-to-men ratio has also been rising constantly. The debate on the causes of this phenomenon, however, is still open and points to environmental factors as potential disease fosterers (Boström and Landtblom, 2015, Koch-Henriksen and Sorensen, 2010).

1.1. Aetiology of multiple sclerosis: multiple causes?

Being a multifaceted disease, the cause of MS has yet to be fully understood and several hypotheses have been put forward so far: from environmental to viral and even vascular reasons (Milo and Kahana, 2010).

1.1.1. Genetics of MS

The observation that a small proportion of MS cases tended to cluster in families triggered the beginning of genetic investigations on this disease. Evidence from twin studies has led to the conclusion that up to 30% of MS heritability (Hawkes, 2013). More recently, genome-wide association studies have shed light on different gene variants implicated in MS. Well established is the causal role of some genes present in the major histocompatibility complex and in particular variants of those genes coding for the human leukocyte antigens (HLA). The allele mainly linked to an increased MS risk is the *HLA-DRB1*15:01*, along with *HLA-DRB1*03:01*, *HLA-DRB1*13:01*, *HLA-DPB1*03:01*. However, different protective variants have also been identified in different populations. One is particularly widespread across samples: the *HLA-A*02:01* allele (Patsopoulos et al., 2013, Sawcer et al., 2011).

In addition to the HLA complex, large genetic studies have also assessed thousands of other single nucleotide polymorphisms (SNPs) in search of associations with increased MS susceptibility. So far 110 new genetic variants have been identified (Beecham et al., 2013), the great majority of which are found in regulatory rather than coding regions (ENCODE Project, 2012). However, considering that the identified SNPs are low-frequency/rare variants and that each single person carries several of them, their simple presence cannot explain on its own the development of a medical condition unless evidence of an association is provided (Sawcer et al., 2014).

This genetic scenario seems to point at a prominently antigen-specific autoimmune cause (Hollenbach and Oksenberg, 2015). Nevertheless, interactions between genetic and environmental factors are thought to play an essential part in the aetiology of MS (a more in depth explanation will be given in the following sections) and its progression by influencing both severity of symptoms and neural damage (Goodin, 2016, Zivadinov et al., 2009).

Furthermore, environmental factors may account for possible epigenetic mechanisms proposed to have a role in the manifestation of MS (van den Elsen et al., 2015). In fact, although Baranzini et al. (2010) found no differences in blood cells of MS-discordant couples of monozygotic twins, epigenetic alterations were reported in the frontal areas of people affected by chronic MS as well as in aged subjects (Pedre et al., 2011). Moreover, these types of alterations observed in lesional tissue were significantly associated with disease duration and dysregulation of oligodendrocyte differentiation. Similar findings were also replicated in a study investigating samples of normal appearing white matter (Huynh et al., 2013). Given

the paucity of investigations into the epigenetics of MS, the role of these changes and the possible therapeutic implications are yet to be clarified (Aslani et al., 2016).

1.1.2. Geographical gradient and vitamin D

Traditionally, the prevalence of MS has been observed to be unevenly distributed around the globe following a peculiar pattern related to latitude, the so-called geographical gradient: the higher the latitude the larger the number of MS cases (Milo and Kahana, 2010). This gradient of distribution, though in reverse, has also been observed in white populations in Australia and New Zealand. Recently, this concept has been criticised and updates have been proposed to accommodate the trend of changes in MS rates. Indeed in Europe and North America the gradient appeared no longer detectable, probably due to changes in lifestyle/environmental factors (Koch-Henriksen and Sorensen, 2011).

The principal influence exerted by latitude relates mainly to the amount of sunlight received: medium level evidence on the relationship between low levels of sun exposure and MS risk has been established (Olsson et al., 2016). In particular, the mainstream investigation in this field focussed on ultraviolet radiations (UVR) yielding interesting findings in many studies of experimental autoimmune encephalitis (EAE) in animal models (Lucas et al., 2015). EAE was observed to be suppressed by a narrow band of UVR with a wave length between 300 and 315 nm (Wang et al., 2013). This occurred without production of vitamin D as found also in another study involving skin biopsy from patients affected by MS, leaving an open question about the mechanisms linking UVR radiations to immunomodulation (Breuer et al., 2014, Lucas et al., 2015). However, UVR action seems to be extensively modulated by the presence of a vitamin-D-related enzyme (25-hydroxyvitamin D3-1a-hydroxylase) indicating an important role of this vitamin in the manifestation of MS (Wang et al., 2016b).

Indeed, in recent years vitamin D has widely been recognised as an important immunomodulatory factor that contributes to the maintenance of optimal immune functionality (Peelen et al., 2011). In fact, vitamin D deficiency has been associated with MS risk (Ascherio et al., 2010) and disease severity (Mandia et al., 2014), while high consumption of food rich in it has been reported to be protective in the Norwegian population (Kampman and Brustad, 2008). Furthermore, vitamin D receptor polymorphisms also appear to impact on immune function and, in turn, the course of MS (Smolders et al., 2009).

Although the effects of vitamin D and UVB light exposure in influencing immune responses have been repeatedly tested, their causal role in the genesis of MS is still not fully understood (Rhead et al., 2016). However, a unique contribution can definitely be ruled out as different regional studies observed rates of MS completely against the geographical gradient hypothesis, suggesting a greater influence of genetic factors. Both in Lapland and New Zealand, places with low levels of sunlight, low prevalence rates of MS are reported among local populations of Sami and Maori (Alla and Mason, 2014, Grønlie et al., 2000). On the contrary, in Sardinia, in the middle of the Mediterranean area, MS figures are significantly higher than the surrounding regions (Barizzone et al., 2015, Pugliatti et al., 2001). Similarly, in Israel, a small country located at a low latitude, differential prevalence of MS can be found in groups with different ethnic origins (Alter et al., 2006). Hence, vitamin D and light exposure, though contributing to specific mechanisms, cannot by themselves account for all the complex disease manifestations.

1.1.3. Viral infections

Among the different causes of MS that have been object of investigation, viral infection is one of the most prominent ones (Ramagopalan et al., 2010). Viral effects on the nervous system relevant to MS pathology may be both direct, such as neuronal damage and demyelination, and indirect by upregulating the level of autoimmunity (van der Star et al., 2012). In particular different ubiquitous human viruses have been associated with MS, namely the Epstein-Barr virus (EPV), the human herpes virus 6 (HHV-6) and the varicella zoster virus (Virtanen and Jacobson, 2012).

The EPV has been strikingly detected in almost 100% of patients with MS; in contrast among people seronegative for EPV infection, the risk of MS is very low (Thacker et al., 2006). Conversely, it was found that the higher the amount of EPV antibodies, the stronger the risk of developing MS (Sundström et al., 2004). However, what appears to be a strong risk factor for MS is history of infectious mononucleosis, i.e. the clinical manifestation of the EBV (Handel et al., 2010, Olsson et al., 2016) which was found to interact with childhood obesity generating impressively high odds of MS incidence and prevalence compared to those unexposed (Hedström et al., 2014).

The HHV-6 has also been implicated in disease course: investigations into MS-caused plaques revealed that this virus was present in lesional white matter (WM) in significantly higher amounts than in normal appearing WM (Cermelli et al., 2003).

Furthermore, activation of the virus during phases of clinical worsening has been observed, with higher rates of viral detection in patients experiencing relapses rather than remissions (Berti et al., 2002). Hence, these pieces of evidence seem to suggest a role of active HHV-6 infection in the clinical course of MS.

Findings on the association between MS and the varicella zoster virus are less clear, although a large population-based study in Taiwan found a higher MS risk in people affected by the virus (Kang et al., 2011). As for the HHV-6, an increased expression of the varicella zoster virus during relapses has also been reported (Sotelo et al., 2014). Despite the currently available evidence on the role of ubiquitous viruses, given their common and widespread distribution among the general population, it appears still difficult to draw definite conclusions on their causal involvement in MS development (Virtanen and Jacobson, 2012).

A possible viral effect has also been suggested by research on human immunodeficiency virus that reported significantly lower risk of MS in patients affected (Gold et al., 2014a). However, only limited and partial evidence has been gathered so far and further replication is needed in order to rule out any possible association.

Finally, endogenous human retroviruses, i.e. those which entered the human genome millions of years ago, were reported active in MS-affected brains. In particular, these retroviruses appeared to affect astrocytes, leading to oligodendrocyte damage and demyelination (Virtanen and Jacobson, 2012), and to be expressed in active MS lesions (van Horssen et al., 2016). However, their detection has not been found to be correlated to MS onset and progression and both experimental and prospective epidemiological studies are currently lacking (Tao et al., 2016).

In conclusion, the debate appears still open both about a possible causal influence of viral factors on MS and on whether one or multiple viral agents may contribute to it (Steiner and Sriram, 2007).

1.1.4. Other environmental factors

Lifestyle could be potentially modulating MS susceptibility as suggested by research on smoking habits. In fact, a meta-analysis by Handel et al. (2011) found that cigarette smoking increases MS risk, while effects on the disease course were not clear. Recently, a large review of systematic reviews pointed out that, apart from positivity to the EBV and history of infectious mononucleosis, only smoking is definitely associated with higher MS rates (Belbasis et al., 2015). Along with active,

passive smoking also affects MS susceptibility, and both interact with genetic predispositions to MS, i.e. variants of the HLA complex (Olsson et al., 2016). Indeed, smoke seems to have a detrimental influence, possibly through lung irritation, on autoimmunity observed across multiple syndromes (Perricone et al., 2016). However, Hedström et al. (2009) found an interesting protective effect of prolonged use of oral tobacco against MS risk. Hence, these findings suggest that nicotine per se, differently from exposure to smoke, might have a positive effect on MS pathology.

In recent years obesity has also been increasingly considered as a possible risk factor for MS. In fact, genetic studies found that several genetic variants involved in high body mass index seem to be also linked to MS risk (Mokry et al., 2016). Moreover, obesity interacts both with genetic susceptibility (Olsson et al., 2016), and childhood obesity (Hedström et al., 2014) dramatically increases MS risk. The main explanation about the possible underlying mechanisms put forward so far highlights the fact that obesity generates a state of chronic inflammation that may, in turn, trigger a cascade of events leading to activation of autoimmunity and MS (Gianfrancesco and Barcellos, 2016).

Finally, a recent line of research investigated the possible negative impact of exposure to different metals found in specific environments: MS prevalence has been associated with exposure to copper in south-western Sardinia (Monti et al., 2016) and to lead and cadmium in Iran (Etemadifar et al., 2016). However, just a few studies of this kind are available and no definite conclusions can be drawn from the evidence available so far.

1.1.5. Vascular factors

Finally, a vascular hypothesis of MS has been among the earliest ones put forward, based on the observation that brain lesions are often close to blood vessels (Rae-Grant et al., 2014). In the second half of the 20th century this hypothesis was discarded. However, it has been recently reconsidered since Zamboni et al. (2009b), using a new venography technique, observed that 100% of MS patients had a condition called chronic cerebrospinal venous insufficiency (CCSVI). This result has been used by Zamboni to advance a new theory of MS having CCSVI as unique and prominent cause and inflammation being a by-product of that.

However, since its inception the concept of CCSVI has been a matter of debate because of a series of criticisms, at times also involving mass media exposure. Apart from problems related to the main authors' conflict of interests, an issue

consistently observed was lack of reproducibility. In fact, the results obtained by Zamboni et al. (2009b) on the CCSVI rate among patients affected by MS have never been replicated by any other investigation (Comi et al., 2013, Rodger et al., 2013). Moreover, recent studies failed to detect differences between patients and healthy people in the prevalence of venous problems according to the CCSVI criteria (Traboulsee et al., 2013, Martin et al., 2016).

In an attempt to defend his theory, Zamboni et al. (2013) identified possible causes leading to the mismatch between their own data and those from other centres, such as high heterogeneity in assessment procedures, equipment used, and training of assessors. Moreover, three meta-analyses have been published so far that found a significant global association between CCSVI and MS, though with several caveats: high inter-studies variability in prevalence of CCSVI, potential conflicts of interests of some authors, reduction or disappearance of association when more conservative analyses were performed (Laupacis et al., 2011, Tsivgoulis et al., 2014, Zwischenberger et al., 2013). Hence, no clear conclusions can be drawn, especially on a possible causal effect of venous insufficiency in MS aetiology. Studies using multimodal imaging to investigate this issue further, in fact, have not reported any significant associations (Tsivgoulis et al., 2015).

Lack of clarity characterises also the effects of the so-called “liberation treatment”, a surgical intervention to solve CCSVI: while considerable improvement of clinical outcome measures was initially reported (Zamboni et al., 2009a), additional research highlighted how those improvements could be mainly due to a placebo effect, given that post-operation improvement was observed only in subjectively reported measures (Siddiqui et al., 2014).

Given the paucity of objective evidence and lack of independent reproducibility, the scientific community has cast doubts on the hypothesis that inflammation may just result from large vessels pathology as it appears far too simplistic an explanation for such a complex disease (Rae-Grant et al., 2014).

However, it is worth noting that, leaving aside the dispute on the concept of CCSVI, research on the cause of MS has also been focusing on the contribution of cerebrovascular factors in the manifestation of pathological changes. In fact, as previously mentioned, perivascular lesions are not rare in MS and an involvement of blood-brain barrier (BBB) disruption has been proposed (Alvarez et al., 2014, Gaitán et al., 2012). This process seems mainly be mediated by leukocytes involvement, that eventually migrate from the bloodstream to the neural tissue where inflammation spreads (Ortiz et al., 2014). Yet BBB damage in MS appears more dynamic and not straightforward to interpret since the presence of vascular

breakdown, usually in the form of cerebral microbleeds as observed in other pathologies of prominent vascular origin, has not been consistently highlighted (Eisele et al., 2016, Zivadinov et al., 2016). Therefore, currently only spurious results have been obtained and a possible role of vascular disruption in MS pathology has not been elucidated yet.

Interestingly, cardiovascular comorbidities in MS have been linked to both faster progression and increased lesion burden (Kappus et al., 2015, Marrie et al., 2010). A recent study has also found overlapping SNPs between MS and cardiovascular risk factors, suggesting common underlying pathological processes (Wang et al., 2016a). In summary, from the above evidence it appears that a relationship between vascular problems and MS cannot currently be ruled out, though the nature and directionality of the relationship remains unexplained.

1.2. Neuropathology of MS

The various manifestations of CNS damage and related symptoms that characterise MS guided researchers towards two main lines of investigation into its pathological substrate: neural inflammation and neurodegeneration (Mallucci et al., 2015).

1.2.1. Inflammatory response and demyelination

Despite lack of certainty about the causes, autoimmunity and CNS inflammation are core features of MS and several lymphocytes have been found implicated in different pathological processes (Dendrou et al., 2015). Autoreactive T cells are those driving demyelination: various subtypes of $CD4^+$ T cells (Elong Ngono et al., 2012, Hellings et al., 2001) as well as $CD8^+$ T cells, whose role is still unclear, that are found more frequently than $CD4^+$ T cells in lesional tissue (Babbe et al., 2000, Frischer et al., 2009) and are considered the major cause of both axonal transection and oligodendrocytes death (Johnson et al., 2007). Autoreactive B cells, whose functioning is still not completely understood, have also been detected in different sites, namely brain parenchyma, meninges, and cerebrospinal fluid (CSF) (Kinzel and Weber, 2016). In particular, in the progressive types of MS, these cells seem to play a major role in meningeal pathology (Howell et al., 2011). Finally, different types of regulatory T cells, cells involved in modulating the activity of other lymphocytes, have also been reported as malfunctioning even though no reduction in their number was found in a recent meta-analysis (Dendrou et al., 2015, Noori-Zadeh et al., 2016).

Two models have been proposed to explain the inflammatory processes characterising MS: CNS-extrinsic and CNS-intrinsic (Dendrou et al., 2015). According to the former, in the first instance autoreactive T cells are activated in the peripheral nervous system and later migrate to the CNS through the BBB and the blood-CSF barrier with B cells and monocytes. Several sources of evidence have highlighted molecular mimicry as the main mechanism driving this process: myelin proteins would be attacked by the immune system because of their resemblance of infectious agents that may have previously promoted autoimmunity (Ji et al., 2010, Hauser and Oksenberg, 2006, Lang et al., 2002, Münz et al., 2009). In the first stage of the disease, peripheral lymphocyte activation subsequently spreading to the CNS is considered predominant. As MS progresses, inflammation settles and becomes chronic in the CNS, leading, in turn, to neurodegeneration (Ellwardt and Zipp, 2014).
In contrast, the latter model views the infiltration of autoreactive leukocytes from the periphery only as a process secondary to others (viral infections and neurodegeneration) occurring in the CNS. Indeed, so-called “pre-active lesions” caused by microglia activation have been observed in normal appearing WM in the absence of lymphocytic infiltration through the BBB and the blood-CSF barrier (van der Valk and Amor, 2009, van Noort et al., 2010).
The peripheral model is considered to be the most plausible one because of its consistency with findings from research on the EAE animal model. Moreover, the lack of any association between MS and genetic variants leading to neurodegeneration independently of inflammation appears as another source of evidence against the centrality of CNS-intrinsic causes of MS (Ben-Nun et al., 2014). However, it is worth mentioning the recent discovery of cerebral branches of the immune system, involved in surveillance and clearance processes, that gives potential support to the CNS-intrinsic model (Aspelund et al., 2015, Heneka et al., 2014, Louveau et al., 2015).
Once activated T cells have migrated into the CNS the myelin sheath surrounding the axons is attacked and demyelination occurs. This process is accompanied by considerable release of pro-inflammatory cytokines that, in turn, will lead to worsening of MS-related pathology. As a first consequence, conduction of neuronal signals is hampered and slowed down. However, as myelin damage spreads, other pathological processes are triggered causing neurodegeneration both in WM and grey matter (GM) (Hauser and Oksenberg, 2006).

1.2.1.1. WM lesions

At a macrostructural level, WM demyelination is manifest in the form of lesions, both in the brain and the spinal cord, that constitute a MS hallmark and a fundamental criterion for diagnosis (Polman et al., 2010). WM lesions can be detected and visualised by means of magnetic resonance imaging (MRI). Indeed, areas of hyperintensity can be highlighted using specific MRI sequences: namely T2 and fluid attenuated inversion recovery (FLAIR). Although lesions are thought to spread randomly across the brain, a peak has been consistently reported in some locations, such as periventricular areas and the centrum semiovale, around the corpus callosum (Dalton et al., 2011, Filli et al., 2012). Moreover, it has been reported how lesions tend to accumulate in regions with high venous coverage and in those with low arterial density, the so-called watershed areas that may be particularly sensitive to pathological processes initiated by MS-related demyelination such as oxidative stress (Haider et al., 2016).

Lesions affecting WM have been classified according to different systems from a pathological perspective. Lucchinetti et al. (2000) focussed on the expression of demyelination and identified four patterns: in patterns I and II, lesions are characterised by the presence of infiltrating T cells, macrophages, ongoing demyelination and variable immunoglobulin depositions; in pattern III and IV, instead, they are mainly characterised by loss of oligodendrocytes and selective myelin preservation. Whether the manifestation of one particular pattern is predominant throughout disease course has not been clarified yet. However, it seems commonly recognised that all types of lesion tend to experience a transition from an active to a final inactive state (Mallucci et al., 2015).

More recently, Kuhlmann et al. (2016) proposed a new lesion classification based on the relationship between the manifestation of inflammatory and demyelinating activities. They divided lesions in three possible categories: active, with spread presence of macrophages and microglia and variable levels of demyelination; mixed, characterised by inflammatory activity limited to the peripheral layers of the lesion; and inactive, without ongoing demyelination and almost no activated macrophages and microglia.

Investigations into demyelination have shed light also on the dynamic of this process, pointing out that lesions can undergo phases of remyelination: the myelin sheath can be regenerated around axons by the activity of the oligodendrocyte precursors cells (Franklin and Ffrench-Constant, 2008). However, remyelination appears to be a precarious process since it has been observed to occur only incompletely and not in all patients (Patrikios et al., 2006). Moreover, newly formed

oligodendrocytes in lesional tissue are more susceptible to further damage than in normal appearing WM, especially in progressive forms of MS (Bramow et al., 2010).
Finally, a subgroup of lesions, named juxtacortical, may manifest in areas of WM extended to the deepest layers of GM. This type of lesions has been observed in equal numbers across different MS types (Sethi et al., 2015) and has been found associated with significant cortical thinning even in early phases of the disease (Pareto et al., 2015). Furthermore, juxtacortical damage, rather than intracortical, seems to have an impact on MS-related cognitive decline, especially in executive functioning (Louapre et al., 2016).
Beyond WM damage, GM and the meninges have also been consistently reported to be affected by inflammation (DeLuca et al., 2014, Klaver et al., 2013, Prins et al., 2015).

1.2.1.2. GM pathology

Along with the well-established WM lesions, GM pathological changes have also been reported. In particular, demyelination appeared to affect GM especially as the course of the disease progresses and becomes chronic (Kutzelnigg et al., 2005, Mainero et al., 2015). This process has been observed throughout the whole brain, both in cortical and deep GM (DeLuca et al., 2014), and recently also confirmed by means of positron emission tomography (Herranz et al., 2016). However, the cerebellum and the spinal cord consistently resulted as the areas in the CNS more strongly affected by demyelination compared to the cortex and deep GM nuclei (Gilmore et al., 2008, Minagar et al., 2013).
Demyelination in GM leads to formation of lesions that have been classified into four types: type I, involving both deep GM layers and WM; type II, otherwise called intracortical because they do not involve either GM surface or underlying WM; type III, are subpial lesions that extend from the pia mater through the most superficial GM layers; and finally type IV, extending from the pial surface throughout the whole cortex (Bø et al., 2003, Calabrese et al., 2009). Interestingly, GM lesions seem to have a negative impact both on physical disability and cognitive impairment (Calabrese et al., 2009, Mainero et al., 2015).
A definite explanation of the mechanisms of formation of GM lesions has not been provided yet and the underlying pathological processes are still poorly understood (Klaver et al., 2013). However, differences in lymphocytic and glial involvement between WM and GM lesions have been consistently observed with the former outnumbering the latter. Several factors may contribute to cause this: later

development of GM lesions, lesser BBB disruption and leakage in GM, and the myelin-led inflammatory response (Prins et al., 2015).

Recently, Mandolesi et al. (2015) investigated the potential role of synaptic dysfunction as the possible missing link between inflammation and GM degeneration, rather than axonal loss. Indeed, neurotransmission of both glutamate and *gamma*-Aminobutyric acid appeared to be altered in MS, especially in the hippocampus. However, this mechanism seems not to be exclusively related to MS, but shared with other neurological diseases (Henstridge et al., 2016).

1.2.1.3. Meningeal inflammation

In recent years, several investigations have highlighted how the meninges are also affected by MS and show signs of inflammation (Dendrou et al., 2015). The more advanced and chronic phases of the disease appear especially characterised by meningeal damage. Indeed, in secondary progressive MS (SPMS), peculiar formations, namely the tertiary lymphoid structures, have been described in patients' meninges comprising lymphocytes (B and T cells) together with dendritic and endothelial cells (Pikor et al., 2016). These aggregates are thought to drive neuropathology as they have been associated with astrocytic dysfunction in SPMS (Howell et al., 2011) and microglia activation in primary progressive MS (PPMS) (Choi et al., 2012).

Moreover, meningeal inflammation has been repeatedly observed to affect the neighbouring cortex and to facilitate spreading of inflammation (Howell et al., 2011, Popescu and Lucchinetti, 2012). Also in the cerebellum, even in the absence of lymphoid-like structures, pathological changes have been reported in relation to subarachnoid inflammation (Howell et al., 2015).

Hence, inflammation in the meninges appears to be associated with GM pathology, especially in the progressive stages. However, accounts of meningeal damage have also been reported early on in the disease development even before WM lesions manifested, thus indicating a possible role in CNS attack and MS onset (Lucchinetti et al., 2011).

1.2.2. Neurodegeneration

The inflammatory response related to MS is accompanied by pathological processes that worsen as the duration of the disease course increases (Stadelmann et al., 2011). Consequently, neurodegeneration becomes a predominant feature of brain damage, especially in progressive MS (Mahad et al., 2015). It is worth noting that

axonal loss is observed during the acute phases of CNS damage, and also early on after disease onset (Trapp et al., 1998). However, it is only after a series of relapses have occurred that inadequate immune responses combined with accumulation of neuronal debris and loss of homeostasis in the neuronal environment lead to the death of neurons (Koudriavtseva and Mainero, 2017, Mandolesi et al., 2015). The pathological mechanisms thought to guide neurodegeneration in MS are reviewed below.

1.2.2.1. Astrocyte activation

In the healthy brain, astrocytes carry out several different protective (e.g. BBB), adaptive, regulatory, and supportive functions that enable normal brain development and functioning (Ludwin et al., 2016). Therefore, defining when reactive astrocytes become detrimental is still regarded as a challenge. Indeed, the role they play in MS pathology is yet not completely understood and it is highly likely that they have both a positive and a negative impact (Ponath et al., 2016, Williams et al., 2007).

It has been suggested that the valence of astrocytic activity may be classified according to the severity and timing of neural tissue injury, since in active lesions astrocytes have been shown to dispose of myelin and cellular debris as well as promote oligodendrocyte regeneration and remyelination (Guo et al., 2016, Ludwin et al., 2016). Moreover, they form perivascular scar-like barriers that prevent further lymphocytes from crossing the BBB (Voskuhl et al., 2009). However, in chronic long-lasting lesions gliosis occurs at a more consistent level, with astrocytes undergoing structural modifications that make them more rigid and lead to the formation of scars that inhibit regeneration (Ludwin, 2006). Hence, the scenario appears complex as recently highlighted by a paper reporting that microglia can activate a subtype of astrocytes that induce death of both neuron and oligodendrocytes (Liddelow et al., 2017). However, these findings were not specific to MS, but nonetheless showed a possible pathway of astrocytic influence on CNS injury.

1.2.2.2. Microglial activation

As for astrocytes, microglia cells also have important functions related to brain development and constant surveillance of brain homeostasis. However, microglia cells have been observed to exert both beneficial and detrimental influences on neural tissue affected by MS pathology: clearance from myelin debris that favours neuronal repair (Bogie et al., 2014, Napoli and Neumann, 2009) and phagocytosis of cells and parts of cells that lead to neurodegeneration (Kettenmann et al., 2011).

Indeed, Peferoen et al. (2015) have recently found that microglia can express both pro- and anti-inflammatory phenotypes in different stages of MS lesions. Furthermore, both in humans and animal models of MS, microglia activation seems to be associated with the formation of inflammatory lesions (Heppner et al., 2005, Ponomarev et al., 2005), oligodendrocyte pathology (Henderson et al., 2009), and axonal degeneration (Singh et al., 2013).

Therefore, it appears that complex interactions occur between oligodendrocytes, astrocytes and microglia both in health and disease. In fact, astrocytes and microglia promote axonal myelination in healthy brains, but when the CNS is attacked they can either foster possible induction of further oligodendrocyte death or facilitate remyelination after acute injury (Domingues et al., 2016). Hence, microglia may have a role in MS-related neural damage as part of a multifaceted picture.

1.2.2.3. Mitochondria dysfunction and virtual hypoxia

Axonal mitochondria represent the fundamental sites of energy production that sustain normal axonal signal conduction and their functioning can be hampered by both demyelination and microglia activation (Mahad et al., 2015). Indeed, the release of reactive oxygen species and nitric oxide are among the reactions triggered by microglia activation observed in MS (Fischer et al., 2012, Gray et al., 2008) and thought to affect mitochondria dysfunction (Larsson, 2010, Stewart et al., 2000). Consistently, several different pathological processes appear to be associated with mitochondrial injury: alterations in mitochondrial DNA, increased production of reactive oxygen species, ion imbalance and cell death (Campbell et al., 2014, Mao and Reddy, 2010).

As a result, respiration occurring in these organelles is downregulated causing virtual hypoxia, i.e. a state of energy deficiency that can be significantly worsened by reduction in oxygen supply to affected tissues or by increased energy demands (Trapp and Stys, 2009). Indeed, the association between mitochondrial dysregulation and neurodegeneration may be due to the redistribution of ion channels on demyelinated axons and, more specifically, sodium channels (Bouafia et al., 2014, Roostaei et al., 2016, Shields et al., 2012, Waxman, 2006). Hence, the overload posed on mitochondria in order to re-establish ionic balance in axons may induce energy failure and a downward spiral of pathological processes ending in neuronal degeneration (McMahon et al., 2012, Trapp and Stys, 2009).

1.2.2.4. Iron accumulation

In the brain iron is naturally stored in the myelin, oligodendrocytes and microglia (Mahad et al., 2015) and, in healthy people not affected by neurological conditions, it accumulates with age (Hametner et al., 2013, Ramos et al., 2014). In the absence of disease, iron plays an important role in maintaining normal levels of myelination as various iron-containing enzymes regulate oligodendrocyte precursor cells (Stephenson et al., 2014). However, when MS-related inflammation occurs and demyelination is triggered, iron is liberated in the intercellular space inducing an increase in toxicity of oxidative reactive species (Hametner et al., 2013).

The brain appears to be differentially affected by iron deposition, with deep GM structures being those experiencing the strongest effect, both in healthy adults and patients with MS from the early phases of the disease (Haider et al., 2014, Khalil et al., 2015, Ramos et al., 2014). Consistently, it has been observed that the basal ganglia and the thalamus are the most damaged GM structures in MS (Lansley 2014). Moreover, their atrophy appears to be associated both with the level of iron load and with degeneration of related WM tracts (Bergsland et al., 2016a) and to induce cognitive impairment (Modica et al., 2014). Therefore, iron accumulation seems to play a role in driving neurodegeneration and related clinical symptoms.

1.2.3. Neuroimaging investigations

The role of MRI in the diagnostic process of MS is crucial, considering the need to highlight brain lesions associated with clinical symptoms (see section 1.3.1). Hence, it comes as no surprise that the literature on neuroimaging studies on MS has flourished throughout the past decades in the attempt to characterise several structural and functional changes that accompany this disease. In this section a brief overview of the aforementioned line of research is provided.

1.2.3.1. Brain microstructure

Investigations on microstructural changes due to MS-related demyelination have especially relied on the use of diffusion tensor imaging (DTI), which allows the assessment of indirect indices of WM integrity, such as fractional anisotropy (FA), based on diffusivity properties of water molecules present in the brain (Le Bihan et al., 2001). Indeed, water diffuses in WM preferentially along the main direction of neural fibres. However, alterations to water diffusivity occur as a result of demyelination or other pathological processes and DTI can detect such changes (Sbardella et al., 2013b).

In a meta-analysis comprising twelve studies, with a total of 495 patients with MS and 253 healthy controls, Welton et al. (2015) observed widespread alterations of WM microstructure, especially in the corpus callosum, periventricular areas and the fornix. Microstructural integrity of thalamic radiations and the fornix correlated with both disability and cognitive impairment. However, while damage to the posterior corpus callosum contributed to worse disability levels, deficits in cognition were related to lower FA in the anterior corpus callosum.

Several studies also investigated the dynamic relationship between lesion formation and microstructural alterations in WM. Some have found that the formation of new lesions, both infratentorial and supratentorial, induces subsequent FA changes in injured WM tracts (Droby et al., 2015a), with a particular vulnerability in both forcipes and the corticospinal tract (Chiang et al., 2016). Deppe et al. (2015) found decreased microstructural integrity in cerebellar normal appearing WM, but no reduction in WM volume. Interestingly, these modifications explained disability levels also when analyses focussed only on patients in the early phase of the disease. Moreover, a longitudinal study carried out on twenty-one patients starting natalizumab treatment found that changes in FA can precede the formation of new gadolinium-enhancing lesions by up to ten months (Ontaneda et al., 2014). Therefore, these results highlight the importance of taking into consideration not only the amount of detectable lesions, but also the status of normal appearing WM. Indeed, microstructural insults induced by inflammation may hamper neuronal signalling way before macroscopic manifestation of lesions and atrophy.

In line with the abovementioned results, decline in WM microstructural integrity has been associated with GM volumetric changes. In particular, thalamic atrophy was correlated with local decrease in FA rather than with total lesion volume (Deppe et al., 2016). Similarly, altered diffusivity properties detected in different WM tracts significantly explain degeneration of GM areas connected by the same tracts both in RRMS and in SPMS (Steenwijk et al., 2015a).

Consistent results were obtained also by means of magnetization transfer imaging: early WM alterations were evidenced in periventricular areas are correlated with severity of disability and predictive of conversion to clinically defined MS (Brown et al., 2017). Also the application of this MRI technique to GM found damage in the outermost cortical layers in several areas possibly caused by subpial demyelination (Rudko et al., 2016). Indeed, Yaldizli et al. (2016) highlighted pathological changes in diffusivity and magnetization transfer ratio in cortical lesions compared to normal appearing GM. However, the comparison of non-lesional GM between patients with MS and healthy controls detected alterations that were particularly accentuated in

the SPMS group. Thus, these studies show that microstructural changes due to MS pathology that also occur in normal appearing brain tissue are clinically relevant, and may shed light on clinical symptoms.

1.2.3.2. Brain macrostructure

The observation of increased rates of brain volume loss associated with MS has been well established. Indeed, Vollmer et al. (2015) found that people with MS lose an average of 0.7% of brain volume per year compared to 0.1-0.3% in healthy age-matched controls. Neurodegeneration, however, does not appear to be evenly spread throughout the brain. In a meta-analysis of 19 studies investigating GM atrophy in MS by means of voxel-based morphometry, some areas were found to be particularly hit by the disease: bilateral thalami, the basal ganglia, primary motor and somatosensory cortices, and the cingulate gyrus (Lansley et al., 2013). Severity of disability correlated not only with the volume of the left sensorimotor cortices but also, as found in a subsequent study, with thickness of the sensorimotor cortices (Steenwijk et al., 2015b). Similarly, degeneration in the visual pathways detected as thinning of the retinal layer by means of optical coherence tomography predicted volume reductions in the occipital cortex (Gabilondo et al., 2014).

Several studies consistently observed that subcortical GM nuclei are most deeply affected by MS pathology and thalamic atrophy is detected even in the pre-symptomatic stages and correlated with total lesion volume (Azevedo et al., 2015). Moreover, Bishop et al. (2017) found that the lower the age at onset, the worse the atrophy in two subcortical areas: the caudate and the hippocampus. After transition to SPMS with subsequent increase of neurodegenerative changes, worsening of volume loss can be observed, compared to the RRMS phase, in both the hippocampus and the cerebellum (Grothe et al., 2016).

Regarding the cause of GM atrophy characteristic of MS, two mechanisms have been proposed: primary degeneration due to GM lesions; and retrograde degeneration secondary to WM lesions leading to axonal transaction (Calabrese et al., 2015). Indeed, neuropathological studies have highlighted how demyelinating lesions occur and accumulate especially in some areas: GM sulci, where they cause oxidative stress that leads to direct GM degeneration; and WM watershed areas characterised by lower arterial blood supply and, therefore, higher vulnerability to degeneration associated with secondary GM volume loss (Haider et al., 2016). Moreover, total WM lesion volume is associated with thinning of different brain

areas, especially involved in cognitive processes: the temporal pole, the entorhinal cortex and the posterior cingulate cortex (Steenwijk et al., 2015b).

The relationship between WM lesions and GM atrophy, however, may not be as straightforward as it seems. In fact, Zimmermann et al. (2015) compared two groups of patients with MS, one with cortical and one with spinal cord lesions, and found that the latter had a larger putamen than the former probably due to compensatory mechanisms within circuits involved in motor control.

In conclusion, it can be argued that MS causes brain atrophy across different brain regions, both cortical and subcortical, that worsens with progression and partially explains physical and cognitive disability.

1.2.3.3. Brain metabolism

The brain of people suffering from MS also undergoes metabolic changes that have been extensively documented by means of positron emission tomography (PET). Most studies used ^{18}F-fludeoxyglucose (^{18}F-FDG), a radiopharmaceutical developed in order to quantify the cerebral metabolic rate of glucose utilization. A common finding is general decrease of glucose metabolism in MS affecting the cortex, deep GM, cerebellum and brain stem (Bakshi et al., 1998, Derache et al., 2006). Moreover, significant hypometabolism has also been observed in the spinal cord, especially in the thoracic and lumbar sections (Kindred et al., 2014). The frontal lobes, however, seem particularly vulnerable and metabolic deficiency in these brain regions and in the basal ganglia has been associated with symptoms of fatigue (Roelcke et al., 1997).

Structural brain damage may also have a relevant impact on glucose metabolism. In fact, Pozzilli et al. (1992) observed that patients with atrophy of the corpus callosum show a significant metabolic rate decrease confined to the left hemisphere yet in the absence of lateralization of lesion distribution when compared to patients without. This decrease appeared, however, generalised and widespread to frontal, parietal and temporal areas. Moreover, Derache et al. (2006) found that total lesion volume influenced metabolism especially of the right thalamus, a deep GM structure particularly affected in MS (Lansley et al., 2013).

In contrast, for WM metabolism the findings are not univocal. Bakshi et al. (1998) reported widespread WM hypometabolism, while Schiepers et al. (1997) found that the glucose metabolic rate of normal appearing WM in MS was comparable to that of healthy people. However, WM lesions showed in most cases an increment in

metabolism compared to normal appearing WM probably due to ongoing inflammation.

More recently, PET imaging has drawn increasing attention as evidenced by the development of new radioligands and their applications to investigate a variety of neuropathological processes associated with MS. In particular, ^{11}C-flumazenil, an antagonist of the central benzodiazepine site located on the gamma-Aminobutyric acid A receptor, has been used to quantify GM integrity (Freeman et al., 2015). Decreased ^{11}C-flumazenil binding was observed across various cortical and subcortical GM areas in patients with both RRMS and PPMS compared to healthy controls. Such findings suggest that this radioligand may be used to assess effectiveness of neuroprotective treatments (Moccia et al., 2017). Moreover, PET has enabled the *in vivo* investigation of neuroinflammation and microglia activation, especially by means of different radioligands binding to the 18-kDa translocator protein, a molecule present mainly on mitochondrial membrane of microglia cells and overexpressed in activated microglia cells (Airas et al., 2018). The ^{18}F-DPA714, a second-generation ligand characterised by significantly higher bioavailability to the brain and signal-to-noise ratio than other rarioligands, has been found to detect areas of local and diffuse neuroinflammation in progressive MS reliably (Hagens et al., 2018). Finally, WM integrity and the dynamics of demyelination have been studied by means of a variety of radioligands and, more recently, by using amyloid PET (Morbelli et al., 2019). Indeed, Pietroboni et al. (2019) found a decrease in ^{18}F-florbetapir binding in normal appearing WM which was associated with reduced WM volume and CSF β-amyloid concentration.

In conclusion, PET imaging appears to detect consistent MS-related changes in metabolic rates, both in GM and WM, as well as molecular alterations which may represent biomarkers of neural damage associated with MS and could be used as outcome measures in future clinical trials.

1.2.3.4. Cerebral blood flow

Given the repeated observation of BBB disruption in MS (Ortiz et al., 2014), changes in cerebral perfusion due to this pathology have been extensively investigated. Studies using single positron emission tomography showed a reduction in perfusion both in GM and WM, especially in frontal areas and this was associated with level of disability (Lycke et al., 1993). In SPMS decreases appear to spread widely across the brain and involve also the temporal lobes, the thalamus, and the basal ganglia (Taghizadeh Asl et al., 2016).

Consistently with these findings arterial spin labelling MRI studies have also shown MS-related hypoperfusion. D'Haeseleer et al. (2013) found that cerebral blood flow was reduced in people with MS compared to healthy controls, but it increased to normal levels after injecting bosetan, i.e. an antagonist of the powerful vasoconstrictor endothelin-1. Indeed, endothelin-1 levels in MS patients were significantly higher than in healthy controls, probably because of an abnormal release by reactive astrocytes present in lesions. Interestingly, a hypercapnia study during which patients inhaled a gas mixture with 5% of carbon dioxide, which acts as vasodilator, found that patients with MS exhibit lower cerebrovascular reactivity than controls in several brain networks: the default mode network (DMN), the somatosensory and fronto-parietal networks (Marshall et al., 2016). Moreover, these deficits correlated with both GM atrophy and total lesion volume especially in the DMN probably due to higher vulnerability to hypoxia caused by reduced cerebrovascular reactivity. In fact, it seems that decreases in cerebral perfusion precede neurodegenerative changes as suggested in a study by Debernard et al. (2014) that found hypoperfusion in RRMS in the absence of significant GM volume loss.

Blood flow alterations, however, seem to be variable especially regarding WM lesions. In a longitudinal study Wuerfel et al. (2004) observed that changes in WM perfusion preceded enhancement and diffusivity changes with an initial increase of cerebral blood flow later declining weeks after enhancement detection. Hence, this study suggests that disease activity and inflammation induces an increased demand of blood flow in lesional tissue that is consistent with findings about metabolic rate changes (Schiepers et al., 1997). Consistently, when perfusion in lesions was compared to that in normal appearing WM, hyperperfusion was noted in the active lesions while inactive ones were hypoperfused (Li et al., 2014). However, more recently Sowa et al. (2015) found a general decrease in blood flow in WM lesions compared to normal appearing WM, but no activity classification and subsequent differential analysis of lesions was performed in this study.

Resting-state functional MRI has also been used to investigate perfusion of different functional networks showing that in SPMS both the DMN and the central executive network showed signs of decline (Ma et al., 2017). Furthermore, these changes correlated with perfusion alterations of both lesional and non-lesional WM rather than WM lesion volume, thus showing that severity rather than the extent of structural damage has an impact on cerebral blood flow. In fact, decline in perfusion of normal appearing WM consistently localises to periventricular areas (Adhya et al., 2006, Varga et al., 2009).

Cerebral perfusion is particularly affected in the progressive forms of MS since patients with PPMS show more marked WM hypoperfusion throughout the whole brain than people with RRMS (Adhya et al., 2006). However, disease activity also plays a major role in driving these changes as found by Bester et al. (2015): highly active patients have reduced cerebral blood volume and flow when compared to patients with low disease activity, even though these perfusion changes do not appear correlated with lesion volume and brain atrophy.

In conclusion, generally in MS cerebral perfusion appears to decline especially in periventricular normal appearing WM and frontal GM, while active lesions show perfusion increases (D'Haeseleer et al., 2015). Axonal loss and brain atrophy do not appear to be the cause of these changes as no significant associations have been found in several studies (Bester et al., 2015, Debernard et al., 2014). On the contrary, it seems plausible that hypoperfusion of some areas may cause structural degeneration (Marshall et al., 2016). Finally, although release of vasoconstrictors by reactive astrocytes may partially explain the observed alterations, currently no definite conclusions can be drawn from the available evidence (D'Haeseleer et al., 2013).

1.2.3.5. Functional connectivity and activation

In recent years many investigations have been carried out with the aim of clarifying which changes occur in functional networks in relation to MS by using resting-state functional MRI. The sensorimotor network in particular attracted particular attention given the prominence of somatosensory and motor symptoms experienced by this clinical population. However, currently available findings are quite variable: Lowe et al. (2008) found no differences in functional connectivity between patients with MS and healthy people while more recent studies have found both decreases (Janssen et al., 2013) and increases in this network (Basile et al., 2014, Faivre et al., 2012), especially in RRMS rather than in SPMS. Indeed, Dogonowski et al. (2013a) adopted a region of interest (ROI) approach and investigated the dorsal premotor cortices. They observed a left-lateralised increase in functional connectivity in RRMS, but not in SPMS, that correlated with EDSS scores.

Consistently, a graph-based study of functional connectivity in MS which analysed different voxel-wise measures of connectivity, i.e. without *a priori* definition of network nodes, found increases in peripheral areas of the sensorimotor network, but decreases in the left premotor cortex and in both primary sensory and motor cortices (Zhuang et al., 2015). Moreover, the observed changes in sensorimotor areas

correlated both with total lesion volume and EDSS scores. A similar scenario characterised by functional changes in both directions was observed also for patients with PPMS (Ceccarelli et al., 2010). Furthermore, during the execution of simple hand movements patients recruited a wider network of areas within and outside the sensorimotor network. This finding is in line with previous observations in people with RRMS executing both active (Rocca et al., 2003) and passive movements (Petsas et al., 2013). Particularly for SPMS stronger activation in primary sensorimotor areas ipsilateral to the moved hand was detected compared to RRMS, correlating with total lesion volume (Petsas et al., 2013). Therefore, the sensorimotor network appears to be affected and to undergo reorganizational changes that, considering the current level of knowledge, may be of a compensatory nature as well as a sign of inefficient neural recruitment during performance of simple motor tasks.

For other brain networks increased functional connectivity was more often reported in the DMN and especially evident in SPMS (Basile et al., 2014, Janssen et al., 2013). However, Bonavita et al. (2011) reported significant decreases in both anterior and posterior DMN. Moreover, these changes were accompanied by increased functional connectivity in areas surrounding the posterior section of the network and more evident in cognitively impaired patients. Changes in both directions were also reported for the visual network, but in general increases of functional connectivity are detected in various networks in RRMS (Faivre et al., 2012). Functional reorganization has also been seen for subcortical nuclei with both the basal ganglia and the thalamus more strongly connected to the sensorimotor network (Dogonowski et al., 2013b). However, decreases in connectivity between the thalamus and frontal, parietal and parahippocampal areas have also been detected, and these appear more pronounced in patients affected by long-standing MS (Liu et al., 2015). Similarly, the striatum also undergoes differential connectivity changes in MS: the dorsal putamen shows increased functional coupling with fronto-parietal areas correlated with disability levels, while the ventral striatum is less connected to the posterior DMN (Cui et al., 2017). Similar findings were observed for the dentate nucleus in the cerebellum showing increased connectivity with fronto-parietal areas that correlates negatively with both total lesion volume and disability levels (Sbardella et al., 2017).

To summarise, functional alterations of several networks occur in relation to MS mainly in the form of increased functional connectivity. These changes, the valence of which is still unclear, seem to be driven by spread of structural brain damage and associated with levels of disability (Sbardella et al., 2015a).

1.3. Clinical features of MS

1.3.1. Diagnosis

The diagnosis of MS relies mainly on the identification of two distinct clinical attacks and on the demonstration of dissemination in time and space of CNS lesions. The McDonald criteria have been devised to reach clinical diagnosis (McDonald et al., 2001) and have been reported to show both high specificity and sensitivity (Dalton et al., 2002, Tintoré et al., 2003). In the past 15 years the McDonald criteria have undergone different revisions by the International Panel on Diagnosis of MS, lastly in 2017 (Polman et al., 2005, Polman et al., 2010, Thompson et al., 2017).

The differential diagnosis with other medical conditions can be carried out in steps in order to rule out non-demyelinating syndromes, non-inflammatory demyelinating diseases and idiopathic inflammatory demyelinating diseases other than MS (Miller et al., 2008). In particular, since the previous revision (Polman et al., 2010), the International Panel stressed the importance of differentiating between MS and neuromyelitis optica and its associated spectrum of disorders. Indeed, differences were observed in disease course, prognosis, and pathology that led the researchers to consider these conditions as separate.

In order to diagnose RRMS at least two different attacks in the absence of fever and inflammation, lasting 24 hours or longer, and manifesting either as new or exacerbation of symptoms must be clearly identified. The attacks can either be reported by patients or objectively observed, and referred to current or past events.

Even though the diagnosis could potentially be made on the basis of clinical history alone, at least one of the attacks should be corroborated by neurological findings, especially by means of MRI. Indeed, CNS lesions are the neuroradiological hallmark of MS and recommendations have been made about their detection to be spread in time and space. Dissemination in time can be demonstrated by showing the presence of a new lesion compared to a baseline MRI scan or by showing the presence of both gadolinium-enhancing and non-enhancing lesions in the same image (Montalban et al., 2010, Rovira et al., 2009). Dissemination in space can be demonstrated with the MRI detection of at least one lesion in at least two different CNS regions: periventricular, cortical/juxtacortical, infratentorial, or spinal cord (Swanton et al., 2006, Swanton et al., 2007). However, some modifications have been made to the MRI criteria to support MS diagnosis. In particular, both dissemination in time and dissemination in space can now be evidenced by the simultaneous presence of gadolinium-enhancing and non-enhancing lesions (Thompson et al., 2017).

For PPMS the criteria are slightly different and comprise a minimum of one year of progression of disease with continuous worsening of symptoms and two out of three objective pieces of evidence including: dissemination in space with at least a lesion in at least one of the periventricular, infratentorial or cortical/juxtacortical areas; dissemination in space of at least two spinal cord lesion; detection of oligoclonal bands by means of CSF analysis, especially in uncertain cases (Filippi et al., 2016, Polman et al., 2010).

Oligoclonal bands highlighted by analysing CSF have been found to be useful to predict a second attack in adults with typical CIS and, therefore, can now be used to overcome the lack of MRI findings in support of dissemination in time. However, some conditions need to be met: typical CIS presentation, evidence of dissemination in space, no better alternative explanation for the symptoms and the absence of other atypical CSF findings (Thompson et al., 2017).

Visual evoked potentials are used to assess the functionality of the visual system, but are not currently among the diagnostic criteria for MS and further evidence is needed in support of their inclusion.

Finally, although the peak age of onset for MS is around 30 years of age, this disease can also manifest in children. However, pediatric MS being out of the scope of investigation of the present PhD thesis, its clinical and radiological characteristics will not be discussed.

1.3.2. MS phenotypes

Recent revisions to previous definitions of the clinical courses of MS (Lublin and Reingold, 1996) have been made with the aims of avoiding overly subjective interpretations of the disease course and of integrating objective biological evidence in the definition of MS phenotypes (Lublin et al., 2014).

Based on the clinical history of the manifestation of MS symptoms, it is possible to distinguish between two broad categories of relapsing and progressive phenotypes. However, common consensus is rising about the consideration of the different MS phenotypes as part of a spectrum of disorders (Ontaneda et al., 2016). In fact, two descriptors have been identified in order to characterise the ongoing disease process: activity and progression. Activity is clinically observed in terms of relapses and by means of MRI with the detection of new either T2 hyperintense or gadolinium-enhancing lesions. Progression, on the other hand, is the steady and objective increase of neurological dysfunction without definite recovery. Consensus has not been reached yet on what MRI indices may contribute to underlie disease

progression. The aforementioned descriptors may be applied to define both relapsing and progressive phenotypes better (Lublin et al., 2014).

1.3.2.1. Clinically isolated syndrome (CIS)

CIS was proposed as one of the MS phenotypes by Lublin et al. (2014) and defined as the first clinical manifestation of inflammatory demyelinating disease potentially leading to MS but not meeting the criteria of dissemination in time. However, the modified McDonald criteria (Polman et al., 2010) have allowed the diagnosis of MS on the basis of a single clinical episode and a single MRI scan on which both enhancing and non-enhancing lesions are shown, thus limiting the identification of CIS cases.

As for the other phenotypes it is also possible to distinguish between non-active and active disease for CIS based on the presence of enhancing lesions on MRI scans. Active CIS is considered to be suggestive of MS conversion since the presence of numerous lesions and oligoclonal bands has been observed to predict disease evolution (Kuhle et al., 2015, Tintoré et al., 2006).

1.3.2.2. Radiologically isolated syndrome (RIS)

RIS is a condition that consists of incidental MRI findings compatible with demyelinating processes but in the absence of any sign or symptom (Lublin et al., 2014). Its prevalence is still debated also in the light of variability in the definition of different lesion subtypes and among different populations. However, it has been consistently reported as very low in the population, with figures lying between 0.05 and 0.2% (Granberg et al., 2013, Lebrun, 2015). Interestingly, it has been found that RIS is more frequent among relatives of people affected by MS than in the general population (De Stefano et al., 2006, Gabelic et al., 2013).

The debate on whether to categorise RIS as an MS phenotype or not is still open, and this condition is thought to be an initial transitory phase to follow up in order to track a possible full manifestation of MS. Indeed, it has been shown that RIS can evolve both into CIS/RRMS and PPMS (Kantarci et al., 2015, Okuda et al., 2014). Male sex and presence of spinal cord lesions were highlighted as the strongest predictors of conversion particularly, combined with age, to PPMS (Kantarci et al., 2015). However, currently RIS is not included among the different MS phenotypes.

1.3.2.3. RRMS

The most common MS phenotype is RRMS. It is diagnosed in about 85% of the total MS patient population (Howard et al., 2016). Diagnosis can either be made from onset, also with a single MRI scan showing enhancing as well as non-enhancing lesions, or result from the evolution of active CIS. Indeed, in order to diagnose RRMS at least two different clinical attacks must be identified and supported by proof of lesions spread in time and space (Lublin et al., 2014).

Patients affected by a relapsing-remitting disease course experience relapses that can last from days to months and are usually accompanied by raising levels of disability. However, each relapse is followed by partial or full recovery from symptoms with subsequent amelioration of general conditions (Lublin et al., 2014). This gives to RRMS a peculiar fluctuating clinical course (

Figure 1.1).

As reported in the last revision of the classification of MS phenotypes, RRMS can be either active or not active depending on the presence of gadolinium-enhancing lesions and/or clinical attacks over a period of time (Lublin et al., 2014).

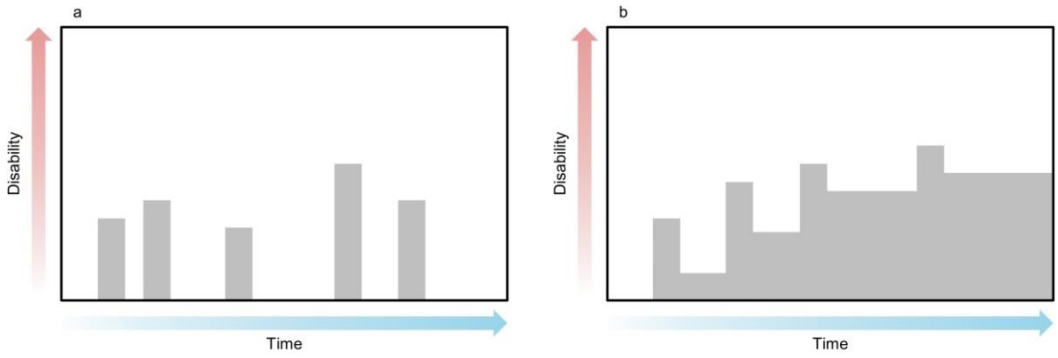

Figure 1.1 Possible clinical evolution of RRMS: a. relapses followed by full recovery; b. relapses followed by partial recovery and accumulation of disability over time.

1.3.2.4. SPMS

After a variable number of cycles of relapses and remissions over a period of 10 to 15 years from onset of RRMS, most patients experience transition to SPMS (Rovaris et al., 2006). Indeed, after 26 years from disease onset up to 90% of patients have been observed to have converted to SPMS (Weinshenker et al., 1989). High age at onset was found to be the strongest predictor of conversion, followed by male gender, time to second attack, and presence of visual/brain stems signs (Confavreux et al., 2000).

Over time the accumulation of brain insults, initially driven by inflammation, evolves into an untreatable neurodegenerative downward spiral paralleled by a steady increase of disability. Although degeneration of brain tissue is the main pathological feature, inflammatory activity may remain as a background process (Figure 1.2). In fact, also progressive MS (both secondary and primary) may be classified as active or not active like RRMS if enhancing lesions are detected (Lublin et al., 2014).

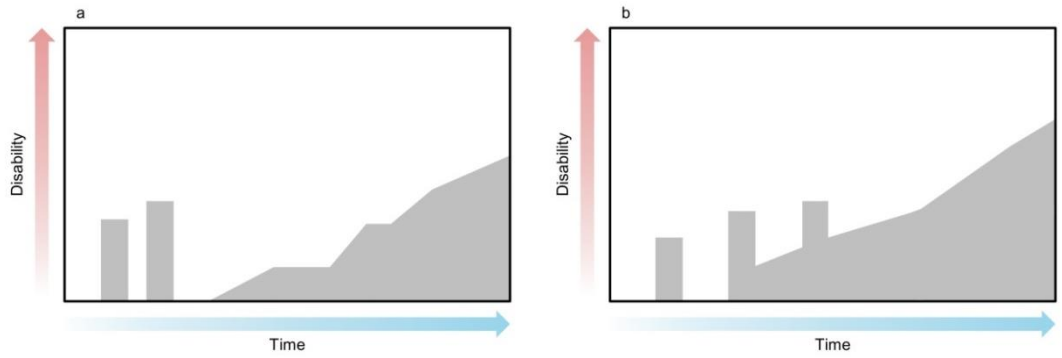

Figure 1.2 Possible clinical evolution of SPMS: a. an initial RRMS phase is followed by disability progression; b. relapses occur during the initial stages of disability progression.

Diagnosis of SPMS can be made only retrospectively by documenting a history of gradual worsening of symptoms over at least 6 months prior to the diagnosis in the absence of clearly defined relapses (Lublin et al., 2014, Rovaris et al., 2006). However, the establishment of the transition from RRMS to SPMS currently still represents a major issue and an agreed definition of SPMS is still lacking (Lorscheider et al., 2016). This is due to several factors: first, conversion is a gradual process that occurs over time; second, pathological processes that characterise progressive courses are triggered during the RRMS phase; and finally, the limitations of imaging biomarkers of progression (Lublin et al., 2014).

1.3.2.5. PPMS

In the MS spectrum, PPMS is the least prevalent phenotype affecting between 10 and 15% of the whole MS patient population (Koch et al., 2009, Miller and Leary, 2007). It is characterised by a steady increase of disability since onset in the absence of any clearly defined clinical attacks (Figure 1.3). From a pathophysiological point of view, this disease course is driven by prominent underlying neurodegenerative processes rather than inflammatory activity (Ontaneda et al., 2016). However, similarly to SPMS, PPMS can also manifest in multiple different ways and show activity along with progression as highlighted by

the newly proposed descriptions of progressive MS: active with progression, active without progression, not active with progression, or not active without progression. This revised classification allowed for better identification of those PPMS cases in whom clinical attacks might occur and led to the elimination of the previously recognised progressive-relapsing MS phenotype now accounted for as active PPMS (Lublin et al., 2014).

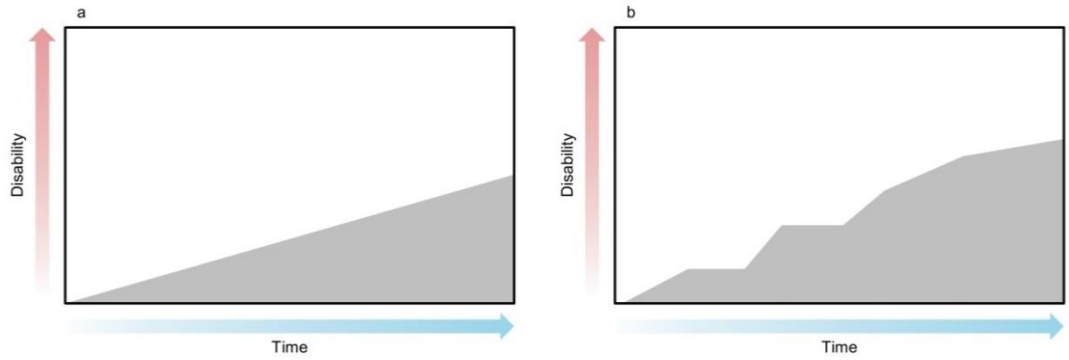

Figure 1.3 Possible clinical evolution of PPMS: a. disability worsens steadily from disease onset; b. disability worsens from disease onset but with periods of stability (plateaus) and temporary mild improvements.

At present, the supposed differences between PPMS and SPMS are still debated and it is widespread opinion that pathologically the two phenotypes can be regarded as fairly similar (Ontaneda et al., 2016). Indeed, contrast-enhancing lesions indicating presence of activity have been reported in PPMS as well as in SPMS (Ingle et al., 2005). Moreover, Frischer et al. (2009) reported that inflammation is consistently found in both progressive phenotypes and linked to axonal injury. These findings combined with the current lack of consensus on any possible biomarkers that would enable us to differentiate between phenotypes leaves this scenario quite unclear. As a result the distinction between progressive forms of MS relies on the obvious absence of previous history of distinct attacks in PPMS (Lublin et al., 2014).

1.3.3. Signs and symptoms

MS being characterised by widespread lesions throughout the whole CNS in sites varying from patient to patient, it follows that the disease symptomatology is multifaceted. In fact, depending on lesion location, different systems can be affected, from sensory perception and motor function to mood and cognition.

Therefore, different scales have been developed in order to assess the complexity of the clinical picture that usually accompanies MS. The examination of the severity

of the general clinical conditions of patients affected by MS is commonly achieved by means of the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). This tool allows a quick assessment of different functional systems, apart from cognition and mood, on a scale from 0 (normal neurological conditions) to 10 (death due to MS). The EDSS has been criticised for its psychometric properties given its low inter-rater reproducibility, low responsiveness to change, and lack of assessment of patient's perception of MS (Hobart et al., 2000). Despite these criticisms the EDSS is currently the most commonly used scale to keep track of the clinical course of this disease.

An alternative scale worth mentioning is the Multiple Sclerosis Functional Composite (MSFC) developed especially with the aim of overcoming EDSS drawbacks as a clinical trial outcome measure of clinical disability (Cutter et al., 1999). The MSFC was the result of a meta-analysis of clinical trials and it was designed to comprise three objective and quantitative measures: the 25-foot walk and the 9-hole peg test for motor function of the leg and the arm respectively, and the Paced Auditory Serial Addition Test (PASAT) as a simple measure of cognitive status. Research has shown contrasting results when comparing the MSFC with other scales of disability: on one hand, it has shown better psychometric properties than the EDSS in relation to both clinical and MRI outcome measures (Rudick et al., 2002). On the other hand, in comparison with other scales it appeared not to be detecting some of the functional changes experienced by patients (Hoogervorst et al., 2001).

All the currently available examination scales have shortcomings, probably due to the variety of manifestations MS can display. For this reason a brief discussion of the most common signs and symptoms characterising MS will follow.

1.3.3.1. Fatigue

Fatigue is a common symptom across a wide range of neurological disorders including MS (Kluger et al., 2013). This symptom, in fact, is reported by 40% to 80% of the patients and is associated with poor quality of life (Amato et al., 2001, Hadjimichael et al., 2008, Lerdal et al., 2007). Even though fatigue has been repeatedly found to be independent of many other debilitating symptoms, a concordant definition still remains elusive as well as the determination of a criterion of clinical relevance (Kluger et al., 2013). A possible explanation may reside in the fact that fatigue can be conceptualised as a multicomponential phenomenon related to different factors contributing to its manifestation.

A first classification of different types of fatigue has been proposed on the basis of the underlying cause: primary fatigue, thought to derive directly from the pathological mechanisms characterising a neurological disorder, and secondary fatigue, considered to be a consequence of other factors such as drugs, sleep disorders etc. Despite possible similarities between the two phenomena, distinguishing primary from secondary fatigue may have beneficial effects both on the investigation of its physiological causes and on treatment choice (Kluger et al., 2013).

Second, various authors have differentiated between the subjective perception of fatigue, usually measured by means of patient-reported measures such as the Modified Fatigue Impact Scale (MFIS) (Fisk et al., 1994b) or the Fatigue Severity Scale (FSS) (Krupp et al., 1989), and performance fatigability, defined as objectively measurable amount of change in task performance over time compared to a reference value. In MS the two constructs have been reported to be independent of each other (Bailey et al., 2007) and according to Kluger et al. (2013) they are determined by different factors: homeostatic and psychological factors are thought to affect fatigue perception, while peripheral and central nervous system anomalies may influence performance fatigability.

In MS in particular, fatigue appears to be related to both cognitive deficits and depression (Brenner and Piehl, 2016). Indeed, various investigations have found that both fatigue perception and fatigability are related to declining alertness (Neumann et al., 2014, Weinges-Evers et al., 2010). However, it is worth mentioning the fact that different operationalisations of cognitive fatigability have been attempted and a comprehensive theorisation has not been put forward yet (Harrison et al., 2016).

Brain changes occurring in patients with MS have also been associated with self-reported fatigue, particularly reduced microstructural integrity of the thalamus (Wilting et al., 2015) and of the connections between the hypothalamus and the locus coeruleus (Hanken et al., 2016). Moreover, atrophy in WM surrounding the thalamus appeared more severe in fatigued patients (Wilting et al., 2015) and both thalamic and cerebellar volume in the early stages of MS significantly predicted worsening of fatigue symptoms (Nourbakhsh et al., 2016). By contrast, investigations by means of functional MRI, instead, have mainly highlighted the involvement of dysfunction in the thalamic-striatal-cortical circuit and a disconnection between thalamus and basal ganglia from the prefrontal cortex, probably associated with deficits in reward-effort calculations (Dobryakova et al., 2013, Engström et al.,

2013). Interestingly, deep GM structures have been found severely atrophic in people with MS compared to healthy subjects (Lansley et al., 2013).

Different hypotheses have been proposed so far to explain MS-related fatigue. In light of the aforementioned cortico-striatal dysfunction, Dobryakova et al. (2015) suggested that an underlying imbalance in the dopaminergic system could account for subjectively reported fatigue. Within this framework high rates of fatigue observed in Parkinson's disease might also be explained (Kluger et al., 2013). Alternatively, Patejdl et al. (2015) proposed that neuroinflammation, in particular some subtypes of cytokines, may play a considerable role in inducing fatigue symptoms as observed in animal models and partially replicated in humans. According to this hypothesis neurons in the area postrema and the vagus nerve detect abnormal levels of cytokines and send signals to the central amygdala inducing the so-called "sickness behaviour". Hence, altered activation of limbic structures and cingulo-insular areas, worsened by widespread cortical and subcortical atrophy, would result in subjective feelings of fatigue (Hanken et al., 2014). However, no definite conclusions have been drawn yet about the neurophysiological mechanisms explaining fatigue probably because of the current limitations related to its operational definition.

1.3.3.2. Motor symptoms

Motor dysfunction in MS can manifest in many different forms. At onset, one of the most commonly reported symptoms is limb weakness, i.e. reduced strength of muscle contraction, usually with a unilateral presentation (Compston et al., 2006). It was observed that weakness can be largely independent of the level of fatigue or peripheral muscle function (Ng et al., 2004). A recent investigation carried out in Australia found that up to 70% of people with MS presents with weakness in at least one muscle group (Hoang et al., 2013). Regarding the neural correlates of this debilitating symptom, lesions to the spinal cord, in particular to descending fibres of the corticospinal tract, are considered the prominent cause even though Reich et al. (2007) observed an association between the presence of weakness and altered diffusivity measure in the cerebral portion of the corticospinal tract.

Another symptom equally related to spinal cord injuries, especially affecting upper motor neurons, is spasticity, i.e. increased muscle stretch reflex at time accompanied by pain (Compston et al., 2006). From a survey carried out on a cohort of 10353 people with MS from the North American Research Committee on Multiple Sclerosis, it emerged that 80% of patients experience spasms mainly in the

lower limbs (Bethoux and Marrie, 2016). This symptom was also associated with worse and more widespread levels of disability. A definite explanation of the neuropathological mechanisms generating spasticity has not been found yet. However, dysfunction of the supraspinal inhibitory pathways may explain hyperexcitability of the stretch reflex (Mukherjee and Chakravarty, 2011). Consistently, Boutière et al. (2016) observed that combining physical rehabilitation with intermittent theta burst transcranial magnetic stimulation over the primary motor cortex induced ameliorations in spasticity with associated reorganizations in functional connectivity (FC) of the contralateral homologous cortex.

By contrast, lesions to the brainstem may involve the oculomotor nuclei or their connections and cause eye movement signs such as nystagmus (involuntary movements) observed in 15-48% of patients with MS (Hickman et al., 2014). Also cerebellar injury is thought to play a causal role in the genesis of alterations of saccadic movements in MS. Whether a relationship between eye movement and other motor impairments exists remains still debated (Moroso et al., 2016). However, both eye and hand motor functionalities have been found to have an impact on the performance in the Symbol Digit Modalities Test (SDMT), a test of visual information processing speed considered a good tool to discriminate between people with and without MS (Hughes et al., 2011, Nygaard et al., 2015).

Motor speech disorders, namely dysarthria, can also arise from brainstem and cerebellar demyelinating pathology in about half of all the patients with MS (Hartelius et al., 2000). The two most common types of dysarthria reported in MS are the spastic type, due to corticobulbar tract injury, and the ataxic type, caused by cerebellar lesions (Rampello et al., 2016).

When the cerebellum is affected by MS pathology different motor and cognitive dysfunctions may arise (Weier et al., 2015). In particular, cerebellar signs balance problems are often observed, and they may be related to a higher rate of falls and consequently have a detrimental impact on the functional status of patients (Sosnoff et al., 2011). Equally limiting for patients' mobility is gait ataxia, i.e. the lack of coordination of lower limbs movements (Compston et al., 2006). Indeed, people with MS have been reported to have greater gait movement fluctuations than healthy controls and this finding significantly correlated with risk of falls (Socie and Sosnoff, 2013).

Finally, tremor has also been documented in a large portion of about 45% of people with MS (Rinker et al., 2015). Cerebellar damage is thought to play a central role in the manifestation of tremor symptoms in MS, though it appears not to provide a comprehensive explanation of this phenomenon (Ayache et al., 2015). Indeed, brain

stem lesions may contribute to this symptom (Ayache et al., 2015) as well as damage in the thalamus given the effectiveness of thalamotomy as treatment for tremor in MS (Mathieu et al., 2007).

1.3.3.3. Autonomic symptoms

Various autonomic symptoms often recur within the clinical landscape of MS (Haensch and Jörg, 2006, Vieira et al., 2015), but few investigations have been carried out so far especially regarding the underlying pathophysiology (Lensch and Jost, 2011). The most commonly observed autonomic dysregulations affect preferentially gastrointestinal, lower urinal and sexual functions (Haensch and Jörg, 2006).

Gastrointestinal symptoms are experienced by more than half of MS patients, though reported figures are highly variable between 40% and 80% (Levinthal et al., 2013, Vieira et al., 2015). In particular, constipation and incontinence are the most common manifestations associated with a wide range of clinical symptoms (Levinthal et al., 2013) that have been reported by people with MS to be affecting their quality of life equally to mobility limitations (Norton and Chelvanayagam, 2010).

Urogenital symptoms have been recently investigated in a large cohort of patients with MS in North America yielding a striking figure of 92% prevalence of neurogenic lower urinary tract dysfunction (Khalaf et al., 2015). As for gastrointestinal dysfunction, the presence of these symptoms has also been linked to decreased quality of life in MS (Browne et al., 2015). The neuropathological correlates appear to be quite variable since cerebral, pontine, but even spinal lesions were observed to be associated with bladder function dysregulation (Charil et al., 2003, Di Benedetto et al., 2008).

Finally, another autonomic symptom with a deep impact on the life of people with MS is sexual dysfunction reported by 60-70% of patients (Celik et al., 2013, Zorzon et al., 2001). Specific symptoms may vary according to gender: indeed men report mainly erectile problems (Zorzon et al., 2001), while women appear to be more troubled by lack of sexual desire (Celik et al., 2013). However, sexual dysfunction represents a complex phenomenon possibly caused at different levels. In fact, aside from primary sexual problems due to MS pathology, also secondary, i.e. other MS-related physical problems, and tertiary causes, namely psychosocial factors, can have a detrimental impact on the sexual function of people with MS (Pintér et al., 2015). Both cerebral lesions, particularly in insular areas (Winder et al., 2016), and pons volume (Zivadinov et al., 2003) have been linked to sexual problems in

patients with MS. However, considering the multifaceted scenario related to the possible causes, it appears plausible that multiple sites of lesion may play a role in the aetiology of this symptom.

1.3.3.4. Sensory symptoms

Sensory symptoms are often among the first to manifest when demyelination spreads and in particular in two sensory modalities: visual and somatosensory. In fact, demyelination of the optic nerve, i.e. optic neuritis, affects up to 70% of people with MS and manifests as an onset symptom in 25% of cases causing visual perceptual problems such as blurred vision, vision loss, and dyschromatopsia (Toosy et al., 2013). A longitudinal study found that 50% of all the optic neuritis cases have evolved into MS 15 years from onset (Optic Neuritis Study Group, 2008). Indeed, optic neuritis has long been established as a core symptom often associated with MS, though found also in other pathologies related to MS like neuromyelitis optica (de Seze, 2013). Moreover, demyelination of visual pathways may have a negative impact on cognitive functions because of degraded sensory signal processing. History of neuro-ophthalmic syndromes in people with MS, in fact, was found to affect performance in tests of processing speed (PS) delivered by means of visually presented material (Costa et al., 2015).

Similarly paraesthesiae, i.e. somatosensory perception alterations, usually manifest in MS as tingling sensations, numbness, and loss of proprioception. They are observed as core symptoms at onset in up to 20% of MS cases (Compston et al., 2006) and are eventually reported by about 40% of patients (Rae-Grant et al., 1999). Demyelination to the afferent somatosensory pathways is thought to be the main underlying cause and was observed to have an impact on both balance and walking functions (Fling et al., 2014, Thoumie and Mevellec, 2002). In particular Fling et al. (2014) found that microstructural degeneration of the right cerebral portion of the proprioceptive pathways affected balance control in people with MS. Consistently, balance problems in a sample of patients with MS were also associated with slowed spinal conduction of somatosensory information, thus indicating spinal injury as the main neural correlate of somatosensory deficits (Cameron et al., 2008).

Moreover, the presence of different manifestations of MS-related painful sensations is recurrent and reported by about 57% to 65% of patients (O'Connor et al., 2007). In general, the cause is thought to be lesions to the spinal cord and the brain stem (Mazhari, 2016).

As for disturbances of the other sensory modalities, little research has been carried out, perhaps because of lower prevalence rates compared to visual and somatosensory symptoms. However, MS-related lesion formation across the brain being a random process, at least based on current knowledge, occasional demyelinating lesions to sensory pathways have been proved to cause deficits in various auditory (Furst and Levine, 2015), gustatory (Doty et al., 2016), and olfactory tasks (Good et al., 2017).

1.3.3.5. Cognitive symptoms

A considerable proportion of people affected by MS complains of cognitive decline, usually affecting multiple domains and often referred to as “brain fog” (Mazanderani et al., 2013). Indeed, figures commonly reported in the literature identify between 43 and 70% of patients as being cognitively impaired (Chiaravalloti and DeLuca, 2008, Rao et al., 1991). The great variability of the abovementioned rates may be due to differences in the tests used to assess cognitive functions and the diagnostic criteria for cognitive impairment set by individual studies. Fischer et al. (2014) have, in fact, highlighted how different approaches were used regarding both the minimum number of cognitive domains affected and the threshold for each domain to be considered impaired in comparison to a population of healthy controls. The situation depicted appears complicated and has not been addressed in a systematic way yet, making it impossible to obtain more accurate figures about cognitive impairment in MS. However, despite current uncertainties, worse cognitive status has been consistently associated with unemployment and lower quality of life (Campbell et al., 2017b, Nunan-Saah et al., 2015). Moreover, cognition could also be particularly influenced by the presence of affective problems and in particular severe depression (Golan et al., 2017).

Whether and to what extent cognitive decline in MS may lead to dementia is still an overlooked issue, probably because of the hesitation to use this term applied to a clinical population of predominantly young and middle aged people rather than older adults (Westervelt, 2015). The only study to date that has investigated the rate of dementia due to MS retrospectively found that 22% of patients met the criteria for dementia diagnosis (Benedict and Bobholz, 2007). However, this field of research remains largely unexplored while cognitive impairment in a broader sense has been intensively studied also in relation to the underlying neuropathology (Chiaravalloti and DeLuca, 2008). Cognitive deficits have been detected from the very early stages and even in the RIS phenotype (Lebrun et al., 2010). Indeed, cognitive

performance seems to be as affected as in patients with MS without major differences between RIS, CIS and MS (Labiano-Fontcuberta et al., 2016, Lebrun et al., 2010). Nevertheless, when RRMS has been contrasted to progressive forms of MS, dominated by neurodegenerative processes, different studies confirmed that the cognitive performance of patients affected by the latter type is significantly worse (Planche et al., 2015, Rosti-Otajärvi et al., 2014).

The cognitive domains mostly affected in MS include slowed processing of information and problems with learning which are the most common deficits observed (figures are variable around 50%), followed by attention and executive functions (Chiaravalloti and DeLuca, 2008, Hämäläinen and Rosti-Otajärvi, 2016). Deficits in PS function have long been established in MS by means of various tasks and appear to be one of the core symptoms (Bodling et al., 2012, Denney et al., 2004, Kail, 1998, Salthouse, 1992, Wojtowicz et al., 2012). Indeed, they have a wide impact on patients' lives: Labiano-Fontcuberta et al. (2016) found that PS deficits are associated with worse depressive symptoms in caregivers.

Although mood and physical problems may have an impact on cognitive functioning, Denney et al. (2004) found that lower PS performance was the only significant cognitive deficit after controlling for levels of fatigue and depressive symptoms when patients were compared to healthy controls. Moreover, slowed cognition has also been observed in the absence of slowed motor function indicating that the two domains can, to some extent, be affected independently from one another (Binétruy et al., 2016).

It has been argued that PS decline in MS resembles that observed in healthy aging (Denney et al., 2004). In this line of research one study investigated the potential relationship between MS and aging but found no interactions between age and disease status, i.e. age-related decline in PS does not appear accelerated by MS (Bodling et al., 2009). However, impairments differ across phenotypes with progressive forms of MS showing worse PS performance than RRMS (De Sonneville et al., 2002). Additionally, deficits in PS-dependent tasks have also been reported in CIS patients suggestive of MS conversion (Viterbo et al., 2013). Further discussion on theories of PS function in MS and about the relationship between PS deficits and decline in other cognitive domains will be extensively treated in Chapter 2.

Working memory is another cognitive function whose different subcomponents have been intensively investigated in MS. In the visuospatial domain, issues appear to be mainly related to the initial processing and encoding of information rather than to the subsequent storage and retrieval stages (Gmeindl and Courtney, 2012). Indeed,

spatial reorienting of attention has been found to be slower in patients with MS compared to controls. Indeed, in verbal working memory, especially studied comparing performance on n-back tasks and on the PASAT, deficits have been observed in the central executive system (Baddeley and Hitch, 1974) and mainly related to speed of information processing (Lengenfelder et al., 2003, Parmenter et al., 2006). Moreover, Covey et al. (2017) found that even in the absence of behavioural differences in accuracy on the n-back task between patients and controls, task-related electrophysiological brain activity appeared altered in MS thus indicating a susceptibility to dysfunction.

Partially related to working memory, attentional capacities are also differentially affected in MS. Investigations using tasks of selective attention, in which the focus of attentional resources is crucial, have shown deficits correlated with level of patients' disability (Adler and Lembach, 2015, Shawaryn et al., 2002b). On the contrary, mixed findings were obtained for divided attention, i.e. the ability to allocate attentional resources to different information sources and to carry out multiple tasks at the same time. While an early study found that divided attention may be impaired when PS demands are high (Paul et al., 1998), recently Williams et al. (2016) found no differences in performance between patients and controls. However, deficits have been consistently reported across sensory modalities and appear amplified in ecological assessment settings, such as virtual reality (Lamargue-Hamel et al., 2015, McCarthy et al., 2005, Tinnefeld et al., 2005, Urbanek et al., 2010).

Declarative long term memory has long been established as one of the core cognitive dysfunctions in MS (Grant et al., 1984). The scientific debate on what memory process is mostly affected by this disease stemmed from contrasting findings related to learning and retrieval stages. Indeed, while Rao et al. (1989) found that retrieval of stored information was prominently impaired in contrast to preserved short term memory retrieval, most studies have highlighted specific deficits in the acquisition of new memories (DeLuca et al., 1994, Grant et al., 1984, Kessler et al., 1992). In line with that, PS deficits have been suggested to influence the ability of patients to process effectively information that needs to be encoded and subsequently stored in long term memory (Beatty et al., 1989, Chiaravalloti et al., 2003).

As for the most complex cognitive abilities, i.e. executive functions, several investigations found deficits in patients affected by MS especially in planning abilities, partially thought to be driven by PS decline (Arnett et al., 1997), and problem solving, especially related to concept formation (Beatty and Monson, 1996, Goverover et al., 2013). Executive deficits may also have a wider impact on

cognitive functioning since they seem to predict lack of retrieval facilitation of self-generated information which is usually remembered better than externally provided information (Goverover et al., 2013). Consistently, abstraction and conceptual issues have been detected by Cerezo García et al. (2015) in a case-control study carried out on 100 people with MS. They found that 71% of patients showed impaired performance in at least 5 out of 16 tests and 3 different executive domains emerged as impaired: abstraction, mental flexibility and inhibitory abilities.

Finally, it is worth mentioning that given the diffuse and random spreading of demyelinating lesions, other neuropsychological deficits have occasionally been reported in MS, such as aphasia (Devere et al., 2000), diagnosed in 17% of the cases (Staff et al., 2009), and limb apraxia, i.e. issues with motor planning not explained by sensory loss or paralysis (Kamm et al., 2012), affecting especially gait (Abou Zeid et al., 2009).

1.3.3.6. Psychiatric and behavioural symptoms

In a meta-analysis, Rosti-Otajärvi and Hämäläinen (2012) highlighted how several symptoms of a psychiatric, affective, and behavioural nature show higher rates in people with MS compared to healthy controls. However, major depressive disorder has long been recognised as the most common psychiatric condition related to MS. Indeed, it is diagnosed in 30% to 50% of all patients and for this reason has featured in a large part of the scientific literature on the mental health of people with MS (Boeschoten et al., 2016, Feinstein et al., 2014). It was found to deeply affect quality of life of patients (Fruehwald et al., 2001) as well as cognitive function (Golan et al., 2017), and to contribute to suicidal ideation, constituting a crucial threat for both ineffective clinical management of disease course and patients' lives (Lewis et al., 2016, Viner et al., 2014). Hasselmann et al. (2016) investigated the differences in clinical phenotypes between MS-related depression and idiopathic major depressive disorder, and found no significant sources of divergence between the two. However, qualitative differences in the manifestation of symptoms have been detected: while the former may be more characterised by irritability and frustration, the latter is usually mainly dominated by feelings of low self-esteem and guilt (Minden et al., 1987).

Contrasting evidence has emerged so far about the prevalence of depressive symptoms across MS phenotypes. No differences were found by Chwastiak et al. (2002) while, more recently, it has been reported that the prevalence of major depressive disorder in progressive MS is almost double than RRMS (Lorefice et al.,

2015). However, severity of disease was significantly correlated with the severity of depressive symptoms, thus pointing at the relevance of the degree of disability for the manifestation of depressed mood (Chwastiak et al., 2002). The neural correlates of depression in MS are yet to be fully understood as variable findings have been published so far, even though different MRI measures have consistently underlined the role of fronto-temporal damage in the genesis of depressive symptoms (Feinstein et al., 2010, Pujol et al., 2000, Zorzon et al., 2002). Hippocampal atrophy, possibly related to dysfunction of the hypothalamic-pituitary-adrenal axis, has also been observed in depressed patients with MS (Fassbender et al., 1998, Gold et al., 2010, Gold et al., 2014b). Additionally, performance on a task of emotional stimuli processing was associated with increased activation of the ventromedial prefrontal cortex (VMPFC) in patients with MS (without depressive or anxious symptoms) compared to healthy controls (Passamonti et al., 2009). The VMPF was also less functionally connected with the amygdala, thus indicating the presence of dysregulation, and possibly susceptibility, in emotion processing even in non-depressed patients. Finally, Bonavita et al. (2017) have recently shown that functional connectivity in different brain networks is altered in depressed patients with MS and specifically in the posterior DMN: a hub whose dysregulation may underlie both depressive and cognitive symptoms.

The prevalence of bipolar disorder among people with MS has been observed to range between 6.5% and 10%, twice as high as in the general population (Carta et al., 2013, Jun-O'Connell et al., 2016, Schiffer et al., 1986). A recent review highlighted that bipolar disorder may also arise prior to MS onset and be particularly characterised by maniac symptoms (Marangoni et al., 2015). Strikingly, when a comparison with the body of literature on MS-related depression is drawn, almost no attempts at characterising the neural correlates of bipolar disorder in MS can be identified. In a case series study only one report of a new orbitofrontal lesion concomitant with a manic attack was noted (Sidhom et al., 2014). Marangoni et al. (2015) found no association with WM lesion location.

Also cases of euphoria, defined as an overly optimistic attitude despite physical and mental conditions, have been observed in patients affected by MS with a prevalence of about 11% to 14%, although earlier investigations reported much higher rates probably due to different clinical definitions (Diaz-Olavarrieta et al., 1999, Duncan et al., 2016, Fishman et al., 2004). Whether and what MS-related brain damage underlies manifestations of euphoria has not been clarified yet, since only one study found the symptom correlated with global GM volume (Sanfilipo et al., 2006).

Moreover, another affective disorder observed in about 10% of patients and distinct from both depressive and anxious symptoms is pseudobulbar affect, i.e. the involuntary manifestation of emotions, especially crying and laughing, in the absence of an evident cause (Feinstein et al., 1997). The aetiology of this disorder is not completely understood and only one study has investigated the underlying neuropathological basis and found a correlation with a widespread pattern of damage between frontal, parietal and brain stem areas (Ghaffar et al., 2008). Indeed, this disorder has been associated with damage to a complex cerebral network involved in emotional processing (King and Reiss, 2013). Interestingly, Hanna et al. (2016) showed that pseudobulbar affect also appears to have a negative impact on verbal memory, but to draw firm conclusions on the clinical implications of these findings further investigations are needed.

Consistently with the aforementioned clinical scenario, social cognition and emotional processing have also been repeatedly found impaired in MS, involving the understanding of others' emotions and mental states (Cotter et al., 2016). A disconnection mechanism has been proposed to explain such deficits as correlations with both WM and GM areas were detected, especially in medial frontal and temporal areas (Mike et al., 2013). Similar results were obtained by Batista et al. (2017) who, however, found that amygdala volume was the best predictor of differences in social cognitive processing between patients and healthy controls. In fact these results complement the functional dysregulation between the amygdala and the VMPFC observed by Passamonti et al. (2009).

Additionally, a recent meta-analysis found that anxiety problems are experienced by 22% of patients with MS (Boeschoten et al., 2016) and appear to affect mood and to be particularly associated with depression (Gay et al., 2017). In particular, within the anxiety spectrum generalised anxiety disorder is the most commonly diagnosed and linked to lower levels of social support and various comorbidities (Korostil and Feinstein, 2007). The cause of anxiety disorders in MS has not been elucidated so far and the neural correlates still remain elusive. Indeed, while Lin et al. (2013) found an association between anxiety measures and the volume of the right middle and superior frontal gyri, most studies failed to detect any correlation, leaving the question still open (Diaz-Olavarrieta et al., 1999, Fassbender et al., 1998, Zorzon et al., 2002).

1.3.4. Treatments for MS

1.3.4.1. Disease modifying treatments

Despite the many advances made in the last decades in investigating different types of drugs, no definite cure for MS has been identified yet. Indeed, only disease modifying treatments (DMTs) are currently available to tackle disease activity. In particular, the aim of DMTs is to reduce severity and number of relapses as well as to slow the course of the disease. The debate on the efficacy of early intervention in improving several MS-related outcome measures is still open. A recent Cochrane review found that early treatment after just the first clinical attack suggestive of MS compared to late treatment reduces the risk of conversion to clinically defined MS (Filippini et al., 2017). However, in general evidence on early treatment compared to placebo appears of low quality especially regarding its effects on disability and quantity of relapses.

Different numbers of DMTs have been approved by the Food and Drug Administration in America and by the European Medicines Agency in Europe and clustered as either first-line, preferentially used just after diagnosis, and second-line treatments, chosen when issues with poor response or tolerability to first-line DMTs arise (Dörr and Paul, 2015). Hereafter the classification according to the European legislation will be used (Table 1.1).

The first type of DMTs ever approved in 1993 for RRMS were the interferons. Type I interferons are cytokines naturally released by eukaryotic cells to contrast pathogens attacking the organisms. For treatment purposes, instead, synthetic type I interferons are produced by means of two different processes: interferon beta 1a through DNA recombination in mammalian cells; while interferon beta 1b is the product of bacterial fermentation (Du Pasquier et al., 2014). The mechanism of action of interferons in MS is highly likely to depend on complex and multifactorial processes involving both direct and indirect effects. In particular, interferons are thought to both upregulate the production of anti-inflammatory agents and concomitantly down-regulate the expression of pro-inflammatory cytokines, as well as reduce cell migration across the BBB (Kieseier, 2011).

Table 1.1 Disease modifying treatments approved for MS.

Active compound	Approved use (European Medicine Agency)	Year of approval
Interferon beta 1a	First-line use	1993
Interferon beta 1b	First-line use	1993
Glatiramer acetate	First-line use	1996
Teriflunomide	First-line use	2012
Dimethyl fumarate	First-line use	2013
Mitoxantrone	Second-line, often used as third-line	2000
Natalizumab	Second-line, first-line for highly active disease	2004
Fingolimod	Second-line, first-line for highly active disease	2010
Alemtuzumab	First line, commonly used as second-line	2013

From a clinical point of view interferon treatment seems to have a positive impact on CIS, reducing the rate of conversion to clinically defined MS (Filippini et al., 2017). However, response to treatment may vary across patients because of the heterogeneity characterising MS. Indeed, a recent study clustered patients in six groups on the basis of the type of cytokines expressed and a differential response to interferon after three months of therapy was observed (Hegen et al., 2016). Interestingly, positive results were also observed regarding attenuation of disability progression in RRMS undergoing interferon treatment (Signori et al., 2016). On the contrary, no improvements were reported for SPMS patients either regarding disability or MRI outcomes (Kuhle et al., 2016).

A recent advancement has seen the licensing of a new formulation of interferon beta 1a combined with polyethylene glycol, named peginterferon. It is characterised by a half-life longer than traditional interferons, thus requiring less frequent injections (Du Pasquier et al., 2014). Recently tested, peginterferon has been found effective regarding reduction of the relapse rate and new T2 lesions (Bhargava and Newsome, 2016). Safety profile and side-effects were comparable to traditional interferons. Therefore, these results appear promising and may have a positive influence on compliance with this type of therapy.

Glatiramer acetate is a heterogeneous polypeptide designed in order to mimic myelin basic protein, i.e. an auto-antigen used to induce EAE. Contrary to expectations, glatiramer acetate exhibited protective effects for EAE, hence starting investigations on its therapeutic potential (Teitelbaum et al., 2017, Weinstock-Guttman et al., 2017). Its mechanism of action, however, is still not fully understood more than 40 years after its discovery. Glatiramer acetate is thought to induce several different processes: turning pro-inflammatory lymphocytes into an anti-

inflammatory state, decreasing production of pro-inflammatory cytokines, and increasing anti-inflammatory cytokines (Buzzard et al., 2012, Du Pasquier et al., 2014). From a clinical point of view, glatiramer acetate was repeatedly proven effective in reducing the number of relapses and disability progression compared to placebo (Qizilbash et al., 2012, Signori et al., 2016). Moreover, it is associated with a few side-effects like rush, bradycardia and chest tightness but these are not considered serious life threats.

Teriflunomide acts by inhibiting the synthesis of pyrimidine, which is fundamental for the creation of new nucleobases. Therefore, it leads to a decrease in DNA copying and, in turn, reduction in proliferation of both T and B cells (Bar-Or et al., 2014). Tests on teriflunomide found that it significantly reduces relapse rate and disability progression (Confavreux et al., 2014), but less effectively than interferons (Vermersch et al., 2013). Despite some side effects it is in general well tolerated and is considered safe (Oh and O'Connor, 2014). However, in animal models it has been observed to exert teratogenic effects, hence women are requested to be negative on pregnancy tests before starting treatment (Fukushima et al., 2007, Oh and O'Connor, 2014).

Dimethyl fumarate is a methyl ester of the fumaric acid, a simple acid found in nature in different plants among which the *Fumaria officinalis*, hence its name. It was first used in the 1950s in the treatment of psoriasis and, only after the discovery of the immunitary aetiopathology of this condition, applications to other autoimmune diseases such as MS were tested (Linker and Haghikia, 2016). Dimethyl fumarate promotes cytoprotection and appears to exert both an anti-inflammatory and a neuroprotective effects (Linker et al., 2011, Du Pasquier et al., 2014). This orally administered drug is associated especially with transient gastrointestinal side effects that disappear within a few months from the beginning of the treatment (Phillips et al., 2015). As the other first-line DMTs, dimethyl fumarate was also found effective in reducing clinical attacks and negative MRI outcomes, namely formation of new lesions (Gold et al., 2012), and atrophy progression at 3 month follow-up (Arnold et al., 2014).

Mitoxantrone is a synthetic anthracendione firstly introduced in medicine as an anti-neoplastic agent and later approved as DMT for MS in 2000 (Cocco and Marrosu, 2014). It affects DNA repair and induces cell apoptosis (Hande, 2008). As a consequence, it exerts a powerful immunodepressant action by reducing the number and, in turn, the proliferation of B cells, T cells, and macrophages (Neuhaus et al., 2005). A Cochrane meta-analysis on mitoxantrone efficacy found significant effects of this drug in reducing both relapse rate and disability progression in

comparison to placebo (Martinelli Boneschi et al., 2013). However, several side-effects associated with mitoxantrone use have been reported along with a teratogenic action that makes it dangerous for pregnant women. Additionally, various severe effects, such as cardiotoxicity, neutropenia and amenorrhea were observed and raised concerns about its safety, leading to a considerable reduction of its use in clinical practice (Torkildsen et al., 2016).

Natalizumab is a recombinant humanised monoclonal antibody that acts as an inhibitor of transmembrane molecules that facilitate adhesion between cells and the extracellular matrix, expressed in white blood cells. As a result white blood cells are prevented from binding to receptors in the inflamed endothelium of the cerebral vasculature thus reducing transmigration across the BBB and CNS inflammation (Léger et al., 1997, Rice et al., 2005, Yednock et al., 1992). Different randomised controlled trials have tested the effectiveness of natalizumab in inducing ameliorations in some of the most commonly investigated outcome measures, i.e. relapse rate and new enhancing lesions reduction (Nikfar et al., 2010). In particular, it appears better than interferon beta 1a and fingolimod in reducing relapse occurrence, and better than alemtuzumab in reducing disability (Kalincik et al., 2017). However, the choice of this DMT is made not without caution given that it appears to increase the risk of progressive multiple leukoencephalopathy, a brain infection caused by the John Cunningham virus (Kornek, 2015). In particular, three main factors contribute to increase this risk: positivity to John Cunningham virus test, prior use of other immunodepressants, and natalizumab use longer than 24 months (Sørensen et al., 2012). Especially the latter raises concerns still unaddressed related to uncertainty about the most appropriate strategy for long-term management of this treatment (Clerico et al., 2017).

Fingolimod was the first oral DMT available for MS licensed in 2010. It is a chemical compound derived from myriocin, a metabolite of the parasitic fungus *Isaria sinclairii* that was already known for its anti-inflammatory properties (Chun and Brinkmann, 2011). In particular, fingolimod acts by preventing naïve and central T cells from migrating outside lymph nodes and circulating in other tissues such as the CNS (Fujita et al., 1994). Effector memory cells, instead, are less affected and continue their activity of immune surveillance in the peripheral tissues (Chun and Hartung, 2010, Pinschewer et al., 2011). Reduction in lesion activity and brain volume loss was observed as effect of fingolimod therapy (Cohen et al., 2010, De Stefano et al., 2017) as well as reduction of relapse rate that appears even stronger than interferon and glatiramer acetate treatments (Roskell et al., 2012). Some side-effects common to other DMTs have been reported, such as an asymptomatic and transient

decrease in heart rate at the beginning of the treatment is commonly reported (Cohen and Chun, 2011).

Finally, alemtuzumab is also included within the category of the recombinant humanised monoclonal antibodies used to treat MS in the form of a single annual infusion. Its target is a specific protein (CD52), present mainly on the surface of both T and B cells (Xia et al., 1993). The effects are detectable as post-infusion depletion of lymphocytes followed by long-lasting changes in peripheral lymphocytic populations (Cox et al., 2005). Alemtuzumab was observed to have positive impact on clinical and MRI outcomes with better results compared to interferon beta 1a and fingolimod, especially in reducing relapse rate (Coles et al., 2012). However, natalizumab appears more beneficial for disability management (Kalincik et al., 2017). Apart from the side-effects common to other DMTs, it is worth mentioning the risk of autoimmune thyroid dysfunction in about a third of patients treated with alemtuzumab (Dörr and Baum, 2016). Other DMTs have been successfully tested so far (such as daclizumab, ocrelizumab, and laquinimod) but considering the aims of the present PhD thesis they will not be discussed further.

1.3.4.2. Autologous hematopoietic stem cell transplantation

Since the mid-1990s a new promising treatment imported from the oncological field has been extensively used in MS: autologous hematopoietic stem cell transplantation (AHSCT). Indeed, between 1995 and 2015 over 800 patients in Europe alone have received this treatment (Kelsey et al., 2016). Accordingly, the first consensus guidelines on the use of AHSCT as therapy for autoimmune diseases were published in 1997 (Tyndall and Gratwohl, 1997).

This treatment comprises four sequential steps: first, the release of hematopoietic stem cells from the bone marrow into the blood stream is induced by giving patients an initial low dose of chemotherapy; second, stem cells are harvested from patients' blood; third, a higher dose of chemotherapy is given to patients in order to ablate their immune system; finally, previously collected stem cells are infused back in order to guide the reconstruction of their immune system (Arruda et al., 2016). It is commonly thought that AHSCT acts by resetting the immune system through three processes: deletion of the autoreactive B and T cells; replacement of the immune system with a new cell repertoire; induction of a new immune homeostasis (Arruda et al., 2016, Collins et al., 2017).

Despite long-standing experience, the great majority of clinical studies published on AHSCT in MS are uncontrolled trials (Sormani et al., 2017b). However, the largest

and most up-to-date observational study of a European cohort highlighted positive results about the use of this therapy in MS (Muraro et al., 2017). Data from 281 patients who received the transplantation showed that after five years about 50% showed no disease progression. Sormani et al. (2017b) in a meta-analysis of fifteen studies including 764 patients showed that the strongest benefit from AHSCT was gained by patients with RRMS and low levels of disability. Interestingly, five years after transplantation about two thirds of patients exhibited no evidence of disease activity.

Regarding MRI outcome measures, although there was a decrease in the rate of new lesion formation, transient brain volume loss was also observed after transplantation (Atkins and Freedman, 2013, Atkins et al., 2016). This phenomenon, described as pseudoatrophy, is thought to be caused by absorption of oedema and decrease in microglial cells rather than actual loss of neural tissue (De Stefano and Arnold, 2015). Indeed, one year after AHSCT brain volume returned to baseline levels (Atkins et al., 2016). A similar trend was detected for cognition by Walker et al. (2014): decreased perfrormance was observed after two months, but followed by a return to baseline levels after two years from transplantation. Moreover, recent reports found increased performance on the PASAT even at 3-year follow-up (Nash et al., 2014, Walker et al., 2016). However, no definite conclusions on the effects of AHSCT on cognitive status and its relationship to brain changes can be drawn, given that all studies currently published do not include a control group. Similarly, general knowledge regarding effectiveness, applicability, and safety of this treatment in the MS scenario is still precarious due to lack of randomised controlled trials comparing AHSCT with available DMTs.

1.3.4.3. Symptomatic treatments

Despite the high prevalence of mood disturbances in MS research, an effective treatment appears lacking. Indeed, only a few studies assessed the effects of pharmacological interventions on depressive (Koch et al., 2011) and anxiety symptoms (Brenner and Piehl, 2016) often reported by people with MS. A couple of trials were carried out on medications, but the most common first-line treatments for depression were not tested (Ehde et al., 2008, Schiffer and Wineman, 1990). Currently, no studies suggest differential effects of available drugs between people with and without MS. In parallel, also the impact of psychotherapy on MS-related depression has been investigated, finding moderate effects (Fiest et al., 2016).

Moreover, definite evidence of effectiveness for pain treatments in MS has not been produced yet, although pregabalin, gabapentin and lamotrigine have all been recommended especially for patients with refractory symptoms (Paolucci et al., 2016). Several other medications have been tested in recent years and results were mixed. In fact, ameliorations of symptoms after treatment with cannabinoids were reported only in some trials (Rog et al., 2005, Svendsen et al., 2004), but not in others (Langford et al., 2013, Wade et al., 2004). Duloxetine was repeatedly found effective in decreasing MS-related pain (Brown and Slee, 2015, Vollmer et al., 2014).

As previously mentioned one of the most common symptoms reported by patients with MS is fatigue. Two main drugs are usually considered in clinical practice to reduce this pervasive symptom: modafinil and amantadine. Modafinil has been originally developed especially to treat conditions of altered alertness such as narcolepsy and, when applied to MS-induced fatigue, results yielded so far by randomised controlled trials have been either null or unclear (Möller et al., 2011, Rammohan et al., 2002, Stankoff et al., 2005). Amantadine, instead, is an antiviral compound originally used in the treatment of parkinsonisms and some flue-related symptoms that has been applied also to treat MS-related fatigue with better outcomes than modafinil (Ledinek et al., 2013). However, Pucci et al. (2007) carried out a meta-analysis on the effects of amantadine raising concerns about the results of the trials analysed due to their small sample sizes and methodological limitations. As a result it seems that no clear-cut conclusions on the effectiveness of pharmacological treatments of fatigue in MS can be drawn. On the contrary, rehabilitative therapies have been found to be more effective than drugs, but most studies were carried out on small samples and with various techniques not always easy to standardise and compare (Asano and Finlayson, 2014).

For cognitive deficits, instead, two approaches have been adopted: testing the secondary effects of DMTs or using interventions to target specifically cognition. So far the use of DMTs has led to weak evidence of effectiveness, probably because of little interest in this issue combined with the investigation of mainly small samples of patients (Roy et al., 2016). Both fingolimod and natalizumab have been observed to stabilise cognition over one year in a similar manner, but without generating any improvement (Utz et al., 2016). Consistently, beneficial effects of natalizumab on attention were found only when a simple test-retest design was used (Kunkel et al., 2015), but not when a placebo group was included (Sundgren et al., 2016). Mixed results are available for interferon beta 1a: while an early study found associated improvements in PS and memory (Fischer et al., 2000), none were subsequently

detected by Patti et al. (2010). Moreover, an observational study conducted with 681 patients who converted to therapy with glatiramer acetate found improvements in several outcome measures among which also cognition (Ziemssen et al., 2014). Hence, no definite conclusion about the cognitive effects of the currently available DMTs can be drawn.

Regarding targeted treatments for cognitive decline in MS different drugs have been co-opted from other pathologies and applied in this field. Indeed, acetylcholinesterase inhibitors, developed especially for Alzheimer's disease, were tested in order to treat memory impairments, quite common among patients with MS. Findings on donepezil efficacy are mixed due to the recent failure of a multicentre randomized controlled trial (Krupp et al., 2011) in replicating the initial promising results (Christodoulou et al., 2006, Krupp et al., 2004). Similarly, rivastigmine was repeatedly found ineffective in improving memory (Mäurer et al., 2012, Shaygannejad et al., 2008). Equally negative results and, for the most severe patients, reversible neurological side effects were observed in relation to the use of memantine, an inhibitor of N-methyl-D-aspartate receptors (Lovera et al., 2010, Peyro Saint Paul et al., 2016, Villoslada et al., 2009). This series of failures may be probably explained by the fact that, even though memory decline is observed in patients with MS, the underlying pathology is different from that typical of other neurodegenerative conditions such as Alzheimer's disease.

Several attempts were also made to treat the more characteristic decline in attentional and PS function observed in people with MS. In particular effects of stimulants were tested both after a single dose and longer treatments. The former approach found that methylphenidate (Harel et al., 2008) and L-amphetamine (Benedict et al., 2008) significantly induce improvements in tests of PS, namely the PASAT and the SDMT. However, long term use of L-amphetamine was found to improve only memory performance but not PS function (Morrow et al., 2009). Morrow et al. (2013), instead, found that lisdexamfetamine dimesylate effectively improved both PS and memory performance. Moreover, promising results were obtained also with fampridine, a potassium channel blocker, which not only consistently improves motor performance, but also PS function (Jensen et al., 2014, Magnin et al., 2015, Triche et al., 2016).

An alternative type of intervention for cognitive decline in MS is cognitive rehabilitation. Two recent extensive reviews highlighted how weak is the evidence gathered so far on the effectiveness of rehabilitative programmes (Amato et al., 2013, Mitolo et al., 2015). In particular most studies suffered from methodological flaws (Amato et al., 2013) and only more recent ones appear promising (Mitolo et

al., 2015). Equally disheartening are the results obtained in a Cochrane meta-analysis by Rosti-Otajärvi and Hämäläinen (2014): randomised and quasi-randomised controlled trials were of low quality and data pooling resulted in non-significant findings. Cognitive rehabilitation for MS, especially focussing of PS and MRI outcome measures, will be more extensively discussed in Chapter 2.

Chapter 2 | Processing speed function and its relationship with brain alterations in MS

As highlighted in Chapter 1, MS is a complex disease characterised by a wide variety of clinical manifestations. In this multifaceted landscape this PhD project will focus on the investigation of cognitive impairment due to MS pathology in an attempt to shed further light on the neural correlates and on potential treatments. More specifically speed of information processing will be studied in detail, given the considerable proportion of patients who are impaired in this function (Hämäläinen and Rosti-Otajärvi, 2016). In fact, ineffective PS is thought to be central in the expression of cognitive symptoms (Costa et al., 2017).

After briefly outlining the evolution and the different lines of research (experimental and clinical) on PS abilities, this chapter will present an overview of the neuropsychological and neuroimaging assessment of this function, also in relation to other cognitive domains. Subsequently, PS function will be analysed within the clinical context of MS and theories put forward about MS-related cognitive decline will be presented. Finally, a systematic review of the literature on the MRI neural correlates of PS function in MS will be carried out, with particular emphasis on studies which have investigated measures of structural and functional connectivity. This review is reported in section 2.2.6, which contains parts of a published paper (Manca et al., 2018). The literature search has been updated to October 2018 in order to include all eight studies published in the field in the past year after the completion of the paper. Quality analysis, figures and tables have been updated accordingly. The original paper can be found in Appendix A, permission for full reproduction granted by the publisher and the co-authors are reported, respectively, in Appendix B and Appendix C.

2.1. PS function: an introduction

The investigation of the speed at which information is processed by the human brain has a long history and it could be argued that it was one of the first cognitive functions ever to be studied. Indeed, the dawn of the endeavour to quantify experimentally mental processes was tightly intertwined with the birth of psychology as a new discipline, independent from philosophy and biology. In particular, the work

of the Dutch physiologist Franciscus Donders assumed crucial importance, since he was the first ever to use reaction times to quantify mental operations by means of the so-called “subtraction method” (Jensen, 2006). Similarly, differential psychologists, starting with Galton, had long been investigating inter-individual differences in reactions times and their assiciation with intelligence (DeLuca and Kalmar, 2007).

Research into PS abilities and, particularly, deficits has also been developed to phenotype the symptoms of different clinical populations. At the beginning of the 20th century Naville (1922) described cognitive slowing, that he termed bradyphrenia, in people affected by encephalitis lethargica. Since then this symptom has been extensively investigated in Parkinson’s disease (Rogers, 1986) and other pathologies characterised prominently by degeneration of either WM or subcortical GM nuclei, for which a debated category has been proposed, i.e. so-called “subcortical dementia” (Albert, 2005).

2.1.1. Neuropsychological assessment of PS abilities

Providing a neuropsychological definition of PS appears essential, though not exempt from difficulties. Indeed, lack of a clear conceptualization of this function has prevented PS-related concepts to be accounted for in most cognitive models. In clinical use, PS is operationally defined as a cognitive function measurable as either “the amount of information processed per unit of time or the time required to process a given amount of information” (DeLuca and Kalmar, 2007).

Despite having direct implications for the choice of tools to select for clinical assessment of PS abilities, this definition presents weaknesses. First, the isolation of a completely pure index of PS abilities is highly unlikely. Indeed, most tasks would engage at least a cognitive domain in which speed of information processing may play a role (Kail and Salthouse, 1994), such as working memory (Mella et al., 2015, Salthouse, 1992), long term memory (Chiaravalloti et al., 2003, Levitt et al., 2006, Sliwinski and Buschke, 1997, Tam and Schmitter-Edgecombe, 2013) and executive functions (Cepeda et al., 2013, McAuley and White, 2011, Urben et al., 2011). Second, Chiaravalloti et al. (2003) showed that although PS has been for long considered a unitary construct it is possible to differentiate between two components: *simple* and *complex PS*. The former is captured by basic measures such as reaction times that are derived from more experimental approaches and are mainly indicative of the status of attentional functions. The latter refers to the speed at which higher order cognitive functions that require more articulated cognitive

operations than simple motor reaction to stimuli are performed. Therefore, this PS function can be measured by means of several classical neuropsychological tests, some of which have been specifically developed in an attempt to characterise better cognitive decline in people with MS. In this section an overview of the most common measures of PS abilities used in clinical settings will be presented to facilitate the understanding of the literature in this field and elucidate the choices made in the studies of this PhD thesis. A comprehensive review of all the tasks involving PS function and especially those developed in experimental psychology research was not among the aims of the present chapter as not considered relevant to the aims of this PhD project.

Tests of PS abilities are included in all of the three batteries of neuropsychological assessment used in MS research: the Brief Repeatable Battery of Neuropsychological tests (Rao et al., 1991), the Minimal Assessment of Cognitive Function in MS (Benedict et al., 2002) and the Brief International Cognitive Assessment for MS (Langdon et al., 2012). The first two batteries include the PASAT (Gronwall, 1977), the most commonly used test to assess PS abilities in people with MS (Costa et al., 2017). In fact, the PASAT is included in the different clinical measures of the MSFC (Cutter et al., 1999), a tool designed to assess comprehensively neurological function in MS. This test essentially assesses verbal working memory by means of auditory presentation of sixty-one random digits (1 to 9) to subjects who are instructed to add each digit (from the second one on) to its preceding one and to provide the answer verbally (Figure 2.1). Each time a number is presented subjects need to update the set of digits kept in their verbal short term memory storage and perform the operation on them. The maximum score on the test is 60. Digits can be presented at different speed levels, most commonly each 3 or 2 seconds, thus allowing the possibility to manipulate the PS load posed on the verbal working memory system and consequently assess PS function (Forn et al., 2008). An alternative version consists of the Paced Visual Serial Addition Test (Nagels et al., 2005), in which numbers are presented visually on a computer screen.

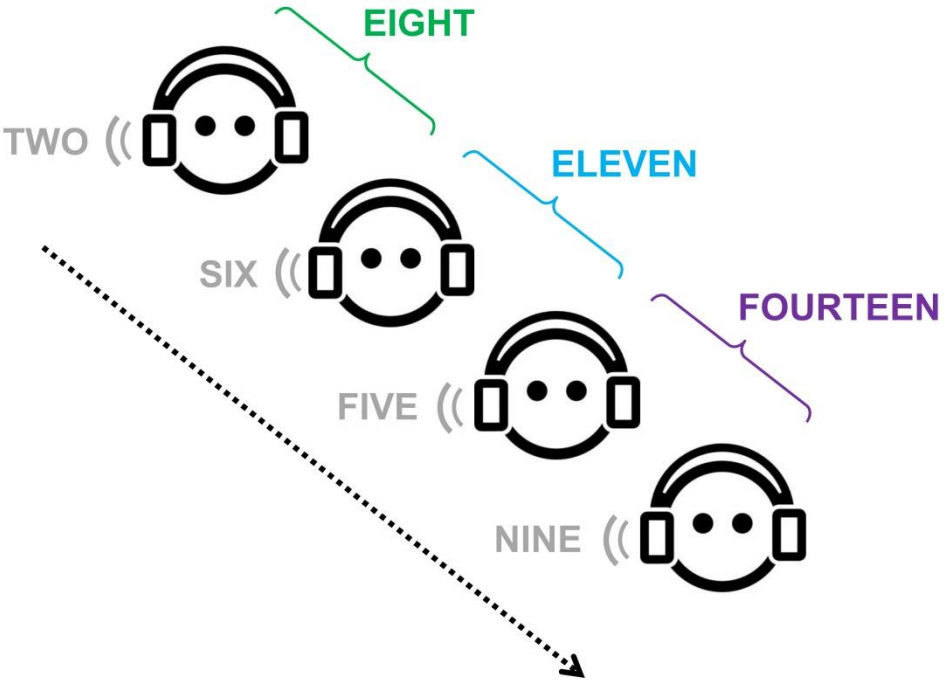

Figure 2.1 Schematic representation of the process of stimuli presentation during the PASAT.

However, despite its wide use, the PASAT presents some drawbacks since it represents a challenging test also for people without cognitive impairments (Brooks et al., 2011), its score can be affected by basic mathematical abilities (Sandry et al., 2016), and it is not well tolerated by people with MS in comparison to the Computerised Test of Information Processing and the SDMT (Walker et al., 2012). Seemingly for this reason the SDMT (Smith, 1982), considered a good tool for cognitive screening in MS (Parmenter et al., 2007b), has been proposed to replace the PASAT as cognitive measure in the MSFC (Drake et al., 2010). Moreover, Costa et al. (2017) noted a recent increase in the number of publications using this test to assess PS abilities in clinical populations. The SDMT is considered a test of visual attention that provides a reliable measure of visual PS (Benedict et al., 2017). It consists of a sheet of paper that presents on the top the so-called key comprising nine symbols associated with digits 1 to 9 and eight lines with fifteen symbols each for a total of 120 (ten of which practice items). The instruction is to pair as many digits to the symbols provided as possible in 90 seconds while proceeding from left to right in each line. The final score is the number of pairs correctly completed (maximum 110).

Other two tests that can be considered alternative versions of the SDMT are the Digit Symbol Coding Test (DSCT) contained in the Wechsler Adult Intelligence Scale (Wechsler, 2008) and the Letter Digit Substitution Test (Jolles et al., 1995).

The DSCT has an exactly reversed design compared to the SDMT since symbols have to be paired to numbers, though with some differences in the time allocated (120 seconds) and the number of items to process (maximum 133). Similarly, the Letter Digit Substitution Test closely resembles the SDMT but with letters instead of symbols, an allocated time of 60 seconds and a maximum score of 125. Despite slight differences across tasks they all require the deployment of visual attentional resources to process the given items quickly and iteratively while inhibiting the surrounding distractors.

For the other clinical measures used to assess PS a major distinction can be drawn between those tapping into verbal or visual/visuospatial functions. Indeed, a verbal task that was initially developed in experimental research (Stroop, 1935) and later adapted for clinical use is the Stroop test (Venneri et al., 1993). This test was shortened and simplified specifically to facilitate its application to cognitive testing of clinical populations. Three distinct subtests make up the shortened version of the Stroop test and all comprise a sheet of paper with 30 stimuli arranged in three columns: names of colours (blue, green and red) printed in black ink for the first part, coloured dots (in the same three colours named in the first subtest) in the second part, and lastly names of colours like in the first part, but printed with ink of a colour incongruent with that represented by the words. Participants are asked to read all the words of the first subtest and to name all the colours in the second part as fast as possible. In the third subtest the instruction is to name as fast as possible the colour of the ink in which each word is printed in, but not to read the words. For all the three parts both execution time and errors are recorded. The PS measure derived from this test is usually the average of the execution time of the first two subtests, but several different PS measures may be calculated from this test (Macniven et al., 2008). The third subtest provides an index of the ability to inhibit automatic responses. It is worth noting that, despite involving eminently verbal processes, the shortened version of the Stroop test poses some demands on attentional functions due to the simultaneous presentation of all stimuli on the same sheet of paper.

Tests that provide more purely verbal measures of PS are semantic and phonemic fluency tasks (Gontkovsky and Beatty, 2006). Both tests are multicomponential as they require linguistic skills, access to and recall from semantic memory long term storage, and different executive functions mainly for phonemic fluency (Alvarez and Emory, 2006, Henry and Crawford, 2004). Instructions for semantic fluency are to report as many items as possible in 60 seconds for each one of three different categories (e.g. cities, animals and fruits) presented one at a time. Therefore,

participants are pushed to recall quickly information from verbal long term memory, thus testing speed of access to their mental lexicon (Shao et al., 2014). In the phonemic fluency task subjects are asked to name as many words starting with a specific letter as possible in 60 seconds. Three letters are given, one for each consecutive trials, and different sets are available, e.g. F-A-S or P-L-F. As already mentioned this task engages more extensively executive functions than semantic fluency but similarly provides an index of complex PS in terms of amount of information processed per unit of time.

Visual PS function can be assessed by two tests of visual search tapping into attentional processes: the Trail Making Test (TMT) (Armitage, 1946) and the Digit cancellation test (Spinnler and Tognoni, 1987). The TMT comprises two parts with 25 stimuli each presented on a single sheet of paper: numbers 1 to 25 in part A, numbers 1 to 13 and letters A to L in part B. The participants are asked to connect as fast as possible and in ascending order all the numbers in part A, and numbers alternated to letters in part B (e.g. 1, A, 2, B, 3, etc). The test score is the amount of time needed to complete each subtest. Time on part A represents the main measure of visual PS that is usually subtracted by the time on part B in order to obtain a measure that captures an executive function named mental set shifting (Miyake et al., 2000).

The Digit cancellation test (Spinnler and Tognoni, 1987) comprises three subtests each one including 130 digits arranged in a matrix of thirteen rows of ten digits each, with the first two rows used for practice. The subjects are asked to cancel out, scanning each row from left to right, one given digit in the first subtest, two in the second one, and three in the third one. The sum of the number of items correctly reported in 45 seconds in each matrix constitutes a measure of complex PS.

Moreover, some computerised measures of PS are available, such as the Computerised Test of Information Processing (Tombaugh and Rees, 2008), well tolerated by people with MS (Tombaugh et al., 2010) and the Useful Field Of View (Ball et al., 1988, Sekuler and Ball, 1986). However, probably because their administration is computer-based the use of these tests with clinical populations has been limited. Moreover, the former comprises three reaction time tasks, thus providing simple PS indices more useful in experimental rather than clinical settings. The latter provides the extent of visual area in which information can be acquired just with one eye fixation. Hence, it is used to evaluate visual perceptual abilities that may have an impact on visual PS and attention and, in turn, activities of daily living such as driving (Goode et al., 1998).

2.1.2. Neural correlates of PS abilities

Many researchers have been interested in understanding how the human brain is able to process information quickly and effectively and how this may contribute to human intelligence. Both clinical and experimental observations led towards a prevalent interest for WM (Filley, 2010). Indeed, if we adopt the famous cognitivist metaphor that views the brain as a computer we can easily conceptualise GM areas as the processing units connected to one another by WM tracts. Therefore, we could argue that WM subserves efficient information transfer throughout the brain allowing integration of information and complementing GM functions. However, it is worth noting that the amount of available processing units may also provide a fundamental contribution to PS capacity (Kail, 1991). For this reason an overview of the different streams of neuroimaging research into PS abilities (limitedly to MRI in line with the purposes of this PhD project) appears useful in order to understand current knowledge.

2.1.2.1. Brain volume and lesions

The association between measures of PS ability and lesional WM volume appears well established in studies on healthy ageing. Indeed, the amount of WM hyperintensities is likely to account significantly for considerable age-related variance in performance on PS tasks (Rabbitt et al., 2007, van den Heuvel et al., 2006). However, some authors failed to replicate this finding (Kerchner et al., 2012), possibly because of differences in the PS measures investigated, namely correct responses (Rabbitt et al., 2007, van den Heuvel et al., 2006) vs mean reaction times (Kerchner et al., 2012). In fact, Walhovd and Fjell (2007) found no significant association between mean reaction times and either WM or GM global volume. On the contrary, intraindividual variability, measured as the standard deviation of reaction times, was significantly correlated with global WM volume independently of age. More specific investigations of WM have pointed out the association between indices of corpus callosum integrity and PS abilities. Bott et al. (2017) comparing the volume of the corpus callosum between fast and slow elderly performers and found the latter group showed significantly smaller volume than the former.

Voxel-based techniques have allowed more detailed correlational analyses of the PS-brain volume relationship. Turken et al. (2008) found that scores obtained by healthy adults on the DSCT were positively associated with the volume of both the superior and inferior longitudinal fasciculus bilaterally (Figure 2.2). The former is a complex WM tract comprising three branches that connect different frontal and

parietal areas (Rojkova et al., 2015) and is involved in attentional processes, while the latter connects occipital and temporal areas and is mainly involved in integration of visual information and in some linguistic functions (Catani and Thiebaut de Schotten, 2008). Moreover, in the same study voxel-based lesion symptom mapping analysis, carried out on a sample of people affected by stroke, highlighted the volume of the left superior longitudinal fasciculus as the neural correlate of PS deficits (Turken et al., 2008). A cluster of WM in the left parietal area, therefore consistent with the position of the superior longitudinal fasciculus, was also found by another study (Kerchner et al., 2012). In contrast, a different scenario was observed by Magistro et al. (2015) in a sample of over 800 young adults: PS was associated with the volume of many different WM tracts, thus leading the authors to conclude that this cognitive function is associated with WM globally rather than to specific fibre bundles. GM volume may be indicative of PS performance as significant correlations were detected in different left-lateralised areas: middle temporal gyrus (Turken et al., 2008), putamen and middle occipital gyrus (Hong et al., 2015).

Hence, WM volume appears to predict PS performance in a variety of studies, but it is still unclear whether this association depends on global or more localised connections. Moreover, between-study differences in methologies, populations investigated and findings pose further limitations to any definite conclusion on the contribution of WM/GM volume reduction to PS function.

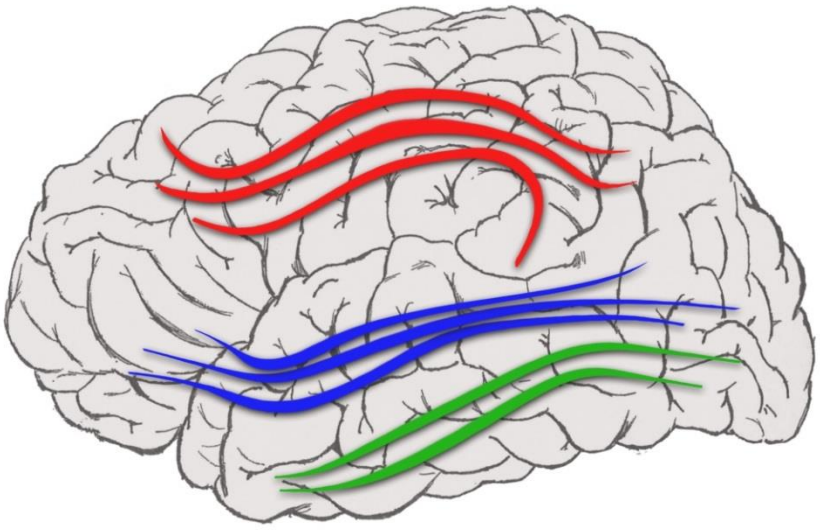

Figure 2.2 Representation of the inferior longitudinal fasciculus (green), superior longitudinal fasciculus (red) and inferior fronto-occipital fasciculus (blue).

2.1.2.2. White matter microstructure

Different approaches have been adopted in DTI investigations ranging from global to ROI and voxel-based measures of WM microstructural integrity. Some authors suggested PS is a general cognitive function supported by WM integrity globally. Indeed, Penke et al. (2010) found that PS performance correlated with DTI indices in 8 different ROIs placed in both commissural and associative WM tracts. Similarly, voxel-based studies reported widespread correlations between DTI indices in tracts encompassing the whole corpus callosum, associative tracts and even the cortico-spinal tract and different PS measures: intra-individual variability of reaction times (Tamnes et al., 2012) and both individual and global PS factors (Kuznetsova et al., 2016).

More focussed analyses highlighted how the status of WM microstructural integrity in the frontal lobes may explain variance in PS performance. Decreases in FA and in the concentration of N-acetylaspartate and choline-containing compounds, considered to be markers of neuronal health status, were associated with slower cognition (Kochunov et al., 2010). Lu et al. (2011) found that only microstructural degeneration in frontal areas and in the genu, but not in the splenium, explained PS capacity in older adults (Figure 2.3). Similar findings were reported by Hong et al. (2015) regarding structural connection integrity between the prefrontal cortex (PFC) and the striatum in older adults aged <70. Moreover, it appears that alterations in the length of WM tracts, especially those connecting the frontal lobes, may contribute to explaining performance on different PS measures (Behrman-Lay et al., 2015).

However, different studies pointed out a few WM tracts, mainly of the associative type, whose microstructural status is consistently observed to be associated with scores obtained on PS tests by participants across the life span. In particular, Ferrer et al. (2013) found that FA in both superior longitudinal fasciculi, but not in the cortico-spinal tracts, significantly explained age-related variance in PS performance in children and adolescents. The same association was found in older adults (Kerchner et al., 2012) and appears consistent with the abovementioned findings from morphometric studies. Furthermore, the inferior fronto-occipital fasciculus (Figure 2.2), a long associative tract connecting the orbitofrontal cortex to the inferior occipital cortex (Catani and Thiebaut de Schotten, 2008), was repeatedly associated with indices of both general PS (Kerchner et al., 2012) and visual PS (Sala-Llonch et al., 2015).

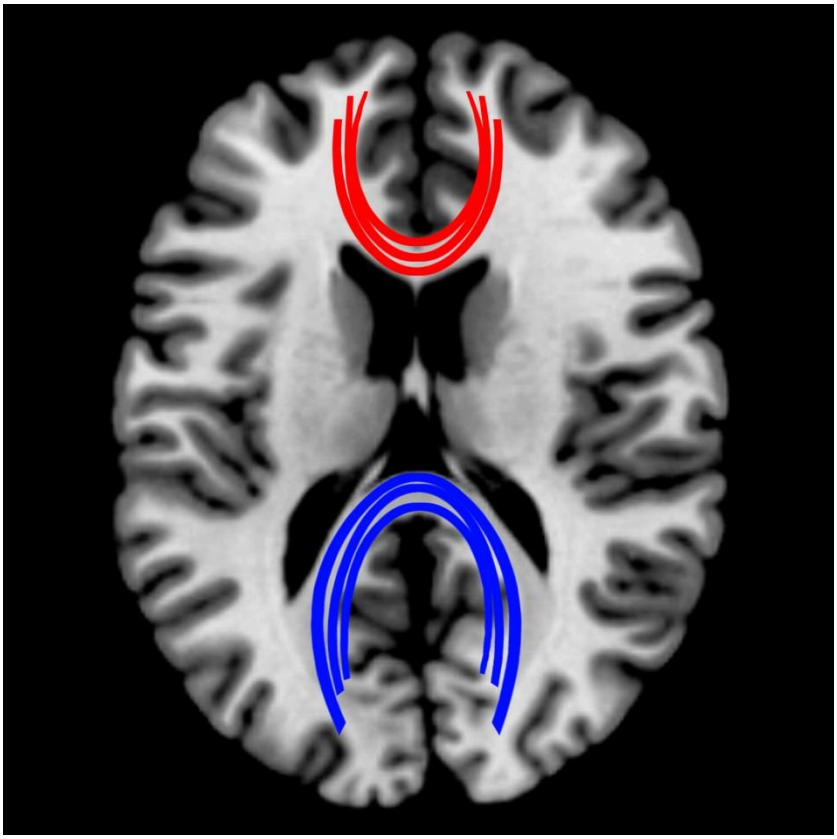

Figure 2.3 Forcipes of the corpus callosum: minor (red) and major (blue).

In conclusion, even though clear and definite conclusions cannot be currently drawn on whether one or a set of specific WM tracts underlie PS abilities in healthy people, the findings described above indicate a preferential involvement of associative WM connections.

2.1.2.3. Brain activity

Over the last decades research into spontaneous low-frequency brain activity at rest by means of MRI (Biswal et al., 1995) and positron emission tomography (Raichle et al., 2001) enabled scholars to disentangle networks of functionally related areas. Several networks, highlighted in Figure 2.4, have been described and investigated extensively in relation to various clinical variables (Menon, 2011, Raichle, 2011). Among these PS has also been found associated with functional connectivity of different networks, namely the DMN, fronto-parietal and salience networks (Shaw et al., 2015). Although these results appear not specific since episodic memory and executive functions showed similar patterns of correlation, voxel-based analysis revealed that PS function mainly correlated with resting-state activity of the right

dorsolateral PFC (part of the fronto-parietal network) and precuneus/posterior cingulate cortex (hub of the DMN).

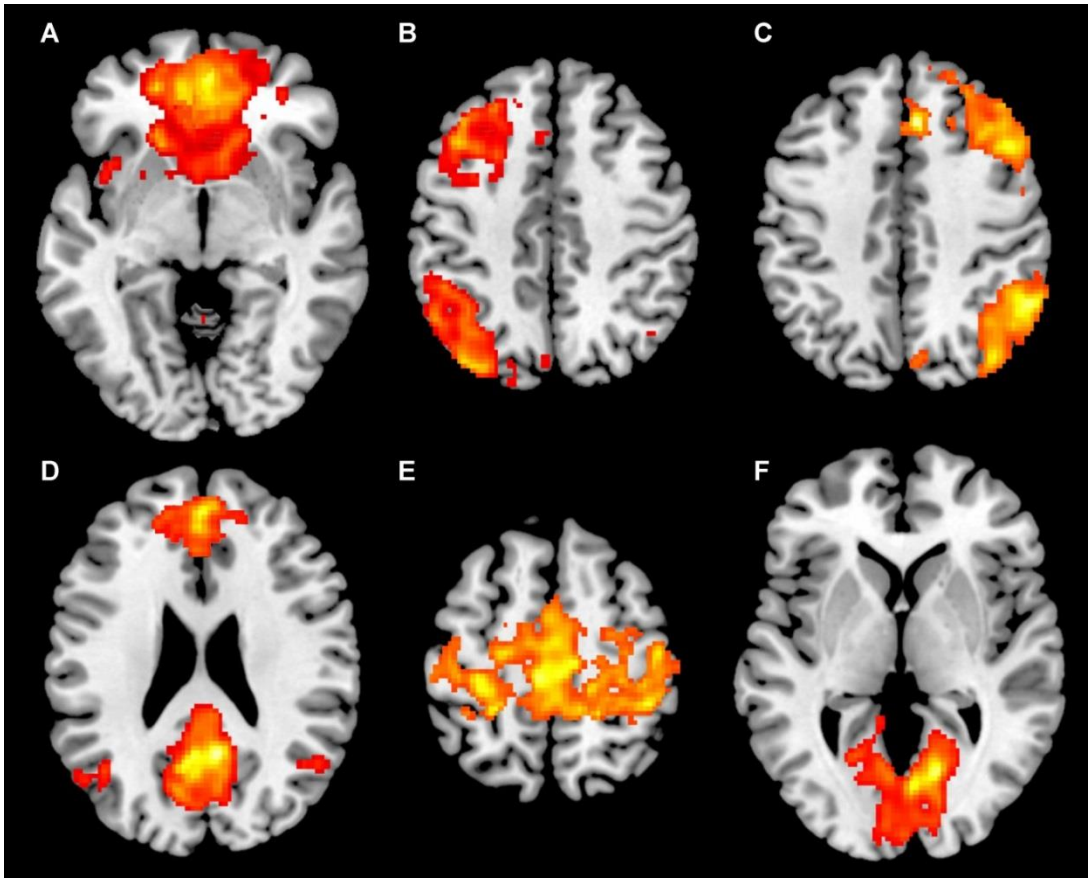

Figure 2.4 Representation of the most common functional brain networks: A) salience network; B) left fronto-parietal network; C) right fronto-parietal network; D) default mode network; E) sensorimotor network; F) visual network.

However, the vast majority of studies into the functional correlates of PS-related cognitive processes were carried out by using task-based investigations. The PASAT has been extensively used as PS test evidencing consistent results across studies in terms of recruitment of left-lateralised frontal and parietal areas (Audoin et al., 2005, Cardinal et al., 2008, Forn et al., 2011), in line with the current knowledge on the neural correlates of verbal working memory (D'Esposito et al., 2000, Petrides et al., 1993). It is worth noting that the activation of premotor, supplementary motor and anterior cingulate cortices emerges as the strongest neural signature, possibly due to the cognitive demands posed by this task (Stuss, 2011).

Other studies on verbal working memory, however, have pointed out the important role of the right PFC in support of fast cognitive performance, in line with the findings by Shaw et al. (2015). Indeed, it was shown that the activation of this area in people

with higher working memory span correlated positively with faster reaction times and that faster responders recruit the right PFC significantly more when performing a task with high vs low working memory load (Prabhakaran et al., 2011, Rypma and Prabhakaran, 2009, Takeuchi et al., 2012).

Rypma et al. (2006), investigated inter-individual differences in visual PS abilities in relation to activity in selected ROIs and found that fast responders exhibited significantly lower activation in the dorsal PFC and higher in the ventral PFC and the posterior parietal cortex compared to slow responders. Stronger connectivity between the dorsal PFC and the parietal cortex was additionally noted for slow performers. The authors interpreted this pattern in terms of neural and cognitive efficiency: if information is processed less efficiently, more executive control is required in order to perform a given PS task, paralleled by greater activation of the dorsal PFC that in turn exerts its influence over the parietal cortex. Comparing brain activity evoked by two commonly used PS tasks, namely the SDMT and the PASAT, the former was shown to be more associated with posterior brain activation in parieto-occipital areas than the latter (Forn et al., 2011). Moreover, increased PS-load on the SDMT induced more extensive recruitment of frontal, parietal and occipital areas bilaterally and activation of the pre-supplementary motor area/anterior cingulate cortex resulted negatively correlated with cognitive performance (Forn et al., 2013). Therefore, these results seem in line with the hypothesis of neural/cognitive efficiency proposed by Rypma et al. (2006).

In conclusion, as already observed for the structural correlates of PS ability, functional investigations have highlighted the involvement of frontal areas, particularly on the right side. However, in light of the findings reported above it appears more likely that PS function may be supported by dynamic interactions between associative hubs in both frontal and parietal lobes rather than by activation of a single brain area.

2.2. PS function in people with MS

2.2.1. PS decline due to MS

It is well established and widely accepted knowledge that PS represents a cognitive domain particularly affected by MS pathology early on in the disease course (Hämäläinen and Rosti-Otajärvi, 2016, Costa et al., 2017). Indeed, deficits in the speed at which people with MS process information have been extensively documented by numerous investigations and across several different cognitive

tasks. Moreover, consistent observations have highlighted a gradient of PS decline across subtypes since people with PPMS and SPMS exhibit more severe impairments compared to people affected by RRMS (Archibald and Fisk, 2000, De Sonneville et al., 2002, Denney et al., 2004, Papathanasiou et al., 2014, Ruet et al., 2013b). However, it must be stressed that progressive subtypes of MS usually present with worse clinical conditions than RRMS, including more extensive cognitive difficulties (Ruet et al., 2013b). Moccia et al. (2015) observed that cognitive status at the time of diagnosis of RRMS, mainly impairment in PS and memory, significantly predicts conversion to SPMS at 10-year follow-up.

Longitudinal studies appear to show a specific vulnerability of this cognitive function across all MS phenotypes as the disease progresses in time (Bodling et al., 2009, Denney et al., 2008, Hankomäki et al., 2014, Strober et al., 2014). In fact, it has been observed that in people with PPMS, compared to healthy controls, only PS abilities decline over time, while no significant changes were detected in problem-solving and verbal memory (Denney et al., 2008). Moreover, PS deficits seem to be the first sign of cognitive impairment to manifest in MS and to worsen faster than other cognitive functions (Van Schependom et al., 2015).

However, some specifications on this matter are needed. In fact, Bodling et al. (2009) found that MS pathology does not interact with age in affecting PS abilities since Stroop-based measures in people with MS and healthy agers seem to follow parallel trajectories of decline. The type of PS indices used may have driven these results as pointed out by Hankomäki et al. (2014) who observed decreases in performance over time only on the SDMT and a cognitively demanding dual task, but not on simple reaction time tasks and on the PASAT. In fact, performance of people with MS has been observed to be differentially affected across different PS measures. Different studies, consistently found that patients performed significantly worse than healthy controls only in those versions of the PASAT with high PS load, usually with a 2-second or even shorter inter-stimulus interval (Forn et al., 2008, Litvan et al., 1988, Parmenter et al., 2006). Moreover, comparing reaction time and rapid serial processing measures different behavioural effects were noted: complexity, compounding and augmentation effects (Hughes et al., 2011). The complexity effect is defined as a steeper worsening of performance for people with MS compared to healthy individuals across increasingly difficult reaction time tasks because the addition of cognitive operations to be performed quickly makes patients' deficits emerge. This effect has been consistently replicated (Bodling et al., 2012, Wojtowicz et al., 2012), although Denney et al. (2011) showed it can be detected only by means of overt, but not covert PS measures. In fact, when no time

limit is set for task performance, patients perform as well as healthy controls. The compounding effect refers to the fact that PS impairments are significantly highlighted by rapid serial processing rather than reaction time tasks. The reason is that in the former type of tasks a cognitive operation, e.g. matching a symbol to a number as in the SDMT, must be performed recursively over trials. The longer the information presented takes to be processed, the more task performance is delayed. Hence, possible overlaps between task execution in one trial and presentation of stimuli for the subsequent one may occur, followed by a consequent drop in performance usually not seen in reaction time tasks. Finally, the augmentation effect consists in a higher rate of cognitive impairment highlighted by means of tests such as the SDMT, which entails the simultaneous presentation of all stimuli associated with increased cognitive demand compared to tests that require processing of a single stimulus at a time. In fact, the prolonged inhibition of many distractors occurring simultaneously to the processing of each relevant stimulus affects the overall processing capacity of people with MS.

In conclusion, decline in PS abilities is believed to play a prominent role in the cognitive symptomatology that characterises MS and a careful selection of tests to assess this function is suggested by several convergent sources of evidence.

2.2.2. Theories and models of PS decline in MS

A range of theories and models have been proposed to explain impairments in PS function mainly observed in older adults and people with MS. However, different aspects are stressed across theories, spanning from the mechanisms underlying general PS performance to the possible trajectories of cognitive decline in MS. As a result, the interpretations provided represent alternative but not always mutually exclusive points of view on this issue.

The *processing-speed theory* by Salthouse (1996) was developed first from observations on age-related differences in cognition in the elderly later applied to people with MS (Chiaravalloti et al., 2013, Genova et al., 2012, Goverover et al., 2007, Leavitt et al., 2011). The main assumption of this theory is that cognitive performance can be limited by several constraints among which speed of processing constitutes a prominent one, especially as age advances. In particular, Salthouse proposed PS constraints can be evidenced by two mechanisms, namely *limited time* and *simultaneity*. The *limited time* mechanism plays a crucial role in cognitive tasks requiring distinct sequential steps of elaboration to be performed in a given amount of time. If processing takes too long in earlier stages, the quality and

quantity of the information available for later ones will be significantly diminished, thus undermining task execution. It follows that the application of challenging time constraints to sequential tasks may expose PS deficits. The *simultaneity* mechanism operates to ensure information is simultaneously available at different cognitive levels involved in complex tasks. Slowed and therefore longer processing may lead to degradation and even loss of information by the time higher-order cognitive processes have taken place. This mechanism is considered particularly relevant for working memory tasks in which information needs to be actively maintained in the short term storage while cognitive operations are carried out on it. If activating the information and subsequently completing the requested operations (e.g. adding two numbers and reporting their sum like in the PASAT) take too long, it is possible that part of the to-be-manipulated information may be lost during cognitive manipulation.

The *neural noise hypothesis* poses particular stress on the theoretical contribution of neural noise to cognitive processing in MS (Kail, 1997, Kail, 1998). According to this theory efficient information processing in the brain depends on both the strength of neural signal and the noise present in neural activity, i.e. random variability in the neural response to a given stimulus. Maintenance of an optimal signal-to-noise ratio is thought to be crucial to fast processing. Age-related changes are hypothesised to occur due to either weaker signal processing, stronger noise, or a combination of both (Crossman and Szafran, 1956, Welford, 1956). Similarly, demyelination due to MS is thought to cause alterations to the signal-to-noise ratio such to drive general cognitive slowing (Bodling et al., 2012, Kail, 1997, Kail, 1998). Yet little research has followed this hypothesis and actual assessment of neural noise in people with MS has not been performed. Hence, whether MS-related PS deficits are associated with signal-to-noise decay still remains an open question.

While the abovementioned theories mainly focussed on which mechanisms may be altered by MS pathology and lead to PS deficits, DeLuca et al. (2004) proposed two alternative models with the aim of explaining the relationship between deficits in PS and working memory and, potentially, the evolution of cognitive decline observed in people with MS. The *relative consequence model* postulates PS decline arises first in MS and constitutes the main cognitive signature of this disease. Subsequently, decreases in working memory capacity are also observed and believed to occur as a consequence of PS deficits. On the contrary, the *independent consequence model* states that PS and working memory can decline simultaneously. Therefore, it implies the breakdown in working memory function does not necessarily result from PS deficits. Although the *relative consequence model* has gained more support than the *independent consequence model* accounts of MS-related cognitive decline in the

absence of PS impairment have been published, thus suggesting people affected by this disorder may present with different cognitive manifestations.

Recently Costa et al. (2017) proposed the *tri-factor model* that, rather than providing an alternative explanation about the role of PS impairment in MS-related cognitive decline, mainly stresses the importance of considering different stages of information processing: perceptual, cognitive and motor. In fact, behavioural performance on PS tests is determined by efficient brain computations at each one of these sequential levels. First, stimuli peripherally detected by sensory organs must be perceived and represented at a cortical level. Second, cognitive operations required to execute a given task are performed on the bits of information perceived. Finally, adequate motor responses are selected and executed. Hence, drops in behavioural performance may be due to slowing in one or more of these three processes.

The tri-factor model appears an attempt to account for the complexity that characterises MS symptomatology and its underlying neuropathology. Moreover, its heuristic value resides in the suggestion that investigating PS function at different levels may shed light on the possible existence of distinct phenotypes either characterised by “purely cognitive” deficits or different combinations of perceptual, cognitive and motor processing impairments. However, if on one hand visual disturbance and decline in visual processing seem to affect visual PS function (Costa et al., 2016), on the other hand, cognitive slowing has been observed in the absence of (Binétruy et al., 2016) or only moderately affected by motor impairments (Bodling et al., 2008). On the contrary, PS decline was found to drive motor slowing in people with MS without motor impairments (Stoquart-Elsankari et al., 2010).

2.2.3. Impact of MS-related PS deficits on other cognitive functions

As written earlier in this chapter, PS is a function that exerts its influence on many different cognitive processes. This may depend on the fact that human cognition does not only suffer from limitations regarding the amount of information that can be processed at a given time point, but by limitations related to speed of processing (Marois and Ivanoff, 2005). As a consequence, an increase in the time required to perform any cognitive operations may crucially impact overall behavioural performance. Hence, the investigation of the wider influences of PS deficits in a disease like MS that is heavily characterised by such cognitive symptoms does appear a relevant enterprise. Indeed a large amount of research has been carried out on this issue.

The relationship between PS and attention has been studied by means of the Attention Network Test (Fan et al., 2002) that allows the assessment of three distinct attentional systems: alertness, i.e. the ability to maintain a state of active attention to respond after the presentation of warning stimuli; orienting, i.e. the ability to shift attention in space; and executive control, i.e. the ability to manage actively conflicting cognitive processes. People with RRMS exhibit slower and more variable response times than healthy controls across attentional tasks with particular deficits in executive control (Wojtowicz et al., 2013). Moreover, intra-individual variability in response times on attentional tasks significantly distinguished patients from controls, thus highlighting a central role of PS abilities. Consistent results were obtained by Roth et al. (2015) who highlighted attention decline in people with RRMS as well as SPMS. However, after statistically controlling for differences in PS capacity between groups, no significant differences in attentional functions between patients and controls remained. Only in the SPMS group residual alertness deficits were shown independently of PS impairment.

In the neuropsychological literature on MS great relevance was given to the interrelation between PS and working memory with the purpose of describing the causal evolution of cognitive impairments. Lengenfelder et al. (2006) found that patients with MS and affected working memory capacity presented longer response times and lower accuracy while performing working memory tasks, especially in relation to high cognitive load. Consistently, manipulation of working memory load appears to induce similar drops in performance on the PASAT and the n-back test (Parmenter et al., 2006, Parmenter et al., 2007a). Indeed, Leavitt et al. (2011) found that PS-impaired patients perform significantly worse on the Keep Track Task (i.e. a computerised working memory test) (Salthouse et al., 1991) than PS-preserved patients. Yet if patients are allowed additional execution time, both groups perform equally. In line with this finding, variance in performance on the Keep Track Task was found to be predicted by PS but not working memory ability (Genova et al., 2012).

Moreover, by manipulating the speed of presentation of stimuli for the PASAT, Demaree et al. (1999) observed that people with MS can reach the same accuracy level of healthy controls if their optimal PS threshold is used when delivering task stimuli. Sweet et al. (2010) found that performance of patients on a verbal n-back task was affected only by the reduction in the time allocated to maintain information in short term memory, but not by increases in the difficulty of stimuli to process. This drop in performance significantly correlated with PS abilities measured by the SDMT and by the PASAT. Hence, interfering with processing time constraints, but not with

the structural capacity of working memory, seems to impact behavioural performance in people with MS.

In contrast with this piece of evidence, recently, Berrigan et al. (2013) showed that the decreases in learning and executive functions experienced by people with MS were predicted by working memory rather than PS ability. The association between PS and working memory was not ruled out by this study, but PS was not observed to mediate the effect of the disease on higher order cognitive functions, thus standing against the *relative consequence model* (DeLuca et al., 2004).

Moreover, learning new information is one of the cognitive domains most commonly observed to decline in MS, perhaps as a consequence of PS impairments. Litvan et al. (1988) found that long term memory function correlated with performance on the PASAT. However, this test assesses working memory and therefore may not be able to distinguish between the independent contribution of PS and working memory to learning. More recently, Chiaravalloti et al. (2013) using different regression models showed how PS was the only significant predictor of learning performance differently from working memory capacity. Consistently, scores on the SDMT appear to predict performance on a visual incidental learning test (Denney et al., 2015).

Finally, the side effects of PS decline on different executive functions have also been extensively investigated. Drew et al. (2009) observed that the PS index of the WAIS (i.e. combination of scores from the Digit Symbol Coding and the Symbol Search tests) predicts performance on tests assessing several different executive functions and especially task-switching, detected by means of the part B of the TMT. However, PS-dependent executive functions seem to be particularly affected in MS, to a greater extent than PS-independent functions (Leavitt et al., 2014). Moreover, after differences in PS ability between people with and without MS are statistically controlled for, patients' performance on tests of PS-dependent executive functions appears no longer impaired when compared to controls (Denney and Lynch, 2009, Leavitt et al., 2014). Indeed, poorer response inhibition abilities due to MS can be predicted by response times in a discrimination task (Denney and Lynch, 2009). Furthermore, evidence of PS influence on cognitive performance has also been observed for planning abilities (Arnett et al., 1997). Indeed, MS patients perform significantly worse than controls only on timed planning tests, e.g. Tower of London (Shallice, 1982), and manifest longer planning time as task difficulty increases. However, differences in planning performance between patients and controls disappear if they are provided with additional time to solve the tasks (Owens et al., 2013).

The investigations here reviewed suggest a clear association between decline in PS function and deficits observed across other several cognitive domains. This relationship appears particularly evident in those studies that controlled for differences between patients and controls in PS abilities and that, by this means, showed how poorer performance in higher order cognition was dependent on slowed processing in attention (Roth et al., 2015), working memory (Demaree et al., 1999, Leavitt et al., 2011), executive functions (Denney and Lynch, 2009, Leavitt et al., 2014), and planning (Owens et al., 2013). These findings appear in line with the *relative consequence model* that states impairment in PS is the primary cognitive force driving decline in other domains (DeLuca et al., 2004). However, all the above mentioned studies adopted cross-sectional designs, thus undermining any definite conclusion about causal relationships in the temporal cascade of MS-related cognitive deteriorations. Moreover, not all studies have found a significant association between deficits in PS and other cognitive functions in MS (Berrigan et al., 2013). This may be due to the high level of heterogeneity that characterises the manifestation of cognitive impairment in MS. In fact, a recent paper that investigated PS and verbal long term memory in 128 people with RRMS found four cognitive phenotypes: more than half of the patients were not impaired in either cognitive domain; only less than 8% were impaired in PS exclusively; 17.2% showed impairments in both domains; and interestingly 18.8% were impaired in memory but not in PS abilities (Leavitt et al., 2018). Therefore, despite extensive evidence about the prominent role of PS in cognitive decline in MS, the presence of subgroups of patients affected by specific PS-independent cognitive deficits must be taken into account. This may be due to differences in lesion location across brain areas and across brain tissues as well as protective factors such as cognitive reserve (Stern, 2002, Sumowski and Leavitt, 2013, Sumowski et al., 2016).

2.2.4. PS and quality of life of people with MS

PS deficits have been shown to affect people with MS at various levels. Impairment in higher order cognition itself may be associated with PS deficits through the mediational role of other symptoms, namely mood and fatigue, though the actual causal relationships have not been clarified yet (Blair et al., 2016, Diamond et al., 2008). Roberg et al. (2012) found that the degree of self-reported slowing of cognitive abilities, although not correlated with objective PS assessment, exerts significant influence on levels of impulsivity, anxiety, and introversion.

Different sources of evidence support the hypothesis that various aspects of health-related quality of life are likely to be affected by PS dysfunction. In particular, mental quality of life of people with MS appears associated with slower cognition, especially with performance on the PASAT, while physical quality of life is consistently predicted by the EDSS scores, i.e. the global level of disability (Barker-Collo, 2006, Baumstarck-Barrau et al., 2011, Shawaryn et al., 2002a). However, one study found contrasting results: a significant relationship between scores obtained on the PASAT and the physical component of quality of life, and an unexpected negative correlation between the SDMT scores and the mental component (Glanz et al., 2010).

Moreover, decreased PS abilities in people with MS were also associated with functional deficits observed in daily life activities (Baumstarck-Barrau et al., 2011), especially those characterised by time constraints, as measured by the Timed Instrumental Activities of Daily Living tool (Goverover et al., 2007, Owsley et al., 2001). This may contribute to explain the high rates of unemployment observed among cognitively impaired individuals in this clinical population. Indeed, PS capacity significantly discriminates employed and unemployed patients (Campbell et al., 2017b) and predicts working status at 7-year follow-up (Ruet et al., 2013a). A recent review of 42 studies including over 30000 people affected by MS found that, along disease severity, fatigue and demographic characteristics, cognitive decline in PS was significantly associated with employment condition (Raggi et al., 2015).

Therefore, it can be reasonably stated that core cognitive deficits that characterise MS can exert multiple negative effects affecting not only cognition but also various aspects of patients' health and lives.

2.2.5. MS-related brain damage and PS function

Despite extensive use of neuroimaging in both clinical (for diagnostic purposes) and research settings (as an outcome measure of treatment trials) involving people with MS, the association between indices of neural and clinical dysfunction is still unclear. This peculiar phenomenon has been named "clinico-radiological paradox" (Barkhof, 2002). Severity of MS symptoms, cognitive decline included, is not necessarily a reflection of the amount of lesions that may be detected on conventional MRI scans. Indeed, lesional burden seen on T2-weighted images appears to affect global cognitive functioning only marginally and it is highly likely to represent an index poorly sensitive to impairment (Mollison et al., 2017). However, more sophisticated analysis may contribute to augment the meaningfulness of

lesional measures by highlighting specific lesion locations particularly associated with cognitive decline (Hackmack et al., 2012).

Studies that focussed specifically on PS found variable associations with indices of lesion burden depending on both MRI and cognitive measures used. The number of WM lesions has been observed to be irrelevant to performance on various PS tests (Deloire et al., 2005, Lebrun et al., 2010, Roosendaal et al., 2009b). This leads to the intuitive speculation that both lesion size and location may play crucial roles in affecting PS function rather than their simple count (Rossi et al., 2012).

On the contrary, several investigations evidenced that total lesion volume (TLV) in general correlates with PS performance (Granberg et al., 2015, Hohol et al., 1997, Khalil et al., 2011, Lin et al., 2008, Mazerolle et al., 2013, Mesaros et al., 2009) and even predicts changes in this function over time (Deloire et al., 2011). Only a minority of studies failed to detect any significant association with PS abilities (Kern et al., 2015, Nocentini et al., 2014). However, distinctions may apply to different PS measures. Indeed, absence of correlations with TLV was mainly highlighted for the PASAT (Audoin et al., 2005, Benedict et al., 2007, Fulton et al., 1999, Morgen et al., 2006, Sanfilipo et al., 2006), especially in versions with low PS load (Sbardella et al., 2013a, Stankiewicz et al., 2011), rather than for the SDMT (Bomboi et al., 2011, Sperling et al., 2001). Consistently, a review of 39 studies found that performance on the SDMT significantly correlated with lesion measures more consistently (70% vs less than 50% of the papers) and more strongly than performance on the PASAT (Rao et al., 2014b).

In line with results on TLV, cortical and WM lesion volumes appear preferentially associated with visual PS function (Mike et al., 2011, Papadopoulou et al., 2013). However, Weier et al. (2014) showed the two most common PS tests used with people affected by MS may be predicted by distinct lesional measures, namely TLV for the SDMT and cortical lesion volume for the PASAT. Detailed analyses showed that regional lesion volumes, mainly localised in frontal areas, seem to contribute differentially to PS deficits (Lazeron et al., 2005, Lazeron et al., 2006). Moreover, the volume of lesions in the corpus callosum (Mesaros et al., 2009) and cerebellum may further contribute to explain MS-related PS deficits (Archibald et al., 2004, Damasceno et al., 2014).

As previously mentioned in Chapter 1, along lesion formation in both WM and GM development of atrophy due to brain tissue loss is a well-established finding in MS research (Lansley et al., 2013, Vollmer et al., 2015). These morphological and volumetric changes have been documented to impact disease evolution and severity of cognitive symptoms (Vollmer et al., 2016). In particular, two different reviews

consistently showed PS abilities correlate with measures of atrophy (Rao et al., 2014b, Vollmer et al., 2016). In line with findings on lesional measures, it has also been observed that performance on the SDMT was affected by brain volume loss in each single study that used this measure, while results for the PASAT were much more variable.

However, reproducibility of findings on correlations between PS performance and simple atrophy measures such as normalised global brain volume or brain parenchymal fraction (i.e. the proportion of WM and GM volumes taken together on total intracranial volume (TIV)) tends to be limited. Indeed, methodological variability in morphometric analysis may explain contrasting findings for a test such as the SDMT (Granberg et al., 2015, Sastre-Garriga et al., 2009). Similarly, although brain parenchymal fraction was found to correlate with different PS tests (Benedict et al., 2007, Mazerolle et al., 2013, Nocentini et al., 2014) and to predict changes in PS function over a period of 7 years (Deloire et al., 2011), several studies failed to detect an association with any measure of PS ability (Baltruschat et al., 2015, Deloire et al., 2005, Lazeron et al., 2005, Lazeron et al., 2006, Sastre-Garriga et al., 2009). An unclear picture emerges from findings on independent analyses of GM and WM volumes. Indeed, Morgen et al. (2006) found patients with MS and deficits in PS had lower GM volume in frontal, parietal and temporal areas bilaterally. Moreover, performance on the PASAT and the SDMT was correlated with GM fraction, i.e. GM volume divided by TIV, both globally (Benedict et al., 2007, Sastre-Garriga et al., 2009) and in specific areas, namely the superior temporal gyrus for the SDMT (Tekok-Kilic et al., 2007) and bilateral orbitofrontal cortex for the PASAT (Sbardella et al., 2013a). Voxel-based analyses seem to indicate a prominent involvement of the frontal lobes since the SDMT scores were correlated with clusters of GM in the right DLPFC (Cerasa et al., 2013, Nocentini et al., 2014) and superior temporal and parietal cortices (Nocentini et al., 2014). Instead, no clear tendencies have been identified regarding the relationship between PS deficits and alterations in WM volume either globally (Papadopoulou et al., 2013, Sanfilipo et al., 2006) or in the corpus callosum (Llufriu et al., 2012, Ozturk et al., 2010).

Detrimental effects of MS-related atrophy have also been investigated in more localised areas and regions of interest. The ventricular fraction, i.e. ventricular volume divided by TIV, has not been found to correlate with scores obtained by patients on several PS tests (Deloire et al., 2005), although one longitudinal study observed changes in this index over a period of 2 years after recruitment predicted PS decline at 7-year follow-up (Deloire et al., 2011). Decreases in the width of the third ventricle have been consistently correlated with deficits PS abilities (Leavitt et

al., 2014, Tekok-Kilic et al., 2007): association that may be driven by thalamic atrophy, previously identified as one of the hallmarks of GM pathology in MS (Lansley et al., 2013). In line with this hypothesis, different studies found that thalamic volume predicted performance on the PASAT (Benedict et al., 2013), the SDMT (Benedict et al., 2013, Bisecco et al., 2017) and tests of PS-dependent executive functions (Kern et al., 2015). Moreover, Bergsland et al. (2016b) observed that atrophy specifically in the left thalamus predicted PS decline over a period of 3 years.

However, associations between PS performance and the volume of several different subcortical GM structures have been observed, such as the occipital cortex, the putamen, and the cerebellum (Bisecco et al., 2017). The role played by the cerebellum remains currently controversial due to inconsistent results observed in the literature (Cerasa et al., 2013, Damasceno et al., 2014, Weier et al., 2014). Similarly not definite results emerged for the hippocampus, a structure notoriously involved in memory functions. Indeed, performance of patients with MS on the SDMT (Koenig et al., 2014) but not on the PASAT (Koenig et al., 2014, Sicotte et al., 2008) was found significantly correlated with bilateral hippocampal volume by one study. Moreover, Köhler et al. (2017) observed that verbal MS-related memory impairments were predicted by both PS decline and by left hippocampal volume reduction. However, whether PS deficits mediate the relationship between hippocampal damage and memory impairments remains unclear.

In conclusion, converging clues from investigations into macrostructural damage in MS highlighted above suggest frontal damage may preferentially affect PS function in people with MS. Nonetheless, the involvement of atrophy in other cortical and subcortical GM structures, as well as in WM tracts connecting them, cannot currently be ruled out.

2.2.6. Brain connectivity and PS function in MS

The lack of consistent association between various indices of macrostructural damage and PS deficits that characterise MS may be due to low sensitivity to cognitive decline for this type of neural marker, especially in the early stages of the disease. Although GM lesions may contribute to the manifestation of some cognitive symptoms (Calabrese et al., 2009), damage to WM fibres that support transmission of neural signal is hypothesised to constitute the main neural correlate of PS decline (Filley, 2010).

Moreover, MS can be said to represent a type of *disconnection syndrome* since WM lesions affect connections between different GM areas (Mesulam, 2012). Reductions in the integrity or even the number of the physical connections within brain networks may alter the effectiveness of signal transmission that, in turn, would lead to limited PS. This process is likely to drive cognitive decline that may be particularly evident when increasing structural damage causes a so-called *network collapse* (Schoonheim et al., 2015).

In consideration of the relevance given to disconnection as a driving mechanism for MS-related cognitive symptoms, it appears necessary to scan the literature that investigated how brain connectivity is associated with PS performance in people with MS systematically. See Appendices B and C for approvals to reproduce the review by Manca et al. (2018) in this section.

2.2.6.1. Methods

A systematic review of neuroimaging studies investigating the relationship between indices of brain connectivity and performance on tasks of PS in MS was carried out. The specific aim was to summarise the current knowledge about the relationship between breakdown in brain connectivity and PS function in people with MS.

A literature search was undertaken in two online databases: PubMed and Web of Science. Studies using DTI and resting-state functional MRI (RS-fMRI) in combination with cognitive PS measures were specifically targeted. The exact strings searched are reported in Appendix A. No time limits were set and all the papers published up to October 2018 were assessed following the steps highlighted in the PRISMA statement (Figure 2.5) (Moher et al., 2009). Additional papers from the reference lists of the selected articles that had not been identified in the literature searches were included. After removal of duplicates, the full text of the remaining articles was inspected and paper selection was performed according to the following exclusion criteria: (1) review articles, (2) theoretical and/or modelling papers, (3) papers related to patients with pediatric-onset MS, (4) papers related to diseases different from MS, (5) animal studies, (6) biological studies, (7) pharmacological studies, (8) papers with no inclusion of PS measures, (9) papers with no use of either DTI or RS-fMRI techniques, (10) papers not in English.

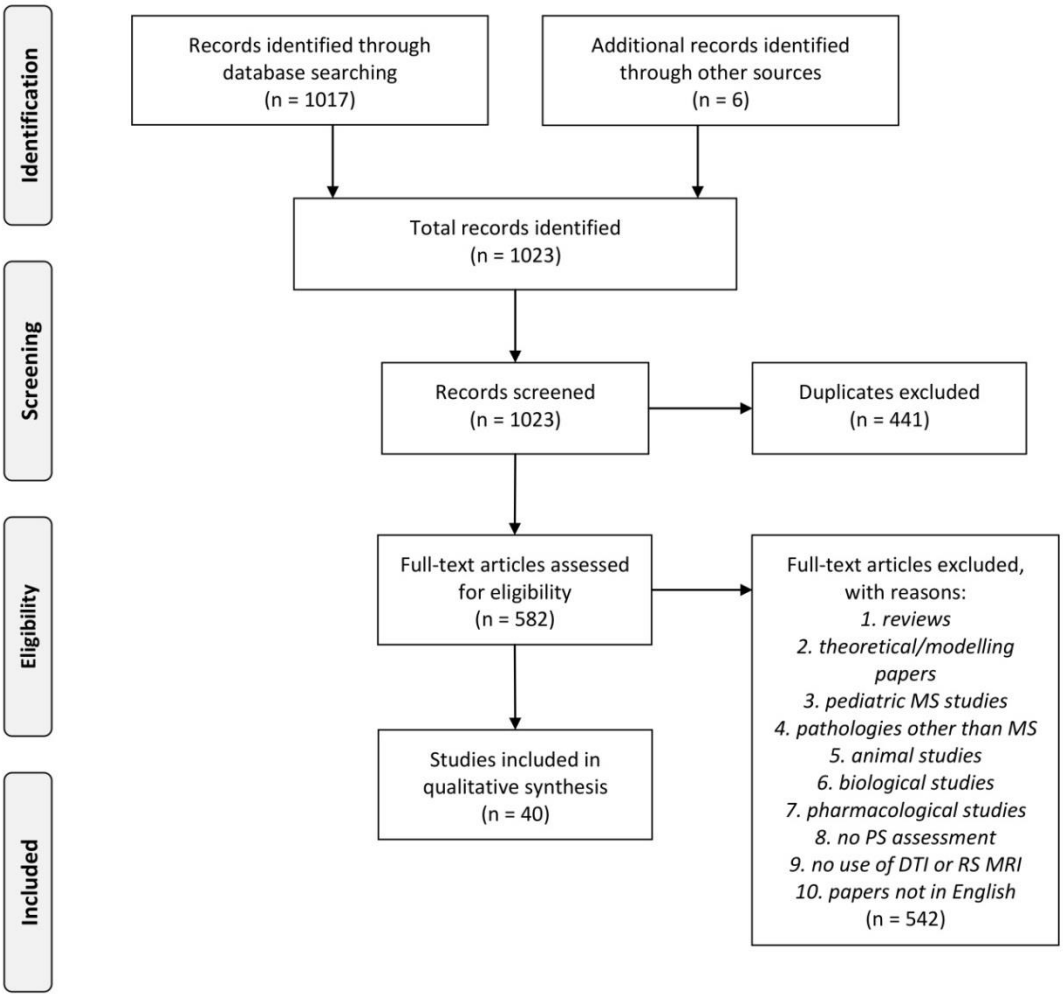

Figure 2.5 Flow chart of the process of study selection.

Papers selected to be included in this review were assessed according to a customised set of criteria, adapted from those used by Welton et al. (2015), that give an indication of their scientific quality and to ascertain possible sources of bias. A checklist of twelve questions was created and organised in five areas: methodology, clinical characteristics, MRI parameters, statistical analysis and results. Particular attention was given to the provision of details about the characterisation of the samples recruited and the analyses performed. A point was assigned for each quality criterion fulfilled. For the criterion assessing sample composition, 2 points were assigned to studies carried out on one or more groups of homogenous MS phenotypes, 1 to studies that included mixed phenotype samples, and no points to those reporting no information about phenotypes investigated. Therefore, the maximum score that could be achieved was 13 points. For more detailed information about quality assessment see Appendix A.

2.2.6.2. Results of the literature search

A total of 1023 papers were identified through online search and review of all the available references. Four hundred and forty-one entries were duplicates and the remaining 478 records were fully screened for eligibility. Fourty papers, all published between 2008 and 2018, met the final selection criteria to undergo review. Twenty-seven studies reported the use of DTI measures to investigate structural connectivity only, 7 studies used RS-fMRI only for functional connectivity, and 6 studies combined DTI and RS-fMRI.

A summary of the quality assessment of the reviewed articles is reported in Table 2.1. Differences in the overall quality of papers between the three MRI categories were analysed using the Kruskal-Wallis test. The analyses showed the differences to be only marginally significant $\chi^2(2) = 6.023$, $p = .049$. After applying Dunn's multiple comparisons test, the only difference that approached significance threshold was the one between studies using only DTI and those combining DTI with RS-fMRI ($p = .06$), with the latter showing higher scores (Figure 2.6). More detailed information on the evaluation of each quality criterion is reported in Appendix A.

Table 2.1 Descriptive statistics for the overall quality assessment of the studies categorised by MRI technique.

MRI technique	Median	Interquartile range	Minimum	Maximum
DTI	9	4	4	12
RS-fMRI	11	2	6	12
DTI and RS-fMRI	11	3	9	13

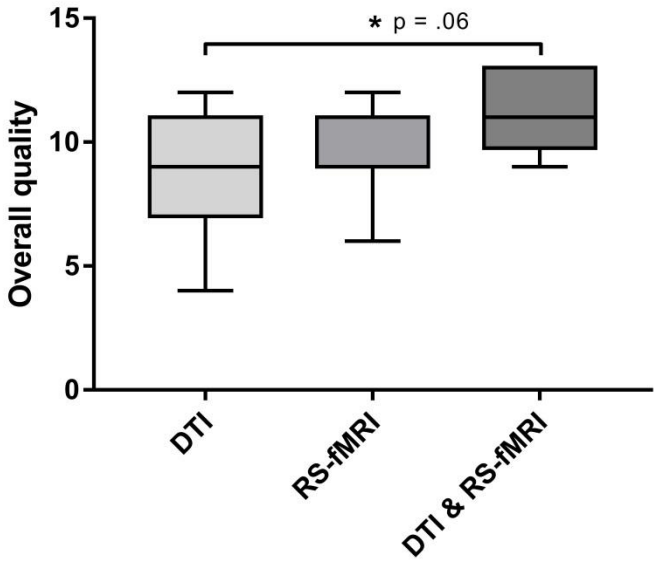

Figure 2.6 Differences in overall quality of the papers reviewed.

These findings show a potential gap in the overall scientific quality between DTI studies and those combining DTI with RS-fMRI measures. Arguably, this may be driven by technological advances and indeed studies using RS-fMRI were in general more recent than the DTI ones. However, it is also possible that studies combining several MRI techniques might have been more thoroughly designed. It must be noted, however, that only a few studies have been carried out with combined methodologies, thus making any conclusions not definitive.

2.2.6.3. Results about structural connectivity

Moderate heterogeneity was seen across studies with respect to sample composition, clinical information, analysis techniques, and covariates of no interest (Appendix A). In particular, despite the fact that the majority of the studies investigated RRMS, nine included patients with different MS clinical courses without specific sub-sample analysis (Benedict et al., 2013, Bergsland et al., 2018, Genova et al., 2013, Koenig et al., 2014, Koenig et al., 2015, Moroso et al., 2017, Ozturk et al., 2010, Roosendaal et al., 2009a, Van Hecke et al., 2010). Progressive MS was underrepresented, with a single study on patients with SPMS (Meijer et al., 2016). Two papers were published on so-called “benign MS” (Bester et al., 2013, Mesaros et al., 2009), while one did not report explicitly the type of MS investigated (Warlop et al., 2009).

In general, information on relapses and medications taken at the time of data collection were reported by most studies, but comorbidities and the presence or absence of fatigue and depressive symptoms were scarcely documented. This, together with lack of clearly stated *a priori* hypotheses lowered the quality of studies using only DTI measures compared to the others reviewed. There was, however, a trend towards improvement with better quality studies found in the most recent investigations of structural connectivity.

Statistical analyses were carried out with different approaches, the most common being the investigation of one or more regions of interest that was used in sixteen out of twenty-seven studies (Benedict et al., 2013, Bergsland et al., 2018, Bester et al., 2013, Bonzano et al., 2008, Kern et al., 2015, Koenig et al., 2014, Koenig et al., 2015, Lin et al., 2008, Llufriu et al., 2012, Mesaros et al., 2009, Moroso et al., 2017, Ozturk et al., 2010, Pokryszko-Dragan et al., 2018, Rimkus et al., 2011, Roca et al., 2008, Roosendaal et al., 2009a). The definition of the regions of interest was mainly *a priori*, though 2 studies defined them according to task-related functional activation (Bonzano et al., 2008) and differences in fractional anisotropy between MS patients and healthy controls (Roosendaal et al., 2009a). Thirteen out of twenty-seven studies did not use multiple comparisons correction strategies (Benedict et al., 2013, Bonzano et al., 2008, Kern et al., 2015, Koenig et al., 2014, Lin et al., 2008, Llufriu et al., 2012, Moroso et al., 2017, Pokryszko-Dragan et al., 2018, Rimkus et al., 2011, Roca et al., 2008, Roosendaal et al., 2009a, Shu et al., 2018, Warlop et al., 2009) and fourteen did not control for any covariate of no interest (Bonzano et al., 2008, Kern et al., 2015, Koenig et al., 2014, Koenig et al., 2015, Lin et al., 2008, Llufriu et al., 2012, Moroso et al., 2017, Ozturk et al., 2010, Pokryszko-Dragan et al., 2018, Rimkus et al., 2011, Roca et al., 2008, Shu et al., 2018, Van Hecke et al., 2010, Warlop et al., 2009). Among those publications that did, age was always included, followed by sex and premorbid cognitive status.

The most consistent difference observed between people with MS and healthy controls in DTI studies was the presence of abnormalities in the corpus callosum. This interhemispheric bundle of fibres appeared to be particularly affected by MS pathology. Additionally, other WM tracts also showed abnormalities including: the superior and inferior longitudinal fasciculus, the cingulum, and the fornix. Most of these are associative WM tracts that mainly support different cognitive functions.

Weak or absent correlation between DTI indices and PS measures was reported in five papers using the PASAT, in particular the 3 sec version (PASAT 3") (Bester et al., 2013, Koenig et al., 2014, Koenig et al., 2015, Llufriu et al., 2012, Sbardella et al., 2013a). This finding, in line with the aforementioned review on atrophy measures

(Rao et al., 2014b), may be due to a lower PS load of the 3 sec version compared to more challenging versions of the same test or to the SDMT. Indeed, Sbardella et al. (2013a) observed that the PASAT 2", but not the PASAT 3", significantly correlated with both fractional anisotropy and mean diffusivity in a widespread network of WM tracts centred on the right inferior longitudinal fasciculus and the left cingulum. Similarly, Riccitelli et al. (2017) found widespread correlations between scores on this test and various DTI indices across many WM tracts. While Sbardella et al. (2013a) and Riccitelli et al. (2017) used a tract-based spatial statistics approach to investigate voxel-wise associations within a skeleton of WM containing only the core of the tracts, lack of correlation between the PASAT 3" and DTI indices was otherwise observed in studies of patients with different MS phenotypes utilising either whole-brain global indices (Warlop et al., 2009) or several regions of interest: anterior thalamic radiations (Bester et al., 2013), corpus callosum (Bester et al., 2013, Llufriu et al., 2012), fornix (Koenig et al., 2014), posterior cingulum and posterior limb of the internal capsule (Koenig et al., 2015). On the contrary, Shu et al. (2018) found that various graph-theory-derived measures indicative of connectivity strength were positively correlated with performance on this test.

In line with the findings from comparisons between people with MS and healthy controls, in studies with mixed MS phenotypes the corpus callosum was the WM bundle most commonly reported to be correlated with the PASAT 3", both in region-of-interest (Lin et al., 2008, Mesaros et al., 2009, Ozturk et al., 2010) and voxel-wise investigations (Bozzali et al., 2013, Dineen et al., 2009, Van Hecke et al., 2010, Yu et al., 2012). However, performance on this test was also noted to correlate with the degree of microstructural integrity of other WM tracts, mainly: the left cingulum (Dineen et al., 2009, Sbardella et al., 2013a, Van Hecke et al., 2010); the superior longitudinal fasciculus, mainly on the left side (Bonzano et al., 2008, Dineen et al., 2009, Van Hecke et al., 2010); and the inferior longitudinal fasciculus bilaterally (Dineen et al., 2009, Sbardella et al., 2013a, Van Hecke et al., 2010). Moreover, less consistent associations with the PASAT were detected in the arcuate fasciculus (Dineen et al., 2009), right posterior thalamic radiations and right sagittal stratum (Yu et al., 2012), hippocampal and cerebellar WM (Bozzali et al., 2013), the lateral portion of the frontal lobes (Roca et al., 2008), and with thalamic mean diffusivity (Benedict et al., 2013). Only one study found that microstructural integrity of the bilateral uncinate fasciculi predicted PS performance assessed combining the PASAT and the SDMT (Kern et al., 2015).

The only study carried out on SPMS did not investigate PS as a distinct domain but divided the patients' sample into cognitively impaired and preserved sub-samples,

based on performance on various tests, among which were the PASAT and the SDMT. Mean global radial diffusivity, among the different DTI measures, emerged as the only significant predictor of cognitive status. However, all DTI indices were found to be significantly different between cognitively impaired and cognitively preserved groups in: the fornix, the superior longitudinal fasciculus, and the forceps major (Meijer et al., 2016).

In contrast, fewer studies investigated the association between structural connectivity measures and performance on the SDMT in people with MS. Among them, only two failed to report any significant correlation in two regions of interest, namely anterior thalamic radiations (Bester et al., 2013) and the corpus callosum (Bester et al., 2013, Llufriu et al., 2012). Consistently, Bergsland et al. (2018) observed that the correlation between SDMT scores and thalamic mean diffusivity values was not significant if thalamic volume was statistically controlled for. Similar to the results on the PASAT, higher structural integrity of the corpus callosum, particularly in the body, appears consistently linked also to higher scores obtained on the SDMT (Mazerolle et al., 2013, Pokryszko-Dragan et al., 2018, Rimkus et al., 2011, Yu et al., 2012) and a similar test of visual PS: the Letter Digit Substitution Test (Roosendaal et al., 2009a). However, DTI indices were more often observed to be correlated with this test in other WM fibre bundles: the fornix, both left-lateralised (Koenig et al., 2014) and bilaterally (Yu et al., 2012); the cingulum, on the right side (Yu et al., 2012) and globally (Koenig et al., 2015); and the posterior thalamic radiations bilaterally (Mazerolle et al., 2013, Yu et al., 2012). In general, results appear quite variable and non-specific (Riccitelli et al., 2017). Less commonly, significant correlations between the SDMT scores and DTI measures were additionally detected by region-of-interest and voxel-wise analyses in: the posterior limb of the internal capsule (Koenig et al., 2015), thalamus (Benedict et al., 2013, Pokryszko-Dragan et al., 2018), middle cerebellar peduncle (Pokryszko-Dragan et al., 2018), bilateral uncinate fasciculi, sagittal stratum (Yu et al., 2012), and the superior longitudinal fasciculus (Mazerolle et al., 2013).

Only two DTI studies, investigated the TMT: the first (Rimkus et al., 2011) reported no correlation in the region of interest of the corpus callosum, while a voxel-wise study found that performance on this test was correlated with fractional anisotropy in different parts of the corpus callosum, the left inferior fronto-occipital fasciculus, right posterior thalamic radiations, and bilateral superior longitudinal fasciculi (Genova et al., 2013). This latter study also found that a PS index derived from the Stroop test correlated with structural connectivity integrity of the corpus callosum, bilateral fornix, and right-lateralised anterior and posterior thalamic radiations.

2.2.6.4. Results about functional connectivity

Seven studies focussed solely on resting-state brain activity and its relation to PS ability in RRMS (Appendix A). Patients' age, duration and severity were quite similar across studies and in general the reported clinical data were more detailed than in DTI studies although details about relapses were missing (Gamboa et al., 2014, Janssen et al., 2013, Lin et al., 2018b). While most studies investigated different cortical and subcortical regions of interest, two studies analysed functional connectivity within the graph theory framework by dividing the brain into GM areas, extracting the average resting-state signal from each area, and finally calculating linear correlation between signals from each pair of GM areas (Gamboa et al., 2014, Lin et al., 2018b). The majority of the studies did not use statistical correction for multiple comparisons (Gamboa et al., 2014, Pravatá et al., 2016, van Geest et al., 2018, Wojtowicz et al., 2014) and five controlled for possible confounding variables (Lin et al., 2018a, Lin et al., 2018b, Pravatá et al., 2016, van Geest et al., 2018, Wojtowicz et al., 2014). However, studies on functional connectivity were of a slightly higher, although not significant, quality compared to the structural connectivity studies given that all were explicitly hypothesis-driven with just one exception (Wojtowicz et al., 2014). The PASAT 3" was the most commonly used test of cognitive PS function, although mainly in combination with other tasks, which resulted in high variability of PS assessment across studies (Gamboa et al., 2014, Janssen et al., 2013).

When functional connectivity was compared between people with MS and healthy controls, reductions were reported in the somatosensory network, medial and lateral visual networks (Janssen et al., 2013), and between posterior and anterior cingulate cortex and right inferior frontal gyrus (Wojtowicz et al., 2014), while different thalamic nuclei appear to undergo multifaceted changes in FC (Lin et al., 2018a). Analysis of static and dynamic FC revealed no differences between patients and controls specifically in the DMN (van Geest et al., 2018), but overall alterations have been observed (Lin et al., 2018b). Consistently, graph-based analysis of functional connectivity revealed how the brains of people with MS tend to reorganise and become more modularised. This means that connectivity between brain areas that are functionally related to one another and form a module tends to increase in MS, while functional connectivity between areas belonging to different brain modules becomes weaker (Gamboa et al., 2014); see Fleischer et al. (2017) for a recent review of graph theory and brain networks in MS. These findings support the view of MS as a disconnection syndrome due to different functionally related areas

becoming more independent from one another and, in turn, hampering information integration across the brain.

Accuracy in a dual-task PASAT 3” was reported to be negatively associated with the general level of network modularity (i.e. reduced between-network connectivity) characterising brains affected by MS: the higher the brain modularisation the worse the PS performance (Gamboa et al., 2014). Using a similar approach, Lin et al. (2018b) found that global changes in both static and dynamic FC are associated with PS as well as executive funtions. Wojtowicz et al. (2014) also found that the higher the intra-individual variability in the semantic search reaction time task of the Computerised Test of Information Processing the lower the functional connectivity between ventro-medial prefrontal cortex and the left frontal pole. No alterations in connectivity of the ventro-medial prefrontal cortex were reported between people with MS and healthy controls, however.

Another region-of-interest study compared functional connectivity changes between a baseline scan acquired just before in-scanner performance of two consecutive blocks of the PASAT 3” and two subsequent scans: one acquired just after completion of the second block and one after 30 minutes. The scores on the PASAT 3” correlated with the decrease of connectivity occurring in the 30 minutes after task performance between the left superior frontal gyrus and the left thalamus (Pravatá et al., 2016). However, PS function was not correlated with functional connectivity of the left superior frontal gyrus at baseline. Similarly, no association was found between scores on this test and FC of thalamic nuclei (Lin et al., 2018a). However, performance on the SDMT correlated negatively with FC between anterior thalamic nuclei and the posterior cingulate and between the lateral thalamic nuclei and the insula. Finally, Janssen et al. (2013) calculated a PS composite score comprehensive of both verbal and visuospatial components and including performance on the PASAT 2”, the PASAT 3”, the letter comparison and the pattern comparison tests. No associations were reported for the composite score with any of the 6 resting-state networks investigated: DMN, executive control, left and right fronto-parietal, cerebellar, and sensorimotor networks. However, using a similar cognitive measure van Geest et al. (2018) observed that dynamic changes in FC of the DMN contributed to predict PS performance along measures of structural brain damage.

2.2.6.5. Results about the combination of structural and functional connectivity

Papers that combined DTI and resting-state analysis were characterised by greater homogeneity in sample composition: five out of six were carried out on RRMS while only one on a mixed sample of PPMS and SPMS (Rocca et al., 2010b). Clinical information was in general extensively reported, apart from the presence of depression. Moreover, all of the studies used the PASAT 3" as test of PS functionality apart from one which investigated also the PASAT 2" (Tona et al., 2014) and a more recent study that compared PS-impaired and PS-preserved patients, classified according to their performance on the SDMT (Meijer et al., 2018). Most studies used statistical correction to account for multiple comparisons, namely Bonferroni and family wise error corrections. Apart from two (Meijer et al., 2018, Zhou et al., 2014), all studies included covariates of no interest in statistical models, mainly age and sex.

Compared to DTI studies, those that investigated both structural and functional connectivity, showed significantly higher quality with two out of five reaching the maximum quality score in our criteria (Tona et al., 2014, Zhou et al., 2016). However, the hypotheses underlying the aims of the studies were not always overtly reported in these papers (Rocca et al., 2010b, Sbardella et al., 2015b, Zhou et al., 2014).

In RRMS DTI findings confirmed those from studies focussing exclusively on this technique, showing lower fractional anisotropy and higher mean, radial, and axial diffusivity globally (Tona et al., 2014) and in the corpus callosum, the inferior and superior longitudinal fasciculi (Sbardella et al., 2015b), thalamic tracts (Zhou et al., 2016), and tracts connecting cortical areas of the DMN (Zhou et al., 2014) compared to controls. Moreover, in SPMS more severe alterations of diffusivity indices were seen in the corpus callosum and the cingulum (Rocca et al., 2010b).

Widespread correlations between scores on the PASAT 3" and fractional anisotropy mainly centred on the corpus callosum were found in one study that used tract-based spatial statistics analysis (TBSS) (Sbardella et al., 2015b). Furthermore, several regions of interest were investigated and were found to be associated with PS performance: the corpus callosum and the cingulum, but not the corticospinal tract and the optic radiations (Rocca et al., 2010b); tracts connecting the posterior cingulate and the precuneus with the right inferior parietal lobule (Zhou et al., 2014); and the anterior thalamic radiations (Zhou et al., 2016). One further study reported no correlations between the two PASAT versions analysed (2" and 3") and global fractional anisotropy and mean diffusivity (Tona et al., 2014). Instead, Meijer et al.

(2018) found that patients with PS deficits compared to those without showed widespread WM damage signalled by lower FA values.

Results of studies of functional connectivity in RRMS were more variable when compared to healthy controls: thalamic connectivity was increased with dorsal and lateral frontal areas, but decreased with medial frontal, medial temporal and occipito-parietal cortices (Tona et al., 2014, Zhou et al., 2016); increased connectivity was found between various pairs of areas part of the DMN (Zhou et al., 2014); and finally decreases in functional connectivity between the left fronto-parietal network and the executive control network were found (Sbardella et al., 2015b). However, a study that compared 130 PS-impaired with 200 PS-preserved patients found that the former group had higher average functional connectivity across the whole brain (Meijer et al., 2018).

When functional connectivity was found to correlate with measures of PS, PASAT 3" score was positively associated with connectivity of the left medial prefrontal cortex and the anterior cingulate (Rocca et al., 2010b) and negatively with the posterior DMN on the left side (Zhou et al., 2014), the executive control network and the medial visual network (Sbardella et al., 2015b), and between the thalamus and distributed cortical and subcortical areas in both hemispheres (Tona et al., 2014). Zhou et al. (2016), instead, found no correlation between thalamic connectivity and performance on the PASAT 3".

Reductions in functional connectivity were also reported in the progressive forms of MS, with slightly different patterns across phenotypes: in the medial prefrontal cortex and the precentral gyrus for SPMS; in the anterior cingulate cortex and the precentral gyrus in PPMS (Rocca et al., 2010b).

Finally, no correlations were observed between measures of structural and functional connectivity by studies that focused on the thalamus (Tona et al., 2014, Zhou et al., 2016). Mean and axial diffusivity in tracts connecting the anterior and posterior portions of the DMN were, however, found to be correlated with their functional connectivity (Zhou et al., 2014), and fractional anisotropy in the corpus callosum and the cingulum correlated with functional connectivity of the anterior DMN (Rocca et al., 2010b).

2.2.6.6. Conclusions

The published literature shows contrasting results on correlations between PS function and measures of functional and structural connectivity. DTI studies have highlighted mainly vague and variable findings. Indeed, when voxel-wise analyses

were carried out, multiple and widespread clusters of WM correlated with PS tasks (Dineen et al., 2009, Genova et al., 2013, Mazerolle et al., 2013, Riccitelli et al., 2017, Sbardella et al., 2013a, Van Hecke et al., 2010, Yu et al., 2012) and the same was observed in the investigation of more specific regions of interest across a range of tests (Bergsland et al., 2018, Bonzano et al., 2008, Kern et al., 2015, Koenig et al., 2014, Koenig et al., 2015, Roca et al., 2008). Lack of correlation with brain connectivity measures was noted more often for the PASAT (especially the 3" version) than the SDMT and various explanations may account for these differences. Firstly, the sensory modality used to present stimuli differs between the two tests: auditory for the PASAT and visual for the SDMT. The latter, in fact, has been reported to be more susceptible to impairment in MS than the former and may better evaluate PS deficits associated with this disease (Costa et al., 2017). Secondly, the way stimuli are presented during test performance differs across tests: for the PASAT stimuli are presented one at a time in sequence, while all the stimuli are presented simultaneously on the same page for the SDMT, thus increasing the demands posed on inhibition of processing of possible distractors. For the PASAT, it has also been suggested that patients may put in place different solving strategies when facing different versions of the PASAT. In fact, Snyder and Cappelleri (2001) reported that patients tend not to perform the task continuously but to skip every third item, thus reducing considerably the difficulty of the test and achieving a higher, though less reliable, score. Finally, it cannot be ignored that the two tests, although both used as PS measures, require the engagement of different cognitive domains: verbal auditory working memory for the PASAT and visual attention for the SDMT. These cognitive functions are long known to rely on activity of different brain areas of both hemispheres (Smith and Jonides, 1999), suggesting these tasks may assess different aspects of the PS function.

Indeed, partially different WM tracts were observed to be related to the tests reviewed. Performance on the PASAT was more associated with the level of microstructural integrity of the left cingulum, the superior longitudinal fasciculus (especially left-lateralised), and the inferior longitudinal fasciculus. In contrast, the SDMT seems to be more associated with DTI measures in bilateral tracts: the fornix, the cingulum and the posterior thalamic radiations. However, the corpus callosum emerged as the WM tract that most consistently correlated with PS performance of people with MS across cognitive tasks. This suggests the importance of multiple WM tracts to support cognitive PS performance across cognitive domains through fast integration of information processed in distributed brain networks.

Despite the variability of results, DTI indices seem to be more consistently correlated with different PS performance than measures of lesion load and parenchymal atrophy. This may result from the fact that microstructural WM damage can spread across fibre tracts (Chiang et al., 2016), and can precede the detection of new macrostructural lesions (Ontaneda et al., 2014). Hence, diffusion indices may be more sensitive in detecting subtle MS pathology leading to decline in PS function than conventional MRI. In fact, apart from commissural fibres (i.e. the corpus callosum) associative WM tracts appear to be more critically involved in PS performance, namely the superior and inferior longitudinal fasciculi and the cingulum. Nevertheless, given the variability in PS tasks used, differential WM involvement may have been detected according to the specific measures used.

In contrast, higher quality and more consistent results were observed in RS-fMRI studies. Functional neuroplasticity seems to be the underlying mechanism supporting cognitive changes, or stability, in the early phases of MS. In fact both people with clinically isolated syndrome and RRMS showed functional connectivity changes, both increases (Lin et al., 2018a, Meijer et al., 2018, Tona et al., 2014, Zhou et al., 2014, Zhou et al., 2016) and decreases (Janssen et al., 2013, Lin et al., 2018a, Sbardella et al., 2015b, Tona et al., 2014, Wojtowicz et al., 2014, Zhou et al., 2016), within various brain networks. Only one study found no changes in static and dynamic FC of the DMN (van Geest et al., 2018). Furthermore, PS performance correlated with functional connectivity alterations in frontal areas, such as the prefrontal and anterior cingulate cortices, and fronto-thalamic connections (Lin et al., 2018a, Pravatá et al., 2016, Rocca et al., 2010b, Sbardella et al., 2015b, Wojtowicz et al., 2014, Zhou et al., 2016). It is also worth noting that, in contrast to DTI studies, almost all those exploring functional connectivity used exclusively the PASAT to measure PS abilities.

Even though a relationship between macrostructural damage and resting-state functional changes in MS appears likely (Droby et al., 2015b), current findings are not consistent. In fact, while some studies observed correlations between total lesion volume and changes of resting-state activity (Zhou et al., 2016) others reported no correlation (Rocca et al., 2010b, Sbardella et al., 2015b). The same discrepancy has been observed about the association between structural and functional connectivity measures, where significant correlations ware found only in a small number of studies (Rocca et al., 2010b, Zhou et al., 2014). Indeed, the relationship between functional and structural brain changes may not be that straightforward in consideration of the fact that if structural connectivity between two areas predicts functional connectivity, the reverse is not necessarily the case, since

functional connectivity can also depend on indirect connections to and from other brain areas (Damoiseaux and Greicius, 2009).

Current knowledge of how MS-related damage to both structural and functional connectivity affects PS function appears incomplete and preliminary. This may be due to methodological shortcomings detected in the reviewed articles. Firstly, most studies, especially those on structural connectivity, were carried out on samples of mixed MS phenotypes. Such lack of differentiation may confound results, particularly since the neuropathology in progressive forms of MS is increasingly recognised to be mainly characterised more by neurodegenerative rather than inflammatory processes (Mahad et al., 2015). Secondly, to date many studies have been carried out using a more explorative approach, often without a clearly defined hypothesis to test, and have been based on a cross-sectional design that does not allow for the assessment of PS decline over time. Finally, a lack of theoretical background on PS decline in MS has emerged from the published literature. The majority of the studies focused mainly on the most common tests of PS that are intrinsically related to various cognitive domains (i.e. working memory for the PASAT and visuospatial attention for the SDMT), neglecting alternative strategies of investigation. These could include better characterisation of the neural correlates of PS deficits in MS considering sensory, cognitive, and motor contributions (Costa et al., 2017) or clarifying any possible influence of PS decline on other cognitive domains when assessing correlations with MRI measures (Genova et al., 2013).

PS performance has been repeatedly found to be impaired in people with MS and to correlate with both structural and functional brain reorganization, in particular degeneration of the corpus callosum (Bester et al., 2013, Bozzali et al., 2013, Genova et al., 2013, Lin et al., 2008, Mazerolle et al., 2013, Mesaros et al., 2009, Ozturk et al., 2010, Rimkus et al., 2011, Rocca et al., 2010b, Roosendaal et al., 2009a, Van Hecke et al., 2010) and altered activity in frontal areas (Pravatá et al., 2016, Rocca et al., 2010b, Sbardella et al., 2015b, Wojtowicz et al., 2014). Nevertheless, the dynamic properties and topography of neural breakdown in MS have yet to be clarified. Recent meta-analyses have been published with the aim of advancing our understanding of brain regions mostly affected by MS. Lansley et al. (2013) showed that GM appears to degenerate in the thalamus, a crucial hub for information distribution across the brain, the basal ganglia, precentral and postcentral gyri, and the cingulate cortex, involved in complex cognitive functions. Furthermore, Welton et al. (2015) highlighted how WM microstructural degeneration could be functionally related: physical disability was found to be mainly related to the posterior corpus callosum and right inferior fronto-occipital fasciculus, while

cognitive decline was mainly linked to the anterior part of the corpus callosum, the thalamus, and the fornix.

The published literature suggests that connectivity of the frontal cortices and between hemispheres is involved in PS function in MS. Interestingly, the *cognitive efficiency* theory (Vernon, 1983) postulates that activity of the prefrontal cortex plays a pivotal role in PS performance as do dynamic interactions with parietal cortices (Rao et al., 2014a, Rypma et al., 2006, Rypma and Prabhakaran, 2009). However, caution is needed when drawing conclusions based on current published evidence in light of limitations related to the lack of *a priori* hypotheses and theoretical definitions of PS function (Costa et al., 2017) and the diffuse practice of including people with mixed MS phenotypes.

In conclusion, whilst reviewed studies have shown significant promise for the use of resting-state functional MRI and DTI to explore the neural substrates underpinning of PS in MS, results to date have not been consistent and further clarifying investigations are necessary. Firstly, more detailed analysis of concepts related to PS function should be brought about in order to provide better theoretical frameworks to the neuroscientific investigation of this domain and its decline due to MS (Costa et al., 2017). Secondly, the differential associations between different measures of PS ability, which may potentially capture different cognitive aspects of this function, and their neural correlates need further characterisation. Thirdly, the use of a longitudinal design, that so far has been largely neglected, may be of help to clarify the interplay between neural and cognitive changes over time and potential maladaptive plasticity in MS. Indeed, Loitfelder et al. (2014) observed that higher activity in the left inferior parietal lobule at 1-year follow-up was negatively correlated with SDMT performance in RRMS. Finally, considering the higher scientific quality observed in studies combining different connectivity measures suggests that adopting multiple neuroimaging approaches may prove to be particularly helpful in tracking PS decline in MS. Combined use of different MRI techniques might allow a more comprehensive approach to mapping connectivity that may help unravel the complexity that characterises MS symptoms. Furthermore, the integration of multimodal MRI and targeted neuropsychological assessment in clinical trials, both pharmacological and non-pharmacological, may provide more detailed outcome measures than simple of enhancing lesions and scales of disease severity. Indeed, interventions targeting cognitive symptoms experienced by people with MS may exert beneficial effects difficult to detect by means of the currently most common outcome measures of neural and cognitive health. However, so far little use has

been done of MRI measures in such trials which almost completely neglected PS function in their design.

2.3. Cognitive interventions and PS function in MS

2.3.1. Cognitive effects

Apart from the pharmacological interventions mentioned in Chapter 1, attempts to tackle MS-related cognitive impairment have been mainly focussed on neuropsychological rehabilitation. However, in the design of such interventions little attention has been given to decline of PS abilities that have been specifically addressed only by a very limited number of studies, usually in combination with other cognitive domains. Three studies focussed particularly on working memory and PS rehabilitation and obtained variable results depending on the different PS measures used (Hancock et al., 2015, Hubacher et al., 2015, Vogt et al., 2009). Vogt et al. (2009) compared two versions of a cognitive rehabilitation programme (high vs low intensity) for people with MS, finding that both induced similar improvements, compared to a non-active group, on various WM and PS measures including also the PASAT and the SDMT. On the contrary, another study using the same programme in a much smaller sample was able to report a significant increase only in the score obtained on the SDMT (Hubacher et al., 2015). Inversely, Hancock et al. (2015) compared two groups of patients receiving alternative versions of the same treatment with different PS and WM difficulties and observed a significant group-by-time interaction only on the PASAT, but not on the SDMT and executive function measures.

In general, it appears that most cognitive interventions tested so far have adopted a multi-domain approach with the aim of rehabilitating impairments in different functions, among which PS. Consistent results regarding positive changes in PS performance, mainly measured with the PASAT, were detected across investigations (Bonavita et al., 2015, De Giglio et al., 2015, Filippi et al., 2012, Mattioli et al., 2010). However, some studies failed to induce significant post-treatment changes in PS function even though beneficial effects were exerted on working memory (Sastre-Garriga et al., 2010) and short term memory (Janssen et al., 2015, Mattioli et al., 2015). Moreover, irrespectively of the rehabilitative approach used all studies that investigated the impact of cognitive interventions on quality of life found no ameliorations in this kind of outcome measure (Mattioli et al.,

2010, Mattioli et al., 2015, Vogt et al., 2009) except for one study (De Giglio et al., 2015).

2.3.2. Neural effects

Only a very small number of studies included neuroimaging outcome measures to test the effects of their protocol. Only Filippi et al. (2012) investigated the impact of cognitive rehabilitation on both brain macrostructure and WM microstructure detecting no significant changes. At a functional level, instead, reorganization was observed to occur in different resting-state networks, namely the DMN (Bonavita et al., 2015, Filippi et al., 2012), and the salience and executive control networks (Filippi et al., 2012). Increases in functional connectivity appear to be induced particularly by non-pharmacological treatments and to correlate with cognitive performance on the PASAT (Filippi et al., 2012) and the Stroop test (Bonavita et al., 2015).

Some studies investigated changes in brain activation during task performance in relation to cognitive interventions. Hubacher et al. (2015) used the n-back task with four people with MS undergoing their rehabilitative programme: no modifications were detected for two participants, while the other two showed changes in brain activation of the fronto-parietal network in opposite directions, thus undermining conclusive interpretations of the study. Sastre-Garriga et al. (2010) recruited fifteen people with MS who underwent cognitive rehabilitation and after completion showed increased activation in two cerebellar clusters while performing the PASAT, compared to a group of five healthy people. Moreover, one study found that engaging in cognitive rehabilitation induced significantly higher Stroop-related activation in the posterior cingulate and the DLPFC in a group of people with MS compared to a non-active group (Filippi et al., 2012).

2.4. General conclusions

In light of the reviewed literature, it appears that lack of clarity about the neural correlates of PS ability in people with MS is still prominent despite its central role in the cognitive symptomatology of this disease. The high level of variability and the few consistent findings on the association between different PS and MRI measures pose strong limitations to drawing any definite conclusion. Moreover, the application of this, even though partial, knowledge to neuropsychological interventions to address cognitive impairment is currently confined to a few studies characterised by

small sample sizes, variable rehabilitative approaches mostly neglecting PS, and the use of different methodologies and outcome measures. As a consequence, the assessment and the comparison of these studies, especially regarding the role of PS in cognitive rehabilitation and possible related neural changes, is still a challenge.

Therefore, it appears necessary to generate hypotheses to characterise both the relationship between PS and indices of brain health (Van Schependom and Nagels, 2017), with particular attention to measures of structural connectivity, and to implement this knowledge in effective cognitive interventions for people with MS to track neuroplastic changes that may underlie cognitive improvements (Kincses and Vecsei, 2018). The present PhD constitutes an attempt to address some of these issues and the specific aims will be enunciated in Chapter 3.

Chapter 3 | Aims and objectives

As highlighted in Chapter 2 current knowledge about how brain degeneration characterising MS is associated with consequent cognitive decline is still only partially understood. The predominant investigation of morphological (e.g. normalised brain volume, callosal volume and area, width of the third ventricle) and global lesional measures indicating brain atrophy development have led to inconsistent results on their impact on PS performance. In fact, although these indices may be useful to track longitudinal neurodegenerative changes they are poorly informative on which neural features may explain the core cognitive deficits observed in people with MS.

Only recently, research has been shifting towards the use of alternative MRI sequences, namely DTI and fMRI, which can provide more detailed information about cognitive performance. Indeed, these MRI techniques have allowed the collection of voxel-based data on the integrity of structural WM connections and on functional alterations of brain activity. In turn, the correlation between cognitive and MRI variables enables researchers to show specific regional associations that may shed light on which brain areas, or network of areas, are more susceptible to insult by MS pathology and therefore clinically relevant. Moreover, such MRI outcome measures may provide a better understanding of the neural correlates of different MS-related symptoms so to inform trials of both pharmacological and non-pharmacological interventions. However, only limited conclusions can be drawn from the current corpus of studies that have investigated the association between PS decline in MS and measures of structural and functional connectivity as highlighted in the systematic review carried out and reported in Chapter 2.

The application of more sophisticated MRI techniques and analyses in this field of research has shed light on the neural consequences of MS pathology leading to the concept of MS as a “disconnection syndrome”. In fact, WM damage to structural connections causes alterations in coordinated activation of functionally related brain areas needed to perform cognitive computations. However, so far investigations on MS-related cognitive decline and on treatment strategies to manage it have exploited this available knowledge only to a limited extent. This is possibly one of the reasons why evidence of effectiveness for both pharmacological and non-pharmacological interventions targeting cognition is still poor.

Therefore, the primary aim of this project is to use different measure of brain connectivity to carry out hypothesis-led investigations about cognitive performance,

especially PS, in people with different MS phenotypes. Moreover, findings from these investigations and from the review of the literature (Chapter 2) will be used to guide the design and the analyses of an evidence-based cognitive intervention for people affected by RRMS. More specifically the objectives of this PhD project are:

1. To investigate the association between structural connectivity integrity and performance on tests of PS and PS-dependent cognitive functions in patients with RRMS.

   Since WM is deeply affected by MS pathology, it is reasonable to hypothesise that disruption of signal transmission between structurally connected brain areas may induce a decrease in the speed at which certain cognitive operations are performed. Moreover, previous studies have reported mixed and inconsistent findings on which WM tracts are more implicated in cognitive tasks of PS and rarely tested any experimental hypothesis. Therefore, a study was carried out on patients with RRMS combining neuropsychological data and DTI scans to ascertain whether associative WM tracts in particular are more strongly and differentially associated with various measures of PS-dependent cognitive performance. The findings of this study are reported in Chapter 4, Experiment 1.

2. To investigate the association between structural connectivity integrity and performance on tests of PS and PS-dependent cognitive functions in patients with SPMS.

   Most studies that tested the link between WM integrity and PS-dependent cognition included either only people with RRMS or a mixed patient sample. Thus, what set of WM tracts supports PS abilities after patients transition to SPMS (when neurodegeneration becomes the predominant pathological process) and whether it resembles that observed in the relapsing-remitting phase (mainly characterised by neuroinflammation) is still an open question. For this reason, a study was carried out on a sample of patients affected by SPMS to test, both at macrostructural and microstructural levels, whether integrity of WM associative tracts account for PS abilities. The findings of this study are reported in Chapter 4, Experiment 2.

3. To investigate the association between functional connectivity of several brain networks and performance on tests of PS and PS-dependent cognitive functions in patients with RRMS.

As for studies on structural connectivity, no definite conclusion can be drawn on what functional networks are more associated with PS abilities of people with RRMS. A few studies consistently observed functional connectivity of frontal areas to support this function. However, a large part of the literature appears more explorative with rare accounts of overt testable hypothesis. Therefore, the relationship between PS performance in a sample of patients with RRMS and some selected brain functional networks was investigated. The hypothesis is that functional connectivity of networks involved in cognitive (especially fronto-parietal) rather than sensory or motor functions (control) may be associated with PS abilities. The findings of this study are reported in Chapter 5, Experiment 3.

4. To investigate the association between functional connectivity of several brain networks and performance on tests of PS and PS-dependent cognitive functions in patients with SPMS.

   Little knowledge has been gained in the past years on the interplay between neural and cognitive changes in SPMS. Hence, how cognitive performance in this MS phenotype is related to functional connectivity of which brain regions remains still a largely unaddressed issue. For this reason a study was carried out on a sample of patients affected by SPMS to ascertain the association between PS function and selected brain networks by testing a set of hypotheses similar to those addressed for the RRMS group. The findings of this study are reported in Chapter 5, Experiment 4.

5. To test the effects of a network-based cognitive stimulation programme on cognition, quality of life, structural and functional connectivity measure in a sample of patients with RRMS.

   Despite the recent advancements in treatment discoveries for MS we are still lacking the definite cure which might stop this disease and its symptoms. On a similar note current strategies to manage cognitive impairment in patients with MS are characterised by weak outcomes and by lack of solid theoretical grounding. Thus, we tested the effects of a hypothesis-based non-pharmacological treatment to tackle cognitive impairments in a sample of patients with RRMS. Indeed, the cognitive stimulation programme used was designed with the aim of modulating the synchronous activation of brain areas across functional networks and, in turn, of promoting information integration. In particular, two alternative versions of the programme (standard

and PS-loaded) were tested in order to clarify whether stressing PS abilities of patients may represent a viable strategy to mitigate cognitive symptoms and boost functional and structural neural changes. The findings of this study are reported in Chapter 6, Experiment 5.

Chapter 4 | Structural neural correlates of PS function in people with MS

As seen in Chapter 2, a moderate number of investigations has been carried out into the associations between measures of PS function, mainly the PASAT, and WM structural integrity in MS. This situation appears mainly motivated by the fact that WM injury is an MS-specific neuropathological hallmark altough GM lesions are increasingly recognised as features of this disease. Indeed, slowing of cognitive abilities, together with executive problems, has been traditionally observed to be characteristic of pathologies mainly affecting WM (Cummings and Benson, 1984, Filley, 2010).

On the contrary, GM lesions may be driving more “cortical” and domain-specific cognitive deficits, depending on the location across the brain. Nevertheless, a contribution of cortical and subcortical GM damage to PS decline observed in MS cannot be definitely ruled out, especially in progressive phenotypes characterised by extensive neurodegenerative processes (Mahad et al., 2015).

As previously highlighted (Manca et al., 2018), the majority of the published studies in this field appears exploratory in nature and overtly stated hypotheses on how and where in the brain an association with PS is expected are rare. In general, no theorethical framework is adopted to clarify PS as a psychological construct and how its measurement may have an impact on the results of clinico-radiological correlations. Indeed, the cause of such variability observed in published results remains elusive and no proper explanation has been put forward regarding the possible roles of underlying differences in cognitive functions and sensory modalities required across tests.

Moreover, potential differences in the structural correlates of PS function/deficits between different MS phenotypes and, therefore, between different disease stages have been utterly neglected. As a matter of fact, altough cognitive impairment has been found more severe in progressive MS, this phenotype has been poorly investigated from a neurocognitive point of view.

4.1. Experiment 1 – Associations between measures of PS function and structural connectivity in people with RRMS

4.1.1. Introduction

At present, a considerable body of knowledge has been accumulated on the changes of PS function which has been mainly investigated in people presenting with the RRMS form of the disease. Findings from many studies seem to suggest a wider role of PS impairment in MS-related cognitive decline in support of the *relative consequence* model (DeLuca et al., 2004). Indeed PS deficits have been linked to worsening in performance across several domains: attention (Roth et al., 2015), working memory (Demaree et al., 1999, Leavitt et al., 2011) and different executive functions (Denney and Lynch, 2009, Leavitt et al., 2014, Macniven et al., 2008, Owens et al., 2013).

Recenelty Leavitt et al. (2018) discussed how cognitive impairment in MS may manifest in a heterogeneous way, since subgroups of patients show decline in memory without signs of PS deficits. Some limitations, however, may be highlighted in this study (Leavitt et al., 2018). First, the third trial of the Stroop test (colour-word mismatch), usually considered a measure of response inhibition, was selected as measure of PS function rather than the first two trials that capture reading and naming speed. Second, memory impairment was diagnosed if performance was found one standard deviation below normative values in at least one out of two tests (Selective Reminding Test and Brief Visuospatial Memory Test-Revised), while PS deficits had to be found in two out of two tests investigated (SDMT and Stroop test). Therefore, an imbalance in the detection of specific cognitive deficits may have been artificially introduced. Although only a very small proportion was observed affected in both PS and memory, the causal relationship between deficits in these two functions cannot be excluded. In fact, it has been shown that when a comprehensive neuropsychological assessment is performed to characterise the cognitive status of patients in detail, memory performance is predicted by PS function (Köhler et al., 2017). In particular, PS deficits may affect encoding of new information (Chiaravalloti et al., 2013) or the efficiency of working memory processing and, in turn, long term memory performance (Sandry et al., 2018).

Nonetheless, it cannot be denied the fact that isolated PS-independent cognitive deficits can be observed in some patients with MS, considering the random distribution of lesions across brain areas and tissues. With reference to this issue, particular interest has been sparked by pathological and imaging studies that

showed how GM is affected as well as WM (Klaver et al., 2013). The presence of significant cortical lesions, therefore, may contribute to explaining specific cognitive deficits such as memory decline (Calabrese et al., 2009). Indeed, the hippocampus has been found to be affected by demyelinating lesions (Dutta et al., 2011, Geurts et al., 2007) which may represent the cause of hippocampal atrophy (Koenig et al., 2014, Sicotte et al., 2008). Koenig et al. (2014) observed that volumetric reduction of the hippocampus in patients with MS was associated with decline in PS performance, although this neurodegenerative finding is highly likely to be involved more selectively in memory dysfunction (Sacco et al., 2015, Sicotte et al., 2008).

Several studies have also investigated the association between different aspects of WM pathology and cognitive decline in MS. In particular the attempt by Rossi et al. (2012) to clarify where in the brain WM lesions are more likely to produce cognitive impairments highlighted the forceps major, i.e. the WM tract connecting the occipital cortices through the splenium of the corpus callosum. Higher quantity of lesions in posterior WM regions was correlated with performance on the SDMT, probably due to the negative impact of brain damage on effective visual processing. Similarly, frontal and parietal lesions have been associated with deficits in sustained attention and working memory (Sperling et al., 2001). Moreover, longitudinal studies seem to support the notion that accumulation of WM lesion over time is associated with worse cognitive outcomes (Ouellette et al., 2018), especially if damage occurs in the left hemisphere (Preziosa et al., 2017).

However, some limitations related to current knowledge on the relationship between WM lesions and cognitive decline in MS must be noted. First, cognitive impairment has been usually treated as a unitary construct and specific investigations into the degree of deterioration across different cognitive functions has been largely neglected. Second, lesional WM tissue has been commonly quantified by means of a global measure, namely TLV. A recent review observed that this index and other measure of brain atrophy have been found to be associated with PS performance in people with MS, though findings differed considerably across cognitive measures of PS (Rao et al., 2014b). Therefore, despite being a useful outcome measure that enables quick estimations of relevant brain damage, TLV provides few or no clues about regional localisation of lesions and, in turn, about which WM bundles are actually damaged. As a consequence, common analysis of WM lesions in general appears to be only marginally informative on decline in specific cognitive domains.

Recently, many studies have used DTI methodology, which provides more sophisticated means than simple lesion analysis to investigate WM involvement in cognition. Indeed, in DTI scans each single voxel carries information about specific

physical properties related to diffusion of water molecules across brain tissues. For this reason, it is possible to investigate between-group differences or associations between diffusivity indices and clinical variables in both lesional and normal appearing WM jointly. The advantage of this type of analysis compared to the investigation of TLV is the possibility of localising brain areas in which variance in diffusivity significantly explains variance in cognitive abilities.

However, as highlighted in the review of the literature about the association between PS performance and structural connectivity in MS (Manca et al., 2018), the majority of DTI investigations carried out so far adopted an ROI approach. Once an ROI of a specific WM tract is identified and delineated on structural images, average diffusivity indices are extracted from it in order to examine correlations with cognitive performance. While this strategy may fit analysis based on *a priori* assumptions, it entails loss of information regarding other non-investigated brain areas. As a consequence, voxel-based analysis appears underrepresented in this field of research since just a few studies used this methodology (Dineen et al., 2009, Genova et al., 2013, Riccitelli et al., 2017, Sbardella et al., 2013a, Yu et al., 2012), in particular TBSS (Smith et al., 2006).

In one study the TMT-A and the Stroop test were used to assess PS function instead of the most common PASAT and SMDT (Genova et al., 2013). These PS measures were correlated with FA in consistent clusters of WM across several tracts comprising the corpus callosum, the corona radiata, posterior and (for the Stroop tests only) anterior thalamic radiations, the superior longitudinal fasciculus and (for the TMT-A only) the inferior fronto-occipital fasciculus.

Widespread patterns of correlations are commonly observed across studies, hinting PS may be a quite complex function with significant overlaps and/or relationships with other cognitive domains. As a matter of fact, PS performance captured by the PASAT (fundamentally a test of working memory but considered the gold standard for PS assessment in MS) emerged to be associated with WM microstructural integrity of several tracts. In particular, scores on the 3-second version of the PASAT correlated with FA in the corpus callosum, a quite consistent and commonly observed finding, the posterior thalamic radiations and the superior longitudinal fasciculus (Dineen et al., 2009, Riccitelli et al., 2017, Yu et al., 2012). Additional significant clusters were detected in the cingulum, inferior longitudinal fasciculus and arcuate fasciculus (Dineen et al., 2009), corona radiata and external capsule (Riccitelli et al., 2017) and sagittal stratum (Yu et al., 2012). This high variability in findings appears mainly due to the paucity of studies, considering that age and disease duration of patients' samples were comparable across studies. On the

contrary, the negative results found by Sbardella et al. (2013a) may be explained by the fact that significantly younger patients were recruited (mean age = 34 years, compared to 40-44 in the other studies) and they had been affected by MS for a shorter period of time (mean duration = 7.4 years, compared to about 10). Indeed, only performance on a more difficult version of the PASAT (2-second inter-stimulus interval) was significantly associated with structural integrity of several of the abovementioned WM tracts, namely in the corpus callosum, the internal and external capsule, the posterior thalamic radiations and the cerebral peduncles. In fact, increased PS load may have induced greater variance in behavioural data, thus allowing significant correlations with MRI measures to emerge (but scores obtained by patients in cognitive tests are not publicly available).

Similarly, the structural correlates of the SDMT were investigated by means of TBSS in two studies (Riccitelli et al., 2017, Yu et al., 2012). Highly convergent findings, partially overlapping with those of the PASAT, were observed in the corpus callosum, the corona radiata, the posterior thalamic radiations, the external capsule and the sagittal stratum. Nevertheless, other tracts were additionally found significantly associated with performance on this test. Therefore, the presented findings support the hypothesis that, independently of the measure used to assess PS abilities, structural damage due to MS resulting in disruption of this cognitive function is likely to occur in a network rather than being confined to a single WM tract.

Our current understanding of which structural network supports PS abilities in MS still remains very limited. Investigation of voxel-based structural connectivity measures may help elucidate whether the association with such network could be detected depending on PS load. Moreover, the use of and comparison between different tests of PS function appears as a fruitful strategy to highlight possible variability in the associated neural correlates. Indeed, PS measures may differ substantially in terms of sensory, cognitive or motor contributions to task execution: differences which remain largely unexplored (Costa et al., 2017).

4.1.2. Methods

4.1.2.1. Participants

A total of forty-five people affected by RRMS (Lublin and Reingold, 1996) who fulfilled the modified McDonald diagnostic criteria for RRMS (Polman et al., 2010) were selected among the forty-eight recruited for a cognitive stimulation intervention reported in Chapter 6. Two patients dropped out of the intervention study and they

were consequently excluded from the analyses. After quality check of the MRI scans, one subject was excluded from the present and the intervention experiments due to abnormally enlarged lateral ventricles evidenced on the T1-weighted scan. In fact, this finding poses challenges regarding the quality of MRI analysis, since significant cerebral morphological alterations may impinge the effectiveness of tissue segmentation.

Inclusion criteria were as follows: age between 25 and 65; EDSS ≤ 6; clinically stable disease for at least three months prior to recruitment; no changes in treatment for at least three months prior to recruitment; visual acuity in the normal range with visual aids (Davis et al., 2009); Mini Mental State Examination (MMSE) score ≥ 24; cognitive complaints; cognitive impairment observed as a score 2 standard deviations below normative values in at least one of the tests included in the neuropsychological battery. Exclusion criteria were as follows: history of major psychiatric disorders including abuse of alcohol or other substances; presence of other concomitant neurological diseases; severe visual impairment; contraindications to MRI.

Ethical approval was obtained from the Regional Ethics Committee of Yorkshire and Humber (Ref No: 12/YH/0474) (Appendix D). All participants were provided with written information material (Appendix E) at least one week before recruitment and gave written consent (Appendix F) to take part in this study.

4.1.2.2. Neuropsychological assessment

A subset of neuropsychological tests from the comprehensive battery administered to test cognitive status of patients taking part in the cognitive stimulation study (Chapter 6) was selected for this experiment. In particular, six tests with prominent PS involvement were identified and scores obtained in the baseline assessment, before randomisation, were used:

- The PASAT (Gronwall, 1977): the procedure described in Chapter 2 (section 2.2.1) was followed. The test was delivered by means of Inquisit version 4.0.3 and during task performance subjects wore a set of headphones to prevent distractions. Firstly, a screen with instructions and examples of the test was displayed, followed by a practice block with ten items. Feedback was provided in order to highlight right and wrong answers. After practice, instructions were verbally repeated to resolve any doubts and two consecutive experimental blocks of sixty-one random digits each were administered at two paces of presentation (3s and 2s) without any feedback provided. During digit

presentation a button with numbers from 2 to 18 were constantly displayed on the screen and patients were asked to use the mouse to click on the number they thought represented the right answer to each trial.

- The DSCT (Wechsler, 2008): the test was delivered using the procedure described in Chapter 2 (section 2.2.1).
- The Stroop test (Stroop, 1935): the short version elaborated by Venneri et al. (1993) reported in Chapter 2 (section 2.2.1) was used. As a measure of PS function (Stroop speed) we calculated the average of completion time in the first two trials (word reading and colour naming).
- The TMT part A and B (Armitage, 1946): the standard version already explained in Chapter 2 (section 2.2.1) was used.
- Phonemic and semantic fluency tests (Lezak, 2004): following the procedure reported in Chapter 2 (section 2.2.1) three letters (F, L, and P) for the former test, and three categories (animals, cities and fruits) for the latter were given to participants in random order.

Participants were asked to self-assess fatigue and depressive symptoms using the MFIS (Fisk et al., 1994a) and the 9-item Patient Health Questionnaire (PHQ-9) (Spitzer et al., 1999) , respectively.

4.1.2.3. MRI acquisition

The outcome measures of the cognitive stimulation study comprised a wide set of MRI scans, acquired at 3T (Ingenia, Philips Healthcare, Best, NL) utilizing a 32-channel radiofrequency head coil. However, only a subgroup of three sequences (baseline acquisition) has been considered in the present study in order to focus on the investigation of the structural neural correlates of PS performance in RRMS:

- Sagittal 3D T1-weighted magnetization prepared rapid acquisition gradient-echo (repetition time = 8.1 ms, echo time = 3.7 ms, slices = 170, slice thickness = 0.94 mm, matrix size = 240 x 222, field of view = 240 x 240 mm^2);
- Sagittal 3D T2-weighted fluid attenuated inversion recovery (FLAIR) (repetition time = 4800 ms, echo time = 289 ms, slices = 326, slice thickness = 1.12 mm, matrix size = 224 x 224, field of view = 250 x 250 mm^2);
- Axial diffusion-weighted echo planar images (repetition time = 3000 ms, echo time = 98 ms, diffusion-encoding gradients b = 0 and 1000 s/mm^2, directions = 32, slices = 48, slice thickness = 2.5 mm, matrix size = 96 x 94, field of view = 240 x 240 mm^2).

4.1.2.4. MRI preprocessing

Macrostructural structural analyses were carried out by means of the Statistical Parametric Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK, http//www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2008a, version 7.6.0 (The Mathworks, Natick, Massachusetts, USA). Firstly, T1-weighted and FLAIR scans were reoriented to the Anterior Commissure-Posterior Commissure line to optimize subsequent preprocessing steps. Secondly, WM lesions were automatically segmented on the reoriented T1-weighted and FLAIR images using the lesion growth algorithm of the Lesion Segmentation Toolbox v1.2.3 (www.statisticalmodelling.de/lst.html). This toolbox was developed for SPM8 and validated on a sample of 53 people with MS (Schmidt et al., 2011). TLV, i.e. the amount of lesional WM, was quantified in millilitres.

Lesion probability maps generated for each subject by the Lesion Segmentation Toolbox were subsequently binarised and normalised to the standard ICBM template by using SPM8. Finally, individual maps were averaged by using the SPM8 toolbox ImCalc in order to obtain a group-specific lesion probability map to localise the strongest lesion concentrations (Riccitelli et al., 2012).

The T1-weighted images were segmented in their native space into three separate tissue classes: GM, WM and CSF. The volume in millilitres of all the segmented images was extracted by means of the MATLAB function “get_totals” and the TIV of each participant was hence calculated as the sum of GM, WM and CSF volumes.

DTI scans were preprocessed and analysed by using FMRIB Software Library v5.0.8 (FSL, http://www.fmrib.ox.ac.uk/fsl). Initially, artefacts commonly encountered in MRI practice, namely eddy currents and head motion, were corrected using the FSL Diffusion Toolbox. The Brain Extraction Tool subsequently allowed the elimination of the non-brain voxels from the corrected images by applying a fractional intensity threshold of 0.5 to DTI images in order to delineate the brain outline. Therefore, a binary brain mask was generated and used to fit the diffusion tensor model at each voxel to obtain maps of FA for each participant.

Hence, the obtained FA images were analysed adopting the widely used tract-based spatial statistics (TBSS) approach in order to overcome alignment and smoothing issues typical of voxel-based morphometry analyses applied to DTI (Smith et al., 2006). A four-step TBSS preprocessing procedure was followed (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS/UserGuide). Firstly, FA images were eroded in order to remove possible outliers derived from the previous diffusion tensor fitting phase. Secondly, all FA images were registered to each one of the others to identify the most representative subject of the sample, i.e. the subject that would require

minimum amount of warping for all the other subjects to be registered to it. Thirdly, each image was non-linearly registered to the sample-specific template, affine-aligned into the MNI standard space (Collins et al., 1994), and averaged. Moreover, during this step the mean FA image was skeletonised, i.e. eroded so that only voxels in the core of WM tracts were maintained for statistical analysis, and individual images projected onto it. Finally, a threshold of 0.2, found to be the optimal value by Smith et al. (2006), was applied to the mean FA skeleton image in order to create the binary skeleton mask, used to prevent any GM and CSF voxels from being accidentally included in the subsequent statistical analysis.

4.1.2.5. Statistical analysis

All the statistical analyses on cognitive and clinical data were carried out using IBM SPSS Statistics Version 21 (IBM, Chicago, IL, USA). Correlations (Spearman's ρ, two-tailed $\alpha = .05$) between cognitive tests were investigated to observe whether measures of PS would be associated, and to what extent, with one another. The same procedure was followed for demographic and clinical variables to ascertain possible relationships between confounding variables.

In consideration of the literature on this issue, a multiple regression model was created to investigate the impact of TLV (global WM damage) on cognitive performance controlling for the effects of other covariates, namely age, education and TIV. Indeed, it was necessary to rule out the effect of ageing processes on cognition that may be independent of any MS pathological changes (Bodling et al., 2009). Similarly, education and TIV were included in the analyses as measures of cognitive reserve and head size (Stern, 2009).

Multiple regression analysis was used to assess the relationship between PS-related performance and FA throughout the WM skeleton controlling for the abovementioned covariates. The *randomise* FSL tool was used to perform non-parametric TBSS analysis and 5000 permutations were carried out for each model in search of both negative and positive associations (Smith et al., 2006). Significant results were reported by using threshold-free cluster enhanced (TFCE) images (Smith and Nichols, 2009). Raw result images were masked with significant ($p < 0.05$) voxels from TFCE images and Montreal Neurological Institute (MNI) coordinates of peaks were extracted from the resulting images. Finally, WM tract labels were identified using the JHU ICBM-DTI-81 White-Matter Labels atlas (Mori et al., 2008).

A primary prediction relates to the fact that FA would correlate positively with cognitive performance: the higher FA the better PS abilities. However, DTI analyses were used to investigate the following more specific questions:

1) *Does PS-load influence the pattern of associations detected between WM microstructural integrity (FA) and scores on the PASAT?*

Two versions of the PASAT (2 sec and 3 sec) were compared in relation to the association with FA values throughout the WM skeleton. The prediction was that the higher the PS load the more extensive the correlation with WM integrity should be.

2) *Do alternative cognitive measures of PS, i.e. the PASAT and the DSCT, correlate with WM microstructural integrity of different tracts?*

WM neural correlates of the PASAT and the DSCT were compared expecting, as already observed in the literature, to find more significant correlations with FA levels for the DSCT.

3) *Is the association between WM microstructural integrity and performance on PS-demanding tests of higher order cognitive functions (fluency tasks, TMT B-A and Stroop inhibition) modulated by the status of PS abilities?*

The same procedure was followed for the abovementioned tests with the aim of controlling for PS abilities and expecting to cause a reduction in the strength of correlations with WM integrity levels.

4.1.3. Results

4.1.3.1. Clinical and cognitive results

All the clinical and cognitive characteristics of the patients and the number of patients impaired in each single neuropsychological test are reported in Table 4.1. From the inspection into clinical characteristics a highly significant positive association emerged between disease duration and TLV: as expectable the longer the disease history, the higher is the amount of WM damage accumulated. Age correlated positively with both variables, thus stressing the influence of a time variable on TLV generation. Instead, severity of fatigue and depressive symptoms were correlated to one another, but not to other demographic or neural characteristics (Table 4.2).

Table 4.1 Clinical and cognitive characteristics of the patient sample (n = 45)

Variable	Mean	SD	Median	Minimum	Maximum	Patients with deficits
Clinical characteristics						
Age (years)	45.2	9.0	45.5	26	65	-
Education (years)	14.4	3.1	13.5	11	25	-
Duration (years)	9.4	7.1	7	1	30	-
EDSS	3.4	1.5	3.5	0	6	-
TIV (ml)	1498.2	194.6	1486.0	1361.2	2070.9	-
TLV (ml)	10.5	13.2	6.0	0	47.9	-
MFIS	48.4	15.7	51.0	11	79	-
PHQ-9	9.17	4.5	9.0	0	23	-
Cognitive tests						
PASAT 3”	37.7	16.6	40	9	59	13
PASAT 2”	22.8	13.9	20	4	55	17
DSCT	62.4	15.6	59	36	96	14
TMT-A (sec)	39.5	15.5	37.5	21	86	6
Stroop speed (sec)	17.6	3.5	17.5	10	27.5	5
TMT B-A (sec)	42.7	24.2	36.5	10	98	16
Stroop inhibition (sec)	16.7	8.3	14.5	4.5	39.5	5
Phonemic fluency	32.8	9.9	31	17	58	1
Semantic fluency	45.5	10.0	43.5	21	67	1

DSCT: Digit Symbol Coding Test, EDSS: Expanded Disability Status Scale, MFIS: Modified Fatigue Impact Scale, PASAT: Paced Auditory Serial Addition Test, PHQ-9: 9-item Patient Health Questionnaire, TIV: Total intracranial volume, TLV: Total lesion volume, TMT: Trail Making Test

Table 4.2 Correlations between clinical variables

	Age	Education	Duration	EDSS	TIV	TLV	MFIS	PHQ-9
Age	-							
Education	-.045	-						
Duration	.357*	-.128	-					
EDSS	-.077	-.349*	.317*					
TIV	.006	.051	-.117	-				
TLV	.311*	.028	**.503†**	.184	-.003	-		
MFIS	.096	.094	.185	.179	-.022	.155	-	
PHQ-9	-.144	.034	.107	.247	.025	.078	**.713†**	-

* $p < .05$ (uncorrected for multiple comparisons)
† $p < .008$ (.05/6 = .008, Bonferroni correction)

EDSS: Expanded Disability Status Scale, MFIS: Modified Fatigue Impact Scale, PHQ-9: 9-item Patient Health Questionnaire, TIV: Total intracranial volume, TLV: Total lesion volume

Instead, correlational analysis on the neuropsychological tests showed how both common PS measures (PASAT and DSCT) are highly correlated to one another and with the other PS-dependent tests despite differences in the underlying cognitive domains they assess (Table 4.3).

Table 4.3 Correlations between cognitive variables

	PASAT 3"	PASAT 2"	DSCT	TMT-A	Stroop speed	TMT B-A	Stroop inhibition	PF	SF
PASAT 3"	-								
PASAT 2"	**.833**†	-							
DSCT	**.465**†	**.469**†	-						
TMT-A	-.365*	-.348	**-.520**†	-					
Stroop speed	-.183	-.158	-.255	.368*	-				
TMT B-A	**-.432**†	-.264	-.368*	.153	.196	-			
Stroop inhibition	**-.481**†	-.379*	**-.478**†	.407*	.281	.388*	-		
PF	.381*	**.481**†	.364*	-.284	-.293	-.247	-.224	-	
SF	**.444**†	**.426**†	.406*	-.122	-.113	-.064	**-.496**†	**.437**†	-

* $p < .05$ (uncorrected for multiple comparisons)
† $p < .005$ (.05/9 = .005, Bonferroni correction)
DSCT: Digit Symbol Coding Test, PASAT: Paced Auditory Serial Addition Test, PF: Phonemic fluency, SF: Semantic fluency, TMT: Trail Making Test

4.1.3.2. TLV results

The WM lesion map is shown in Figure 4.1, with the highest lesion probability detected in the corona radiata.

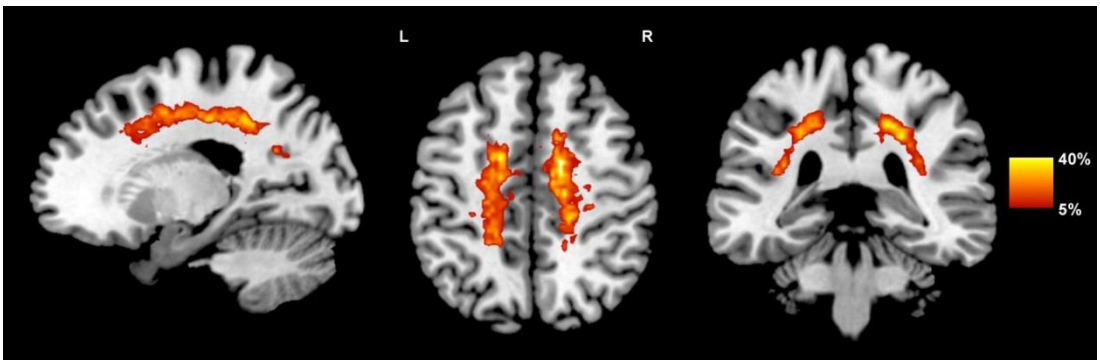

Figure 4.1 Group-level lesion probability map

Multiple regression assumptions were tested to ensure they were fully met: A) linear relationship and B) homoscedasticity by plotting the residuals against the predicted values; C) normality of the residuals by visually inspecting their histogram and P-P plots; D) independence of residuals by means of the Durbin-Watson test (values between 1 and 3); E) multicollinearity by ensuring values of the variance inflation factor and of the tolerance statistics were, respectively, below 5 and above 0.2.

Subsequently, multiple regression analysis highlighted significant models only for the DSCT ($F(4, 37) = 3.7$, $p = .012$) with an R^2 of .29 and the semantic fluency task

($F(4, 37) = 4.7$, $p = .004$) with an R^2 of .34. However, only the DSCT test was significantly predicted by the TLV (B = -.41, p = .028) but not by all the other covariates, meaning that every millilitre of TLV accumulated corresponds to a loss of .41 total score on the DSCT. On the contrary, semantic fluency scores were significantly predicted by education (B = 1.15, p = .011) and TIV (B = .015, p = 0.37). Therefore, particularly significant appears the influence of education on semantic fluency performance, since a one-year increment in education levels corresponds to an increase of 1.15 in the amount of items generated on the semantic fluency task.

4.1.3.3. DTI results

The analyses carried out to answer the first research question (comparison between DTI correlates of PASAT 3" and of PASAT 2") revealed no significant correlations between either PASAT versions and WM microstructural integrity. However, the DSCT correlated with FA in several cluster mainly in the body of the corpus callosum, the left inferior fronto-occipital fasciculus and bilateral anterior thalamic radiations (Figure 4.2 and Table 4.4). With reference to the second research question, these findings appeared to suggest that different measures of PS abilities may show differential associations with structural connectivity. Yet they provide no clarification on the reason why these differences may occur. In fact, the PASAT and the DSCT differ in several ways, the cognitive functions engaged during task performance (verbal working memory vs visuospatial attention), the sensory modality of stimulus presentation (auditory vs visual). Moreover, while stimuli are presented in series during the PASAT, all the DSCT stimuli are simultaneously presented on a paper sheet, hence becoming sources of distraction.

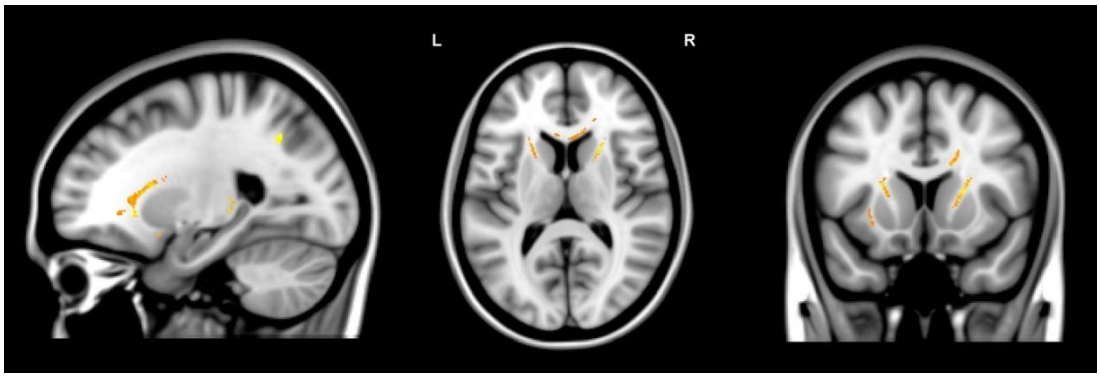

Figure 4.2 Clusters of WM in which FA positively correlated with DSCT scores (p < .05)

Table 4.4 Positive correlations between the DSCT and FA ($p < .05$)

Cluster extent	r	Side	White matter tract	*t* value	MNI coordinates		
					x	*y*	*z*
520	.657	L	Inferior fronto-occipital fasciculus	4.32	-23	22	-3
		L	Inferior fronto-occipital fasciculus	4.23	-28	9	-12
		L	Inferior fronto-occipital fasciculus	3.95	-22	25	-4
		L	Anterior thalamic radiations	3.81	-22	14	13
		L	Inferior fronto-occipital fasciculus	3.47	-24	21	-1
		L	Anterior thalamic radiations	3.43	-23	19	9
439	.543	R	Forceps minor	3.28	4	24	5
		R	Forceps minor	3.22	12	20	18
		R	Forceps minor	3.04	16	25	17
		R	Forceps minor	2.98	9	24	10
		R	Forceps minor	2.94	14	24	15
		R	Forceps minor	2.89	13	22	17
265	.659	R	Anterior thalamic radiations	4.41	19	11	9
		R	Anterior thalamic radiations	4.09	22	12	10
		R	Anterior thalamic radiations	4.03	22	15	10
		R	Anterior thalamic radiations	3.98	23	17	10
		R	Anterior thalamic radiations	3.76	17	6	8
		R	Anterior thalamic radiations	3.52	16	8	8
151	.579	L	Anterior thalamic radiations	3.74	-24	-31	-1
		L	Anterior thalamic radiations	3.74	-19	-29	6
		L	Anterior thalamic radiations	3.70	-19	-31	5
		L	Inferior fronto-occipital fasciculus	3.60	-31	-30	5
		L	Anterior thalamic radiations	3.56	-18	-32	3
		L	Anterior thalamic radiations	3.45	-23	-31	5
147	.592	L	Forceps minor	3.75	-14	34	-6
		L	Forceps minor	3.59	-16	37	-5
		L	Forceps minor	3.27	-12	31	-5
		L	Forceps minor	3.06	-12	31	-1
		L	Forceps minor	3.00	-15	35	-2
		L	Forceps minor	2.80	-13	33	-1
85	.557	R	Body of corpus callosum	3.10	15	13	26
		R	Body of corpus callosum	3.08	18	13	31
		R	Body of corpus callosum	2.92	18	14	29
		R	Body of corpus callosum	2.84	13	10	26

For this reason two other PS measures both involving stimuli visually presented on a paper sheet to be processed either verbally (Stroop speed) or visuospatially (TMT-A) were investigated in association with FA. Considering that the two tests share the same sensory (visual) and executive (distractor suppression) demands, it was hypothesised that possible differences in neural correlates may be due to the type of cognitive processing needed (verbal vs visuospatial). Only performance on the TMT-A was correlated with FA in clusters of WM in the same tracts observed for the DSCT, though significant clusters were especially right-lateralised (Figure 4.3 and Table 4.5).

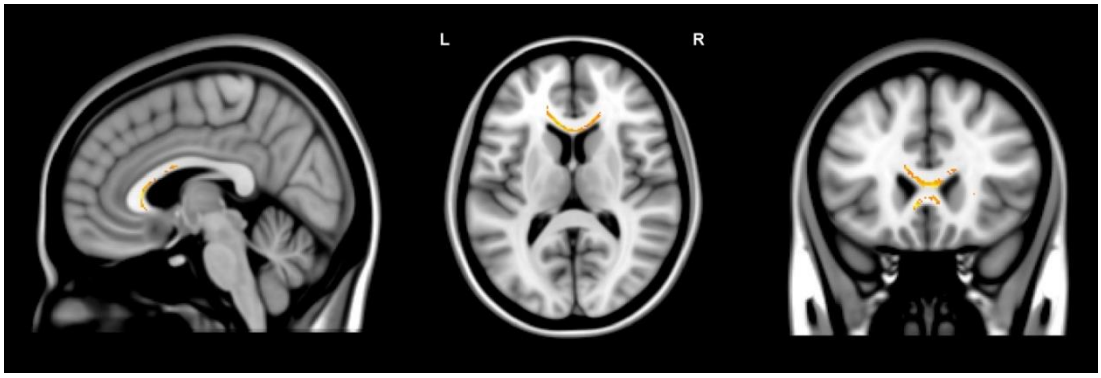

Figure 4.3 Clusters of WM in which FA negatively correlated with TMT-A scores (p < .05)

Table 4.5 Negative correlation between the TMT-A and FA (p < .05)

Cluster extent	r	Side	White matter tract	*t* value	MNI coordinates		
					x	*y*	*z*
1360	-.573	R	Forceps minor	5.26	5	24	7
		R	Forceps minor	5.02	5	25	5
		L	Forceps minor	3.97	-9	26	9
		R	Forceps minor	3.94	1	21	-1
		L	Forceps minor	3.91	-6	23	-3
		R	Forceps minor	3.85	9	27	3
142	-.561	R	Inferior fronto-occipital fasciculus	4.02	26	35	-1
		R	Inferior fronto-occipital fasciculus	3.79	23	33	-4
		R	Inferior fronto-occipital fasciculus	3.68	25	27	0
		R	Inferior fronto-occipital fasciculus	2.79	30	35	3
54	-.570	R	Anterior thalamic radiations	3.71	21	19	2
		R	Anterior thalamic radiations	3.57	20	16	3
		R	Anterior thalamic radiations	3.36	17	13	3
		R	Anterior thalamic radiations	3.18	19	14	2

Finally, analyses carried out on the more complex cognitive tests characterised by PS demands in order to answer the third research question failed to show any significant association with indices of WM microstructural integrity.

4.1.4. Discussion

In this study no significant correlations were observed between FA values and any of the PASAT versions investigated, independently of PS load. This finding was against the expectation that increasing task difficulty by reducing the time to manipulate verbal material would highlight, in DTI analysis, those WM tracts crucial to support performance on the PASAT as found by Sbardella et al. (2013a). However, in Chapter 2 it was already noted that not all the studies that have investigated this test found it to be reliably correlated with measures of WM microstructural integrity (Bester et al., 2013, Koenig et al., 2014, Koenig et al., 2015, Llufriu et al., 2012, Warlop et al., 2009). Consistently, Rao et al. (2014b) found that

PASAT performance of people with MS correlated significantly with measures of atrophy and TLV only in about 50% of the studies reviewed.

On the contrary, DSCT scores correlated with FA values in several frontal WM tracts such as the forceps minor and the anterior thalamic radiations, whose integrity has been previously associated with deficits in PS abilities in clinical populations showing WM damage due to cerebrovascular problems (Duering et al., 2013, Duering et al., 2014) and to colour naming (Stroop test) speed abilities of bilingual adults (Mamiya et al., 2018). These findings appear complementary to those of Bisecco et al. (2017) who observed how attention and PS functions are affected by MS-related thalamic atrophy. Indeed, electrical stimulation of the mediodorsal nucleus of the thalamus was shown to impact visual attention/working memory processes (Peräkylä et al., 2017).

The analysis highlighted the involvement of the inferior fronto-occipital fasciculus, a long associative tract connecting the frontal to occipital, and to some extent also parietal, cortices (Hau et al., 2016, Wu et al., 2016). Its functional relevance appears complex and not yet fully elucidated since several studies have shown this WM tract may support fast naming (Rollans et al., 2017), sensorimotor integration (Sarubbo et al., 2013), attentional (Herbet et al., 2017b) and even semantic processes (Almairac et al., 2014, Herbet et al., 2017a).

Consistently, scores obtained by patients on the DSCT were the only ones significantly predicted by the total amount of WM damage. Hence, signalling an association between cognitive functions involved in performance of the DSCT and WM structural integrity possibly due to the need for integration of information across different brain areas to execute this task effectively (He et al., 2009).

This point may help with the interpretation of the difference observed in clinical-radiological correlations between the PASAT and the DSCT. In fact, DSCT performance may depend on interhemispheric processing of information to a greater extent than PASAT performance does. As a consequence, DSCT-related cognitive processes may exhibit higher probability of being affected by insults to structural connections and accordingly explain the sensitivity of this test to cognitive impairment in people with MS (Parmenter et al., 2007b).

In fact, the visual stimuli used in the DSCT are minute letters and symbols processed visually through the left fusiform gyrus (Starrfelt and Gerlach, 2007, Vogel et al., 2014). However, visual attentional processes, commonly found impaired in MS (Gmeindl and Courtney, 2012) and crucially managed by the right fronto-parietal network (Capotosto et al., 2011, Coull et al., 1996), are needed to execute this task successfully. This hypothesis appears consistent with findings that

performance on the SDMT, an alternative version of the DSCT, is associated with more bilateral brain activation than the PASAT (Forn et al., 2011).

On the contrary, the PASAT, being a test of verbal working memory (Paulesu et al., 1993), has been proven to rely mainly on left-lateralised brain activation (Audoin et al., 2005, Cardinal et al., 2008) in line with the well-established specialisation of the left hemisphere for processing of verbal stimuli (Hervé et al., 2013). Greater bilateral frontal activation is only observed when working memory load, and in turn task difficulty, is significantly increased (Rypma et al., 1999).

However, although no major differences in motor execution can be highlighted between the abovementioned tests (at least in the procedures implemented for this study), significantly higher levels of distractibility characterise the DSCT rather than the PASAT. In fact, the simultaneous presentation of all the visual stimuli on the same paper sheet poses higher demands on attentional control (Lustig et al., 2006) that can influence performance on complex measures of PS (LaPointe et al., 2005, Randolph et al., 2017). Furthermore, it must be noted that experimental stimuli are presented through two different sensory modalities: auditory for the PASAT and visual for the DSCT; the latter being usually the most affected by MS pathology (Iragui et al., 1986) even in attentional tasks (McCarthy et al., 2005).

For these reasons, further analyses were performed on two time-based PS measures with the aim of minimizing discrepancies between sensory modalities involved: the TMT-A and the Stroop speed index, both requiring processing of visual stimuli presented simultaneously and, therefore, characterised by similar levels of distractibility. Although the motor responses required by the two tests were dissimilar (hand for the TMT vs mouth for the Stroop), the main and crucial difference is thought to be represented by the underlying cognitive processing: visuo-spatial and verbal respectively. As a result, the detection of the same WM tracts observed to be associated with the DSCT was replicated also in the analysis of the TMT-A only, though mainly right-lateralised. Thus, suggesting that in the RRMS phase, visuo-spatial PS function appears more extensively dependent on integrity of structural connectivity than its verbal counterpart.

Finally, to assess the impact of PS decline on higher order cognitive functions, investigations were made into different tests with PS demands. No significant associations with FA for any of the tests, assessing both executive and linguistic functions, were observed in contrast with the findings by Genova et al. (2013). A possible explanation for these negative results may be found in the observation that the patients recruited for this experiment had generally preserved cognitive status, characterised by only mild impairments. Indeed, patients had been in stable

remission for at least three months before recruitment and previous studies highlighted how cognition is usually, and expectedly, more preserved in such phases rather than while patients are experiencing a relapse (Giedraitiene et al., 2018). Reduced variance both in cognitive performance and diffusivity levels due to disease stability might not allow the detection of significant cognitive-radiological associations. Currently, only a handful of investigations have compared cognitive functioning of people with RRMS while in relapse vs remission phases and mainly limited cognitive assessment to the SDMT (Benedict et al., 2014, Morrow et al., 2011, Pardini et al., 2014). Moreover, none of these studies investigated the neural markers associated with cognitive changes across disease stages, apart from longitudinal evaluation of enhancing lesions (Pardini et al., 2014). It follows that the proposed hypothesis cannot be more than speculative and would need support by further longitudinal research with aims, however, different from those of this PhD work.

In conclusion, from the results of this study it emerges that microstructural damage in frontal WM associative tracts may be a marker of PS impairment in RRMS. This appears particularly the case when PS function is assessed by means of measures requiring integrated processing of information and, in turn, recruitment of widespread functional networks.

4.2. Experiment 2 – Associations between measures of PS function and structural connectivity in people with SPMS

4.2.1. Introduction

Research on the cognitive manifestations of MS has generally attributed little attention specifically to those people presenting with SPMS (D'Amico et al., 2016). This situation has probably been caused by the fact that this phenotype is considered the natural evolution of RRMS (Weinshenker et al., 1989). Moreover, although in general transition to the secondary progressive phase is considered to occur when steady functional deterioration is observed gradually over a period of at least 6 months in the absence of any relapse (Lublin and Reingold, 1996), diagnostic uncertainty of transition remains high (Katz Sand et al., 2014). As a consequence, most investigations have not distinguished between RRMS and SPMS when comparing cognitive performance of patients and healthy controls. Additionally, only a few studies have investigated whether differential degrees of impairment exist across MS phenotypes (D'Amico et al., 2016). Nevertheless, it appears well-established that cognitive deterioration is more severe in SPMS than RRMS, globally and particularly in PS abilities (Archibald and Fisk, 2000, De Sonneville et al., 2002, Huijbregts et al., 2004, Papathanasiou et al., 2014). De Sonneville et al. (2002) noticed complex PS function is particularly affected by disease severity that is higher in progressive phenotypes, though the effect size of this difference has been estimated to be small (Papathanasiou et al., 2014).

Understandably, the amount of knowledge currently accumulated on the neural correlates of cognitive deficits in SPMS is even scarcer than that available for the RRMS phenotype and non-specific. Camp et al. (1999) used a global index to assess overall cognitive decline in patients with both primary and transitional progressive MS and observed that it correlated with TLV and cerebral volume. Moreover, the corpus callosum area calculated on a mid-sagittal T1-weighted image was associated with cognitive performance of patients with SPMS, both on tests of memory and processing speed (Papathanasiou et al., 2017). Instead, Riccitelli et al. (2011) highlighted by means of VBM analysis that cognitively impaired patients have more atrophy in fronto-temporal areas, the hippocampus and the thalamus than those with preserved cognition. Finally, only a couple of studies examined microstructural differences associated with cognitive decline in patients with SPMS by means of TBSS analysis on DTI data, yielding quite inconsistent results due to paucity of data (Francis et al., 2014, Meijer et al., 2016). In fact, Francis et al. (2014)

found that overall cognitive deficits were associated with lower FA in the left posterior thalamic radiations, corpus callosum and right sagittal stratum, while Meijer et al. (2016) observed reduced WM microstructural integrity in the fornix, the superior longitudinal fasciculus and the forceps major.

From this brief review, a clear picture emerges that shows how structural neural damage associated with PS deficits in SPMS have been completely neglected. Some DTI studies included a few patients with this phenotype in their analysis, but only as part of mixed groups in which people affected by RRMS were numerically predominant (Benedict et al., 2013, Genova et al., 2013, Koenig et al., 2014, Koenig et al., 2015, Ozturk et al., 2010, Roosendaal et al., 2009a). Therefore, currently no substantial information is available to hypothesise whether PS abilities in the SPMS stage are supported by the same WM tracts observed for patients with RRMS. Indeed, although the border between these two MS phenotypes may be blurred during the transitional phase, it has been shown that inflammatory activity is significantly reduced in SPMS while different neurodegenerative processes become predominant (Calabrese et al., 2015, Mahad et al., 2015), driving volume loss significantly more severe than in RRMS (Fisher et al., 2008, Grothe et al., 2016, Mallik et al., 2015, Sampat et al., 2009) and associated with worsening of global disability (Ge et al., 2000).

Therefore, in light of the abovementioned differences observed between patients with RRMS and SPMS both in cognitive and brain changes, it seems advisable to study how these two elements are interrelated also in SPMS. In particular, alongside investigating the association between cognitive performance and WM microstructure as in Experiment 1, VBM will be used to ascertain whether PS abilities are associated with macrostructural indices (i.e. regional brain volumes).

4.2.2. Methods

4.2.2.1. Participants

Thirty-one patients affected by SPMS according to criteria by Lublin and Reingold (1996) who have been relapse-free for at least 3 months were consecutively recruited at the MS clinic of the IRCCS Fondazione Ospedale San Camillo (Venice, Italy). Patients who reported cognitive complaints had to show preserved global cognitive status, screened by means of the Raven's Coloured Progressive Matrices (Basso et al., 1987), and the absence of neurological or psychiatric comorbidities.

This study was carried out according to the Declaration of Helsinki and was approved by the Institutional Review Board of the IRCCS Fondazione Ospedale San

Camillo (Venice, Italy) (Protocol N. 11/09 version 2) (Appendix G). Written informed consent was obtained from each study participant.

4.2.2.2. Neuropsychological assessment

Cognitive performance of patients was assessed by means of a selected neuropsychological battery. Some tests were used in Experiment 1, namely the Stroop test, the TMT, phonemic and semantic fluency tasks, with the addition of:

- Raven's Coloured Progressive Matrices (Basso et al., 1987): thirty-six figures missing a part were presented to participants who were asked to complete each of them with a piece selected among a choice of six within a maximum execution time of 10 minutes. This test measures abstract reasoning skills and the absence of major intellectual deficits is indexed by an adjusted score > 17.5.
- Digit Cancellation Test (Spinnler and Tognoni, 1987): the administration procedure is that explained in Chapter 2 (section 2.2.1).

Scores obtained by patients on three timed tests that enable the evaluation of the speed of different cognitive processes (the TMT part A, the Stroop speed and the number of items detected on the Digit Cancellation Test) were z-transformed and averaged to calculate a composite index (PS_{CI}) as a measure of PS function. Previously to this step, the score on the Digit Cancellation Test had been inverted so that it quantified PS abilities analogously to the other two tests, i.e. the higher the score the worse PS performance. The PS_{CI} was calculated by combining three simple tests routinely used in clinical practice but nonetheless characterised by a substantial cognitive component (more complex than in simple reaction time tasks) in order to minimise possible confounding effects of peripheral motor impairments on test results. Indeed, it appears difficult, if not almost impossible, to disentangle completely the assessment of sensory, cognitive, and motor components in PS tasks (Costa et al., 2017). Moreover, raw scores on the TMT B-A, Stroop inhibition, phonemic and semantic fluency tests were used to investigate cognitive abilities characterised by substantial PS load as in Experiment 1.

4.2.2.3. MRI acquisition

Patients were scanned on a 1.5 T Philips Medical Systems Achieva scanner (Best, the Netherlands) with a standard head coil. The structural MRI protocol all patients underwent included:

- Sagittal 3D T1-weighted turbo field echo (repetition time = 7.4 ms, echo time = 3.4 ms, slices = 280, slice thickness = 0.6 mm, matrix size = 256 x 256, field of view = 240 x 240 mm^2).
- Coronal 3D T2-weighted FLAIR (repetition time = 8000 ms, echo time = 125 ms, slices = 30, slice thickness = 4.5 mm, matrix size = 260 x 232, field of view = 148 x 192 mm^2).
- Axial diffusion-weighted echo planar images (repetition time = 8280 ms, echo time = 70 ms, diffusion-encoding gradients b = 0 and 800 s/mm2, directions = 32, slices = 45, slice thickness = 3 mm, matrix size = 96 x 96, field of view = 240 x 240 mm^2).

4.2.2.4. MRI preprocessing

The same procedures as in Experiment 1 were followed for preprocessing of WM lesions and DTI scans. VBM analyses (Ashburner and Friston, 2000) were carried out in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK, http//www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2008a, version 7.6.0 (The Mathworks, Natick, Massachusetts, USA). Firstly, reorientation to the Anterior Commissure-Posterior Commissure was performed for all the T1-weighted images to facilitate the following steps. Secondly, as in Experiment 1 three tissue classes (GM, WM and CSF) were segmented on the T1-weighted images in their native space, in order to extract their volumes by means of the MATLAB function "get_totals", and, limitedly to GM and WM, normalised to the MNI standard coordinate system (Collins et al., 1994). Moreover, this step included modulation of normalised images, i.e. a spatial scaling process to compensate for possible volumetric alterations induced by normalisation (Good et al., 2001). Finally, modulated normalised GM and WM images were smoothed to correct possible normalisation inaccuracies: an isotropic Gaussian kernel of 8 mm was applied so that smoothed intensity value of each voxel was transformed in the locally weighted average of the values of the surrounding ones (Good et al., 2001).

4.2.2.5. Statistical analysis

All the statistical analyses on cognitive and clinical data were carried out using IBM SPSS Statistics Version 21 (IBM, Chicago, IL, USA). A procedure similar to Experiment 1 was followed, at first in order to investigate the associations between clinical and cognitive variables (Spearman's ρ, two-tailed $\alpha = .05$). Consequently,

the impact of the amount of TLV on cognitive performance was investigated using multiple regression models which included age, education and TIV as covariates. The regression model was applied to VBM and subsequently to TBSS analyses to ascertain both macrostructural and microstructural correlates of PS-related cognitive performance in SPMS. Predictions were made that higher PS performance would correlate positively with higher WM volumes and FA values. In particular, the aim of this experiment was to answer the following research questions related to the structural neural correlates of PS:

1) *Does PS function in people affected by SPMS rely mainly on WM rather than GM volume?*

VBM analysis was used to answer this question by correlating scores on the selected PS-related cognitive tests and GM as well as WM maps (Tyler et al., 2005). PS ability was expected to be associated more extensively with regional WM volumes. Only clusters that survived statistical correction for multiple comparisons at a Family Wise Error (FWE) threshold of $p < .05$ were considered. GM areas containing significant peaks highlighted by the analysis were identified by means of the Talairach Daemon (http://www.talairach.org/daemon.html), after converting their coordinates from the MNI to the Talairach reference system (Talairach and Tournoux, 1988). Instead, WM tracts were identified using the JHU ICBM-DTI-81 White-Matter Labels atlas (Mori et al., 2008).

2) *Are PS abilities in SPMS supported by a specific structural network of WM tracts?*

This point was tackled by using DTI scans to ascertain where in the brain FA levels were associated with PS_{CI} and PS-demanding tests. The higher FA values the better PS performance was expected. Analyses were performed in FSL, by means of the procedure reported in Experiment 1.

3) *Is the association between performance on PS-demanding tests and measures of brain volume and WM microstructural integrity modulated by the status of PS abilities in SPMS?*

Similarly to the procedures previously used (VBM and diffusivity analysis), analogous regression models were generated for PS-demanding tests but adding the PS_{CI} scores among the covariates to control statistically for PS demands. The exoectation was that the association between PS-demanding tests and

neuroimaging measures would be absent or reduced after accounting for PS_{CI} scores.

4.2.3. Results

4.2.3.1. Clinical and cognitive results

The full clinical profile of the sample of patients is summarised in Table 4.6.

Table 4.6 Clinical, cognitive and volumetric characteristics of the sample (n = 31)

Variable	Mean	SD	Median	Minimum	Maximum	Patients with deficits
Clinical characteristics						
Age (years)	54.8	11.5	54	29	70	-
Education (years)	10.3	2.8	11	5	13	-
Duration (years)	16.3	8.5	14	3	31	-
EDSS	6.5	1.2	7	3.5	8	-
FSS	5.0	1.2	5	2.7	7	-
TIV (ml)	1685.1	170.4	1685.6	1341.3	2044.4	-
TLV (ml)	23.5	18.9	21.7	0.8	82.0	-
Cognitive tests						
PS_{CI}	0.0	1.0	-0.1	-1.1	2.4	-
Stroop inhibition (sec)	28.3	15.0	20.5	5.5	66	8
TMT B-A (sec)	131.9	151.0	80.0	5	663	16
Phonemic fluency	28.1	11.2	31	8	48	6
Semantic fluency	38.0	11.6	35	20	61	6

EDSS: Expanded Disability Status Scale, FSS: Fatigue Severity Scale, PS_{CI}: Processing speed composite index, TIV: Total intracranial volume, TLV: Total lesion volume, TMT: Trail Making Test

Weak associations were detected between clinical and demographic variables (Table 4.7). These associations did not survive Bonferroni correction for multiple comparisons ($p < .05/7 = .007$).

Table 4.7 Correlations between clinical variables

	Age	Education	Duration	EDSS	FSS	TIV	TLV
Age	-						
Education	-.364*	-					
Duration	.193	-.138	-				
EDSS	-.385*	.292	.360*	-			
FSS	-.151	.447*	.057	.371	-		
TIV	-.124	.235	.013	.108	.201	-	
TLV	-.243	.023	.023	.246	.315	.230	-

* $p < .05$ (uncorrected for multiple comparisons)

EDSS: Expanded Disability Status Scale, FSS: Fatigue Severity Scale, TIV: Total intracranial volume, TLV: Total lesion volume

Associations between cognitive variables were highlighted between the PS_{CI} and most of the other PS-dependent tests, especially the fluency tasks which were strongly correlated to one another (Table 4.8).

Table 4.8 Correlations between cognitive variables

	PS_{CI}	Stroop inhibition	TMT B-A	Phonemic fluency	Semantic fluency
PS_{CI}	-				
Stroop inhibition (sec)	.378*	-			
TMT B-A (sec)	.375	.332	-		
Phonemic fluency	**-.498†**	-.068	-.231	-	
Semantic fluency	**-.560†**	-.271	.058	**.608†**	-

* p < .05 (uncorrected for multiple comparisons)
† p < .01 (.05/5 = .01, Bonferroni correction)

PS_{CI}: Processing speed composite index, TMT: Trail Making Test

4.2.3.2. TLV results

The highest peaks of WM lesion probability were mainly detected in the superior corona radiata, periventricular and occipital areas (Figure 4.4).

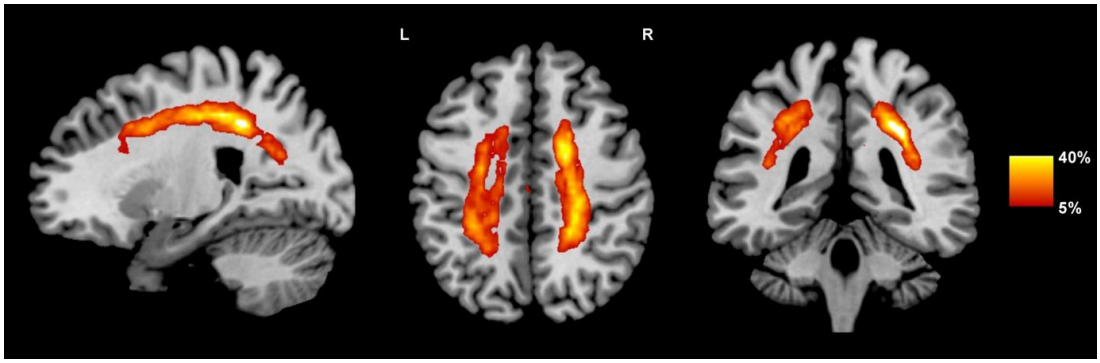

Figure 4.4 Group-level lesion probability map

First, it was checked that all assumptions for regression were met as in Experiment 1 in order to proceed with the analysis for all cognitive tests. It emerged that, while the PS_{CI} was not significantly predicted by any of the covariates investigated, the multiple regression model significantly explained response inhibitory performance on the Stroop test ($F(4, 26) = 3.2$, $p = .032$) with an R^2 of .33. Moreover, TLV represented the only significant predictor (B = .428, p = .004) and showed a negative impact on inhibition abilities: every additional millilitre of TLV was associated with an increase in the time for response inhibition of almost half second. Finally, although the whole model did not significantly explained scores obtained on

the semantic fluency test, yet TLV emerged as a significant predictor of cognitive performance (B = -.308, p = .012).

4.2.3.3. VBM results

In relation to the first research question, no significant associations with WM volume were detected for the PS_{CI}, the phonemic fluency test, the TMT-A and the Stroop inhibition test. Only the semantic fluency task was found to correlate with regional volumes of bilateral WM clusters mainly involving the superior longitudinal fasciculus, the corpus callosum and the anterior thalamic radiations (Figure 4.5 and Table 4.9).

Similar findings were observed in the VBM analysis on GM maps where only the semantic fluency task was observed to correlate significantly with several regional GM volumes. In particular, the significant clusters were located in occipital and temporal areas, the posterior cingulate cortex and the caudate (Figure 4.5 and Table 4.9).

After statistically controlling for PS performance (research question 3), significant associations were still detected between semantic fluency scores and: volumes of WM tracts mentioned above, though in smaller clusters, and the volume of a GM clster in occipito-temporal areas (Table 4.9).

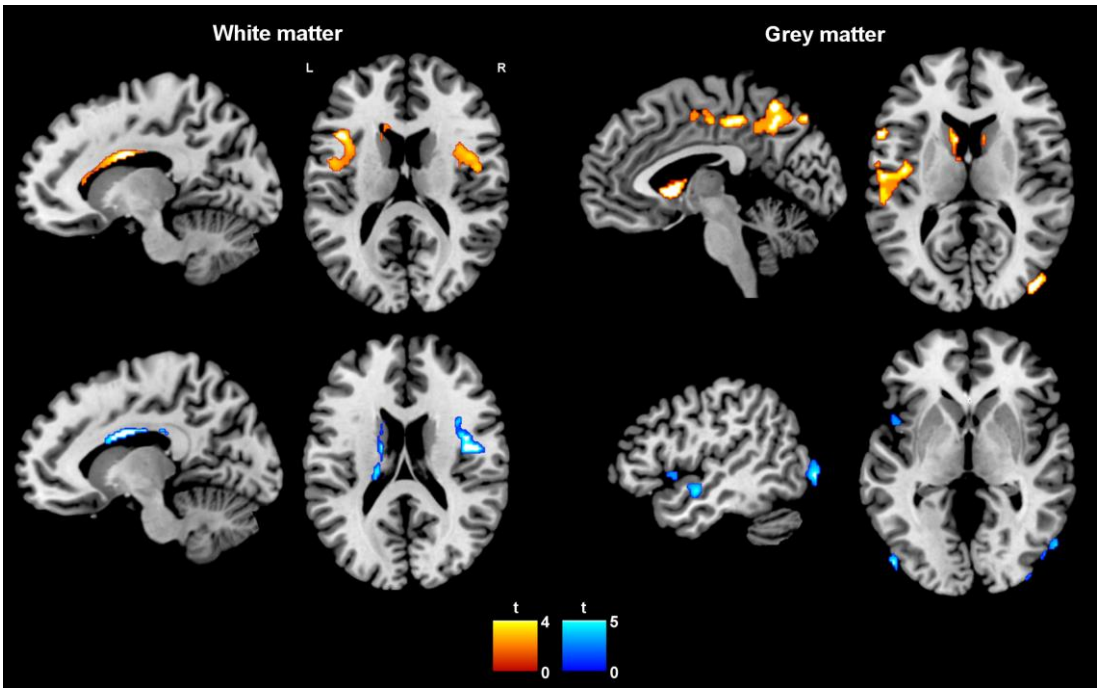

Figure 4.5 WM (left) and GM regions (right) positively correlated with the semantic fluency task before (red) and after (blue) correcting for PS abilities (p < .05 FWE)

Table 4.9 Positive association between semantic fluency and GM/WM regional volumes before and after controlling for the PS_{CI} ($p < .05$ FWE)

Cognitive variable	Cluster extent	r	Side	Brain region	*t* value	MNI coordinates *x*	*y*	*z*
				White matter tracts				
SF	364	.635	L	Anterior thalamic radiations	6.16	-16	-2	24
			L	Body of corpus callosum	4.32	-8	-12	28
			L	Forceps minor	3.96	-14	26	10
	1180	.634	L	Uncinate fasciculus	5.81	-42	16	16
			L	SLF	5.30	-34	12	20
			L	SLF	4.88	-32	-4	40
	334	.613	R	SLF	4.72	46	-6	18
			R	SLF	4.68	42	2	18
			R	SLF	4.19	42	-14	36
PS_{CI}-corrected SF	281	.620	L	Anterior thalamic radiations	5.38	-16	-2	24
			L	Body of corpus callosum	4.28	-12	-14	30
			L	Anterior thalamic radiations	4.20	-18	-20	24
	235	.626	R	SLF	4.37	42	4	16
			R	SLF	4.36	48	-6	18
			R	SLF	3.99	36	12	24
				Grey matter regions				
SF	504	.639	R	Temporal pole (BA 38)	5.99	16	14	-36
			R	PHG (BA 28)	4.21	16	-14	-38
			R	PHG (BA 34)	4.11	8	-8	-24
	456	.639	R	MTG (BA 19)	5.48	44	-84	14
			R	IOG (BA 18)	5.46	40	-92	-12
			R	IOG (BA 19)	4.74	50	-82	-10
	272	.667	L	Caudate	5.06	-6	12	0
			L	Caudate	4.07	-10	8	14
			L	Caudate	3.97	-10	-2	14
	718	/715	L	Precuneus (BA 7)	4.92	-2	-56	54
			L	Precuneus (BA 7)	4.88	-2	-64	48
			L	Precuneus (BA 7)	4.42	-12	-76	52
	835	.654	L	STG (BA 22)	4.86	-48	10	-2
			L	STG (BA 42)	4.44	-58	-14	14
			L	PreCG (BA 44)	4.34	-58	14	10
PS_{CI}-corrected SF	211	.708	L	IOG (BA 18)	6.11	-42	-92	-12
			L	MOG (BA 19)	5.45	-50	-84	-2
	466	.667	R	MOG (BA 19)	5.81	44	-84	12
			R	IOG (BA 18)	5.37	40	-92	-12
			R	ITG (BA 37)	4.65	58	-72	-2
	223	.646	L	STG (BA 21)	5.49	-52	-6	-12
			L	STG (BA 22)	3.92	-48	10	-2
			L	Temporal pole (BA 38)	3.65	-40	6	-16
	215	.637	R	Temporal pole (BA 38)	5.27	16	14	-36
			R	PHG (BA 36)	4.43	14	6	-40
			R	PHG (BA 35)	4.00	16	-14	-36

BA: Brodmann area, IOG: Inferior occipital gyrus, ITG: Inferior temporal gyrus, MOG: Middle occipital gyrus, MTG: Middle temporal gyrus, PHG: Parahippocampal gyrus, PreCG: Precentral gyrus, SF: Semantic fluency, SLF: Superior longitudinal fasciculus, STG: Superior temporal gyrus

4.2.3.4. DTI results

WM microstructure analysis carried out to answer the second research question showed PS_{CI} scores were associated with integrity of the corpus callosum and different frontal tracts, namely the anterior thalamic radiations and the inferior fronto-occipital fasciculus. Instead, performance on all cognitive tests was differentially associated with FA in several WM tracts (Figure 4.6), though for the TMT B-A the significance threshold had to be increased to p = .1 in order to probe the strongest peaks of correlation which emerged in a cluster in the forceps minor. Indeed, FA in the corpus callosum was positively associated with performance on all tests despite widespread patterns of correlations, especially for the semantic fluency and the Stroop inhibition tasks. Nonetheless, some of the strongest associations were consistently found in the inferior fronto-occipital fasciculus as observed for the PS_{CI} (Table 4.10). Additionally, the superior longitudinal fasciculus emerged as significantly involved in PS-demanding cognitive performance across tests.

For semantic fluency and Stroop inhibition, a similar pattern survived after statistically correcting for the PS_{CI} but in smaller clusters (research question 3). However, a shift of the most significant peaks of association towards more posterior occipito-temporal tracts, i.e. the forceps major and the inferior longitudinal fasciculus, clearly emerged for both tests. For phonemic fluency no significant results were observed after controlling for PS abilities.

Table 4.10 Correlation between cognitive tests and FA (p < .05)

Cognitive variable	Cluster extent	r	Side	White matter tract	*t* value	MNI coordinates		
						x	*y*	*z*
PS_{CI}†	1705	-.705	L	Anterior thalamic radiations	5.42	-20	27	30
			L	Body of corpus callosum	4.78	-13	14	26
			L	Anterior thalamic radiations	4.75	-22	29	21
			L	Body of corpus callosum	4.48	-13	7	30
			L	IFOF	4.46	-24	28	8
			L	Body of corpus callosum	4.40	-7	7	27
SI†	38191	-.647	R	SLF	5.91	42	-45	9
			L	IFOF	5.76	-29	-72	4
			R	Body of corpus callosum	5.49	12	19	22
			L	Anterior thalamic radiations	5.39	-16	-10	-1
			L	Forceps major	5.31	-27	-74	4
			R	Forceps minor	5.20	11	28	13
PS_{CI}-corrected SI†	29954	-.586	R	SLF	6.14	43	-46	8
			R	SLF	6.07	43	-46	10
			L	Forceps major	5.50	-29	-72	4
			L	Forceps major	5.12	-29	-71	6
			L	ILF	5.02	-44	-38	-7
			L	Forceps major	5.00	-27	-69	2

Table 4.10 *(continued)*

TMT B-A*†	18	-.642	L	Forceps minor	3.85	-10	30	11
			L	Forceps minor	3.66	-8	29	10
			L	Forceps minor	3.39	-5	27	10
PF‡	212	.699	L	SLF	4.71	-17	6	34
			L	SLF	4.19	-17	12	34
			L	SLF	3.93	-16	1	37
			L	Body of corpus callosum	3.67	-15	19	25
			L	SLF	3.65	-16	9	35
			L	SLF	3.48	-17	15	32
	205	.644	L	SLF	4.70	-29	3	7
			L	SLF	4.58	-28	3	9
			L	IFOF	3.97	-25	18	0
			L	SLF	3.91	-30	-4	11
			L	SLF	3.69	-29	-1	10
			L	IFOF	3.66	-26	14	0
SF‡	18771	.693	L	SLF	7.58	-18	7	35
			L	SLF	7.37	-17	6	37
			R	Body of corpus callosum	6.44	6	-24	28
			L	SLF	5.86	-41	11	8
			L	Body of corpus callosum	5.86	-10	2	31
			L	SLF	5.64	-17	1	37
	144	.484	R	IFOF	3.10	39	-27	-3
			R	IFOF	3.02	39	-28	-1
			R	IFOF	2.98	32	-21	-2
			R	IFOF	2.67	35	-28	0
			R	IFOF	2.61	37	-32	2
			R	IFOF	2.49	40	-32	-3
PS_{CI}-corrected SF‡	4420	.670	L	SLF	6.74	-18	7	37
			L	SLF	6.36	-18	7	35
			R	Body of corpus callosum	6.14	6	-24	28
			L	Body of corpus callosum	5.77	-15	-5	36
			L	SLF	5.42	-17	-18	37
			L	SLF	5.38	-17	-16	36
	1332	.577	L	ILF	4.27	-32	-60	24
			L	ILF	4.17	-32	-61	26
			L	IFOF	3.66	-31	-60	19
			L	IFOF	3.52	-33	-55	17
			L	ILF	3.48	-27	-59	19
			L	SLF	3.37	-37	-51	29

* $p < 0.1$

† negative correlation

‡ positive correlation

IFOF: Inferior fronto-occipital fasciculus, ILF: Inferior longitudinal fasciculus, PF: Phonemic fluency, PS_{CI}: Processing speed composite index, SF: Semantic fluency, SI: Stroop inhibition, SLF: Superior longitudinal fasciculus, TMT: Trail Making Test

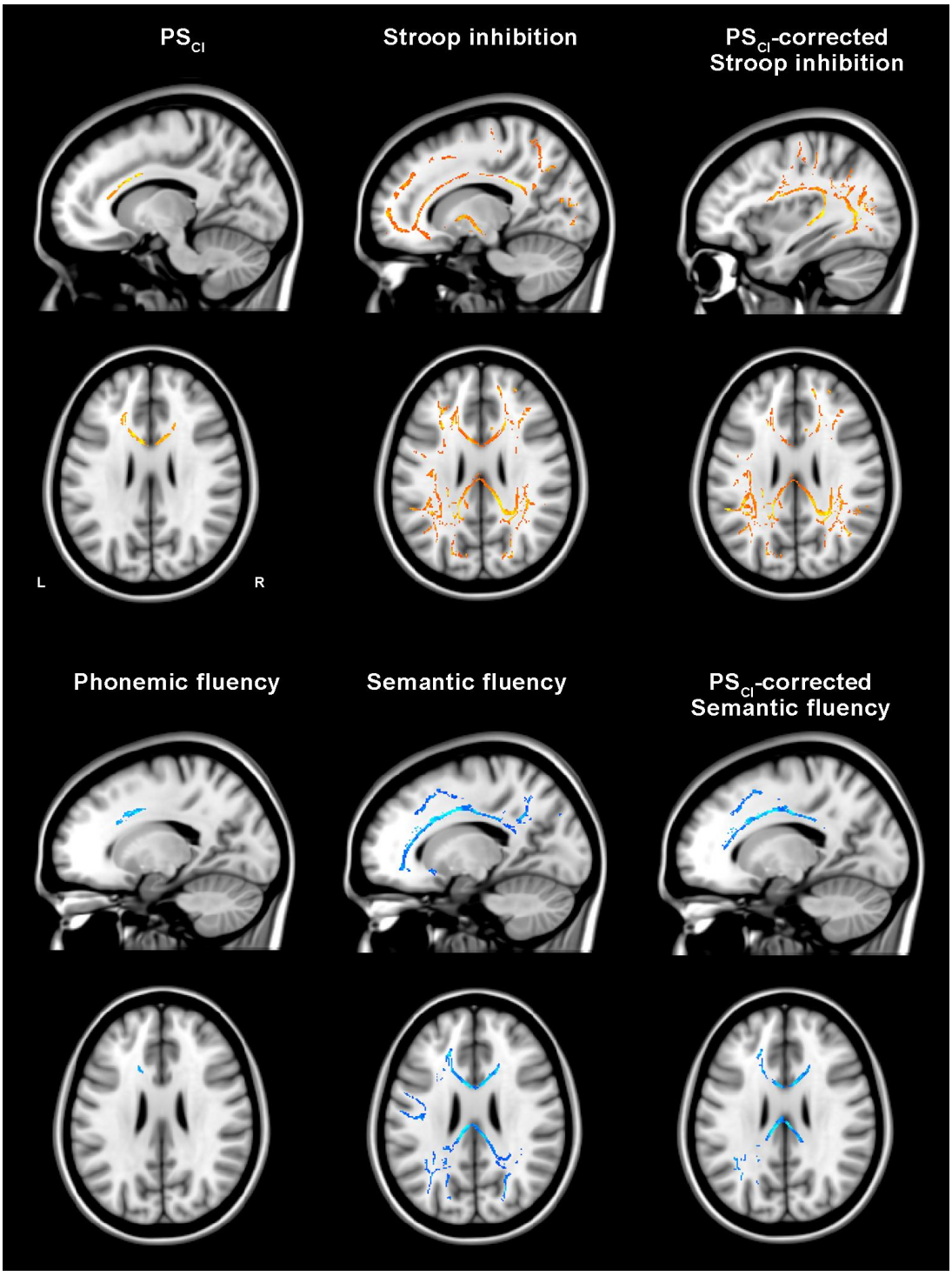

Figure 4.6 Patterns of correlation (orange negative, blue positive) between FA and the PS_{CI}, phonemic fluency, and semantic fluency and the Stroop inhibition scores before and after correcting for PS abilities ($p < .05$)

4.2.4. Discussion

In this study the combination of VBM and TBSS analyses provided new insights on the association between measures of structural brain integrity and cognitive performance of patients with SPMS on tasks characterized by substantial PS load.

In particular, the investigation of WM microstructure showed that PS function was positively associated preferentially with the level of integrity of commissural and frontal associative WM tracts: the body of the corpus callosum, the anterior thalamic radiations and the inferior fronto-occipital fasciculus. Furthermore, performance on the PS-loaded cognitive tests investigated was associated with FA values in analogous WM tracts, in particular with the corpus callosum, which resulted significantly involved in each single test, and the inferior fronto-occipital fasciculus, detected for the Stroop and the fluency tasks. Hence, these two WM tracts, along with the anterior thalamic radiations, appear to represent the structural network supporting PS-loaded cognitive performance in people affected by SPMS, in line with previous findings in ageing (Borghesani et al., 2013, Kerchner et al., 2012, Salami et al., 2012). Additionally, the superior longitudinal fasciculus was found to be associated only with the more complex tests (Stroop and the fluency tasks), but not with the index of PS, possibly because of their greater global cognitive load beyond PS (Kincses et al., 2011, Turken et al., 2008). This might explain why performance on Stroop inhibition and semantic fluency tasks was significantly predicted also by TLV. In fact, the highest lesion probabilities were observed in fronto-parietal WM areas.

After statistically controlling for PS ability, the correlation between FA and the Stroop and semantic fluency tests only survived in smaller clusters of WM mainly localised in posterior occipital (forceps major) and occipito-temporal (inferior longitudinal fasciculus) tracts involved mainly in visual perceptual processes (Catani and Thiebaut de Schotten, 2008). Therefore, the PS load that characterizes these tests appears, as expected, to modulate the detection of significant correlations with clusters of WM in more frontal and cognitively salient tracts.

VBM analysis was used with the aim to investigate whether regional atrophy of either WM or GM could also capture PS decline, since the secondary progressive stage of this disease is heavily characterized by neurodegenerative processes (Mahad et al., 2015) driving considerable levels of brain atrophy (Eshaghi et al., 2018). In our battery of tests, only the scores obtained on the semantic fluency task emerged significantly correlated with both GM and WM volumes. Moreover, this unique association was found with TLV, thus confirming semantic fluency may be particularly sensitive to WM damage. Indeed, semantic cognition is supported by a distributed network both regarding storage of semantic knowledge (Patterson et al., 2007) and deployment of sematic control processes (Jefferies, 2013). On the contrary phonemic fluency is a task requiring prevalently executive control

processes associated with the frontal lobes that undergo macrostructural degeneration later on in the disease course (Eshaghi et al., 2018).

The findings of this study suggest that in this clinical population fast cognitive processing is supported by structural connections that enable integration of information across each hemisphere and especially between frontal lobes and other GM structures. Among the various WM tracts likely to be crucial for cognitive PS abilities, of particular interest is the inferior fronto-occipital tract whose level of microstructural integrity was found to correlate with all the PS-related tests investigated in this study. In fact, the inferior fronto-occipital fasciculus has been found to subserve complex functions related to semantic cognition, though characterized by interhemispheric differences (Herbet et al., 2017a, Khan et al., 2014), and to contribute to attention orienting (Herbet et al., 2017b).

In conclusion, this study provides new insights into the structural correlates of PS-related cognitive performance in patients with SPMS, a phenotype that has been mostly neglected by researchers. Further investigations on the functional networks connected by the WM tracts observed in these analyses are needed to characterise more extensively the relationship between neural and cognitive changes in SPMS. Indeed, currently little is known about functional reorganization in the SPMS phenotype since most investigations focused on RRMS and the Default Mode Network only (Raichle et al., 2001).

4.3. General discussion

The findings from Experiment 1 showed that performance mainly on measures of visual and visuo-spatial rather than verbal/auditory PS seem to be correlated with integrity of structural connections in people affected by RRMS. In particular, the structural correlates highlighted in this study differ, apart from the corpus callosum, from those most consistently observed to be associated with visual PS abilities measured by the SDMT across the studies reviewed in Chapter 2: the fornix, the cingulum and the posterior thalamic radiations. By contrast, the forceps minor, the anterior thalamic radiations and the inferior fronto-occipital fasciculus emerged from analyses in Experiment 1 for both the DSCT and the TMT-A.

First, these discrepancies may be partially explained by the exiguous number of studies on the structural correlates of the SDMT in RRMS. Indeed, a certain degree of variability in results across investigations is normally expected due to inevitable subtle differences in the samples recruited and methodologies used (ROI vs voxel-based). Moreover, the exploratory nature and the widespread lack of hypothesis

testing observed in most studies can be seen as a probable factor contributing to less critical discussion of the results obtained. In light of these limitations, it appears difficult to consider the current knowledge as a definite frame of reference.

It may also be argued that the results found in Experiment 1 appear to have higher functional relevance than those summarised in the literature review when considering their implications for PS function (Manca et al., 2018). In fact, although some of the mentioned fibre bundles may contribute to performance on the DSCT (or the SDMT), they may support functions not necessarily related to speed of information processing. First, the fornix has been extensively studied both in monkeys (Gaffan and Wilson, 2008) and humans (Aggleton and Brown, 2001) and its role in mnestic function, of visual and visuo-spatial types, is widely recognised. Second, the cingulum is a quite complex tract, possibly subdivided into different and functionally specialised sections like the adjacent cingulate gyrus (Bubb et al., 2018). Although its frontal portion is likely to be involved in executive/attentional processes that may contribute to PS, current DTI analytic techniques do not allow an effective disentangling of cingulum components. Third, the posterior thalamic radiations may subserve visual perceptual functions, necessary in order to perform the SDMT, but not clearly involved in PS-demanding cognitive processes (Schmahmann and Pandya, 2008). On the contrary, as reported in the discussion to Experiment 1 evidence has been accumulated on the prominent role of frontal connectivity in supporting fast cognitive operations. Indeed, executive control processes may contribute to support performance of challenging tasks requiring information to be processed quickly (Amann et al., 2010, Benedict et al., 2013, Bisecco et al., 2017). The lesser is the damage to frontal structures the more executive support can be deployed to perform cognitive tasks in conditions experienced as challenging by both patients and healthy people (Bonnet et al., 2010, Forn et al., 2013).

Notably, in Experiment 2 very similar tracts have been found associated with PS abilities of people who transitioned to SPMS, with the addition of the superior longitudinal fasciculus. However, differently from Experiment 1 the significant findings emerged mainly for PS-demanding tests requiring verbal rather than visuo-spatial processing. Although a direct comparison between the two experiments cannot be performed due to fundamental differences in characteristics of the samples (native languages and cultures that impact cognitive performance), cognitive measures used and MRI parameters (3T vs 1.5T), a qualitative interpretation may be attempted. Indeed, it appears that tasks assessing visuo-spatial abilities may be particularly sensitive early on in the disease course due to a

particular vulnerability to this pathology. Indeed, alerting deficits may be observed that may induce additional burden on attentional control mechanisms (Crivelli et al., 2012, Roth et al., 2015). Thus, variance in scores on tests of visual PS function relying on integration of information across hemispheres may be significantly explained by variance in structural damage induced by neuroinflammation to related WM tracts.

However, as the disease progresses to SPMS and neurodegeneration spreads throughout the brain, also performance in tasks that heavily rely on GM integrity, such as semantic fluency, may be impaired. Indeed, it has been shown semantic knowledge is stored in several different cortical areas functionally connected in a network to the left temporal pole that is thought to "contain" amodal representations of concepts (Patterson et al., 2007). Therefore, tests like semantic fluency may be particularly sensitive to a combination of widespread WM damage (disrupting integration of information) and GM atrophy (impacting on memory storage) as detected in the analyses of Experiment 2. Consistently, Preziosa et al. (2017) have recently been investigating how increased lesion accumulation in the left hemisphere, prominently specialised in processing of information of verbal type, appears to be the significant predictor of progression of cognitive decline over a period of 5 years. Thus, even though analysis on single tests were not carried out in their study, these findings suggest that in more advanced stages of the disease language-based tests requiring integration of information may be good markers of changes in cognitive functions.

As mentioned above, the differences in methodologies observable between the two studies in this chapter relating to discrepancies in neuropsychological tests and MRI parameters used limit any direct quantitative comparisons. Indeed, the cross-sectional nature of these investigations poses challenges for the interpretation of differential findings across the two MS phenotypes. Therefore, the fact that performance on different PS-demanding tests was associated with microstructural damage in similar WM tracts for the two samples needs further clarification and no definite conclusions can be currently drawn. Additionally, the technique used for processing of DTI data (i.e. namely TBSS) may be seen as a partial limitation since it constrains the analysis to the core of WM tracts, thus potentially missing smaller or more complex bundles. In fact, despite allowing voxel-based analysis, resolution of individual tracts is limited and tractography analysis may provide complementary insights. However, the resolution of the DTI scans collected could not enable such analysis to satisfying standards.

Nevertheless, these experiments showed that hypothesis-based use of voxel-based analysis combined with neuropsychological tests can provide more detailed information on the structural correlates of PS function in MS than the investigation of TLV. In fact, detection of enhancing lesions predicts the absence of practice effect over time on the SDMT (Fenu et al., 2018), significant microstructural changes may affect normal appearing WM before any macrostructural damage could be detected (Ontaneda et al., 2014). Similarly, the formation of new lesions has been observed to have wider impact on both structural (Chiang et al., 2016) and functional connectivity (Droby et al., 2015b) that may explain clinical evolution of symptoms, among which also cognitive ones.

In conclusion, the results of these two experiments together mainly support the conceptualization of MS as a disconnection syndrome (Mesulam, 2012), with cognitive symptoms arising from network disruption (Schoonheim et al., 2015). It may be argued, therefore, that effective execution of PS-challenging cognitive tasks require brains affected by MS to deploy executive control processes reliant on brain networks centred on the frontal lobes to a greater extent than in healthy brains. This appears in line with cognitive (Vernon, 1983) and neuroimaging (Rypma et al., 2006, Rypma and Prabhakaran, 2009) findings on efficient and fast cognitive processing in healthy people. Therefore, preservation of frontal connections that allow control processes and integration of other cognitive functions appears crucial for supporting execution of PS-demanding tasks.

Chapter 5 | Resting-state functional neural correlates of PS function in people with MS

Research carried out to investigate how PS function in people with MS depends on measures of functional connectivity is very limited compared to the amount of studies on structural connectivity. In fact, only in recent years the identification and investigation of networks of functionally related areas have become more represented in the field of functional MRI. Less than twenty years ago Raichle et al. (2001) described the DMN, which represents the most widely studied among all brain networks in relation to PS performance in MS (Janssen et al., 2013, Rocca et al., 2010b, Sbardella et al., 2015b, Zhou et al., 2014). In particular, most papers currently available on this topic mainly used the PASAT as PS measure (Manca et al., 2018). Therefore, it appears difficult to hypotesise whether the association between alterations in this network and PS decline is specific or whether damage to other functional networks may play a major role.

Current characterisation of the involvement of functional networks in cognitive symptoms in MS appears in general insufficient, especially regarding SPMS. High variability of findings emerged across and within studies, since Sbardella et al. (2015b) found that performance on the PASAT was related to functional connectivity of two out of the eleven networks investigated: the executive control network, expectedly due to well established frontal activation during working memory tasks, and the medial visual network, despite no visual processing is involved in such test. The lack of consistency seen so far may be explained by the very limited number of studies that have addressed this topic not without substantial methodological discrepancies (ROI-based, network-based and graph-theory-based approaches). Moreover, it is likely that the randomness of lesion manifestation may pose a particular challenge to the investigation of the association between complex brain networks and one single measure of PS function. Indeed, it has been recently shown that MS-related structural damage to both WM and GM has an impact on functional connectivity in different ways (Tewarie et al., 2018). Hence, alterations observed in patients are likely to be complex, widespread and dynamic.

Additionally, it is worth mentioning that different authors may define the same networks in slightly different ways, especially due to the methods used to separate them, potentially introducing further sources of variability. Indeed, the most common analysis technique used is the so-called Independent Component Analysis (ICA):

the blood-oxygen-level dependent signal acquired from the whole brain, at rest, is fed into statistical analyses carried out with the aim of disentangling signal implied to be generated by independent sources (Calhoun et al., 2001). In doing so, brain activity from each voxel is correlated with brain activity in each other voxel, in order to highlight those clusters whose resting low-frequency fluctuations occur synchronously, i.e. functionally related networks of areas (Raichle, 2011). In fact, these networks detected in the resting brain happen to co-activate while performing specific tasks of either sensory, motor, or cognitive kind.

This approach emerges as predominant in the field and it has been applied to the study of brain alterations in various pathologies, since it offers a window into the brain functional architecture without requiring participants to engage in task performance and it is easily applicable to intervention studies as outcome measure (Barkhof et al., 2014). For these reasons, network-based analysis was chosen to investigate in detail the functional correlates of PS function in people affected by two different phenotypes of MS with the aim of obtaining complementary findings to those on structural connectivity reported in Chapter 4 (Enzinger and Fazekas, 2015). It must be stressed that, although structural and functional connectivity may be associated, their relationship is not bidirectional (Damoiseaux and Greicius, 2009). Therefore, the investigation of the functional correlates of PS abilities in this chapter may be guided by the results of the structural connectivity analysis, but with no expectations of a perfect match between structural and functional findings.

5.1. Experiment 3 – Associations between measures of PS function and functional connectivity in people with RRMS

5.1.1. Introduction

A widely investigated aspect associated with MS pathology, but observed also in other diseases to different degrees and in various manifestations, consists in the alteration and reorganization of several functional brain networks (Cruz-Gomez et al., 2014, Rocca et al., 2012b). These changes express predominantly as decreases of functional connectivity, although increases have been reported to a lesser extent.

In particular, different studies focussed their investigations on the role that the DMN may have in MS-related cognitive impairment. This network of areas usually deactivates when engagement in goal-directed behaviours is required (Raichle et al., 2001). Therefore, a failure in shifting activation from the DMN to other task-related networks may impact negatively on cognitive performance. Bonavita et al. (2011) showed that in cognitively impaired patients alterations in different hubs of the DMN occur compared to cognitively preserved individuals with MS: decreased resting-state activity was observed in the posterior cingulate and the left inferior parietal lobule, while increases were noted in the posterior cingulate more posteriorly. Similar results were also obtained by applying graph-theory analysis, which comprises an initial parcellation of the brain in anatomically distinct areas (nodes) and the generation of the network of connections (edges) on the basis of the correlation between the resting-state signal of each couple of nodes. The comparison between patients with and without cognitive symptoms showed that the presence of deficits is associated with loss of nodes mainly in left-lateralised areas such as the superior frontal gyrus, the anterior cingulate gyrus, the precuneus and the thalamus (Rocca et al., 2016). Moreover, the DMN appears to become more central across the brain organization at rest in cognitively impaired patients, suggesting a dysfunction in this network that may be associated with inability to shift brain activation toward other task-related networks (Eijlers et al., 2017).

However, as already mentioned in previous chapters, there may be problems with the interpretation of the results from studies that distinguish between cognitively impaired and preserved patients on the basis of failure on a set number of tests, without discriminating functions assessed by specific tests. In fact, across patients within a group, impairments may be present in different cognitive domains, which rely on the integrity of distinct brain networks. Moreover, patients supposed to have intact cognition may show, though to a lesser extent (e.g. failure on a single test),

signs of decline that may be ignored. For these reasons, confounders might have been introduced in the results currently available. Additionally, most studies focussed their analysis exclusively on the DMN, thus preventing speculations on the possibility that disruption of other networks may be significantly affecting cognitive performance.

From the results of the literature review it emerged that more specific investigations into the commonly observed PS deficits have generated quite variable and inconsistent results so far. The PASAT has been extensively used as a measure of PS function and performance of people with RRMS on this test appears to be associated with functional connectivity of several distinct brain networks: the DMN (Zhou et al., 2014), thalamic connectivity by Tona et al. (2014) but not by Zhou et al. (2016), executive control and medial visual networks (Sbardella et al., 2015b). Hence, current knowledge of the resting-state functional correlates of PS abilities in MS seems limited. Indeed, the results by Sbardella et al. (2015b) are unexpected and counterintuitive considering the lack of visual information processing during performance on the PASAT. Although recruitment of non-cognitively related brain areas (such as the primary visual cortex) might represent a compensatory mechanism needed to support cognitive efficiency in other pathologies (De Marco et al., 2017), visual function and organization of cortical visual networks are often affected in MS because of optic neuritis (Janssen et al., 2013, Tewarie et al., 2017). As a consequence, it seems less likely that in MS visual areas may be recruited to maintain or improve cognitive performance. Indeed, the negative correlation detected between PASAT scores and connectivity of the visual network (Sbardella et al., 2015b) may highlight a phenomenon of maladaptive plasticity (Stern, 2009).

Consistent findings across studies point at a preferential involvement of the left hemisphere in supporting PS functions in MS. Zhou et al. (2014) observed that performance on the PASAT correlated with functional connectivity between the posterior DMN and the left medial temporal lobe. Instead, in a study involving repeated measurements of brain activity, a drop in functional coupling between left superior frontal gyrus and left thalamus was observed after the execution of the PASAT, although no significant correlation emerged between performance on the test and functional connectivity of the left superior frontal gyrus (Pravatá et al., 2016). Additionally, intraindividual variability on a semantic search reaction time task correlated with the strength of connectivity between the anterior hub of the DMN and the left frontal pole (Wojtowicz et al., 2014). It is worth noting that the predominant localization of findings mainly in the left hemisphere may be explained by the fact that most PS tests used required processing of verbal information. In particular, the

role of the left fronto-parietal network in cognitive decline caused by MS is still unclear since it appears altered also in cognitively preserved patients, while cognitive decline may be due to more widespread disruption (Cruz-Gomez et al., 2014).

On the contrary, the only study among those included in the abovementioned review that used a composite index of PS function found no correlations with any of the investigated networks (Janssen et al., 2013). Moreover, Gamboa et al. (2014) showed by means of graph-based analysis that performance on a modified version of the PASAT was predicted by the level of functional modularity, i.e. the degree of integration of information across brain networks. This means that PS function may be affected particularly by disconnection between different functionally specialised networks and subnetworks that, in the absence of pathology, cooperate to enable efficient information processing. It follows that the interplay between PS-dependent cognitive performance and measures of resting-state brain activity may be less straightforward than expected, on the basis of the results reported by multiple studies that compared cognitively impaired and preserved people with MS.

Nevertheless, involvement of sensory and motor networks is not likely or may be detected for tests such as the TMT, prominently dependent on motor responses and processing of visual information that are often compromised in MS. It is expected that cognitive decline in PS function may be due to altered functional connectivity of cognitive networks (including the DMN). The clarification of the role of two networks appears particularly compelling: the salience network (Seeley et al., 2007), whose function is related to assessment of the level of experienced salience, i.e. relevance for the organism, in order to guide subsequent goal-directed behaviours; and the fronto-parietal network, which may be divided in left and right, controlling the coordination of such goal-directed behaviours (Coull et al., 1996). Indeed, although these three networks (DMN, salience network and fronto-parietal network) perform different functions, they are to some degree connected to one another and successful task execution depends on the coordination of their activation and deactivation patterns (Sridharan et al., 2008). Therefore, PS performance in people affected by MS may depend on dysfunction in one or more of these networks and investigation of this issue is the aim of the experiments reported in this chapter.

5.1.2. Methods

5.1.2.1. Participants

Forty-two out of forty-eight people affected by RRMS (Lublin and Reingold, 1996) who fulfilled the modified McDonald diagnostic criteria for RRMS (Polman et al., 2010) were selected from the cohort recruited for the intervention reported in Chapter 6. Inclusion and exclusion criteria are the same used in Experiment 1 (Chapter 4). Three participants were excluded for the same reasons highlighted in Experiment 1. Three additional patients who emerged as outliers during resting-state MRI scan preprocessing for head motion parameters were excluded to avoid artefacts in subsequent analysis.

Ethical approval was obtained from the Regional Ethics Committee of Yorkshire and Humber (Protocol STH17001 version 4.0) (Appendix D). All participants were provided with written information material (Appendix E) at least one week before recruitment and gave written consent (Appendix F) to take part in this study.

5.1.2.2. Neuropsychological assessment

This study used the same short battery of tests used in Experiment 1 comprising tests with prominent PS involvement (scores from the baseline assessment): the PASAT (3s and 2s), the DSCT, the Stroop test, the TMT and the phonemic and semantic fluency tests.

5.1.2.3. MRI acquisition

The MRI protocol used in this study was acquired at 3T (Ingenia, Philips Healthcare, Best, NL) utilizing a 32-channel radiofrequency head coil. Analyses were carried out specifically on:

- Axial T2*-weighted Echo Planar Imaging (repetition time = 2600ms; echo time = 35ms; slices = 35; volumes = 200; slice thickness = 4 mm; matrix size = 96 x 94, field of view = 230 x 230 mm^2).

5.1.2.4. MRI preprocessing

First, all resting state scans were initially slice-time corrected in order to compensate for the delay in time of acquisition between slices of each brain volume (100 volumes for each of the two sessions). Second, each session was realigned independently by using the 4th Degree B-Spline Interpolation option. This step was needed in order to correct for possible head movements occurred in between the

acquisition of different volumes. Indeed, mean volumes were created as reference and parameters of linear and rotational head motion were estimated. Graphical reports were visually inspected to ensure linear and rotational head movements would not exceed, respectively, ±3 mm and ±3° to avoid negative consequences on subsequent analyses. Third, realigned images were normalized using the first realigned volume of the first session as source image to match the default echo-planar template available in SPM 8, and voxel size was isotropied at 2.0 × 2.0 × 2.0 mm. Normalization of resting-state scan was applied in order to account for differences in head size and shape and to facilitate subsequent statistical analyses. Fourth, a band-pass filter was applied to normalized scans with the aim of removing noise frequencies not believed to result from the expression of neural activity. This was carried out using the REST toolbox (www.restfmri.net) (Song et al., 2011). Similar to the majority of studies on BOLD signal, a low-pass filter was set at 0.1 Hz to eliminate frequencies generated by physiological mechanisms and a high-pass filter was set at 0.008 Hz to remove low-frequency scanner drifts (Fox and Raichle, 2007). Finally, band-pass filtered volumes were spatially smoothed with a 6 mm^3 full-width at half maximum Gaussian kernel to account for any possible inter-subject differences remaining after normalization, and to improve signal-to-noise ratio.

After pre-processing, group-level ICA was performed on all baseline resting state fMRI scans using the GIFT toolbox for SPM8 (GIFT v1.3i; mialab.mrn.org/software/gift) (Calhoun et al., 2001) to identify several sample-specific functional networks for further analyses. The general aim of an ICA is to separate sources of signal assumed to be independent that have been mixed together in the acquisition of the signal itself.

An initial principal component analysis was carried out to reduce the number of sources of signal variability. The Infomax algorithm was then chosen to perform the ICA on the group and the number of components to extract was set at twenty, commonly accepted as a standard value that allows the detection of the main functional brain networks known and avoids excessive dissociation of signal sources (Wang and Li, 2015). Finally, the ICA procedure ended by reconstructing participant-specific spatial maps of each one of the twenty components estimated. This study focused on six different networks already investigated in the literature: four extensively invoved in cognitive processes, i.e. the default mode network, the salience network, the right and left fronto-parietal networks, and two control networks associated with sensory and motor functions particularly affected in MS, i.e. the visual network and the sensorimotor network. The z-score maps of these

networks were visually identified and extracted from all individual sets of components for statistical analysis.

5.1.2.5. Statistical analysis

Multiple regression models were created to investigate the association between all the above mentioned PS-dependent tests and each one of the six functional networks extracted from the sample of RS-fMRI scans. For sake of consistency with Experiment 1 and to allow complementary interpretations of the results on structural connectivity, the same covariates were statistically controlled for in each model: age, years of education and TIV.

Only clusters that survived statistical correction for multiple comparisons at a FWE threshold of $p < .05$ were considered. GM areas containing significant peaks highlighted by the analysis were identified by means of the Talairach Daemon (http://www.talairach.org/daemon.html), after converting their coordinates from the MNI to the Talairach reference system (Talairach and Tournoux, 1988).

In consideration of the results obtained from DTI analyses which pointed at a prevalent role of frontal, in addition to interhemispheric, structural connectivity in support of PS function in MS a main research question was first addressed:

1) *Are PS abilities of people with RRMS based on visuo-spatial rather than verbal cognitive operations associated with functional connectivity of the left and right fronto-parietal networks?*

Indeed, the hypothesis was tested that DSCT and the TMT-A scores were expected to correlate with functional connectivity of networks mainly anchored to the frontal lobes. However, based on previously published findings focussed on the role of DMN dysregulation in MS-related cognitive decline, it appeared necessary to extend investigations to other cognitive networks and address the following question:

2) *Is PS function in people with RRMS associated with functional connectivity of the default mode or the salience networks rather than that of fronto-parietal networks?*

Since the DMN, the salience network and the fronto-parietal ones have been observed to be functionally connected to one another, it does not appear trivial to hypothesise that one or more of these networks may contribute to sustain PS-demanding cognitive performance in this clinical population.

Additionally, regression analysis was used to investigate the association between PS performance and functional connectivity of two control networks, namely the visual and the sensorimotor networks, and to answer the following research question:

3) *Is performance on PS-demanding tests specifically dependent on functional connectivity of cognitive rather than perceptual and motor networks in people with RRMS?*

The different cognitive measures used in this study were selected to assess PS-demanding cognitive performance in conditions with little or no demand posed on motor and sensory functions. Therefore, it was hypothesised that no correlations should be detected between PS cognitive performance and functional connectivity of these two networks.

5.1.3. Results

5.1.3.1. Clinical and cognitive results

All the clinical and cognitive characteristics of the patients, who consist in a subsample of those included in Experiment 1, are reported in Table 5.1.

Table 5.1 Clinical and cognitive characteristics of the patient sample (n = 42)

Variable	Mean	SD	Median	Minimum	Maximum	Patients with deficits
Clinical characteristics						
Age (years)	44.6	8.8	45.0	26	65	-
Education (years)	14.0	2.7	13.0	11	19	-
Duration (years)	9.7	7.2	7.5	1	30	-
EDSS	3.4	1.6	3.5	0	6	-
TIV (ml)	1503.2	184.9	1486.0	1361.2	2070.9	-
TLV (ml)	10.6	13.4	6.0	0	47.9	-
Cognitive tests						
PASAT 3”	39.0	15.9	42.5	9	59	11
PASAT 2”	23.4	13.9	21	4	55	15
DSCT	62.7	15.8	61	36	96	14
TMT-A (sec)	39.5	15.8	37.5	21	86	6
Stroop speed (sec)	17.6	3.6	17.7	10	27.5	5
Phonemic fluency	32.2	9.5	31	17	58	1
Semantic fluency	45.3	10.1	43.5	21	67	1

DSCT: Digit Symbol Coding Test, EDSS: Expanded Disability Status Scale, PASAT: Paced Auditory Serial Addition Test, TIV: Total intracranial volume, TLV: Total lesion volume, TMT: Trail Making Test

5.1.3.2. Resting-state fMRI results

First, correlations between scores of all the cognitive tests and functional connectivity maps of the fronto-parietal networks were investigated. Connectivity of the right fronto-parietal network was not correlated with PS-dependent cognitive performance on any of the tests. However, unexpected results were highlighted for the left fronto-parietal network: neither the DSCT nor the TMT-A scores were found to be associated with this network, against predictions made on the basis of the results observed in Experiment 1. These tests, in fact, were thought to be sensitive cognitive measures that could capture alterations in brain connectivity, also at the functional level. Results related to tests of verbal PS function were mixed: both PASAT versions yielded null results, while Stroop speed scores were negatively correlated with functional connectivity between the left fronto-parietal network and the right posterior cingulate cortex (Table 5.2 and Figure 5.1).

Table 5.2 Correlations between performance on PS-dependent tests and functional connectivity of the left fronto-parietal network ($p < .05$ FWE)

Cognitive variable	Cluster extent	r	Side	Brain region	*t* value	MNI coordinates		
						x	*y*	*z*
Stroop speed*	73	-.628	R	PCC (BA 31)	5.26	28	-44	30
			R	PCC (BA 31)	4.05	28	-52	28
Phonemic fluency†	115	.605	R	SFG (BA 8)	4.86	4	32	56
			R	SFG (BA 8)	4.10	2	36	46
Semantic fluency†	100	.481	R	PCG (BA 4)	5.06	60	-6	22
			R	PCG (BA 6)	4.14	54	-8	34
			R	PCG (BA 6)	3.73	46	-8	32

* negative correlation

† positive correlation

BA: Brodmann area, PCC: posterior cingulate cortex, PCG: Precentral gyrus, SFG: superior frontal gyrus

Consistently, the total scores obtained by patients on the fluency tasks were directly associated with functional connectivity between the same network and different frontal areas and, in particularly, the prefrontal cortex for the phonemic fluency task and the motor and premotor cortices for the semantic fluency task (Table 5.2 and Figure 5.1).

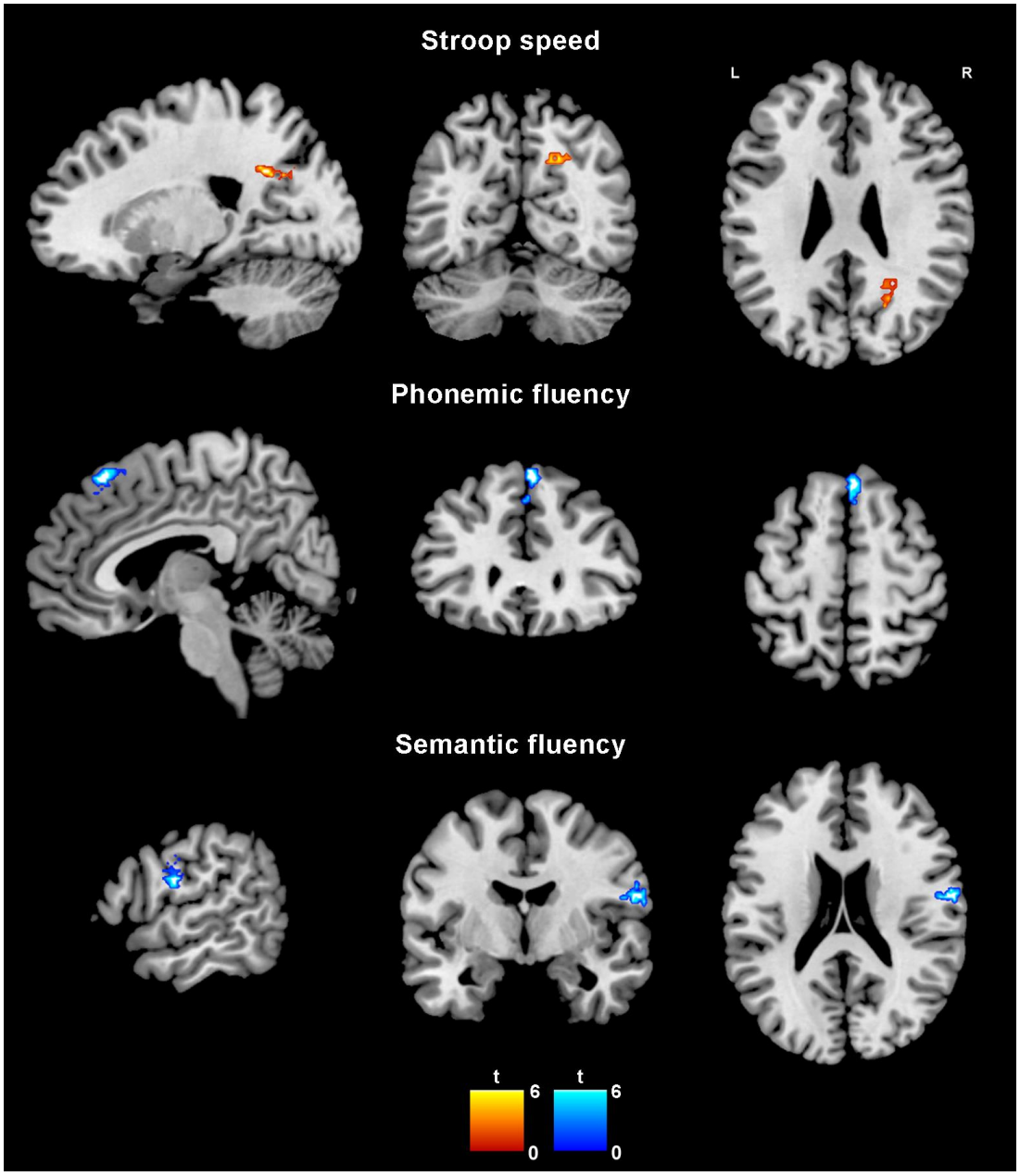

Figure 5.1 Negative (red) and positive (blue) correlations between PS-dependent performance and functional connectivity of the left fronto-parietal network ($p < .05$ FWE)

Second, the same analytic procedure was applied to other previously mentioned cognitive networks: the default mode network whose connectivity with the cerebellum was found to be negatively associated with performance on the semantic fluency task; and the salience network, found to be associated with both the PASAT (only 3s version) and the TMT-A. In particular, the PASAT scores were negatively correlated with connectivity between the salience network and the posterior cingulate cortex, while the TMT-A scores were positively correlated with functional coupling with a network of frontal, parietal and cerebellar areas (Figure 5.2 and Table 5.3).

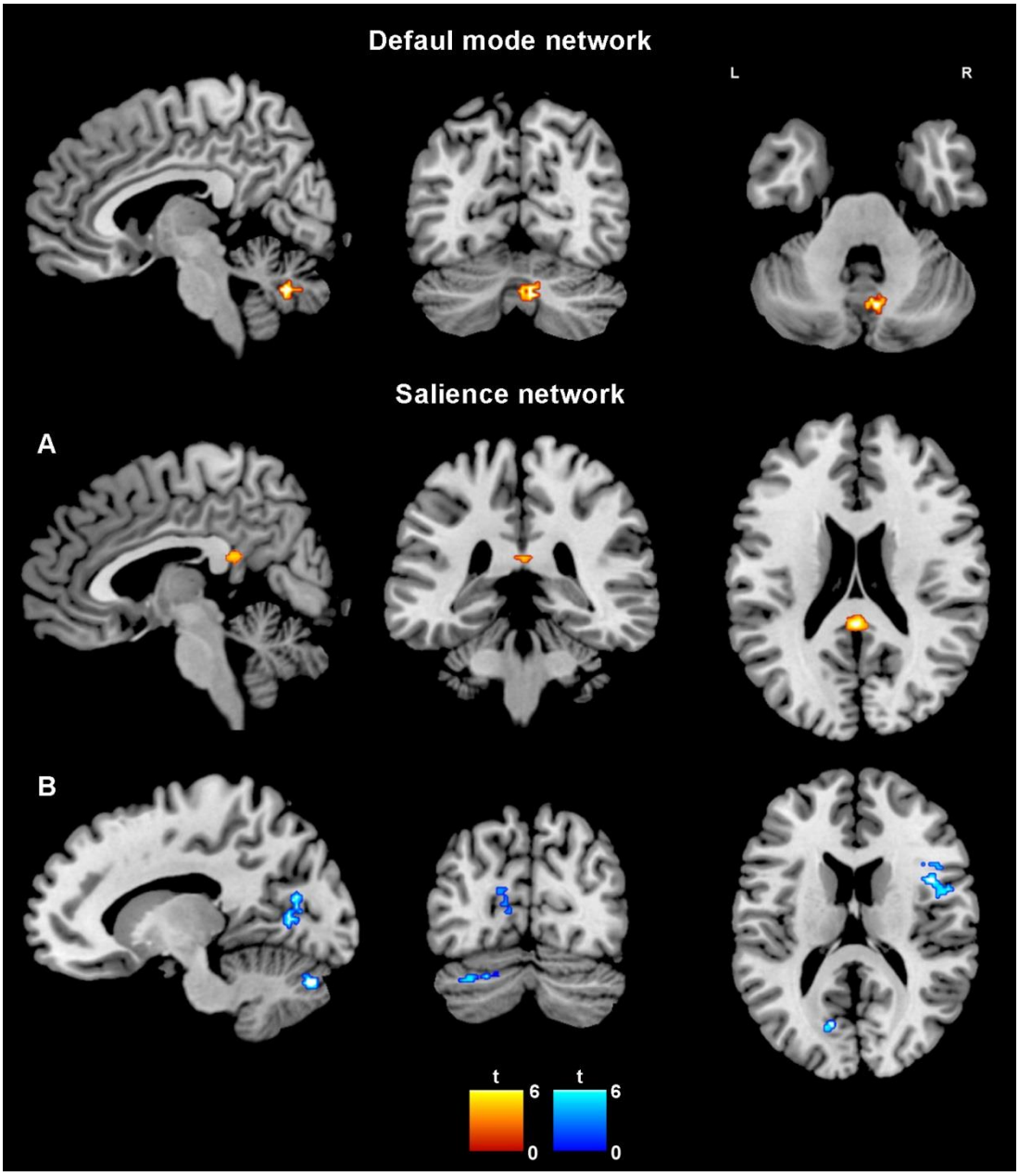

Figure 5.2 Negative (red) and positive (blue) correlations between semantic fluency scores and functional connectivity of the default mode network (top row) and between functional connectivity of the salience network and performance on the PASAT 3" (A) and TMT-A (B) ($p < .05$ FWE)

Table 5.3 Correlations between performance on PS-dependent tests and functional connectivity of the default mode and salience networks (p < .05 FWE)

Cognitive variable	Cluster extent	r	Side	Brain region	*t* value	MNI coordinates		
						x	*y*	*z*
Default mode network								
Semantic fluency*	69	-.541	R	Nodule	5.82	8	-64	-32
			R	Uvula	4.21	0	-64	-36
			R	Declive	4.08	20	-60	-28
Salience network								
PASAT 3"*	76	-.634	L/R	PCC (BA 23)	6.22	0	32	20
TMT-A†	91	.745	L	PCC (BA 30)	5.07	-16	-68	4
			L	Cuneus (BA 18)	4.48	-12	-72	16
			L	Lingual gyrus (BA 18)	4.32	-8	-72	4
	102	.678	R	Declive	4.97	-14	-78	-26
			R	Declive	4.69	-22	-76	-28
			R	Declive	4.07	-30	-78	-30
	140	.714	R	IFG (BA 44)	4.84	46	10	20
			R	IFG (BA 44)	4.26	52	4	12
			R	IFG (BA 45)	4.20	46	18	18

* positive correlation

† negative correlation

BA: Brodmann area, IFG: inferior frontal gyrus, PASAT: Paced Auditory Serial Addition Test, PCC: posterior cingulate cortex, TMT: Trail Making Test

Finally, control analyses were carried out on the visual and the sensorimotor networks with the expectation not to detect any significant associations between PS performance and connectivity of either networks. However, TMT-A scores correlated with functional coupling of the sensorimotor network with motor areas, positively, and with the right inferior frontal gyrus, negatively (Table 5.4 and Figure 5.3).

Table 5.4 Correlation between TMT-A scores and functional connectivity of the sensorimotor network (p < .05 FWE)

Cognitive variable	Cluster extent	r	Side	Brain region	value	MNI coordinates		
						x	*y*	*z*
Positive correlation								
TMT-A	398	.676	R	PCG (BA 6)	5.80	48	-16	26
			R	PCG (BA 43)	4.97	60	-10	10
			R	PCG (BA 4)	4.94	64	-10	28
	315	.604	L	PCG (BA 43)	5.19	-54	-6	14
			L	Insula (BA 13)	5.05	-44	-12	26
			L	PCG (BA 4)	4.22	-60	-4	28
Negative correlation								
	73	-.687	R	IFG (BA 44)	5.25	48	6	16
			R	IFG (BA 44)	4.28	54	12	18
			R	IFG (BA 9)	4.13	46	14	24

BA: Brodmann area, IFG: inferior frontal gyrus, PCG: Precentral gyrus, TMT: Trail Making Test

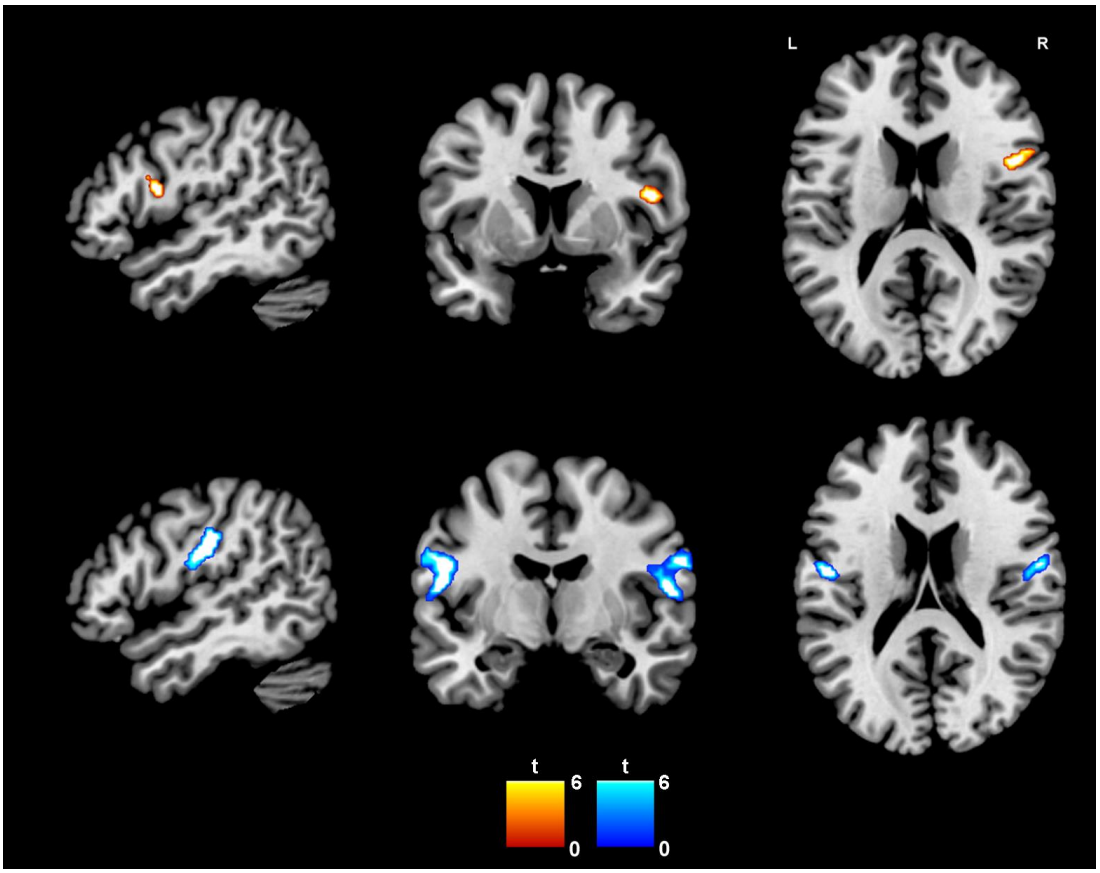

Figure 5.3 Positive (red) and negavtive (blue) correlations between TMT-A scores and functional connectivity of the sensorimotor network ($p < .05$ FWE)

5.1.4. Discussion

Coherently with findings from Experiment 1 that highlighted a strong association between integrity of frontal structural connectivity PS function in RRMS, functional connectivity of the left fronto-parietal network was found associated with several of the PS-dependent test considered in this study. This network supports attentional and executive processes that are necessary to coordinate other cognitive functions (Coull et al., 1996). Associations were observed both for elementary PS measures, namely the Stroop speed, and more complex ones, i.e. the fluency tasks. Moreover, it was systematically noticed across tests that better PS performance was associated with stronger functional connectivity between the left fronto-parietal network and either bilateral (phonemic fluency) or right-lateralised (Stroop speed and semantic fluency) frontal and parieto-limbic areas. These findings support the hypothesis that efficient integration of information across both hemispheres in several areas involved in cognitive control and motor planning contributes to faster processing. Indeed, the dorsal posterior cingulate, found to be linked to performance

on the Stroop speed index, correlates at rest with attentional networks and may play a role in attention allocation (Leech et al., 2011).

On the contrary, the right fronto-parietal network was not associated with any PS-demanding tasks, independently of the cognitive processes/modalities engaged. In particular, differently from what emerged from Experiment 1, no significant correlations were detected with scores on either the DSCT or the TMT-A, mainly dependent on visual and visuo-spatial processes associated prevalently with right frontal and parietal activations. Therefore, the left lateralisation observed in these findings may support the hypothesis of a stronger role of this hemisphere in PS performance. Alternatively, it may be due to the fact that all of the above mentioned tests fundamentally require processing of verbal information mainly elaborated by areas in the left hemisphere.

The investigation of the DMN showed that only the semantic fluency test was negatively correlated with the strength of functional connectivity between this network and the right cerebellum. This result may be interpreted as the need for a functional dissociation between the DMN, not active during active task performance, and a cerebellar area consistently observed to contribute to execution of this test (Gurd et al., 2002, Schlosser et al., 1998). However, none of the PS measures were correlated with resting-state activity of the DMN, contrarily to what reported by some studies (Wojtowicz et al., 2014, Zhou et al., 2014). It is worth highlighting that Janssen et al. (2013) found no correlations between a robust PS composite score and functional connectivity of any of the functional networks investigated, DMN included.

On the contrary, the salience network appeared significantly associated with two PS measures: the PASAT 3" and the TMT-A. In particular, negative effect on performance across both tasks of increased coupling at rest between this network and various bilateral frontal, cerebellar and posterior cingulate areas involved in different executive (Sestieri et al., 2014, Vallesi, 2014) and visuospatial processes (Maguire, 2001) was observed. Hence, it appears that a functional dissociation between the salience network and, on one hand, the posterior DMN and, on the other hand, areas of the right fronto-parietal network may facilitate PS performance. Indeed, these networks are negatively correlated to one another (Uddin et al., 2009) and the integrity of the salience network has been observed to be a predictor of DMN functionality after traumatic brain injury (Bonnelle et al., 2012). Therefore, the salience network may play a crucial role in enabling efficient deployment of attentional resources that allow effective information processing.

Bidirectional correlations emerged between completion time on the TMT-A and connectivity of the sensorimotor network, possibly due to the importance of motor function in the execution of this test. More specifically, higher completion times were predicted by higher connectivity with areas within the network and by lower connectivity with the right inferior frontal gyrus. These findings seem to suggest that functional segregation of the sensorimotor network and lack of integration with executive networks may impact motor execution as well as psycho-motor PS function.

It can be argued that the results obtained in this study show that different functional networks anchored to frontal hubs (the salience and the left fronto-parietal ones) appear to be associated with PS performance in people with RRMS. This is in line with what expected on the basis of the results obtained in Experiment 1 and hypothesised in the first research question investigated. However, discrepancies have emerged between the two experiments with respect to the measures of PS abilities detected as significantly associated with such frontal areas. In fact, while in Experiment 1 mainly visuo-spatial PS abilities seemed sensitive to variable degrees of microstructural WM integrity, in Experiment 3 different measures of PS function based on elaboration of verbal material were mainly highlighted in the analyses. This situation may be explained by the fact that the match between structural and functional connectivity is not straightforward and perfectly bidirectional (Damoiseaux and Greicius, 2009). Moreover, MS pathology may differentially affect these two neural levels, thus influencing the associations that can be detected. Indeed, Tona et al. (2014), by adopting an ROI approach, found that performance on the PASAT correlated only with thalamic functional connectivity but not with DTI indices. The exact opposite scenario has been observed by Zhou et al. (2016) hence hinting that current knowledge on the interplay between cognitive performance and different connectivity measures affected by MS is not conclusive.

Additionally, a quite considerable level of variability was seen in relation to the fact that not all PS-dependent measures investigated were found to be associated with the same functional networks. The most compelling explanation of such variable results appears to rely on the diversity of cognitive functions underlying the PS measures collected. This means that different cognitive tests may not be interchangeable in the assessment of the same function and may depend on different neural correlates. Another caveat may be in the fact that networks' resting-state activity probably enables only partial characterisation of the status of a cognitive function that is essentially dynamic, such as quickly performing cognitive processes (van Geest et al., 2018).

In line with the latter point, the findings from Experiment 3 suggest that integrity of PS function in RRMS may depend on functional connectivity strength between different networks rather than on a single one. Indeed, Gamboa et al. (2014) found that MS-caused lack of communication between functionally specialised brain modules and may affect fast cognitive operations that depend on integration of different types of information across brain areas and hemispheres. On the contrary, functions that are eminently lateralised in one hemisphere rely less on information transfer because performed by more localised networks (Simon-Dack et al., 2015). Therefore, understanding the relevance of balance between functional segregation and integration across brain networks seems to be a relevant issue for further investigating PS function decline in MS.

5.2. Experiment 4 – Associations between measures of PS function and functional connectivity in people with SPMS

5.2.1. Introduction

It has been established that people affected by RRMS generally experience a transition to SPMS about 10 years after disease onset (Rovaris et al., 2006). Therefore, the two phenotypes can be said to constitute a clinical continuum in the evolution of the disease that may prove challenging to disentangle, especially with respect to the transition phase (Larochelle et al., 2016). However, differences in neural and clinical manifestations allow the distinction of these phenotypes, both qualitatively and quantitatively. Indeed, Faivre et al. (2016) applied graph-theory-based analysis to study longitudinally disease progression showing that decreases in brain functional connectivity, i.e. disconnection between different brain areas, are linked to steady increase in disability levels. Similarly, from a clinical point of view cognitive symptoms have been observed to present to a greater extent in people with SPMS compared to those with RRMS, especially regarding PS function (Archibald and Fisk, 2000, De Sonneville et al., 2002, Denney et al., 2004, Papathanasiou et al., 2014, Ruet et al., 2013b).

Nevertheless, the use of functional neuroimaging techniques has received little attention so far in the investigation of the neural features that characterise SPMS and its phenotypical expression (Brown and Chard, 2016). Most efforts to clarify the neural correlates of cognitive impairment in this disease, in fact, have been focussed on RRMS. Small groups of people with SPMS have often been included alongside, but rarely examined independently. Therefore, the scarcity and variability of the literature in this field limits any consideration of the currently available findings that are to be considered preliminary.

Two approaches have been used to study functional reorganization related to cognitive dysfunction in SPMS: analysis of task-related and resting-state brain activity. Loitfelder et al. (2011) found people with SPMS, despite being more cognitively impaired, performed as well as people with CIS on a go-no go discrimination task. However, stronger and more widespread cerebral activations were observed for people with SPMS across prefrontal, parietal and temporal associative areas also in the least challenging experimental condition. This finding was interpreted in terms of functional compensation needed in order to sustain cognitive performance in more advanced stages of the disease. Rocca et al. (2012a) showed altered patterns of connectivity of the right cerebellum with fronto-parietal

areas during the performance of incongruent trials of the Stroop task for people with SPMS compared to those affected by RRMS. The observed functional alterations were associated with worse cognitive performance in the SPMS group. Taken together, these results seem to suggest that different processes of reconfiguration of brain activity may occur, but the specific valence of these changes in relation to cognitive performance remains unclear. Moreover, mainly executive functions have been investigated, while other cognitive abilities more commonly observed impaired, such as memory and PS, received little attention.

Resting-state functional MRI analyses revealed mixed results regarding the direction of changes in functional connectivity of the DMN. In fact, both decreases (Rocca et al., 2010b) and increases (Basile et al., 2014) in resting-state activity of this network have been reported for people with SPMS compared to healthy controls. In particular, it was found that these changes appeared more pronounced in SPMS than in RRMS. Nonetheless, both studies revealed a similar involvement of the anterior cingulate cortex in PS performance: total score of correct items (Rocca et al., 2010b) and errors on the PASAT 3" (Basile et al., 2014). The relationship between the DMN and the salience network, whose core hub is represented by the anterior cingulate, appears to be crucially supporting PS abilities in SPMS. However, the investigation of how fast information processing might be potentially affected by disruption in the salience network or of other important cognitive networks, namely the two fronto-parietal networks, has been utterly neglected. Only Basile et al. (2014) showed that increased functional connectivity of the sensorimotor network in SPMS is significantly higher than in healthy controls but lower than in RRMS. Yet these changes were not found to be associated with performance on the PASAT.

In consideration of the findings outlined above the scenario appears fragmented, highly inconsistent and minimally helpful for explaining the functional correlates of cognitive slowing that characterises people who have transitioned to SPMS. Hence, this study will address this issue to investigate which functional networks (cognitive, sensory or motor) may be associated with PS-dependent cognitive performance in this poorly studied MS phenotype.

5.2.2. Methods

5.2.2.1. Participants

Twenty-five people affected by SPMS according to criteria by (Lublin and Reingold, 1996) from the cohort recruited for Experiment 2 were included in this study. One individual was excluded after preprocessing due to issues related to high degree of

head motion, while five participants were not able to complete the full MRI assessment.

This study was carried out according to the Declaration of Helsinki and was approved by the Institutional Review Board of the IRCCS Fondazione Ospedale San Camillo (Venice, Italy) (Protocol N. 11/09 version 2) (Appendix G). Written informed consent was obtained from each study participant.

5.2.2.2. Neuropsychological assessment

The same neuropsychological assessment carried out in Experiment 2 was used in this study, comprising scores on: the Digit Cancellation Test, the Stroop test and the TMT (used to create the PS_{CI}) and the addition of the phonemic and semantic fluency tests.

5.2.2.3. MRI acquisition

Patients were scanned on a 1.5 T Philips Medical Systems Achieva scanner (Best, the Netherlands) with a standard head coil in order to collect functional MRI scans:

- Axial T2*-weighted Echo Planar Imaging (repetition time = 2000 ms, echo time = 50 ms, slices = 20, slice thickness = 6 mm, matrix size = 72 x 71, field of view = 230 x 120 mm^2).

5.2.2.4. MRI preprocessing

The same preprocessing procedure as explained for Experiment 3 was followed for resting-state scans to obtain network images for statistical analysis.

5.2.2.5. Statistical analysis

The analysis of the association between PS performance and functional connectivity across functional networks was carried out following the same procedure used in Experiment 3.

Structural connectivity analysis that showed frontal connections, especially left-lateralised, emerged as correlates of PS abilities in people with SPMS. Therefore, a first main question was elaborated:

1) *Are PS abilities of people with SPMS differentially associated with functional connectivity of the two fronto-parietal networks?*

The prediction is that scores on fluency tasks would be preferentially associated with connectivity of the left fronto-parietal network. Moreover, considering that connectivity of the DMN was found to be altered and potentially detrimental for cognitive functioning in the SPMS phase, like in Experiment 3, additional analyses were carried out on the default mode and the salience networks to answer the following research question:

2) *Is PS function in people with SPMS associated with functional connectivity of other malfunctioning brain networks mainly involved in cognitive processing?*

Indeed, as the disease progresses it seems likely that widespread brain damage may impact negatively on the functional reorganization of the brain leading to failure in information integration across several networks. If this were the case, PS function would be expected to be associated with functional connectivity across a plurality of networks others than the fronto-parietal ones.
Finally, the visual and the sensorimotor networks were investigated to clarify the following issue:

3) *Is performance on PS-demanding tests specifically dependent on functional connectivity of cognitive rather than perceptual and motor networks in people with SPMS?*

This choice was made because, given the general exacerbation of neurological symptoms in SPMS, compared to RRMS, it cannot be ruled out that changes in information processing at levels other than those prominently cognitive may affect PS performance.

5.2.3. Results

5.2.3.1. Clinical and cognitive results

The clinical profile of the sample of patients, a subsample of those included in Experiment 2, is summarised in Table 5.5.

Table 5.5 Clinical, cognitive and volumetric characteristics of the sample (n = 25)

Variable	Mean	SD	Median	Minimum	Maximum	Patients with deficits
Clinical characteristics						
Age (years)	55.8	11.0	56	36	70	-
Education (years)	10.0	2.6	9.5	5	13	-
Duration (years)	16.6	8.0	15.5	5	30	-
EDSS	6.5	1.1	7	4.5	8	-
FSS	4.9	1.2	4.7	2.7	7	-
TIV (ml)	1659.0	179.1	1659.2	1341.3	2044.4	-
TLV (ml)	22.9	18.4	20.6	1.2	82.0	-
Cognitive tests						
PS_{CI}	0.1	1.0	-0.2	-1.1	2.4	-
Stroop speed	17.9	5.6	16.7	10.5	37.0	7
TMT-A/DCT	0.6	1.0	-0.1	-1.3	2.9	15
Phonemic fluency	28.8	12.0	32	8	48	5
Semantic fluency	38.8	12.2	35.5	20	61	5

DCT: Digit Cancellation Test, EDSS: Expanded Disability Status Scale, FSS: Fatigue Severity Scale, PS_{CI}: Processing speed composite index, TIV: Total intracranial volume, TLV: Total lesion volume, TMT: Trail Making Test

5.2.3.2. Resting-state fMRI results

The analyses carried out on the PS_{CI} and the fluency tasks yielded, unexpectedly, only null results across all brain networks independently of their functional role: either prominently cognitive or sensory/motor.

Failure to detect any significant correlations prompted supplementary analysis on the PS_{CI} by disentangling two distinct components: verbal (Stroop speed) and visuo-spatial (TMT-A + Digit Cancellation Test). Parallel analyses were performed to investigate whether more basic PS measures than fluency tasks would be associated with functional connectivity of the expected networks differentially depending on the modality of information processing. The expectation, based on findings from Experiment 2, was that the verbal PS component would correlate more significantly than its visuo-spatial counterpart.

Indeed, as expected Stroop speed scores correlated negatively with connectivity of the left fronto-parietal network with the right inferior frontal gyrus (Table 5.6 and Figure 5.4). Additionally, verbal PS abilities emerged to be positively associated with functional connectivity of the anterior cingulate cortex in the salience network (Table 5.6 and Figure 5.4).

Table 5.6 Correlations between performance on the Stroop speed index and functional connectivity of the left fronto-parietal and salience networks (p < .05 FWE)

Cognitive variable	Cluster extent	r	Side	Brain region	*t* value	MNI coordinates *x*	*y*	*z*
				*Left fronto-parietal network**				
Stroop speed	90	-.810	L	IFG (BA 47)	6.31	-36	22	-12
			L	IFG (BA 47)	5.09	-36	22	-2
				Salience network†				
	108	.784	L	ACC (BA 33)	4.95	-8	8	30
				ACC (BA 24)	4.82	-4	12	30

* negative correlation

† positive correlation

ACC: posterior cingulate cortex, BA: Brodmann area, IFG: inferior frontal gyrus, PASAT: Paced Auditory Serial Addition Test

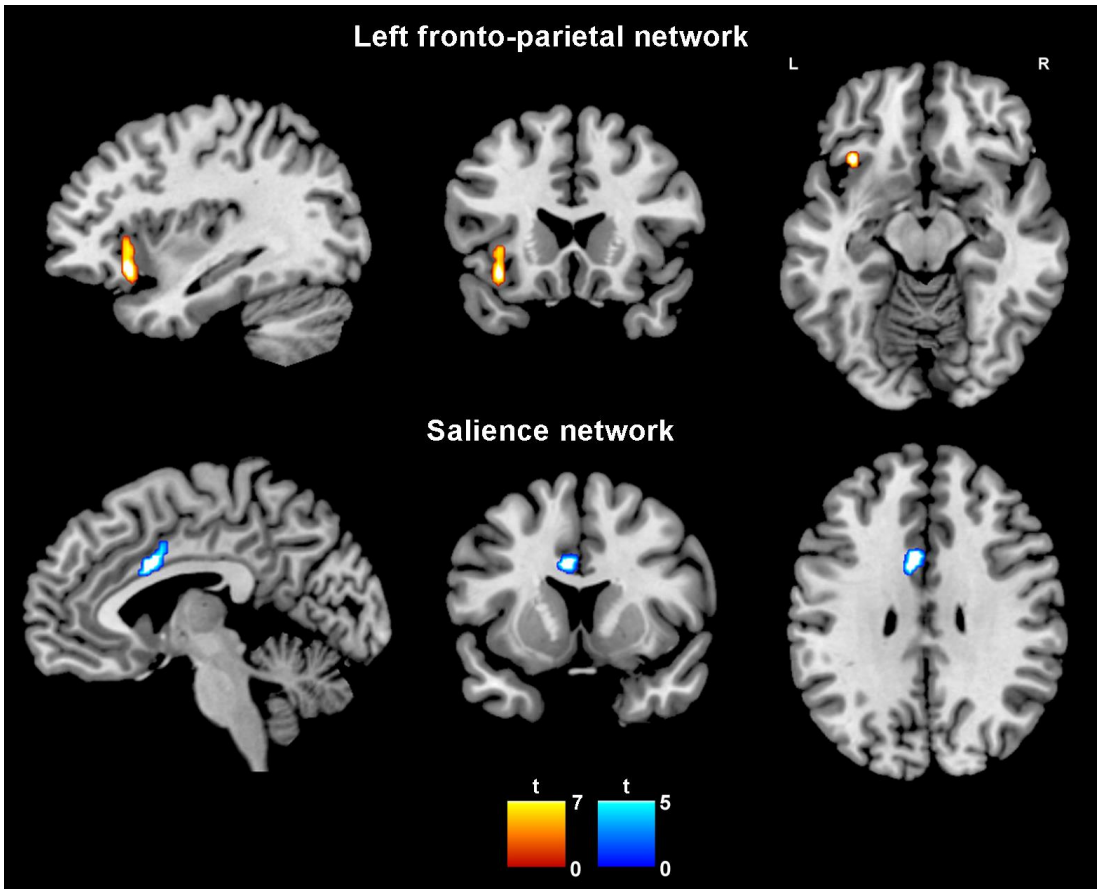

Figure 5.4 Correlations (negative in orange and positive in blue) between Stroop speed scores and functional connectivity of the left fronto-parietal and salience networks (p < .05 FWE)

5.2.4. Discussion

The results of this experiment showed that only one of the different PS measures investigated (the Stroop speed index) was significantly correlated with functional connectivity of the left fronto-parietal network as emerged also from analysis on the

RRMS cohort. However, differently from Experiment 3, slower performance on the Stroop test was associated with lower functional connectivity between this network and the left inferior frontal gyrus: an area associated with oral verbal production and semantic processing (Hagoort, 2005, Sowman et al., 2012). Moreover, these results appear compatible with those on the structural correlates of PS. Indeed, in Experiment 2 scores on the same test were shown to be associated with microstructural integrity levels of the inferior fronto-occipital fasciculus, a WM tract connecting the inferior frontal gyrus to occipital areas and found to be involved in semantic functions (Sarubbo et al., 2013).

The analyses carried out to answer the second research question revealed that also connectivity of the salience network with the rostral anterior cingulate cortex (part of the DMN) was associated with patients' PS abilities. In particular the stronger the association between these two networks the slower was cognitive performance. Interestingly, this finding has already been repeatedly observed in the literature on DMN alterations in SPMS (Basile et al., 2014, Rocca et al., 2010b). However, the analysis carried out in this experiment on the DMN maps showed no significant associations with PS performance. The interpretation of the discrepancy between this result and the findings of the studies reported above remains uncertain. Indeed, they both included the anterior cingulate as part of the DMN although this structure is considered a central hub of the salience network (Seeley et al., 2007). However, it cannot be ignored that brain networks are functionally associated with one another (Sridharan et al., 2008) and, hence, fast cognitive processing may be affected by between- rather than within-network disconnections, e.g. decoupling between the default mode and salience networks.

On the contrary, no involvement of motor or sensory networks was highlighted, thus showing how PS decline detected also in simple tasks may be attributed mainly to cognitive causes. Indeed, in Experiment 3 only scores obtained on the TMT, a test that relies on eye-hand coordination, were associated with connectivity of the sensorimotor network. Indeed, functional analyses showed no significant results for the index of visual PS replicating the results on structural connectivity. This may suggest that PS-dependent tests based on processing of verbal material might be more sensitive to neural disruption in later stages of MS.

However, the investigation of the fluency tasks failed to detect associations with functional connectivity of any of the investigated networks contrary to expectations based on the results of Experiment 2. First, a possible explanation may be found in the fact that the accumulation of structural damage is the main cause driving cognitive symptoms (Preziosa et al., 2017) and functional alterations in MS (Droby

et al., 2015b). Therefore, reorganization of functional brain architecture may occur in the early stages of the disease and contribute to support cognitive performance (Faivre et al., 2016). The increasing severity of brain insults may lead to the depletion of these adaptations and produce a drop in variance both in cognitive performance and functional connectivity that may, in turn, prevent the identification of associations between cognitive and neural variables. Second, it must be stressed that the relationship between static structural connectivity and dynamic functional connectivity appears not straightforward and our current understanding of it is still highly limited (Fjell et al., 2017, Tsang et al., 2017). Third, it cannot be ignored the fact that the limited sample size of this study (a subsample of all patients recruited for Experiment 2) may have negatively affected statistical power in the present study.

From these analyses the left fronto-parietal network seems to represent the main functional neural correlate of verbal PS abilities exhibited by people with SPMS in line with previous findings on structural connectivity. Nevertheless, functional differentiation between the DMN and the salience network emerged as a crucial aspect related to cognitive functioning in this clinical population.

5.3. General discussion

The results of the experiments reported in this chapter point towards a central role of the left fronto-parietal network in supporting performance on tests requiring fast information processing, e.g. the Stroop speed index. In fact, this network emerged consistently in the analyses carried out on two cohorts of patients affected by RRMS and SPMS and across tests characterised by PS demands. Yet the implications of its involvement may assume differential significance in distinct phases of the disease as suggested by the associations observed in the two studies: fast performers exhibited higher functional coupling between the left fronto-parietal network and the right posterior cingulate, in the RRMS group, and with the left inferior frontal gyrus in the SPMS group. Therefore, it may be argued that the left fronto-parietal network supports PS abilities by interacting with areas involved in allocation of attentional resources in early MS (Leech et al., 2011). As the disease and cognitive impairments progress, interactions with hubs involved in cognitive control of information processing become more important (Jefferies, 2013). However, a longitudinal assessment of both cognitive and neuroimaging features should be implemented to test this hypothesis.

Although this network is mainly thought to be involved in language-based processes, e.g. verbal working memory (Yamashita et al., 2015), it is likely to be engaged in various cognitive functions. Indeed, together with its counterpart in the right hemisphere it supports not only attentional, but also mnestic processes performed on different types of materials (Naghavi and Nyberg, 2005). Indeed, prefrontal and parietal areas comprise associative cortices with several connections across the brain concerned with integrating information and modulating other cortical activity. Interestingly, disruption of functional connectivity of this network has been associated with severity of cognitive fluctuations, i.e. spontaneous alterations in attention and arousal, observed in patients affected by dementia with Lewy bodies (Peraza et al., 2014).

Nevertheless, Cruz-Gomez et al. (2014) have previously highlighted alterations not only in the left but also in the right fronto-parietal network, as well as in the salience network, in patients with MS and cognitive impairments. Hence, decline is likely to be linked to functional neural correlates not confined to a single brain network. In fact, the salience network and, to much lesser extent, the DMN have emerged to be associated with performance on different PS-dependent tests in both experiments reported in this chapter. Indeed, the quick evaluation of stimuli salience may speed up responses to them. Moreover, it is worth noting that the salience network seems to overlap substantially with the so-called cingulo-opercular network (Muller et al., 2016) that is believed to maintain tonic alertness (Sadaghiani and D'Esposito, 2014) and thus potentially important to facilitate efficient information processing.

Convergent findings in these and previously published studies, both in MS and other pathologies, show how different networks may be involved in basic and complex attentional functions and their disruption may affect PS function. However, whether one of these networks has a specific and prominent role in fast cognitive processing over other networks cannot be clarified with these analyses.

What clearly emerges is the need to push investigations further in the attempt to overcome a series of limitations currently present in the literature. First, there is a strong need for more extensive characterisation of disease-stage-dependent alterations in functional neural networks in MS phenotypes and their involvement in manifestation of cognitive symptoms. Indeed, several studies have shown cognitive impairments due to MS are likely to be dependent on scattered disruptions in communications between brain areas beyond the most commonly studied DMN (Meijer et al., 2017, Nejad-Davarani et al., 2016, Rocca et al., 2018). Second, the use of multi-domain tests, that require integration of information across brain areas and networks in a fast way, may represent a fruitful strategy to contribute to the

assessment of cognitive status in people in different stages of MS. Third, the implementation of longitudinal studies may help a deeper understanding of the complex intertwined evolution of functional and structural connectivity changes due to MS pathology, as well as their relevance for cognition (Park and Friston, 2013).

The clarification of the above issues may significantly contribute not only to the understanding of the neural correlates of cognitive symptoms experienced by people affected by MS, but also to the identification of possible MRI markers to be employed in clinical trials. Indeed, it appears necessary to develop objective and reliable outcome measures to test the mechanisms of action and effectiveness of treatments to manage cognitive health in this clinical population.

Chapter 6 | Cognitive rehabilitation to modulate cognitive performance and brain connectivity in people with RRMS

6.1. Experiment 5 – Application of a network-based cognitive rehabilitation programme in a sample of people with RRMS

6.1.1. Introduction

In the past decades, as cognitive manifestations associated with MS became increasingly recognised, the management of these symptoms has captured attention of clinicians and researchers. DMTs and previously developed medications for treating MS have been only marginally effective (see Chapter 1). Non-pharmacological interventions targeted for cognition have been proposed more recently, but this kind of approach has not been always characterised by high quality, and often detailed descriptions of the form of intervention used have been lacking or insufficient (Mhizha-Murira et al., 2017).

The debate on whether cognitive rehabilitation might or might not be a viable strategy to improve cognitive performance in MS is still open. In general, findings appear inconsistent (Mitolo et al., 2015), though computerised training may have the potential to improve memory functions (Dardiotis et al., 2018). Beneficial effects seem to occur across a wider spectrum comprising patients' metacognitive abilities, awareness of disease status, and quality of life (Klein et al., 2017).

The best rehabilitative approach has yet to be defined and it is possible that individual differences and needs may guide the decision-making process. Most interventions which have been tested were individualised, but group-based cognitive rehabilitation was found to be effective in treating cognitive decline due to MS (Rilo et al., 2016). Another unsolved issue relates to the duration of the effects, if any exist. Studies that included follow-up assessments found that post-training improvements in performance on PS and verbal fluency tasks was maintained after six months (Stuifbergen et al., 2018) and even after two years (Mattioli et al., 2016) from completion of the treatment.

As already highlighted in Chapter 2, although PS deficits are commonly observed in patients with MS, to date only a handful of rehabilitative programmes have been specifically designed to train PS and working memory abilities (Hancock et al., 2015,

Hubacher et al., 2015, Vogt et al., 2009). As a result, findings appear only partially comparable across studies due to methodological differences and definite conclusions cannot be drawn. However, reappraising the literature reported in Chapter 2, that shows how a strong the relationship between PS and other cognitive functions is in both people with and without MS, it may be argued that rehabilitating the ability to perform cognitive operations quickly may induce generalised improvements. In fact, a recently published pilot study (Chiaravalloti et al., 2018) found that PS training can induce improvements not only in the pace of information processing, but also in verbal short term memory and timed instrumental activities of daily living.

Previous investigations on PS ability training have been mainly carried out on healthy subjects and used tasks tapping into visuospatial divided attention (Takeuchi et al., 2011). Hence, most studies found improvement in this function, as seen by means of pupillometry (Takeuchi and Kawashima, 2012). Additionally, PS training induced increases in self-reported measures of quality of life and timed instrumental activities of daily living. In a cohort of young adults, Takeuchi et al. (2011) found that rehabilitating basic PS abilities induced not only improvements in simple PS measures, but also in complex arithmetic tasks as well as PS-dependent intelligence measures. However, more recently it was noted that neither working memory nor PS training for healthy adults older than those recruited by Takeuchi et al. (2011) resulted in significant increases in any of the cognitive domains investigated, PS included (Lawlor-Savage and Goghari, 2016). On the other hand, Bayesian analysis of the effects of home-based working memory training seems to suggest the absence of transfer gains to other cognitive functions (De Simoni and von Bastian, 2018). These and other recent findings currently leave many questions related to cognitive rehabilitation still unanswered.

Another scarcely explored aspect resides in the inclusion of MRI outcome measures as part of the objective evaluation of the effects of non-pharmacological interventions for cognitive impairment due to MS. Although resting-state functional MRI has already been shown to provide reliable results in MS (Pinter et al., 2016), only a restricted set of studies can be tracked down in the literature that adopted such approach in combination with neuropsychological testing. Therefore, the use of advanced imaging, considered a promising tool to investigate treatments for MS, appears only marginally explored in its applications to cognition (Mahajan and Ontaneda, 2017).

Weak modulatory effects of rehabilitation targeting specifically episodic memory emerged in only two studies: Ernst et al. (2016) found training-induced increased

activity in fronto-temporal areas that did not survive statistical correction for multiple comparisons; in contrast, Huiskamp et al. (2016) noted no behavioural improvements but increased activation in the fronto-parietal network during performance of the n-back task for the experimental group, even though working memory was not trained. One poorly designed study, including only six participants in the training and four in the control group, investigated the effects of a rehabilitation programme based on Baddeley's working memory model (Hubacher et al., 2015). Qualitative comparisons across individual post-treatment brain changes showed increases in activation over fronto-parietal cortices while performing the n-back task only for one subject and decreases for another one. However, four out of six participants who underwent the training showed no changes in brain functioning different from those in the control group.

Most rehabilitative programmes combined exercises targeting different domains such as attention, executive functions and processing speed. In particular, the RehaCom package appears the most commonly used platform to deliver cognitive rehabilitation (www.schuhfried.at) (Bonavita et al., 2015, Cerasa et al., 2013, Filippi et al., 2012, Parisi et al., 2014a, Parisi et al., 2014b). Different MRI outcome measures have been included across studies, though only a couple explored structural changes. Filippi et al. (2012) investigated the effects of a twelve-week-long training on brain structure in a group of ten people with MS finding no volumetric or WM diffusivity changes. Similarly, Campbell et al. (2017a) found no significant rehabilitation-related changes using magnetic transfer imaging.

More variegated results emerged from analysis of brain activity either during task performance or at rest. However, in general it appears that brain activation in people affected by MS can be increased by cognitive exercises. In particular, post-treatment increases in activation during tasks of working memory were detected in the cerebellum (Cerasa et al., 2013, Sastre-Garriga et al., 2010) and parietal cortices (Campbell et al., 2017a, Cerasa et al., 2013). Stroop-induced brain activity has been found to be particularly enhanced in the posterior DMN and the left DLFPC (Filippi et al., 2012).

Furthermore, changes in resting-state brain architecture have been noted across various functional networks. Increases in functional connectivity of the posterior DMN were found in two studies (Bonavita et al., 2015, Filippi et al., 2012), aside a reduction in the correlation between this network and superior frontal areas (Bonavita et al., 2015). Similarly, the salience network appears to be modulated by cognitive rehabilitation, especially connectivity of the anterior cingulate cortex (Filippi et al., 2012, Parisi et al., 2014b). In fact, increased synchronicity between this area

and the right inferior parietal lobule has been found to sustain cognitive performance of people with MS two years after completion of treatment (Parisi et al., 2014a). Moreover, variations in functional connectivity have been observed across other cognitive, i.e. thalamic (De Giglio et al., 2016), frontal executive and cerebellar (Filippi et al., 2012, Pareto et al., 2018), as well as more perceptual networks, i.e. visual and auditory (Pareto et al., 2018). Although these publications appear to suggest that cognitive rehabilitation may induce neuroplastic changes in people with MS, the clinical significance of these results currently remains difficult to acknowledge fully. In fact, several criticisms shall be taken into account that may limit the generalisation of any interpretations. First of all, to date the number of studies that included MRI outcome measures is relatively small. Moreover, the rehabilitative approaches adopted have mainly been symptomatic (Ernst et al., 2016, Hubacher et al., 2015, Huiskamp et al., 2016) and in general devoid of any *a priori* hypotheses regarding the mechanisms of action. In addition, a range of methodological flaws can be highlighted: 1) all studies recruited small samples of patients and, therefore, current findings can be considered only limited (Cerasa et al., 2013, De Giglio et al., 2016, Ernst et al., 2016, Filippi et al., 2012, Hubacher et al., 2015, Huiskamp et al., 2016, Parisi et al., 2014a, Parisi et al., 2014b); 2) the choice of the control group does not always appear appropriate, e.g. people not affected by MS who did not undergo the same cognitive rehabilitation (Pareto et al., 2018, Sastre-Garriga et al., 2010); 3) in some cases results of MRI analyses appear weak as they did not survive correction for multiple comparisons, probably because of lack of statistical power (Ernst et al., 2016, Hubacher et al., 2015); 4) interaction analyses comparing neural changes between groups have not always been performed, favouring more qualitative analyses (Bonavita et al., 2015, Ernst et al., 2016, Hubacher et al., 2015); 5) in general no active control group has been included in the studies. Hence, current knowledge must be interpreted with caution and no clear conclusions can be drawn on whether cognitive rehabilitation yields consistent changes in people with MS.

In consideration of the shortcomings listed above, the aim of this study was to apply multi-modality MRI to test the effects of a hypothesis-based cognitive rehabilitation programme and to characterise associated neuroplastic changes in people affected by RRMS (Tomassini et al., 2012). Specifically, two research questions were investigated:

1) *Does engaging in multidomain rehabilitative exercises developed to stimulate cross-network functional connectivity (De Marco et al., 2016) have an impact on neural reorganization and cognitive performance?*

Since functional disconnection has been suggested to be the main mechanism underlying cognitive decline in MS (Schoonheim et al., 2015), counteracting this process was hipotesised to exert beneficial effects for patients.

2) *Does engaging in multidomain rehabilitative exercises with high PS demands generate stronger and more generalised neurocognitive effects than in a condition with low PS demands?*

Indeed, it has been shown that PS training for people with MS might generated cognitive improvements in untrained functions such as memory (Chiaravalloti et al., 2018).

6.1.2. Methods

6.1.2.1. Participants

Forty-eight patients affected by RRMS (Lublin and Reingold, 1996) who fulfilled the modified McDonald diagnostic criteria for RRMS (Polman et al., 2010) were recruited at the MS clinic at Sheffield Teaching Hospitals NHS Foundation Trust (UK). Inclusion and exclusion criteria were the same as in Experiment 1 (Chapter 4). Pharmacological treatment had been stable for all patients over the whole year before recruitment: 22 were not receiving any DMTs, 11 were receiving interferone beta 1a, 7 natalizumab, 6 fingolimod, 1 glatiramer acetate and 1 dimethyl fumarate.

6.1.2.2. Procedure

Ethical approval was obtained from the Regional Ethics Committee of Yorkshire and Humber (Ref No: 12/YH/0474) (Appendix D). At the time of clinic visit, all participants who met the inclusion criteria and were willing to take part in the study were provided with written information material after a brief introduction on the study requirements (Appendix E) (Figure 6.1). One week later they were contacted via telephone to confirm their willingness to volunteer. A first appointment was booked during which participants gave written consent to take part in the study (Appendix F) and the full neuropsychological assessment was carried out. An MRI scanning session was carried out within three days from then neuropsychological

assessment. Subsequently, from week 2 to week 5 patients were assigned without concealed allocation to either a non-active control group or to one of two treatment groups: standard cognitive rehabilitation programme or PS-loaded programme. The treatment started the week following the baseline assessment. Two patients dropped out after seven and eight sessions of cognitive rehabilitation respectively. Additionally, one patient from the control group was excluded after observing abnormally enlarged ventricles, thus leaving the total number of participants who completed the study to forty-five (fifteen in each group). Neuropsychological and MRI assessment were repeated the week following the end of the rehabilitation programme or after four weeks of usual care for participants in the control group.

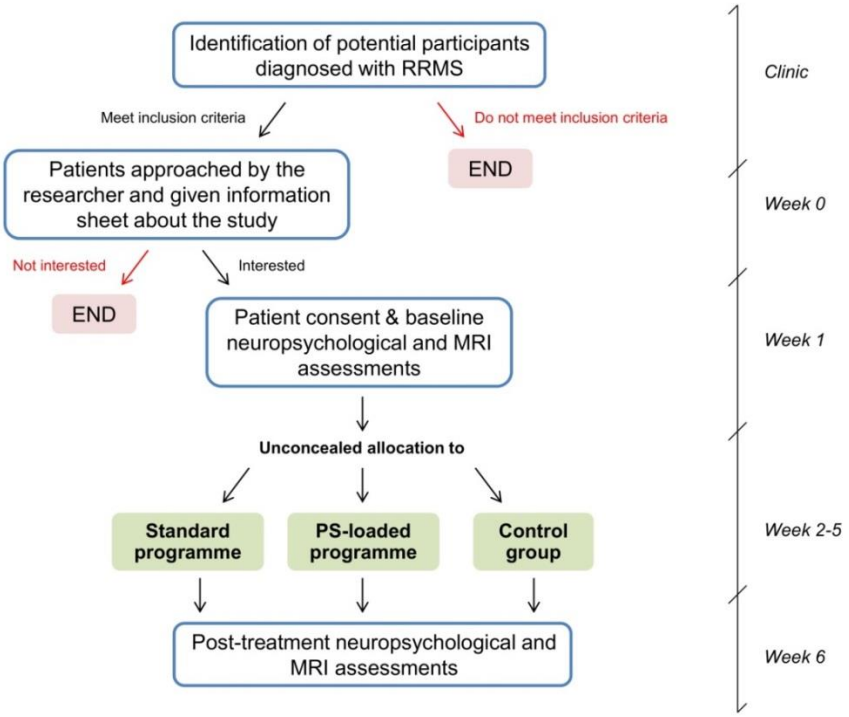

Figure 6.1 Study design

6.1.2.3. Cognitive rehabilitation programme

Participants in the treatment groups received the intensive programme of cognitive rehabilitation over twenty sessions: five days a week for four consecutive weeks. Participants were allowed to catch up with any missed sessions at the end of the four weeks. Each treatment session lasted for about one hour and was performed under the supervision of a neuropsychologist. The non-active control group received no rehabilitation, but care as usual, e.g. DMTs, physiotherapy and engagement in activities of patient groups: all these activities involved different types of social contact but not with the researcher.

The rehabilitation programme was computer-based and comprised tasks specifically designed to target multiple cognitive domains by promoting the synchronous co-activation of multiple brain areas. The expectation was to counteract functional disconnection usually observed in several neurological conditions including MS. All tasks were administered through the E-Prime Software, Version 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA). The program was not tailored to individual performance and the difficulty level was fixed across each of the experimental groups. In particular, the standard cognitive rehabilitation exercises were developed and adapted from previously used methodology (De Marco et al., 2016, De Marco et al., 2018) with four clusters according to the most prominent cognitive domain: PS/attention, working memory, semantic knowledge and logical reasoning.

Four tasks were designed to exercise PS and sustained attention abilities considered to be fundamentally affected by MS (Costa et al., 2017) and related to cognitive efficiency (Rypma et al., 2006) (Figure 6.2). Indeed, it has been reported that faster and more efficient individuals exhibit less activation in prefrontal regions and higher activations in parietal cortices (Rypma and Prabhakaran, 2009). Thus, the evidence from these studies shows how fronto-parietal interactions appear to be central for fast cognitive processing.

The tasks used were the following:

- *Verbal simple reaction time*: after an initial fixation cross was presented for 1000 ms and then a blue capital A was displayed at the centre of the computer screen for 150 ms. Participants were instructed to press the “0” key as fast as they could any time they saw the stimulus. One hundred and sixty stimuli were presented in two blocks of eighty each.
- *Visual simple reaction time*: this task is similar to the previous one, but the stimulus presented was a blue square (1 cm x 1 cm).
- *Verbal choice reaction time*: eighty blue capital As and eighty Bs were displayed at the centre of the computer screen for 150 ms and participants were instructed to press the “1” key as fast as they could in response to A and to press “2” in response to B. One hundred and sixty stimuli were presented in two blocks of eighty each.
- *Visual choice reaction time*: this task is equivalent to the previous one but stimuli presented comprised either red or blue squares (same as in the simple reaction time task). The “1” key was to be pressed in response to red squares while “2” in response to the blue ones.

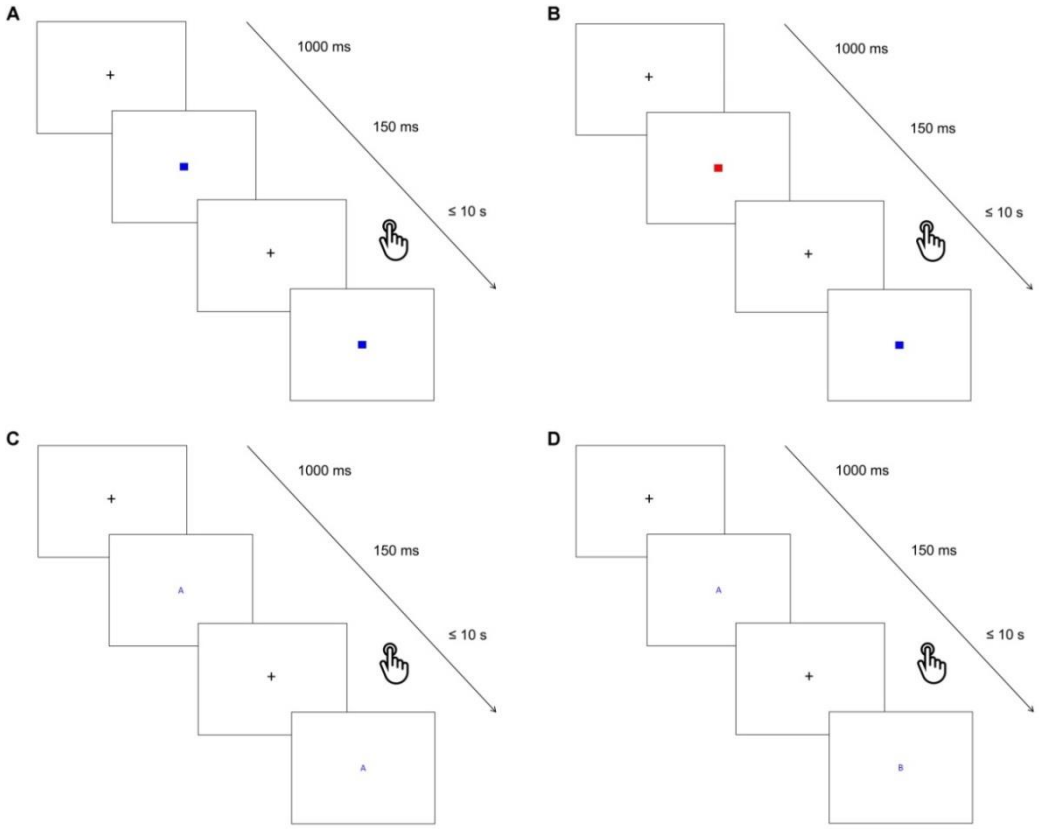

Figure 6.2 PS and sustained attention tasks: A. simple visual reaction time; B. choice visual reaction time; C. simple verbal reaction time; D. choice verbal reaction time

For all these tasks an interstimulus interval of 10 seconds was set in order to allow participants to respond to each stimulus. However, the delivery of a new stimulus was triggered by each response.

Four tasks were aimed to exercise mainly semantic retrieval and control mechanisms that are widely accepted to be reliant on a network of associative areas scattered across temporal, frontal and parietal lobes bilaterally (Binder et al., 2009, Jefferies, 2013, Patterson et al., 2007). Nevertheless, the abovementioned semantic processes served as a scaffolding to develop integrated tasks that required working memory and inhibitory processes on both verbal and visual stimuli. Indeed, deficits in such functions have been extensively documented in MS (Henry and Beatty, 2006). Therefore, it is assumed that exchange and integration of information across multiple cerebral networks, prominently involved in cognitive computations rather than motor or sensory abilities, is heightened by engaging with these tasks that may favour functional connectivity changes. A description of the semantic tasks used is provided and shown in Figure 6.3:

- *Change calculation*: first a fixation cross was presented for 1000 ms followed by the image of a banknote (£5, £10, £20 or £50) or a £2 coin to remember was displayed on the computer screen for 5000 ms. Then the images of four different items were presented for 5000 ms and participants had to scan through them to detect the only item that could be bought with the amount of money previously presented. Finally, a list of four amounts of money were presented and the participants had to choose (by pressing the key of the corresponding number 1 to 4) which one represented the change he/she would receive after paying for the affordable item with the note/coin originally presented. Seven consecutive trials were presented in each session.
- *Lexical odd one out*: a fixation cross was presented for 1000 ms at first and then the ending of a word was displayed 5000 ms, followed by a list of four possible word beginnings only three of which could make a word if completed with the previously given ending. The participants had to report the number associated with the only resulting non-word by pressing the relative key (1 to 4). Eight consecutive trials were presented in each session.
- *Semantic odd one out*: after a fixation cross lasting 1000 ms four words were displayed simultaneously, three of which belonged to one semantic category. Participants were instructed to find the only word which did not fit the target category. However, one of the other three words represented a distractor being semantically related to the odd one. Ten consecutive trials were presented in each session.
- *Semantic inhibition*: in each trial (four in total) after a fixation cross displayed for 1000 ms, a sentence was presented with no time limit to allow the participants to read it carefully and memorise it. As instructed, when they felt ready they had to press the space bar to show four images on the screen: three of them semantically related to the sentence and one not. The aim was to report the latter.

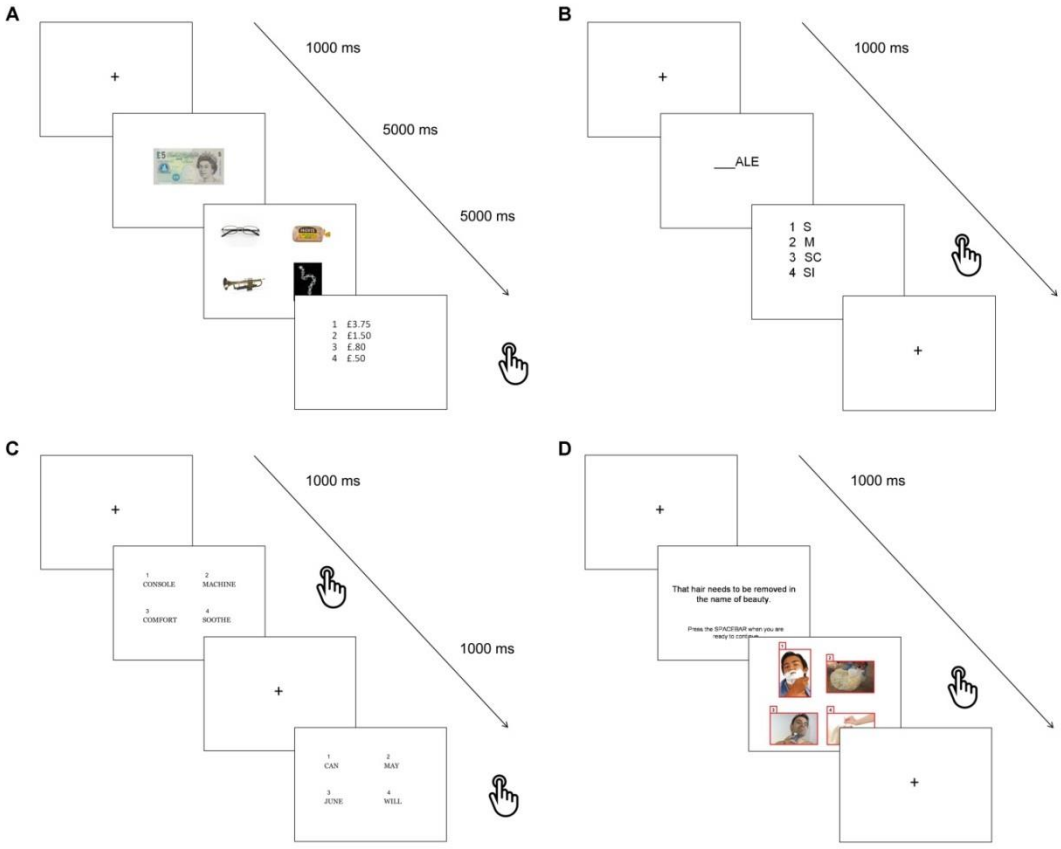

Figure 6.3 Lexical-semantic tasks: A. change calculation; B. lexical odd one out; C. semantic odd one out; D. semantic inhibition

Similarly, logical reasoning tasks were created based on semantic material that could train higher order cognitive functioning on both verbal and visual material (Figure 6.4). Indeed, complex and sequential cognitive computations are performed during exercises of deductive reasoning, namely material exploration, evidence gathering and information integration (Fangmeier et al., 2006). Therefore, different brain networks are thought to be stimulated with this type of exercises:

- *Verbal sequence completion*: after an initial presentation of a fixation cross (1000 ms), a set of different words was displayed in each trial (five in total) with two on the left side of the screen being connected by an arrow and related to one another. In the middle of the screen a word only was present with an arrow and a blank space for a word to be picked from two options on the right side of the screen so that the same relationship of the first couple is reproduced. However, both options were semantically related to the words in the middle of the screen, so that inhibitory processes had to be used in order to detect the most relevant one in the given context.

- *Visual sequence completion*: this test is similar to the previous one apart from the fact that images were presented instead of words.
- *Sentence completion*: after an initial presentation of a fixation cross (1000 ms), a sentence was presented on the screen with a missing word and four options below it, two of which semantically related to the sentence. The participants had to select the most appropriate word to obtain semantically correct sentences (five trials in total).
- *Scene completion*: in five consecutive trials an array of five images, one in the middle and four smaller ones at the corners of the computer screen. The participants were instructed to select the image most logically/semantically associated with the target one in the middle.

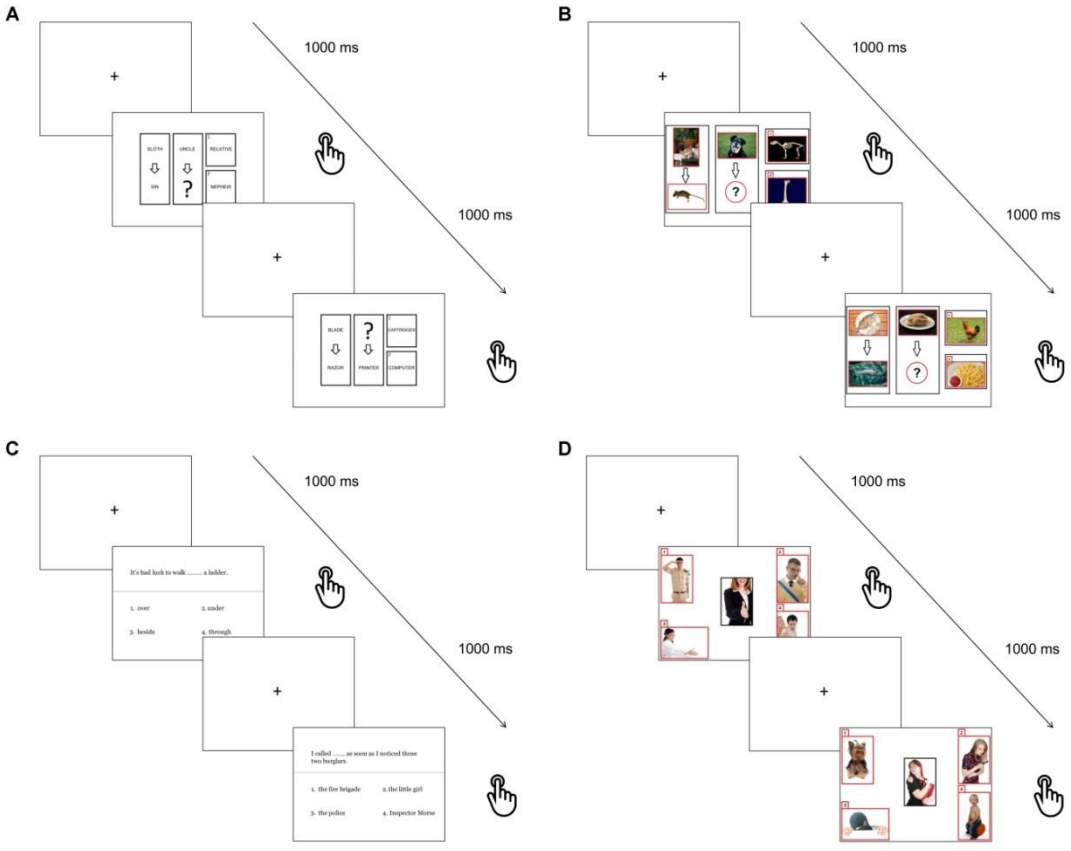

Figure 6.4 Reasoning tasks: A. verbal sequence completion; B. visual sequence completion; C. sentence completion; D. scene completion

The PS-loaded programme included exactly the same exercises of the standard one. However, all tasks but those based on reaction times were modified by setting a maximum amount of time to respond to each trial. The allocated time in each individual task was equivalent to median reaction time obtained with a pilot study

conducted in the preliminary phase of the study. Seven additional people with RRMS were recruited from the same clinic to ensure that at least 80% of the responses in each task were correct. Moreover, from session 6 on (throughout weeks 2, 3 and 4) the maximum response time allocated to each task was progressively decreased of 100 ms in each session. The aim was to gradually exercise participants' PS ability, in light of its prominent role played in cognitive functioning of people with MS, and to test whether this strategy could produce stronger effects than the standard rehabilitation approach.

6.1.2.4. Neuropsychological assessment

A comprehensive battery of tests and questionnaires was used to evaluate different aspects of cognitive functioning of all participants at baseline and after either completion of the rehabilitation sessions or four weeks of usual care.

Global cognitive functioning was assessed by means of the common MMSE (Folstein et al., 1975) and only patients with a score ≥ 24 were recruited as previously mentioned. The definition of this threshold allowed the inclusion of people with just mild cognitive impairment. Indeed, severely compromised cognitive function may affect the ability of participants to engage effectively in the rehabilitation tasks and, in turn, the possibility of gaining benefits from them.

Verbal memory was extensively assessed using several tests:

- the PASAT (Gronwall, 1977): procedure was describe in Chapter 2, two interstimulus intervals of 3 sec and 2 sec were used;
- the Digit Span Test (Wechsler, 2008): a series of digits are verbally presented to the participant that has to report them either in the same order (forward condition to assess verbal short term memory capacity) or reverse (backward condition to assess verbal working memory capacity). First two digits are presented and as the participants report them correctly the amount of digits is increased of a unit at a time till two consecutive errors are made. Two alternative lists were used and counterbalanced between baseline and post-treatment assessments;
- the Logical Memory Test (Wechsler, 2008): a brief story is verbally presented to the participant and he/she is instructed to report all the details that they could remember immediately after. The story is read a second time and recall is requested after a ten minute delay during which the participant is engaged in other tasks not tapping memory or verbal processes to test long term episodic memory for organised verbal material (the maximum score is 25);

- the Buschke Selective Reminding Test (Buschke, 1973): this test assesses learning of verbal material by presenting a list of twelve words to the participants and asking them to report as many words as they can. This is repeated for twelve times, but from the second trial on only the words that have not been mentioned in the previous trial are read. Yet participants have to aim to report all of the twelve words (the maximum score is 144). Moreover, after a 30 minute delay spent on other tasks the participants are asked to freely recall the list again;
- the Phonemic and Semantic fluency tasks (Lezak, 2004): to assess lexical knowledge and semantic memory retrieval as explained in Chapter 2.
- the Corsi Block-Tapping Test (Corsi, 1972): this test is used to assess visuo-spatial memory in two conditions by using a tray with nine cubes on it. First, similarly to the Digit Span Test, the examiner points at some cubes (starting with two) and asks the participant to point at these cubes in the same order. The amount of cubes is gradually increased until the participant makes two consecutive errors: the final score is considered a measure of visuo-spatial short term memory capacity (span). Two alternative lists were used and counterbalanced between baseline and post-treatment assessments. Second, the examiner points at a sequence of eight cubes and the participant is asked to reproduce it (supraspan conditions). Eighteen trials are available to this aim and the sequence is repeated by the examiner before each attempt. However, the test stops if the participant reproduces the sequence correctly for three times consecutively. This condition evaluates visuo-spatial learning abilities (the maximum score is 29.16);
- the Rey-Osterreith Complex Figure Test (Osterreith, 1944): in order to asses visuo-constructive abilities, the participant is provided with a complex figure to copy. After a ten minute delay, filled with other non-visuo-spatial tasks, the participant is asked to recall the figure to evaluate visual long term memory (the maximum score is 36).

A selection of tests of attention and PS were included to ascertain performance on these domains, which are consistently reported as affected by MS pathology:

- the Digit Cancellation Test (Spinnler and Tognoni, 1987): this is a visual search task that allows the evaluation of selective visual attention as described in Chapter 2;
- the TMT – part A (Armitage, 1946): this test allows the evaluation of both visuo-spatial attentional and PS abilities (see Chapter 2 for description);

- the Stroop speed index (Stroop, 1935): calculated as the average of the completion time of the first two trials of the Stroop test (see Chapter 2), this index provides a measure of PS for automatic responses;
- the DSCT (Wechsler, 2008): test of visual PS extensively used in MS described in Chapter 2.

Moreover, executive functions were assessed by means of some of the abovementioned tests, namely:

- the TMT (Armitage, 1946): the difference in completion time between part B and part A gives an index of task-switching executive abilities;
- the Stroop inhibition index (Venneri et al., 1993): the difference between the completion time of the third trial of the Stroop test and the Stroop speed index is thought to measure the ability to inhibit automatic responses efficiently and provides a measure of executive inhibitory control.

Finally, participants were additionally asked to fill in four questionnaires to self-assess a range of different symptoms associated with MS as well as perceived levels of quality of life:

- the MFIS (Fisk et al., 1994a): this scale is used to ascertain the severity of fatigue, the most common symptom experienced by people with MS;
- the PHQ-9 (Spitzer et al., 1999): this short questionnaire gives information about depressive symptoms in patients;
- the 7-item Generalised Anxiety Disorder (GAD-7) scale (Spitzer et al., 2006): a common questionnaire used to evaluate symptoms related to anxiety;
- the Multiple Sclerosis Quality of Life (MSQoL-54) Instrument (Vickrey et al., 1995): a 54-item questionnaire developed specifically to investigate the impact of MS on quality of life, both physical and mental.

Motor performance was assessed for patients in the active groups by comparing simple and choice reaction times (average of visual and verbal) from the first and last sessions of the programme. Only correct trials were considered.

6.1.2.5. MRI acquisition

A comprehensive MRI scanning session was performed at baseline and post-treatment. The acquisition protocol included T1-weighted, T2-weighted FLAIR, DTI

and T2*-weighted resting-state functional MRI scans. Acquisition parameters were previously described in Experiment 1 (Chapter 4) and Experiment 3 (Chapter 5).

6.1.2.6. MRI preprocessing

Structural and DTI images were preprocessed following the procedure already explained in Experiment 1. Resting-state functional MRI scans were preprocessed as described in Experiment 3. In particular, three specific pairwise ICAs were run on resting-state scans to extract functional networks to be fed into subsequent analyses.

6.1.2.7. Statistical analysis

All demographic, clinical, cognitive and self-reported characteristics of the sample underwent a first inspection to check the normality of distribution within each of the three groups. Moreover, homogeneity of variance across groups was assessed for each variable by means of the Leven's test. Therefore, possible differences across the three treatment groups in demographic and clinical variables, normally or not normally distributed, were investigated respectively using analysis of variance (ANOVA) and Kruskal-Wallis test. Subsequently, analysis of covariance was used to check for differences in cognitive performance controlling for demographic/clinical variables that resulted significantly different across groups. Given the high amount of cognitive and self-reported measures (twenty-six in total) results were statistically corrected for multiple comparisons by applying the Bonferroni correction to the significance threshold that was set at $p < .002$ (.05/26 = .002). Independent sample t-test was used to compare reaction time measures between the two active groups.

Similarly, baseline DTI and resting-state preprocessed scans were compared across the three groups to ascertain whether there were any differences either in WM microstructural integrity or functional network connectivity at the stage of recruitment.

The aim of assessing the effects of the cognitive rehabilitation programmes on cognitive functioning, structural and functional connectivity was pursued by means of three repeated measures models. These models were created to test for interaction effects between two two-level factors, namely *time*, as a within-group factor, and *treatment group*, as a between-group factor. In particular, two groups at a time were investigated: 1) Standard programme vs Control, to test the effects of the multidomain cognitive exercises (to answer research question 1); 2) PS-loaded programme vs Control, to test the effects of PS-demanding multidomain cognitive

exercises; 3) Standard programme vs PS-loaded programme, to test directly whether engaging in rehabilitative exercises with high PS demands can exert stronger effects than the condition with low PS demands (to answer research question 2). This choice was made not to reduce the degrees of freedom in the analysis, in consideration of the limited sample size of the recruited groups. For the cognitive outcome measures the same corrected significance threshold $p < .002$ was used, while for DTI and resting-state analysis the same thresholds (set level uncorrected and cluster FWE-corrected) as already described in Experiment 1 and Experiment 3 were adopted. Partial η^2 was calculated to quantify the effect size of cognitive changes observed. Additionally, paired t-test was used to assess within group changes in cognitive performance.

6.1.3. Results

6.1.3.1. Cognitive results

Analyses carried out on demographic and clinical characteristics of the participants revealed that only educational levels significantly differed across groups (Table 6.1). In particular, Dunn's pairwise tests showed that patients allocated to the control group had significantly fewer years of education than those in the PS-loaded group (test statistics = -15.27, p = .004 adjusted with Bonferroni correction), but not than those in the Standard treatment group. For this reason education was included as a covariate in subsequent analysis.

Table 6.1 Clinical and demographic characteristics of the sample

Characteristic	Standard programme (N = 15)	PS-loaded programme (N = 15)	Control (N = 15)	F	p
Demographic					
Age (years)	45.40 (10.55)	45.73 (8.61)	42.73 (7.27)	.51	.604
Age at onset (years)	36.80 (9.92)	37.13 (8.40)	32.73 (6.86)	1.25	.297
Education (years)	14.07 (2.63)	16.47 (3.5)	12.67 (1.88)	10.68*	.005
Clinical					
Disease duration (years)	8.60 (5.38)	8.60 (7.05)	10.00 (7.40)	.31*	.856
Relapses (n. in last 12m)	0.13 (0.35)	0.60 (1.06)	0.27 (0.59)	3.09*	.213
EDSS	3.23 (1.83)	2.87 (0.90)	4.14 (1.38)	2.83	.070
TIV (ml)	1511.81 (150.95)	1452.25 (196.25)	1497.02 (244.79)	.63*	.729
TLV (ml)	11.96 (16.60)	9.28 (10.70)	10.19 (12.36)	.36*	.837

* Kruskal-Wallis test

EDSS: Expanded Disability Severity Scale, TIV: total intracranial volume, TLV: total lesion volume

No differences across groups on cognitive or self-reported variables were observed after applying Bonferroni correction to the analysis of covariance (Table 6.2).

Table 6.2 Baseline cognitive and self-reported characteristics of the sample

Characteristic	Standard programme (N = 15)	PS-loaded programme (N = 15)	Control (N = 15)	F	p
Global cognitive status					
MMSE	28.80 (1.26)	28.67 (1.88)	28.47 (1.19)	1.12	.335
Verbal working memory					
PASAT 3"	35.93 (17.79)	36.87 (19.90)	39.53 (14.41)	1.25	.299
PASAT 2"	21.40 (14.98)	23.73 (12.90)	23.40 (15.51)	.67	.517
Digit Span - forward	6.00 (0.84)	6.47 (1.68)	6.33 (0.98)	1.13	.334
Digit Span - backward	4.53 (1.06)	4.93 (1.53)	4.73 (1.10)	.99	.380
Verbal long term memory					
LMT - immediate recall	13.40 (3.52)	13.93 (3.90)	12.73 (3.26)	.37	.861
LMT - delayed recall	14.87 (3.89)	17.13 (3.54)	15.00 (2.90)	1.21	.324
BSRT - total	90.33 (17.43)	100.93 (13.30)	102.33 (15.87)	2.31	.063
BSRT - delayed recall	8.13 (2.53)	6.80 (2.76)	7.47 (2.56)	.98	.439
Phonemic fluency	28.40 (9.17)	38.93 (9.58)	32.07 (9.14)	.17	.844
Semantic fluency	43.73 (9.47)	52.87 (9.22)	43.47 (9.65)	3.56	.038
Visuo-spatial memory					
Corsi test - span	4.87 (1.19)	4.73 (1.22)	4.93 (1.16)	.25	.782
Corsi test - supraspan	25.00 (3.82)	25.64 (3.46)	26.11 (2.68)	.81	.451
Rey Figure - copy	34.80 (1.37)	33.47 (2.53)	34.80 (1.37)	.83	.444
Rey Figure - delayed recall	14.53 (4.26)	18.27 (6.38)	18.16 (3.77)	1.08	.348
PS/visuo-spatial attention					
Digit Cancellation test	50.87 (7.38)	52.00 (6.00)	54.27 (4.83)	.56	.577
TMT-A (sec)	44.33 (14.45)	36.00 (9.88)	36.47 (16.91)	1.18	.316
Stroop speed (sec)	17.83 (3.87)	17.53 (2.65)	16.33 (3.07)	.43	.824
DSCT	62.20 (16.56)	62.60 (13.24)	64.20 (16.87)	.05	.998
Executive functions					
Stroop inhibition (sec)	16.27 (8.63)	16.10 (5.99)	15.40 (7.62)	1.04	.363
TMT B-A (sec)	43.87 (24.03)	45.73 (27.61)	36.53 (21.07)	.31	.736
Self-reported measures					
MFIS	43.53 (16.34)	53.07 (16.34)	48.47 (17.10)	.74	.597
PHQ-9	8.93 (5.65)	10.87 (5.79)	8.40 (3.60)	.37	.856
GAD-7	6.87 (6.08)	6.13 (4.53)	7.20 (5.06)	.31	.903
MSQoL-54 - Physical	51.68 (20.25)	44.81 (16.63)	52.02 (19.79)	.46	.806
MSQoL-54 - Mental	58.64 (17.52)	53.38 (18.83)	64.53 (19.79)	1.21	.325
Motor performance					
Simple reaction times*	149.28 (41.04)	182.10 (51.32)	---	1.91	.067
Choice reaction times*	374.39 (61.68)	401.04 (78.17)	---	1.02	.315

BSRT: Buschke Selective Reminding Test, DSCT: Digit Symbol Coding Test, GAD-7: 7-item Generalised Anxiety Disorder, LMT: Logical Memory Test, MFIS: Modified Fatigue Impact Scale, MMSE: Mini Mental State Examination, MSQoL-54: 54-item Multiple Sclerosis Quality of Life; PASAT: Paced Auditory Serial Addition Test, PHQ-9: 9-item Patient Health Questionnaire, TMT: Trail Making Test

* Independent sample t-test

A trend towards a significant difference, however, was noted for the semantic fluency test. Post-treatment data and within-group changes are shown in Table 6.3.

Table 6.3 Cognitive and self-reported characteristics of the sample after completion of the intervention and paired t-test showing within-group changes (in bold t-tests surviving Bonferroni correction p < .002)

Characteristic	Standard programme (N = 15)	t	PS-loaded programme (N = 15)	t	Control (N = 15)	t
Global cognitive status						
MMSE	29.33 (1.05)	-1.95	29.27 (1.83)	.69	28.73 (1.03)	-.74
Verbal working memory						
PASAT 3"	41.87 (16.35)	-3.02	39.80 (16.33)	-1.39	43.07 (13.90)	-1.84
PASAT 2"	26.93 (16.00)	**-3.87**	26.40 (14.96)	-1.26	28.33 (15.83)	-2.00
DS - F	6.60 (0.99)	-2.81	6.53 (1.19)	-.19	6.07 (0.70)	1.29
DS - B	4.93 (1.49)	-2.75	5.27 (1.33)	.00	4.60 (0.91)	.49
Verbal long term memory						
LMT - IR	14.27 (3.61)	-1.12	13.73 (4.46)	.28	11.40 (3.68)	1.86
LMT - DR	17.73 (3.08)	**-4.16**	16.33 (4.27)	.68	14.20 (5.28)	.84
BSRT - total	115.80 (12.76)	**-8.96**	110.60 (15.44)	**-3.72**	107.73 (15.37)	-1.56
BSRT - DR	8.93 (2.37)	**-4.78**	8.73 (2.55)	-1.35	8.13 (2.26)	-1.72
PF	38.80 (10.53)	**-9.25**	43.73 (12.03)	-2.17	36.87 (10.49)	**-4.00**
SF	55.93 (13.91)	**-6.19**	55.73 (8.78)	-1.30	44.67 (8.68)	-.94
Visuo-spatial memory						
Corsi test - span	5.47 (1.24)	-2.95	5.00 (1.36)	-0.81	5.40 (0.91)	-1.70
Corsi test - SU	27.04 (1.94)	-2.08	25.76 (3.66)	-.95	27.50 (1.42)	-1.77
RF - copy	35.00 (1.00)	-.54	34.20 (2.31)	-.95	34.93 (1.03)	-.40
RF - DR	22.47 (4.94)	**-8.03**	18.80 (6.90)	-.33	22.20 (5.17)	-3.50
PS/visuo-spatial attention						
DCT	55.07 (5.24)	-2.69	54.47 (5.89)	**-4.24**	54.47 (5.36)	-.13
TMT-A (sec)	35.67 (14.36)	**3.87**	33.67 (9.55)	1.09	34.00 (13.89)	1.03
SS (sec)	15.50 (2.72)	3.11	16.63 (3.12)	2.34	17.13 (3.80)	-1.09
DSCT	69.07 (16.92)	-2.88	67.00 (15.29)	-2.84	70.87 (21.75)	-2.97
Executive functions						
SI (sec)	12.10 (5.46)	2.44	14.97 (5.47)	.74	13.87 (4.68)	1.34
TMT B-A (sec)	35.53 (23.48)	1.91	39.87 (19.82)	.82	29.60 (12.45)	1.14
Self-reported measures						
MFIS	32.40 (15.98)	3.65	44.93 (17.64)	2.42	49.07 (18.10)	-.20
PHQ-9	6.53 (3.96)	2.33	8.60 (5.34)	2.91	8.73 (4.03)	-.38
GAD-7	4.33 (3.31)	1.83	5.87 (5.07)	1.71	5.27 (3.86)	1.34
MSQoL-54 - P	61.80 (21.21)	-3.40	44.45 (17.81)	.14	50.38 (22.08)	.82
MSQoL-54 - M	66.58 (19.87)	-1.28	56.03 (24.42)	-2.14	59.30 (19.28)	1.49
Motor performance						
SRT	151.74 (41.04)	-.29	187.67 (47.93)	-.34	---	---
CRT	333.63 (60.73)	2.10	379.79 (93.23)	1.00	---	---

BSRT: Buschke Selective Reminding Test, CRT: Choice reaction time, DCT: Digit Cancellation Test, DR: Delayed recall, DS: Digit Span (B: backward, F: forward), DSCT: Digit Symbol Coding Test, GAD-7: 7-item Generalised Anxiety Disorder, IR: Immediate recall, LMT: Logical Memory Test, MFIS: Modified Fatigue Impact Scale, MMSE: Mini Mental State Examination, MSQoL-54: 54-item Multiple Sclerosis Quality of Life (P: physical, M: mental); PASAT: Paced Auditory Serial Addition Test, PF: Phonemic fluency, PHQ-9: 9-item Patient Health Questionnaire, RF: Rey Figure, SF: Semantic fluency, SI: Stroop inhibition, SRT: Simple reaction time, SS: Stroop Speed, SU: supraspan, TMT: Trail Making Test

The standard programme induced the strongest within-group effects, especially in memory. Pairwise repeated measures models were used to investigate differential effects of the rehabilitation programmes on cognitive performance (Table 6.4).

Table 6.4 Cognitive changes resulting from the three group-by-time (2 x 2) repeated measures models (in bold: tests surviving Bonferroni correction p < .002)

Characteristic	**Standard vs Control**		**PS-loaded vs Control**		**Standard vs PS-loaded**	
	F	p	F	p	F	p
Global cognitive status						
MMSE	1.42	.243	.15	.700	.60	.445
Verbal working memory						
PASAT 3"	.30	.587	.92	.347	2.87	.102
PASAT 2"	.35	.558	2.43	.130	2.36	.136
Digit Span – forward	7.61	.010	.01	.914	1.98	.170
Digit Span – backward	6.35	.018	.28	.599	1.49	.233
Verbal long term memory						
LMT - immediate recall	3.13	.088	.81	.377	1.34	.256
LMT - delayed recall	9.40	.005	.01	.918	4.78	.038
BSRT - total recall	**19.21**	**< .001**	2.34	.138	**12.22**	**.002**
BSRT - delayed recall	8.88	.006	.23	.637	3.77	.063
Phonemic fluency	9.42	.005	.54	.469	2.90	.100
Semantic fluency	**18.96**	**< .001**	.05	.820	**13.03**	**.001**
Visuo-spatial memory						
Corsi test - span	.27	.608	.22	.642	3.94	.057
Corsi test - supraspan	.09	.769	1.64	.211	1.76	.196
Rey Figure - copy	.05	.824	.29	.594	.01	.930
Rey Figure - delayed recall	6.69	.015	1.64	.212	< .01	.988
PS/visuo-spatial attention						
Digit Cancellation test	3.00	.095	2.40	.133	.27	.605
TMT-A (sec)	3.55	.071	1.31	.262	2.30	.141
Stroop speed (sec)	6.30	.018	2.55	.122	.65	.428
DSCT	.03	.860	.15	.704	.60	.446
Executive functions						
Stroop inhibition (sec)	3.90	.059	.10	.758	.79	.381
TMT B-A (sec)	.28	.602	.02	.894	.17	.686
Self-reported measures						
MFIS	5.30	.029	1.55	.224	1.32	.261
PHQ-9	4.42	.045	3.79	.062	< .01	.987
GAD-7	.03	.860	.02	.894	.21	.647
MSQoL-54 - Physical	10.66	.003	.17	.679	4.74	.038
MSQoL-54 - Mental	4.97	.034	2.04	.164	.01	.914
Motor performance						
Simple reaction times	---	---	---	---	.04	.844
Choice reaction times	---	---	---	---	1.46	.238

BSRT: Buschke Selective Reminding Test, DSCT: Digit Symbol Coding Test, LMT: Logical Memory Test, MFIS: Modified Fatigue Impact Scale, MMSE: Mini Mental State Examination, MSQoL-54: 54-item Multiple Sclerosis Quality of Life; PASAT: Paced Auditory Serial Addition Test, PHQ-9: 9-item Patient Health Questionnaire, TIV: total intracranial volume, TLV: total lesion volume, TMT: Trail Making Test

These models showed significant interactions between treatments and time. In particular, the Standard programme induced significantly stronger improvements than usual care on two measures of verbal memory: total recall on the Buschke Selective Reminding Test (Figure 6.5) and the semantic fluency test (Figure 6.6). The effect size resulted high for both tests as shown by a partial η^2 index of .42 and .41 respectively.

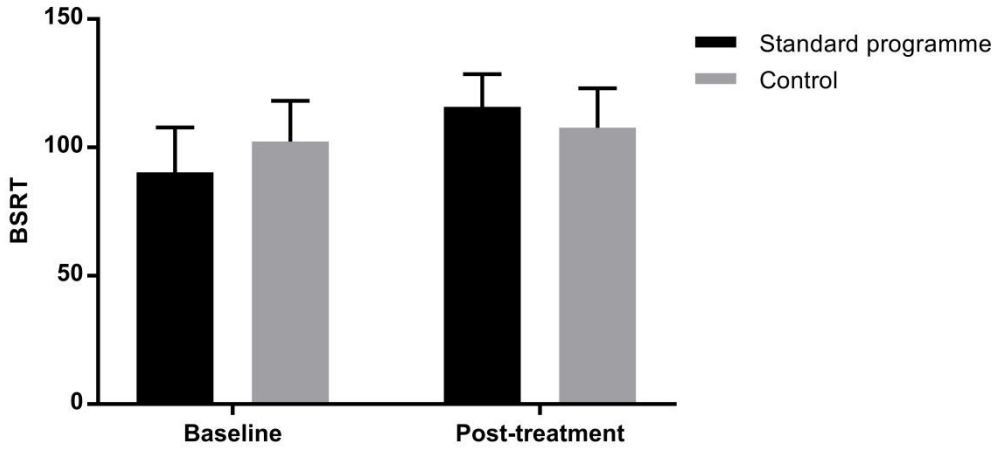

Figure 6.5 Changes in the total number of items reported on the Buschke Selective Reminding Test in the Standard programme and Control groups

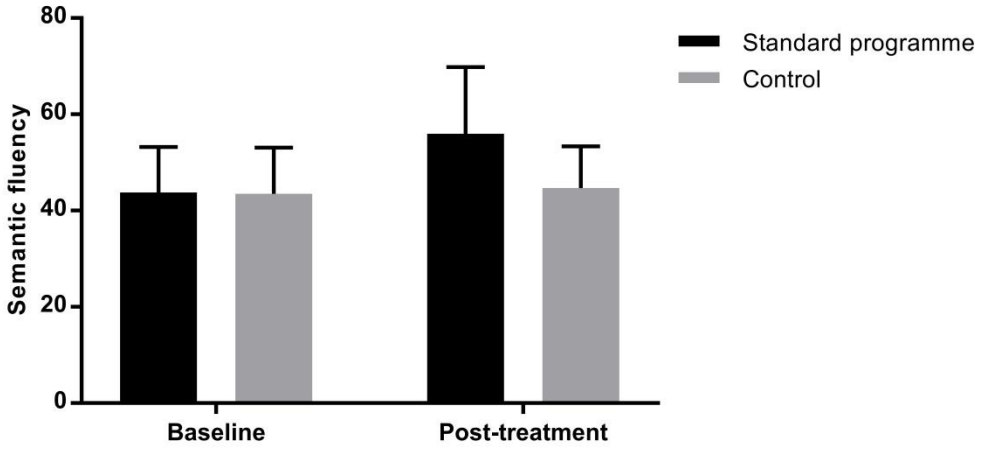

Figure 6.6 Changes in the total number of items reported on the semantic fluency test in the Standard programme and Control groups

Additionally, some improvement (approaching significance threshold) in physical quality of life was seen post-treatment compared to the control group. No significant interactions emerged from the contrast between the PS-loaded programme and the

control groups, even before applying the Bonferroni correction. Hence, these quantitative results show that this version of the treatment appears not to influence cognitive performance differently from usual care. Finally, the direct contrast between the two active groups showed results coherent with those reported above. In fact, participants who underwent the Standard programme showed an increase in cognitive performance significantly higher than that observed in those who underwent the PS-loaded programme exactly on the same tests as found in the first contrast (Table 6.4), i.e. the total recall on the Buschke Selective Reminding Test (Figure 6.7) and the semantic fluency test (Figure 6.8). The effect size for both tests resulted moderately high with observed partial η^2 values of .31 and .33 respectively.

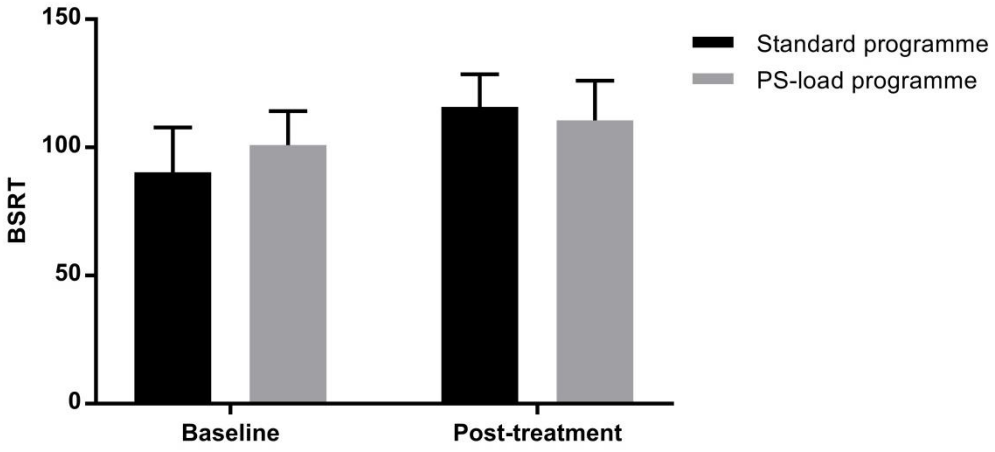

Figure 6.7 Changes in the total number of items reported on the Buschke Selective Reminding Test in the active treatment groups

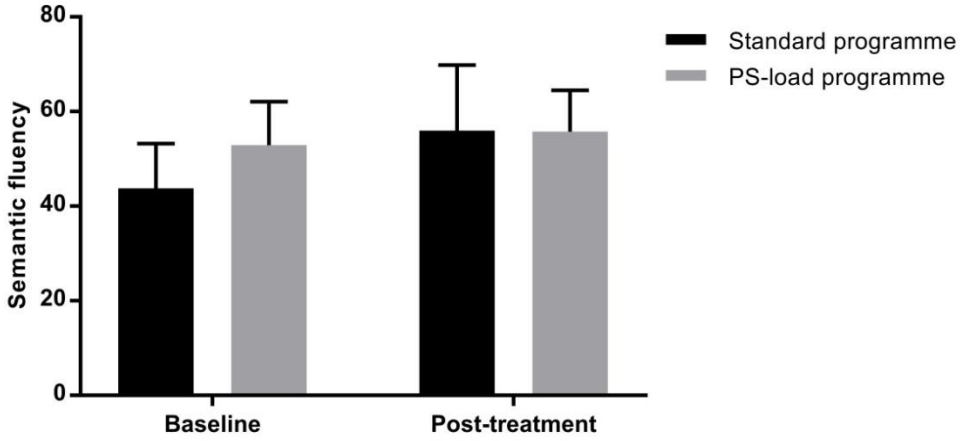

Figure 6.8 Changes in the total number of items reported on the semantic fluency test in the active treatment groups

6.1.3.2. Structural connectivity results

Analysis carried out on DTI scans at baseline highlighted no differences in FA between any of the groups. Similarly, no longitudinal intervention-related FA changes were detected in any of the three mixed models.

6.1.3.3. Functional connectivity results

Interaction analyses highlighted a significant modulation of functional connectivity only in the salience network when comparing the Standard programme group with both of the other patient groups. These changes, however, were observed in different directions (both increases and decreases) within various brain areas (Table 6.5). In particular, patients undergoing the Standard programme showed a decrease in functional connectivity in the anterior cingulate. This decrease was significantly higher than that observed in those patients who did not engage in cognitive rehabilitation (Figure 6.9). In the comparison between the Standard and the PS-loaded programmes the Standard group experienced decreased connectivity in the left putamen and thalamus, but increased in temporo-occipital areas (Figure 6.10).

No significant changes were detected for any of the other functional networks included in the analysis.

Table 6.5 Significant changes in functional connectivity of the salience network resulting from the mixed repeated measures models (p <.05 FWE)

FC changes	Cluster extent	Side	Brain region	*t* value	MNI coordinates *x*	*y*	*z*
			Standard vs Control				
Decrease	110	R	Anterior cingulate (BA 24)	4.90	20	-6	42
		R	Anterior cingulate (BA 24)	4.81	12	-2	40
		R	Anterior cingulate (BA 32)	4.03	18	6	42
			Standard vs PS-load				
Decrease	119	L	Putamen	5.30	-28	-22	-2
		L	Thalamus	3.81	-16	-16	-2
Increase	160	R	Fusiform gyrus (BA 19)	4.79	36	-70	-18
		R	Fusiform gyrus (BA 37)	4.37	34	-58	-24
		R	Middle occipital gyrus (BA 18)	4.06	30	-82	-12

BA: Brodmann area

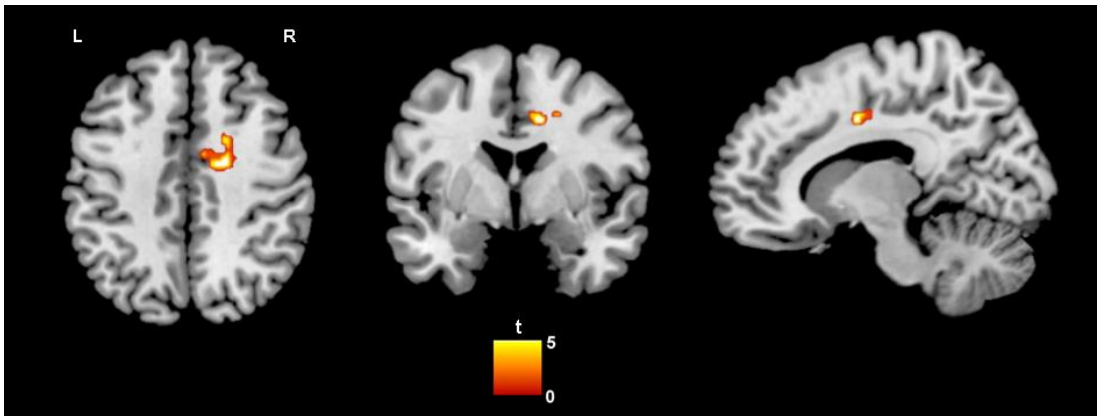

Figure 6.9 Decreases in functional connectivity of the salience network: Standard programme vs Control ($p < .05$ FWE)

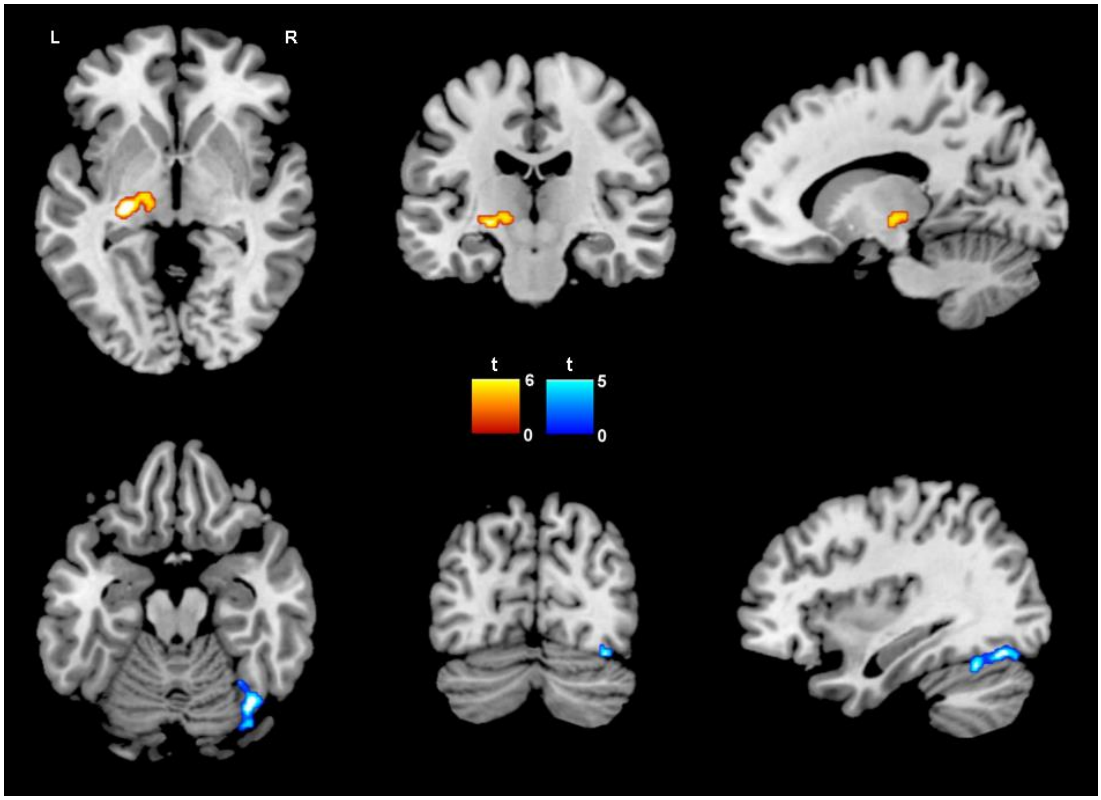

Figure 6.10 Decreases (red) and increases (blue) in functional connectivity of the salience network: Standard programme vs PS-loaded programme ($p < .05$ FWE)

6.1.3.4. Supplementary results

After observing parallel significant interactions in cognitive and resting-state functional connectivity in the comparison between the Standard treatment and both the Control and PS-loaded programme groups, associations between measures of cognition and connectivity were tested for the group that showed cognitive

improvements. Two approaches were adopted: a region-of-interest analysis and a subtraction-map analysis.

The first type of analysis was performed by saving in SPM the three clusters where significant changes in functional connectivity occurred (Table 6.5) as regions of interest. The average resting-state signal within each region was then extracted from both baseline and post-treatment maps of the salience network by means of MarsBaR (Brett et al., 2002). Subsequently, treatment-induced changes in connectivity as well as in clinical measures scores (Buschke Selective Reminding Test, semantic fluency and physical quality) were calculated by subtracting baseline from post-treatment values. Finally, correlations between cognitive/quality of life and functional connectivity difference scores were investigated.

The second approach required the calculation of subtraction maps by means of the SPM function ImCalc: baseline salience network maps were subtracted, for each individual who underwent the Standard cognitive rehabilitation, from the post-treatment ones. In a second step, regression models were run in SPM to investigate the association between difference scores on measures of cognition/quality of life and the subtraction maps.

Either approaches failed to highlight any significant associations between improvements in cognition or physical quality of life and changes in functional connectivity in the salience network.

Finally, given the findings of an association between cognitive performance and functional connectivity of the DMN and the left fronto-parietal network reported in the previous chapter, a decision was made to replicate the latter analysis (subtraction maps) to investigate the potential role of these networks in supporting cognitive improvements. Indeed, the increase in performance on the semantic fluency task was positively associated with greater functional connectivity of the left hippocampus within the DMN (FWE-corrected $p < .05$) (Table 6.6 and Figure 6.11).

Table 6.6 Positive associations between changes in functional connectivity of the DMN and improvements on the semantic fluency task for the Standard rehabilitation group ($p < .05$ FWE)

Cluster extent	Side	Brain region	*t* value	MNI coordinates		
				x	*y*	*z*
81	L	Hippocampus	9.05	-32	-30	-12
		Hippocampus	6.90	-28	-26	-12

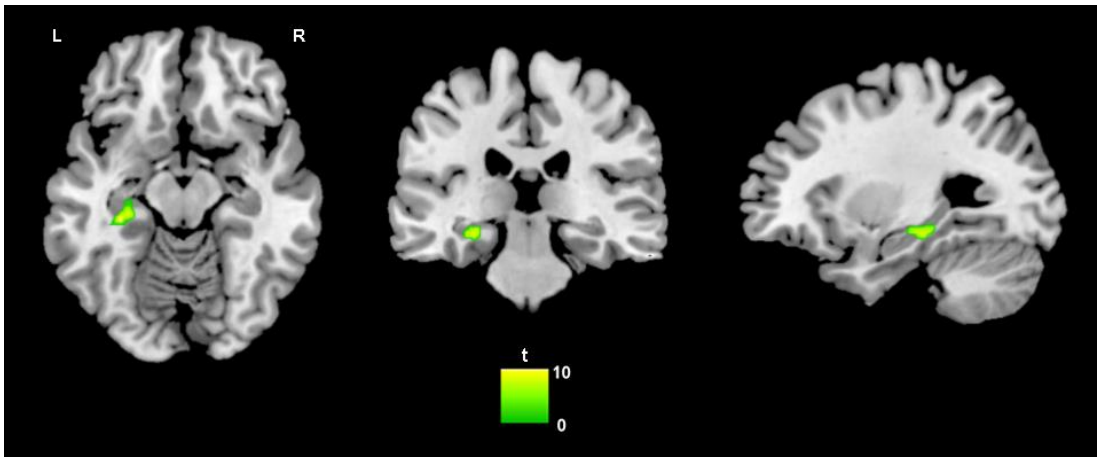

Figure 6.11 Positive associations between improvement on the semantic fluency task and increased functional connectivity of the DMN in the Standard rehabilitation group (p <.05 FWE)

6.1.4. Discussion

6.1.4.1. Cognition

The results of the study presented in this chapter appear in line with the idea that using tasks fostering integration of information supported by multiple brain networks may be a viable strategy to improve cognition in people with RRMS. In fact, the multi-domain rehabilitation exercises that patients underwent involved the synchronous engagement of various functions across a range including: PS, sustained attention, working and short-term memory, learning, lexical-semantic processing, inhibitory abilities and logical reasoning. The intensive pace of the standard programme aimed at stimulating the coordination of the neural systems supporting these functions resulted in improvements, compared to controls, especially in semantic processing (semantic fluency) and verbal long-term memory functions (Buschke Selective Reminding Tests). Effects on these functions have been observed in some previous studies (Bonavita et al., 2015, Filippi et al., 2012, Rilo et al., 2016). However, high variability across findings and even lack of effects (Campbell et al., 2017a) can be traced in the literature. Moreover, it is worth noting that most of the published studies have adopted a symptomatic approach and no transfer effects were in general found. Indeed, only Chiaravalloti et al. (2018) recently showed how PS training may positively influence memory functioning.

In this study post-treatment improvements were observed in verbal long term memory. This function is often impaired in MS, perhaps because of a combination of retrieval and encoding deficits (Lafosse et al., 2013). Although memory was not specifically targeted by the exercises, beneficial effects on this function may arise as a consequence of generally improved efficiency of information processing (Sandry

et al., 2018). Indeed, decline in mnestic function has been suggested to be partially dependent on loss of interhemispheric integration of information due to corpus callosum agenesis (Paul et al., 2016) and shrinkage observed in amnestic mild cognitive impairment and Alzheimer's disease (Qiu et al., 2016). Therefore, improved efficiency due to strengthened functional connectivity of regions involved in supporting cognitive processes (i.e. semantic processing) necessary to perform well on long term memory tasks might have played a role. Moreover, there were improvements in some aspects of semantic memory that depends on a distributed neural system and, thus, possibly prone to benefit from improvements in distributed information processing (Patterson et al., 2007).

On a different note, it must be noted that previous applications of some of the rehabilitation exercises used in this study have yielded no significant effects on cognitive functioning of healthy older adults (De Marco et al., 2016) and people affected by mild cognitive impairment (De Marco et al., 2018). However, the version that was used in this study included modifications to the original set of exercises. These changes were aimed at reducing the engagement of lexical processing in favour of more intensive practice of executive attentional/executive functions, more consistently impaired in MS. Indeed, they appear to have been beneficial and have triggered gains in cognitive performance. Additionally, patients with MS were significantly younger than those previously tested (De Marco et al., 2018), as MS onset occurs much earlier in life than cognitive decline due to neurodegeneration. Hence, although experience-dependent neuroplasticity has been observed also in older adults (Park and Bischof, 2013), younger brains may retain higher neuroplastic abilities resulting in significant post-rehabilitation improvements in performance. Furthermore, the impact on response to treatment due to differences in neuropathological damage between MS and primary neurodegenerative conditions cannot be completely ruled out. In fact, although it is believed MS shares some of the neurodegenerative mechanisms linked with Alzheimer's disease (Lassmann, 2011), neurotransmission does not appear to be severely affected. MS-induced synaptic alterations are reversible (Mandolesi et al., 2015) and, therefore, it might be suggested that neurons affected by this pathology are in a better position than those pressed by neurodegenerative conditions to form new connections and synaptic links in response to intensive training.

Paired t-tests showed that the Standard programme induced improvements on a number of tests wider than those observed for the other two groups. These changes were observed mainly in tests of memory, as well as working memory and attention. However, the significance of the findings of this study emerges from the analyses

carried out on the second experimental group who underwent the PS-loaded version of the rehabilitation programme. In fact, these patients showed no changes in their cognitive performance after treatment when compared to the non-active control group. Nevertheless, the contrast between the two active groups showed how the standard programme induced significant improvements on exactly the same neuropsychological tests already observed in the comparison with the untreated controls.

First, it may be suggested that the modified programme appears ineffective because the increased PS-load made the tasks too challenging for patients. Indeed, no adaptations were applied on the basis of individual PS abilities. However, the time cap had been decided after group-based preliminary findings on patients' performance from the pilot study. Although adjusted to be suitable for most patients with MS, this time-limited condition might have prevented efficient information processing or strategy acquisition for those with more severe PS deficits and, in turn, resulted in lack of overall gains in performance. The practical implication of these findings is that, in order to be effective, cognitive rehabilitation should allow patients all time needed to complete processing of stimuli and decision making to the full. This is to ensure that all the necessary underlying processes are duly engaged and can foster functional rewiring. Second, the comparison between the active groups strengthens the evidence about the potential of the Standard programme to tackle cognitive decline in MS effectively. Indeed, patients in the two groups performed the same tasks across the same number of sessions and interacted with the researcher in analogous ways. It follows that divergent effects on cognitive outcome measures do not appear to depend on differences in treatment exposure across experimental conditions (apart from the imposition of pressure on information processing in the PS-loaded programme version), thus ruling out some unspecified placebo effects triggered by the personal attention received as a result of engaging with the trial. In fact, no changes in self-reported levels of depression and anxiety were detected across treatment groups.

Therefore, on one hand prompting patients with MS to perform cognitive tasks by engaging multiple functions simultaneously appears to lead to improvements in their cognitive health. On the other hand, however, reducing the time allocated to process information with the aim of stimulating quicker answers seems to be an ineffective strategy.

6.1.4.2. Structural connectivity

The analysis carried out on DTI scans showed no effects of cognitive rehabilitation on WM microstructural integrity: both active groups had no greater changes in FA than those observed in the control group. Previously, only one study has used a similar methodology in a sample of patients with RRMS (Filippi et al., 2012). Despite methodological differences in the length of the rehabilitation programme (twelve weeks vs four weeks) and the type of imaging analysis carried out, a similar lack of modulation of structural connectivity by means of cognitive rehabilitation was observed. Two studies do not probably constitute enough sources of evidence to discard the idea that non-pharmacological interventions for MS-related cognitive impairment may be able to induce structural neuroplastic changes. However, they pose a challenge for future investigations since neither high-intensity nor low-intensity multi-domain cognitive rehabilitation appeared to influence WM microstructure.

One study has been able to detect short-term experience-dependent microstructural plasticity in MS. A randomized controlled trial of a video-game-delivered balance training intervention in people with MS (Prosperini et al., 2014) observed significantly increased FA and decreased RD in the superior cerebellar peduncle twelve weeks after training, though these changes were not maintained at the 3-month follow-up. Therefore, these findings suggest that also in brains affected by a demyelinating disease like MS, neuroplasticity may still occur at a microstructural level. Whether these effects can be triggered by cognitive interventions and whether treatment exposure over time plays a role can only be ascertained with further research. Indeed, the study presented in this chapter might have failed to induce microstructural changes bacuase it included only twenty sessions in four weeks. However, longer treatments may not necessarily stimulate such changes (Filippi et al., 2012).

In fact, a few studies explored the effects of cognitive rehabilitation on neural structural connections and found contrasting results. Training of logical reasoning in a sample of healthy participants without neurological disease has been associated with changes in both mean and radial diffusivity in a widespread network including frontal and parietal areas (Mackey et al., 2012). It is worth noting that the age of the participants recruited by Mackey et al. (2012) was around 21 years, an age at which myelination of associative tracts is still an ongoing process and, in turn, the brain is more plastic (Lebel et al., 2008). However, a modulation of DTI indices in parietal areas that correlated with improvements in PS abilities following multi-domain cognitive training was found in healthy elderly people (Cao et al., 2016). On the

contrary, no effects on WM microstructural integrity have been seen in stroke patients who underwent a 6-week working memory training (Nyberg et al., 2018). Consequently, such variety of findings currently leaves open the question about whether improvements in structural brain connections can be induced by cognitive rehabilitation, both in MS and other neurological conditions.

6.1.4.3. Functional connectivity

The investigation of the effects exerted by cognitive rehabilitation on functional brain organisation clearly showed a modulation of connectivity of the salience network only. These changes occurred in response to the Standard cognitive programme and were observed in comparison to both PS-loaded treatment and standard care, thus matching the findings at the cognitive level. The salience network is functionally associated with networks differently involved in various cognitive control functions (Cauda et al., 2013) and appears to have a mediational role in the transition from an internally focussed to a more externally directed activity (Sridharan et al., 2008).

It may be argued that engagement in the multi-domain exercises included in the rehabilitation programme might have been particularly reliant on the use of this network. In particular, it was found that after completing the standard treatment patients showed decreased functional connectivity in the anterior cingulate cortex, an important hub of the salience network (Seeley et al., 2007). Previous studies reported a change in the opposite direction (Filippi et al., 2012, Parisi et al., 2014a), that seemed to be associated with maintenance of cognitive improvements two years after treatment completion (Parisi et al., 2014b). However, this discrepancy may be due to the different form of treatment used by Filippi et al. (2012) that included exercises for attention, PS and executive functions. Instead, the programme used in this study involved also intensive practice of lexical-semantic processing across exercises. Additionally, treatment exposure was noted to be significantly different: while in this study an intense pace was followed, patients recruited by Filippi et al. (2012) completed a total of thirty-six sessions, with three sessions a week spread over twelve weeks. Finally, differences in sample characteristics may have played a role as the patients who took part in the study by Filippi et al. (2012) appear less educated and had MS for a longer period of time than those recruited for this study.

The role of anterior cingulate plasticity in supporting post-treatment cognitive improvements appears interesting because thickness of this brain area has previously been linked to different fluency tasks (Geisseler et al., 2016). However,

the supplementary analysis in this study highlighted how gains on the semantic fluency test induced by the standard programme were associated with increased connectivity of the left hippocampus. This brain area, considered a part of the DMN (Buckner et al., 2008), has been long known to be involved in declarative retrieval, both semantic and episodic (Burianova et al., 2009, Prince et al., 2005, Venneri et al., 2018). Moreover, functional coupling between the hippocampus and the semantic network has been observed during performance on the semantic fluency task (Glikmann-Johnston et al., 2015).

The comparison between the two active groups showed changes in connectivity of the salience network in both directions. In the Standard treatment group a decrease was found in the connectivity of a left-lateralised cluster comprising the thalamus and the putamen. Both these deep GM nuclei are believed to be part of the salience network (Seeley et al., 2007) as well as of the so-called cingulo-opercular network (Muller et al., 2016, Sadaghiani and D'Esposito, 2014, Sestieri et al., 2014). These regions usually undergo shrinkage in MS (Lansley et al., 2013), probably due to their susceptibility to WM damage (Kuceyeski et al., 2015). The severity of atrophy of these structures was also consistently found to predict cognitive impairment in people with MS (Fujiwara et al., 2017), especially in attentional functions and PS (Bisecco et al., 2017, Tortorella et al., 2013). Hence, the behavioural effects observed may be interpreted as an effect of a more efficient reshaping of the salience network.

After treatment completion patients in the Standard group showed increased coupling with areas across the right fusiform and middle occipital gyri. These are visual associative areas that have been seen to be active during performance on the SDMT (Forn et al., 2013), but also to be over-recruited by patients with MS while performing tasks that would not require their contribution (Loitfelder et al., 2011, Rocca et al., 2010a, Sweet et al., 2004). Therefore, the strengthening of the association between the salience network and perceptual areas may emerge as a compensatory change aimed at improving information processing. In fact, it has been suggested that the salience network not only detects salient perceptual stimuli, but favours access of such stimuli to attention and working memory systems for further processing (Menon and Uddin, 2010).

The scenario that emerges from these findings could be interpreted within the framework of the disconnection hypothesis proposed to explain MS symptomatology (Fleischer et al., 2017). The group who had the Standard treatment which resulted in cognitive improvements seems to experience a reorganization of an important functional network that holds a coordinating role by decreasing its internal functional

connections and strengthening those with cortices outside the network. Therefore, these modulations seem to counteract the modularisation of brains affected my MS pathology by reinforcing connections between brain areas and facilitating integration of information. In turn, this may result in beneficial effects on clinical outcome measures, such as cognition. However, no correlations were found between the variations observed in functional connectivity within areas of the salience network and in cognitive performance. A possible explanation may reside in lack of statistical power since only a small number of patients had this form of the treatment. Alternatively, it cannot be ruled out that the effects observed at the two levels (behavioural and neural) may have occurred independently from one another and that the observed neural changes had little or no influence on cognitive performance. Moreover, the network-based approach used might not be the best strategy to detect the neural counterpart of behavioural improvements as they could emerge subsequently to modifications in connectivity between different networks or even between subsets of network nodes. However, this finding seems to suggest that it is possible that inter-network communication changes may play a prominent role as significant associations were detected between improvements in semantic fluency performance and increases in functional connectivity of the left hippocampus within the DMN.

Finally, arguments similar to those exposed in section 6.1.4.1 may be extended to the divergent functional connectivity findings from those of previous studies that used the original version of the cognitive rehabilitation programme (De Marco et al., 2016, De Marco et al., 2018). Indeed, a modulation of the DMN has not been replicated in this clinical population. However, the modifications applied to the cognitive exercises and the different demographic, cognitive, and neurological profiles of the two patient samples may have driven such discrepancies. In particular, the programme was integrated and modified to tackle more intensively sustained attention and executive processes, which are more in the domain of the salience network where changes were indeed observed in this study.

6.1.4.4. General

A series of strengths of this study can be highlighted that differentiate it from previous investigations. First, the cognitive programme used has been developed on the basis of a hypothesis, i.e. stimulating the brain by means of multi-domain exercises that tap into different functional networks should enhance neural connections and lead to cognitive improvements. Moreover, a second hypothesis

concerning the fact that increasing cognitive PS demands may induce even larger improvements was also investigated. On the contrary, so far no other studies overtly referred to, tested or proposed theories or models about how cognitive rehabilitation should be in MS, and all focussed on training only specific symptoms. Second, an active control group was included in the study design to enable a direct comparison between alternative treatments aside of standard care. Indeed, the Standard programme elicited improvements on the same neuropsychological tests in comparison to both of the other groups. Hence, it is suggested that the observed effects may be due to rehabilitation-related mechanisms rather than some sort of placebo effect due to exposure to a new care setting and the interaction with the researcher. Third, the use of multiple MRI outcome measures is particularly important, though limitedly explored in the literature, as an objective means to assess possible mechanisms of action of cognitive rehabilitation. Fourth, the inclusion of self-reported measures of clinical symptoms and quality of life ensure that possible behavioural effects may not confounded by these factors.

Yet some limitations must be noted, such as the size of the samples that is to be considered small, as only fifteen patients were included in each treatment group. This situation partially depended on the study design itself since the number and frequency of hospital visits to attend daily sessions had an impact on people willingness to take part in the study. However, the experimental nature of the investigation could not allow high degrees of adaptability in order to maintain treatment conditions as similar as possible across participants. Exploring home-delivered computerised interventions may overcome this issue in future investigations. Given these conditions, a possible selection bias towards overall more educated and motivated participants than those recruited in other studies may not be completely ruled out. The PS-loaded exercises were not subjected to adaptations to individual cognitive abilities, thus possibly limiting the potential effects of the treatment. Finally, hemispheric dominance and handedness were not recorded, although the researchers involved in the study recall that most patients were right-handed. Additionally, no objective measure of motor performance is available for all patients. Only reaction times were collected for patients in the two active groups who showed a comparable performance since no significant between-group differences were observed. However, all patients recruited had no upper limb motor deficits and the motor action required by all exercises in the programme was a simple key press. Therefore, any influence of motor performance and handedness on either the cognitive or neural results observed would be negligible. Indeed, no

modulation of psychomotor speed on either simple or choice reaction times was observed following cognitive rehabilitation.

In general, this study shows that the original hypothesis of rehabilitation by boosting neural connectivity may be effective as a form of non-pharmacological treatment for people with stable RRMS well grounded in a solid theoretical framework. Nonetheless, the strategy selected to modulate PS demands in the rehabilitative programme and test the neurocognitive consequences of this modifications did not exert the expected effects. In fact, as PS deficits are often observed in patients with MS it was forecasted that practising fast information processing could facilitate cognitive gains. Future investigations into alternative approaches to clarifying this issue may be trialled by using adaptive training. This may be realised by introducing the possibility to individualise exercise PS-demands so that escalation to more difficult (i.e. faster) sessions would depend on the accuracy of each participant's previous performance.

Use of advanced brain imaging analysis techniques, e.g. graph theory, is an area to be developed further to test how non-pharmacological interventions might induce structural neural adaptations. Volumetric changes might be difficult to observe in response to a short-term, although intense, treatment in brains already affected by different types of damage, including neurodegeneration. However, changes at a microstructural level may be better detected in terms of connection strength across network. Nevertheless, considering that cognitive rehabilitation acts on brain functioning, it follows that more significant effects should be detected (as in the case of this study) by assessing brain activity, even at rest. However, it is possible that only more detailed techniques characterised by higher resolution, e.g. new generation 7T MRI scanners, will enable the ascertainment of whether clinically meaningful practice-dependent neuroplasticity actually occurs in this clinical population and to what extent.

Chapter 7 | General discussion

Cognitive impairment is one of the many symptoms that MS may present with and is increasingly recognised as a major source of functional burden for people affected by this condition. Suboptimal performance across a variety of domains has been associated with a negative impact on quality of life (Glanz et al., 2010). In fact, the majority of the people affected by MS are diagnosed around 30 years of age, a period of life which represents the peak of productivity both in personal and professional contexts. Therefore, it is understandable that effortful processing of information and reduced attentional functions can have a detrimental impact on intellectual outputs and emotional status. As a result, cognitively impaired people with MS are highly likely to face work capacity reduction and even unemployment. Therefore, better and more articulated understanding of the causes that lead to cognitive decline in MS emerges as a pressing issue that needs to be targeted with effective treatments.

Shedding light on how core cognitive deficits relate to brain imaging indices at different levels appears particularly crucial. For decades, scholars have been wondering why patients with similar levels of brain damage show variable degrees of functional impairments, i.e. the so-called clinical-radiological paradox. Cognitive reserve may represent a helpful concept that provides a justification for such paradox by considering inter-individual differences in the efficiency of neurocognitive functioning due to exposure to life experiences and their role in shaping the clinical manifestations of MS (Stern, 2009). However, recent technical advancements in the acquisition and analysis of brain imaging have enabled more detailed investigations of the relationship between severity of neural damage and clinical symptoms (Droby et al., 2015a, Welton et al., 2015). Deeper characterisation of predominant structural and functional pathways to cognitive impairment in MS would also contribute to guiding the design and assessment of different types of interventions.

Pharmacological treatments currently used in clinical practice are mainly DMTs designed to intervene during the relapsing-remitting phase and meant to reduce the recurrence of relapses. However, no definite treatment is currently available to stop this disease, although encouraging results have been observed with stem cell transplantation (Sormani et al., 2017a). Moreover, it must be noted that cognitive symptoms are usually an overlooked issue in clinical trials and that the EDSS score, the main outcome measure of disease severity, is heavily reliant on motor dysfunction. So far, only a few investigations have been carried out on the effects of

DMTs on cognitive functioning. The results of these studies have been variable. Also drugs repurposed from related clinical fields and trialled to treat specific cognitive symptoms in MS have mainly led to failure. Lack of knowledge about the factors (biological or otherwise) that might mitigate cognitive dysfunction in people with MS and about the evolution of these symptoms over time may have contributed significantly to lead research in this field to such a dead-end. Similarly, non-pharmacological interventions for cognitive deficits experienced by people with MS have only achieved partial results. In particular, solid theoretical foundations appear to be lacking in the design of most interventions that, in turn, mainly represent explorative attempts to induce cognitive improvements. Moreover, integration of MRI outcome measures has been pursued only by a small number of studies even though this brain imaging technique plays a major role in diagnosis and routine check-ups.

Experiment 1 and 2 were designed to assess how patients' cognitive performance, particularly in conditions with high PS load, was modulated by degradation of structural brain connections. In fact, it is widely believed that slowed information processing is among the most common symptoms observed during neuropsychological assessments in people affected by MS. Since PS is a multifaceted function, slower cognitive computations may be observed across several related functions, namely attention, working memory and learning. Indeed, a wide variety of psychological constructs imply not only structural (i.e. how much information can be processed) but also operational capacity (i.e. how or how fast the information can be processed). Therefore, different neuropsychological tests of PS skills used in clinical settings can be fruitfully used to unravel possible associations with neural measures detected by means of MRI.

The study of a sample of people with RRMS showed that visual PS measures correlated with microstructural integrity of the anterior corpus callosum, the anterior thalamic radiations and the inferior fronto-occipital fasciculus. All of these WM tracts connect areas of the frontal lobes with other subcortical and cortical hubs that are mainly associated with attentional and executive functions. The same tracts, among others, were found underpinning cognitive performance also in the SPMS group with the difference that associations were mainly observed for PS measures involving verbal processes. Although only qualitative comparisons can be made between the two experiments, results strongly suggest that a distributed network of structural connections mainly located in frontal areas support fast information processing in MS. Therefore, demyelination of both interhemispheric and associative WM fibres that enable cognitive control processes to be performed by the frontal lobes appears

to limit PS abilities in these patients. In fact, this pathological process may pose limitations to functional compensatory phenomena consistently observed as more extensive frontal activation needed to sustain optimal performance in demanding conditions (Forn et al., 2013). In MS and several other diseases, increased frontal recruitment is seen in conditions that are considered not challenging for healthy individuals and for this reason is thought to signal reduced neural efficiency and increased cognitive effort (Chiaravalloti et al., 2015). Hence, it can be argued that damage to structural connections of frontal areas usually involved in boosting cognitive performance may reduce efficient communication within these same areas and result in serious limitations to any compensatory mechanisms.

In Experiment 3 and 4 the contribution of different functional networks to PS-demanding cognitive processes was investigated in both RRMS and SPMS. In particular, considering the results found in Experiment 1 and 2 fronto-parietal networks were expected to emerge as core correlates of fast information processing. Several brain networks were investigated with the aim to explore those known to be involved in different cognitive functions, in consideration of the fact that these networks do communicate with one another (Sridharan et al., 2008). Indeed, although specific cognitive processes have been linked to definite cerebral anatomical locations, it is now widely accepted that the brain works by engaging networks encompassing different areas in order to perform complex functions. In parallel, sensory and motor networks were also investigated to highlight and rule out possible non-cognitive contributions to indices of PS function (Costa et al., 2017).

In line with expectations, measures of verbal PS resulted mainly associated with functional connectivity of the left fronto-parietal network in both groups of patients. Additionally, the salience network and, limitedly to patients with RRMS, the DMN were found to be implicated in PS abilities measured by different neuropsychological tests. However, TMT-A scores correlated with connectivity of the sensorimotor network, possibly because of the major reliance of this test on the execution of upper limb movements. As already seen at the structural level, it is likely that PS performance depends also on the integrity of different functional networks and on the communication between them. In particular, the involvement of left fronto-parietal network may depend on attentional and executive processes required to perform the tasks included in the assessment independently of the modality of stimuli that need to be processed. However, integrity of the salience network appears to have an impact on fast cognitive processing. A reason may resides on the fact that this set of areas processes environmental stimuli and attaches valence to them and, in turn, prioritises access of the most relevant stimuli to higher

functions, mainly performed by frontal and parietal associative areas (Menon and Uddin, 2010). Therefore, current knowledge of this network may suggest it plays an important role in ensuring information processing occurs smoothly. Whether the malfunctioning of one of these networks or of an hub belonging to such networks play a pivotal role in affecting fast information flow across the brain affected by MS cannot be definitely established with these experiments. Further hypothesis-driven investigations particularly focussed on connectivity of those considered the principal hubs of such networks should be brought about to clarify this issue.

So far, most studies carried out on this topic concentrated their attention mainly on the most widely known network, i.e. the DMN (Janssen et al., 2013, Rocca et al., 2010b, Wojtowicz et al., 2014, Zhou et al., 2014). In contrast with this line of research, Experiment 3 and 4 did not highlight a prominent involvement of the DMN, only found to be associated with scores obtained by patients with RRMS on the semantic fluency test. Different reasons could account for such results: first, this network is particularly involved in memory-related and internally-generated processes rather than more general speed of information processing (Sestieri et al., 2011); second, it is believed that the DMN negatively affects goal-directed cognitive performance if its deactivation fails to occur (Anticevic et al., 2012). Hence, this network may exert only indirect influences on the speed at which information is processed. It is worth noting that no exhaustive comparisons with the previous literature can be made due to lack of investigations into the relationships between functional connectivity of other networks and cognitive performance in MS.

Experiments reported in Chapters 4 and 5 suggest that the combined use of multiple cognitive measures with different PS involvement may provide useful insights for the understanding of the MS-related neural changes associated with performance on this function. Indeed, considering that MS pathology may affect various cognitive and non-cognitive functions (Costa et al., 2017) and that performance on different PS tests partially depends on other cognitive functions, the investigation of a single measure of PS abilities may provide only a partial view of the picture. In contrast, hypothesis-based comparisons between multiple PS measures appear necessary in order to ascertain the potential contributions of different neural circuits to such function.

A possible limitation associated with the cognitive measures used in the experiments in this thesis relates to their ecological validity, i.e. the ability of cognitive measures to predict patients' functional impairment in real life. Indeed, the ecological validity of neuropsychological assessment for patients with MS remains an overlooked issue (dasNair et al., 2018). Research has shown that tests of

memory and attention designed to have enhanced ecological validity predict MS-related functional imparments better than standard tests, althought both types are correlated with severity of impairment (Higginson et al., 2000). Moreover, it has been observed that environmental distractions (similar to those present in real life settings) during task performance may enhance the detection of PS deficits in people with MS (Randolph et al., 2017). However, performance on common tests of PS function, as well as global cognitive performance (Goverover et al., 2016), appears to be associated with scores on the Timed Instrumental Activities of Daily Living task (Goverover et al., 2007) and the Test of Everyday Cognitive Ability (Charvet et al., 2018), both tests particularly sensitive to MS-specific functional impairments. Therefore, although further investigations are needed to produce more clinically relevant results on how neural changes impact cognition in MS, it seems reasonable to suggest that commonly used cognitive tests have moderate ecological validity.

Findings of the first four experiments outline a complex scenario characterised by multiple levels of disconnection underlying PS abilities observed in different MS phenotypes. In this framework, counteracting MS pathological processes by means of connectivity restoration appeared a reasonable strategy to attempt in Experiment 5 with the aim of reducing the extent of cognitive impairments. Differently from other approaches used before, this experimental work was based on the hypothesis that enhancing the communication between different neurocognitive systems that rely on networks of functionally (and structurally) related areas could boost performance. Hence, a previously tested cognitive rehabilitation programme (De Marco et al., 2016) comprising a set of multi-domain exercises was partially adapted to the cognitive profile typically observed in MS. The effects of this programme were not only compared to usual care, but also to a modified version with increased PS-load implemented in all exercises. The second hypothesis behind this intervention was that speeding up the abovementioned inter-network communication would produce even broader neural and cognitive effects.

The relapsing-remitting phenotype appeared to be the natural target of this intervention due to the expectation of lower degree of neurodegeneration, in comparison to SPMS, that might have negatively impacted on treatment outcomes. Similarly, higher levels of disability, both cognitive and physical, experienced in advanced stages of the disease were considered a significant obstacle to compliance to high-intensity treatments. Moreover, the limited number of patients presenting with the secondary progressive phenotype could have affected the

recruitment phase and should be carefully considered in order to design trials with enough statistical power and appropriate to the needs of this population.

Among these two hypotheses, only the first one was supported by the results of this experiment. In fact, engagement in cognitive rehabilitation without time constraints led patients to experience cognitive improvements significantly larger than those seen in the other two groups. These changes were observable on tests of learning and semantic processing. Although long-term memory was not specifically targeted by the programme, the effects seemed to transfer to this function that substantially relies on the manipulation of semantic representations that are known to depend on a distributed neural system. At the neural level, neuroplastic effects were detected in parallel to cognitive improvements in the same contrasts, i.e. standard treatment vs control and standard vs PS-loaded treatment. Only functional connectivity changes were detected limitedly to the reorganisation of the salience network itself and with areas outside the network, since increased coupling was seen between the salience network and more posterior visual areas.

However, the clinical relevance of these results remains partially elusive. In fact, the salience network is known not to be involved in mnestic functions or semantic processes. Speculations could be made that increased connectivity between this network and occipito-temporal areas involved in visual object recognition may enhance information processing and saliency detection. As a consequence, information encoding and later recall may be enhanced. Nevertheless, no correlations were detected between the observed cognitive improvements and changes in functional connectivity of the salience network.

Post hoc analyses on the possible links between cognitive and neural changes showed that the increased number of items reported by people in the standard treatment group on the semantic fluency task was associated with higher functional connectivity of the left hippocampus, a part of the DMN. This result appears plausible and in line with current knowledge regarding the neural correlates of semantic memory functioning (Burianova et al., 2009), although no modulation of this network was seen across treatment groups. A possibility is that multiple networks have been modulated by the intervention and analyses focussed on individual networks may not fully capture the extent of the effects exerted. Improved inter-network connectivity may meaningfully contribute to sustaining efficient cognitive performance more than functional reorganization within single networks.

The experiments carried out offer some insights on how different levels of brain organization relate to PS-demanding performance in people with MS who usually suffer from slowing in cognitive abilities. Moreover, evidence that cognitive

rehabilitation can trigger both behavioural and neural changes has been shown in experiment 5. However, as previously mentioned each one of these studies has limitations and further research is necessary to clarify the underpinning of these positive changes. Advanced imaging techniques, in particular more extensive applications of ultra-high field MRI at 7T, hold the potential to improve spatial resolution of image acquisition significantly, and to enable more detailed analysis of lesions due to MS. Indeed, so far cortical and subcortical lesion contribution to cognitive decline in MS has gained limited and variable results. Similarly, the use of novel diffusion-weighted sequences such as high angular resolution diffusion imaging (Frank, 2001) and neurite orientation dispersion and density imaging (Zhang et al., 2012), now increasingly available in clinical contexts, provides alternative models more accurate than that based on the diffusion tensor. These images can be fed to tractography analysis resulting in the reconstruction of detailed tri-dimensional WM fibre tracts (by means of spherical deconvolution methods) and improved quantification of macro- and micro-structural alterations that can be linked to clinical symptoms. Nonetheless, limitations to the applicability of this type of analysis must be considered since probabilistic fibre tracking relies on directionality of water diffusivity in the brain, i.e. on FA values in each single voxel. It follows that abrupt variations in FA due to the presence of demyelinating lesions can affect the efficient reconstruction of WM tracts and lead to false positives (Ciccarelli et al., 2008).

Additionally, the development of new methodologies to analyse MRI data must necessarily move forward with a multidisciplinary effort that would combine technical-mathematical insight with clinical knowledge. In particular, the application of graph theory and network-based analysis are increasingly regarded as new tools to uncover alterations in brain functional and structural organization due to pathological processes. Such approach has already been applied to MS (Fleischer et al., 2017) and with apparent good reason, considering that MS lesions randomly distribute across the brain causing damage to structural connections and functional alterations. However, more sophisticated and hypothesis-driven investigations may shed light on specific pathways that are crucially involved in MS-related cognitive symptoms. Moreover, biological and environmental factors that may contribute to mitigate cognitive decline have received so far little attention, especially their impact on connectivity measures. The implementation of this kind of analysis in studies with longitudinal designs will enable the tracking of the evolution of clinically relevant changes over time.

Regarding treatments for cognitive deficits in MS, two possible strategies may be further explored. One relies on the better characterisation of the effects exerted on cognitive performance by the most promising DMTs. Currently, how most of the available pharmacological treatments affect brain structure and functioning has been very limitedly explored by means of advanced MRI techniques. In fact, global measures of atrophy and lesion volume are fast and, to some extent, clinically informative outcome measures in clinical trials. However, exploration of more sophisticated MRI sequences and dedicated analyses may clarify the wider impact that DMTs have on the brains affected by MS and even factors causally involved in treatment responsiveness. In particular, an intervention for MS that appears worth more thorough investigations is transplant of autologous stem cells due to its recognised potential.

An alternative to medications is the development of effective non-pharmacological interventions. Integration of the wealth of knowledge about MS-induced cognitive impairments and their neural correlates with theories on neurocognitive functioning should be pursued to tackle these symptoms. Additionally, the use of non-invasive neurostimulation has also been drawing attention in the past decades in the field of neuroscience. Several techniques, above all transcranial direct current and transcranial magnetic stimulation, have been tested in the treatment of a wide range of clinical manifestations in MS. Significant results have been highlighted in a recent review about improvement of cognitive as well as motor performance (Iodice et al., 2017), thus motivating further research on the potential effects of protocols combining synergistically cognitive rehabilitation with neurostimulation.

Another possible pathway to explore with the aim of improving the delivery of cognitive interventions is the personalised home-based approach involving technological devices (Lavorgna et al., 2018). In fact, adopting an e-health approach could help improving the clinical management of this patient population that presents with a quite variable range of symptoms. From a research point of view recruitment of patients for clinical trials could be facilitated by delivering interventions by means of applications for digital devices and allowing more degrees of flexibility to meet participants' needs. Using this type of approach, more difficult populations, especially those people affected by progressive and more severe forms of the disease, would have the opportunity to be more easily included in trials specifically designed on their clinical profile. Finally, this approach would enable the testing of treatment effects in an everyday life context and would provide insights into the real applicability of such interventions and their ecological validity.

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APPENDIX A

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Review Article

Brain connectivity and cognitive processing speed in multiple sclerosis: A systematic review

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ABSTRACT

Background: Processing speed (PS) decline is the most commonly observed cognitive deficit in people with multiple sclerosis (MS) resulting in a significant impact on quality of life. Despite its importance, knowledge of the underlying neural substrates is lacking.
Objective: As MS is increasingly recognised as a disconnection syndrome, our aim was to carry out a systematic literature review to clarify the relationship between PS performance and MRI measures of structural and functional brain connectivity in people with MS.
Search methods: A literature search was carried out on PubMed and Web of Science that included publications predating September 2017. Additional articles were added after inspection of the reference lists of all selected papers.
Data extraction: All selected papers were categorised in three sections according to the MRI measures investigated, independently or both. Quality assessment was carried out using a customised set of criteria.
Results: Thirty-two articles met the inclusion criteria and were included in the review. Microstructural integrity of the anterior corpus callosum and functional connectivity of frontal areas were more consistently found to correlate with PS performance, though high variability of findings was observed across studies. Several methodological flaws emerged from the reviewed literature.
Conclusions: Despite the observed trends, no definite conclusions can be drawn on the relationship between brain connectivity and PS decline in MS given the limitations of the current literature. Future investigations may benefit from theoretical and methodological advances to clarify how MS-related brain damage affects patients' cognition.

1. Introduction

Multiple sclerosis (MS) is an immune mediated disease characterised by an abnormal immune response targeting the central nervous system and causing both axonal demyelination and neuronal loss. The clinical course is variable, but can be categorised based on the degree of disease activity and disability progression rate into relapsing-remitting (RRMS), primary progressive (PPMS) and secondary progressive (SPMS) [1]. About 40 to 70% of people with MS experience cognitive impairment that may significantly impact both their quality of life and employment [2–5]. The cognitive domain that is most consistently affected in MS is processing speed (PS) [6,7]. PS is usually assessed by measuring the amount of information processed in a unit of time or the time needed to process a given amount of information [8,9].

Deficits in PS may have a broad influence on cognitive performance in people with MS. Indeed, memory and learning impairment is associated with impaired PS function [10–12]. Similarly, working memory and attention functions were predicted by performance in PS tasks that were also observed to be the best measure to discriminate people with MS from healthy controls [13–17].

Deficits in executive functions [18] and, more specifically, in planning [19,20] and interference inhibition on the Stroop test [21,22] are associated with PS across the various clinical courses of MS. Several studies investigated the impact of PS decline on cognition in people with MS. It was observed that in tasks of working memory [14,23], response inhibition [21], planning [20], task switching [24], and attention [25], after statistically controlling for PS performance, the differences between people with MS and healthy controls disappeared.

Despite extensive investigation into cognitive impairment in MS, its relationship with specific aspects of neural damage, especially in

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relation to PS function, is not clear yet. A meta-analysis of seven diffusion tensor imaging (DTI) studies found that cognitive decline in general was associated with lower fractional anisotropy, i.e. a measure of integrity of structural connectivity, in various tracts involved in different cognitive functions [26]. Another meta-analysis of thirty-nine studies [27] showed a strong correlation between measures of cognitive PS, namely the Paced Auditory Serial Addition Test (PASAT) and the Symbol-Digit Modalities Test (SDMT), and indices of white matter (WM) lesion volume and atrophy. However, most of the reviewed studies were carried out on samples of patients with mixed MS phenotypes using MRI measures that are global indicators of neurodegeneration and not linked to functionally defined brain regions. Only one study investigated the association between MS lesion location and cognitive impairment and found that lesions occurred with greater frequency in the splenium and forceps major of the corpus callosum in cognitively impaired patients [28].

MS is increasingly recognised as a "disconnection syndrome" where widespread WM damage hampers communication between brain regions in a non selective manner [29–35]. In line with this view the aim of the present review was to evaluate correlations between PS function in MS and measures of structural and functional connectivity.

2. Methods

A systematic review of neuroimaging studies investigating the relationship between indices of brain connectivity and performance on tasks of PS in MS was carried out. The specific aim was to summarise current knowledge about the relationship between breakdown in brain connectivity and PS function in people with MS.

A literature search was undertaken in two online databases: PubMed and Web of Science. Studies using DTI and resting-state functional MRI (RS-fMRI) in combination with cognitive PS measures were specifically targeted. The exact strings searched are reported in Appendix A – Table A.1. No time limits were set and all papers published up to September 2017 were assessed following the steps highlighted in the PRISMA statement (Fig. 1) [36]. Additional papers from the reference lists of the selected articles that had not been identified in the literature searches were also included. After removal of duplicates, the full text of the remaining articles was inspected and paper selection was performed according to the following exclusion criteria: (1) review articles, (2) theoretical and/or modelling papers, (3) papers related to patients with pediatric-onset MS, (4) papers related to diseases different from MS, (5) animal studies, (6) biological studies, (7) pharmacological studies, (8) papers with no inclusion of PS measures, (9) papers with no use of either DTI or RS-fMRI techniques, (10) papers not in English (Fig. 1).

Papers selected to be included in this review were assessed according to a customised set of criteria, adapted from those used by Welton and colleagues [26], that give an indication of their scientific quality and to ascertain possible sources of bias. A checklist of twelve questions was created and organised in five areas: methodology, clinical characteristics, MRI parameters, statistical analysis and results. Particular attention was given to the provision of details about the characterisation of the samples recruited and the analyses performed. A point was assigned for each quality criterion fulfilled. For the criterion assessing sample composition, 2 points were assigned to studies carried out on one or more groups of homogenous MS phenotypes, 1 to studies that included mixed phenotype samples, and no points to those reporting no information about phenotypes investigated. Therefore, the maximum score that could be achieved was 13 points. For more detailed information about quality assessment see Appendix B – Table B.1

3. Results

A total of 820 papers were identified through online search and review of all the available references. Three hundred and forty-two entries were duplicates and the remaining 478 records were fully screened for eligibility. Thirty-two papers, all published between 2008 and 2017, met the final selection criteria to undergo review. Twenty-three studies reported the use of DTI measures to investigate structural connectivity only, 4 studies used RS-fMRI only for functional connectivity, and 5 studies combined DTI and RS-fMRI.

A summary of the quality assessment of the reviewed articles is reported in Table 1. Differences in the overall quality of papers between the three MRI categories were analysed using the Kruskal-Wallis test. The analyses showed that differences were significant $\chi^2(2) = 6.497$, $p = 0.039$. After applying Dunn's multiple comparisons test, the only difference that remained significant was the one between studies using only DTI and those combining DTI with RS-fMRI ($p = 0.035$), with the latter showing higher scores (Fig. 2). More detailed information on the evaluation of each quality criterion is reported in Appendix B – Tables B.2-B.4.

These findings show a gap in the overall scientific quality between DTI studies and those combining DTI with RS-fMRI measures. Arguably, this may be driven by technological advances and indeed studies using RS-fMRI were in general more recent than the DTI ones. However, it is also possible that studies combining several MRI techniques might have been more thoroughly designed. It must be noted, however, that only a few studies have been carried out with combined methodologies, thus making any conclusions not definitive.

3.1. Structural connectivity

Moderate heterogeneity was seen across studies with respect to sample composition, clinical information, analysis techniques, and covariates of no interest (Table 2). In particular, despite the fact that the majority of the studies investigated RRMS, eight included patients with different MS clinical courses without specific sub-sample analysis [37–44]. Progressive MS was underrepresented, with a single study on patients with SPMS [45]. Two papers were published on so-called "benign MS" [46,47], while one did not report explicitly the type of MS investigated [48].

In general, information on relapses and medications taken at the time of data collection were reported by most studies, but comorbidities and the presence or absence of fatigue and depressive symptoms were scarcely documented. This, together with lack of clearly stated a priori hypotheses lowered the quality of studies using only DTI measures compared to the others reviewed. There was, however, a trend towards improvement with better quality studies found in the most recent investigations of structural connectivity.

Statistical analyses were carried out with different approaches, the most common being the investigation of one or more regions of interest that was used in fourteen out of twenty-three studies [37,38,40,42–44,46,47,49–54]. The definition of the regions of interest was mainly *a priori*, though 2 studies defined them according to task-related functional activation [51] and differences in fractional anisotropy between MS patients and healthy controls [37]. Eleven out of twenty three studies did not use multiple comparisons correction strategies [37,40,42,44,48–54] and twelve did not control for any covariate of no interest [38,39,42–44,48–54]. Among those publications that did, age was always included, followed by sex and premorbid cognitive status.

The most consistent difference observed between people with MS and healthy controls in DTI studies was the presence of abnormalities in the corpus callosum. This interhemispheric bundle of fibres appeared to be particularly affected by MS pathology. Additionally, other WM tracts also showed abnormalities including: the superior and inferior longitudinal fasciculus, the cingulum, and the fornix. Most of these are associative WM tracts that mainly support different cognitive functions.

Weak or absent correlation between DTI indices and PS measures was reported in five papers using the PASAT, in particular the 3 s version (PASAT 3″) [42,43,46,53,58]. This finding, in line with the aforementioned review on atrophy measures [27], may be due to a lower PS load of the 3 s version compared to more challenging versions

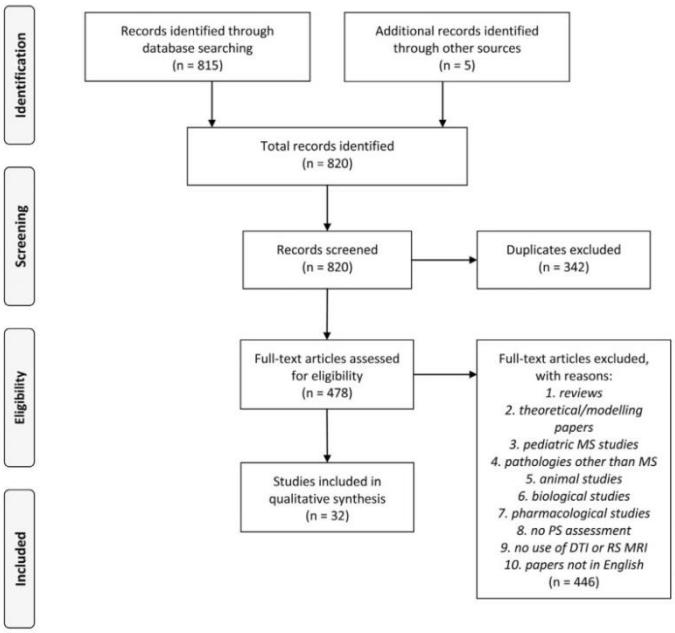

Fig. 1. Flow chart outlining the study selection process.

Table 1
Descriptive statistics for the overall study quality assessment, categorised by MRI technique.

MRI technique	Median	Interquartile range	Minimum	Maximum
DTI	9	4	4	12
RS-fMRI	10	6	6	12
DTI and RS-fMRI	12	3	10	13

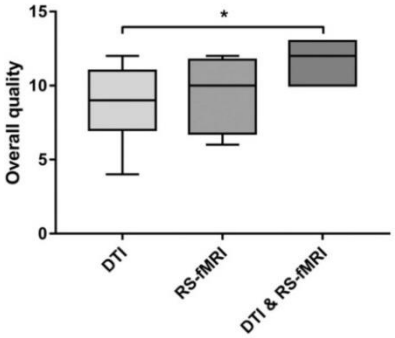

Fig. 2. Differences in the overall quality of reviewed papers.

of the same test or to the SDMT. Indeed, Sbardella et al. [58] observed that the PASAT 2″, but not the PASAT 3″, significantly correlated with both fractional anisotropy and mean diffusivity in a widespread network of WM tracts centred on the right inferior longitudinal fasciculus and the left cingulum. While Sbardella and colleagues [58] used a tract-based spatial statistics approach to investigate voxel-wise associations within a skeleton of WM containing only the core of the tracts, lack of correlation between the PASAT 3″ and DTI indices was otherwise observed in studies of patients with different MS phenotypes utilising either whole-brain global indices [48] or several regions of interest: anterior thalamic radiations [46], corpus callosum [46,53], fornix [42], posterior cingulum and posterior limb of the internal capsule [43].

In line with the findings from comparisons between people with MS and healthy controls, in studies with mixed MS phenotypes, the corpus callosum was the WM bundle most commonly reported to be correlated with the PASAT 3″, both in region-of-interest [38,47,49] and voxel-wise investigations [29,39,55,56]. However, performance on this test was also noted to correlate with the degree of microstructural integrity of other WM tracts, mainly: the left cingulum [29,39,58]; the superior longitudinal fasciculus, especially on the left side [29,39,51]; and the inferior longitudinal fasciculus bilaterally [29,39,58]. Moreover, less consistent associations with the PASAT were detected in the arcuate fasciculus [29], right posterior thalamic radiations and right sagittal stratum [55], hippocampal and cerebellar WM [56], the lateral portion of the frontal lobes [50], and with thalamic mean diffusivity [40]. Only one study found that microstructural integrity of the bilateral uncinate fasciculi predicted PS performance assessed combining the PASAT and the SDMT [54].

The only study carried out on SPMS did not investigate PS as a distinct domain but divided the patient' sample into cognitively impaired and preserved sub-samples, based on performance on various tests, among which were the PASAT and the SDMT. Mean global radial diffusivity, among the different DTI measures, emerged as the only significant predictor of cognitive status. However, all DTI indices were found to be significantly different between cognitively impaired and cognitively preserved groups in: the fornix, the superior longitudinal

Table 2
Characteristics of studies on structural connectivity.

Study	Sample	Age (years)[a]	Duration (years)[a]	EDSS[a]	PS measure	Covariates	Analysis	Results about correlations
Lin et al. [49]	36 RRMS: 9 CI 27 CP 13 HC	37 (7.5) 37.7 (7.2) 34 (29–40)[b]	10 (4.5) 7.2 (6.4)	3.7 (1.5) 2.7 (1.2)	PASAT 3″	None	ROI of the CC	PASAT 3″ negatively correlated with apparent diffusion coefficient in the CC
Roca et al. [50]	12 RRMS 12 HC	32.5 (8.0) 31 (8.5)	N.R.	< 2	PASAT 3″	None	ROIs: FL, FM, OF, cingulate	PASAT 3″ positively correlated with FA only in the FL region
Bonzano et al. [51]	23 RRMS 18 HC	32.5 (4.2) n.r.	6.9 (3.2)	1.6 (0.8)	PVSAT-100	None	ROI of the left SLF	PVSAT-100 positively correlated with mean FA in the left SLF
Dineen et al. [29]	41 RRMS 27 HC	43.5 (31–56)[b] 36.4 (28–55)[b]	10.5 (3–28)[b]	3 (1.5–6.5)[b]	PASAT 3″	Age, IQ, EDSS	TBSS	PASAT 3″ positively correlated with FA in the CC, left SLF and cingulum, right ILF, and bilateral AF and optic radiations
Mesaros et al. [47]	54 BMS 21 HC	46.4 (35–63)[c] 45.7 (25–66)[c]	22.5 (15–39)[c]	1.5 (0–3)[c]	PASAT 3″	Age, sex	ROI of the CC	PASAT 3″ positively correlated with mean FA and negatively with mean MD in the CC
Roosendaal et al. [37]	30 MS: 5 CIS 21 RRMS 4 SPMS 31 HC	40.6 (9.1) 40.6 (9.9)	3.6 (3.5)	3 (0–6.5)[b]	LDST	Age	ROI of clusters of lower FA: MS < HC	LDST positively correlated with mean FA and negatively with mean RD in the left body of the CC
Warlop et al. [48]	15 MS	37.6 (21–49)[c]	5.1 (3.4)	2.5 (2.2)	PASAT 3″ SDMT	None	Whole brain	PASAT 3″ not correlated with any measure; SDMT negatively correlated only with global RD
Ozturk et al. [38]	69 MS: 35 RRMS 20 SPMS 14 PPMS 29 HC	45 (22–66)[c] 40 (22–58)[c] 51 (40–66)[c] 50 (29–66)[c] 34 (22–63)[c]	10 (0–42)[c] 6 (0 – 22)[c] 19 (4–37)[c] 8 (1–42)[c]	3.5 (0–7)[c] 3 (0–6)[c] 6.5 (3.5–7)[c] 3.5 (1.5–6.5)[c]	PASAT 3″	None	ROI of the CC	PASAT 3″ positively correlated with mean FA in the CC, especially anterior body, in all MS phenotypes
Van Hecke et al. [39]	20 MS: 10 LD 10 HD 10 HC	43 (9) 41 (7) 42 (10)	12 (7) 11 (5)	2 (1) 6 (1)	PASAT 3″	None	Voxel-wise	PASAT 3″ correlated with FA, MD and RD in: left cingulum and ILF, bilateral CR, FMI, genu of the CC, SLF, interna and esterna capsulae
Rimkus et al. [52]	23 RRMS 13 HC	31.9 (9.2) 27.7 (5.4)	2.4 (1.4)	1.4 (1.2)	SDMT TMT	None	ROI of the CC	SDMT negatively correlated with mean FA of the CC; TMT not correlated with any measure
Llufriu et al. [53]	21 RRMS 12 HC	37.2 (6.9) 35.2 (7.4)	9.5 (5.4)	2 (0–6)[b]	PASAT 3″ SDMT	None	ROI of the CC	PASAT 3″ and SDMT not correlated with any measure
Yu et al. [55]	37 RRMS 20 HC	40.9 (10.1) 34 (10.3)	9.3 (9.5)	2.2 (0–4)[b]	PASAT 3″ SDMT	Age	TBSS	PASAT 3″ positively correlated with FA in: right PTR, right SS and CC; SDMT positively correlated with FA in: CC, right CR and cingulum, left external capsule, bilateral PTR, SS and UF
Benedict et al. [40]	75 MS: 50 RRMS 24 SPMS 18 HC	46.4 (9) 42.1 (11.5)	11. (7.5)	3.5 (0–6.5)[b]	PASAT 3″ SDMT	Age, education	TBSS and ROI of the thalamus	Mean thalamic MD was third predictor, after age/education and thalamic volume, of scores on PASAT 3″ and SDMT
Bester et al. [46]	26 BMS 24 HC	53.4 (7.1) 51.6 (11.2)	25.8 (9.6)	1.5 (0–3)[b]	PASAT 3″ SDMT	Age, sex	ROIs: CC and ATR	PASAT 3″ and SDMT not correlated with FA and MD either in the CC and the ATR bilaterally
Bozzali et al. [56]	25 RRMS 25 HC	34.5 (8.6) 31.8 (8.1)	7 (2–16)[b]	2 (0–4.5)[b]	PASAT 3″	Age, sex, T2-LL, n. of voxels	TBSS and ACM	PASAT 3″ positively correlated with ACM in: anterior CC, IX cerebellar lobule bilaterally, and right hippocampus
Genova et al. [41]	25 MS: 22 RRMS 2 SPMS 1 PPMS 15 HC	44 (7.9) 36.3 (10.3)	9.6 (7)	N.R.	TMT-A CWIT	Age	TBSS	TMT-A positively correlated with FA in: left body and splenium of the CC, left IFOF, right FMI and PTR, bilateral CR, FMA and SLF; CWIT positively correlated with FA in: left body of CC, right ATR, PTR, splenium and FMI, bilateral fornix and SLF
			8.1 (6.9)	2.2 (0–6)[b]	SDMT	Age	TBSS	

(*continued on next page*)

Table 2 (*continued*)

Study	Sample	Age (years)[a]	Duration (years)[a]	EDSS[a]	PS measure	Covariates	Analysis	Results about correlations
Mazerolle et al. [57]	20 RRMS 20 HC	42.4 (6.3) 42.5 (7.8)						SDMT correlated positively with FA and negatively with MD/RD in: body and genu of CC, PTR, SLF; no correlations with AD
Sbardella et al. [58]	36 RRMS 25 HC	34 (8) 31 (6)	7.4 (6.1)	2.5 (1–4.5)[b]	PASAT 2″ PASAT 3″	Age, sex, T2-LL	TBSS	PASAT 2″ correlated positively with FA in widespread WM tracts and negatively with MD in right cerebral peduncle, right ILF, and left cingulum; PASAT 3″ not correlated with any measure
Koenig et al. [42]	53 MS: 45 RRMS 7 SPMS 20 HC	44.3 (8.9) 41.3 (9.7)	8 (1 – 33)[b]	1.5 (1–6.5)[b]	PASAT 3″ SDMT	None	ROI of the fornix	PASAT 3″ not correlated with any measure; SDMT correlated with all diffusion measure of the fornix, more strongly on the left side
Kern et al. [54]	27 RRMS 20 HC	37.9 (8.2) 34.1 (9.4)	N.R.	2.5 (1.1)	PASAT and SDMT combined	None	ROIs: cingulum, fornix, UF	Mean FA of bilateral UF significantly predicted PS performance; mean RD of bilateral UF correlated with PS scores
Koenig et al. [43]	57 MS: 44 RRMS 13 SPMS 17 HC	44.6 (8.4) 42.7 (10.1)	11 (1–33)[b]	2.5 (1–6.5)[b]	PASAT 3″ SDMT	None	ROIs: posterior cingulum, PLIC	PASAT 3″ not correlated with any measure; SDMT negatively correlated with MD and RD (mean of left and right) in PLIC and posterior cingulum
Meijer et al. [45]	30 SPMS: 12 CI 18 CP 32 HC	51.9 (8.1) 55 (7.4) 40.6 (12.9)	19.3 (8–30)[b] 25 (9–48)[b]	6.5 (4–8.5)[b] 6.2 (5.5–8.5)[b]	PASAT 3″ SDMT combined to assess CI	Age, sex, IQ	TBSS	Global mean RD significantly predicted global cognitive impairment
Moroso et al. [44]	37 CIS 32 MS 36 HC	36 (19–59)[b] 42 (29–59)[b] 36 (21–60)[b]	4.25 (1.98)[d] 106.11 (61.44)[d]	1 (0–6)[b] 3 (0–8)[b]	SDMT	None	ROIs: cerebellar peduncoli, VI, VIIb, VIIIa, VIIIb, crus I, and crus II lobules	SDMT correlated positively with FA in the left VI, the right VIIIa and VIIIb lobules, middle and inferior cerebellar peduncles; SDMT correlated negatively with MD in the vermis crus II, middle and left superior cerebellar peduncles

ACM: anatomical connectivity maps, AD: axial diffusivity, AF: arcuate fasciculus, ATR: anterior thalamic radiations, CC: corpus callosum, CI: cognitively impaired, CP: cognitively preserved, CR: corona radiate, CWIT: Colour Word Inhibition Test, EDSS: Expanded Disability Status Scale, FA: fractional anisotropy, FL: fronto-lateral, FM: fronto-medial, FMA: forceps major, FMI: forceps minor, HC: healthy controls, ILF: inferior longitudinal fasciculus, IFOF: inferior fronto-occipital fasciculus, IQ: intelligence quotient, LDST: Letter Digit Substitution Test, MD: mean diffusivity, OF: orbito-frontal, PASAT: Paced Auditory Serial Addition Test, PLIC: posterior limb of the internal capsule, PPMS: primary progressive multiple sclerosis, PTR: posterior thalamic radiations, PVSAT: Paced Visual Serial Addition Test, RD: radial diffusivity, ROI: region of interest, RRMS: relapsing-remitting multiple sclerosis, SDMT: Symbol-Digit Modalities Test, SLF: superior longitudinal fasciculus, SPMS: secondary progressive multiple sclerosis, SS: sagittal stratum, T2-LL: lesion load on T2 images, TBSS: tract-based spatial statistics, TMT: Trail Making Test, UF: uncinate fasciculus, WM: white matter.

N.R. = not reported.

[a] Mean (SD).

[b] Median (Range).

[c] Mean (Range).

[d] Duration in month.

fasciculus, and the forceps major [45].

In contrast, fewer studies investigated the association between structural connectivity measures and performance on the SDMT in people with MS. Among them, only two failed to report any significant correlation in two regions of interest, namely anterior thalamic radiations [46] and the corpus callosum [46,53]. Similar to the results on the PASAT, higher structural integrity of the corpus callosum, particularly in the body, also appears consistently linked to higher scores obtained on the SDMT [52,55,57] and a similar test of visual PS: the Letter Digit Substitution Test [37]. However, DTI indices were more often observed to be correlated with this test in other WM fibre bundles: the fornix, both left-lateralised [42] and bilaterally [55]; the cingulum, on the right side [55] and globally [43]; and the posterior thalamic radiations bilaterally [55,57]. Less commonly, significant correlations between the SDMT scores and DTI measures were additionally detected by region-of-interest and voxel-wise analyses in: the posterior limb of the internal capsule [43], thalamus [40], bilateral uncinate fasciculi, sagittal stratum [55], and the superior longitudinal fasciculus [57].

Only two DTI studies investigated the Trail Making Test: the first [52] reported no correlation in the region of interest of the corpus callosum, while a voxel-wise study found that performance on this test was correlated with fractional anisotropy in different parts of the corpus callosum, the left inferior fronto-occipital fasciculus, right posterior thalamic radiations, and bilateral superior longitudinal fasciculi [41]. This latter study also found that a PS index derived from the Stroop test correlated with structural connectivity integrity of the corpus callosum, bilateral fornix, and right-lateralised anterior and posterior thalamic radiations.

3.2. Functional connectivity

Four studies focussed solely on resting-state brain activity and its relation to PS ability in RRMS (Table 3). Patients' age, duration and severity were quite similar across studies and, in general, the reported clinical data were more detailed than in DTI studies although details about relapses were missing [30,59]. While most studies investigated different cortical and subcortical regions of interest, one study analysed functional connectivity within the graph theory framework by dividing the brain into 116 grey matter (GM) areas, extracting the average resting-state signal from each area, and finally calculating linear correlation between signals from each pair of GM areas [30]. The majority of the studies did not use statistical correction for multiple comparisons [30,31,60] and only two controlled for possible confounding variables [31,60]. However, studies on functional connectivity were of a slightly higher, although not significant, quality compared to the structural connectivity studies, given that all were explicitly hypothesis-driven with just one exception [60] (Appendix B – Table B.3). The PASAT 3″ was the most commonly used test of cognitive PS function, although mainly in combination with other tasks, which resulted in high variability of PS assessment across studies [30,59].

When functional connectivity was compared between people with MS and healthy controls, reductions were reported in the somatosensory network, medial and lateral visual networks [59], and between posterior and anterior cingulate cortex and right inferior frontal gyrus [60]. Consistently, graph-based analysis of functional connectivity revealed how the brains of people with MS tend to reorganise and become more modularised. This means that connectivity between brain areas that are functionally related to one another and form a module tends to increase in MS, while functional connectivity between areas belonging to different brain modules becomes weaker [30]; (see Fleisher et al. [61] for a recent review of graph theory and brain networks in MS). These findings support the view of MS as a disconnection syndrome due to different functionally related areas becoming more independent from one another and, in turn, hampering information integration across the brain.

Accuracy in a dual-task PASAT 3″ was reported to be negatively associated with the general level of network modularity (i.e. reduced between-network connectivity) characterising brains affected by MS: the higher the brain modularisation the worse the PS performance [30]. Wojtowicz et al. [60] also found that the higher the intra-individual variability in the semantic search reaction time task of the Computerised Test of Information Processing, the lower the functional connectivity between ventro-medial prefrontal cortex and the left frontal pole. No alterations in connectivity of the ventro-medial prefrontal cortex were reported between people with MS and healthy controls, however.

Another region-of-interest study compared functional connectivity changes between a baseline scan acquired just before in-scanner performance of two consecutive blocks of the PASAT 3″ and two subsequent scans: one acquired just after completion of the second block and one after 30 min. The scores on the PASAT 3″ correlated with the decrease of connectivity occurring in the 30 min after task performance

Table 3
Characteristics of studies on functional connectivity.

Study	Sample	Age (years)[a]	Duration (years)[a]	EDSS[a]	PS measure	Covariates	Analysis	Results about correlations
Janssen et al. [59]	28 RRMS 28 HC	46.4 (8.8) 45.6 (9.2)	10.6 (5)	3.9 (1.2)	Composite score: PASAT 2″, PASAT 3″, LCT, PCT	None	ROIs: 6 RS networks	Composite score not correlated with FC of any of the 6 networks
Gamboa et al. [30]	16 MS: 8 CIS 8RRMS 20 HC	35.3 (8.3) 29.9 (7)	N.R.	≤2.5	Dual task PASAT 3″	None	Modularity of the global FC network	Accuracy in the dual task PASAT 3″ correlated with global network modularity
Wojtowicz et al. [60]	18 RRMS 16 HC	42.1 (7.4) 43.1 (7.8)	7.5 (1–28)[b]	2.2 (1–3.5)[b]	CTIP	ISD of RT	ROI: DMN	ISD on the SSRT negatively correlated with FC between vmPFC and left frontal pole
Pravatà et al., [31]	22 RRMS: 11 NF 11 WF 12 HC	40 (5.8) 46.6 (9.3) 41.4 (8)	6 (4.4) 9.5 (3.8)	1.4 (0–3)[b] 2.5 (0–3.5)[b]	PASAT 3″	EDSS, duration	ROIs: SFG, caudate, thalamus	PASAT 3″ not correlated with FC of the left SFG, but positively correlated with post-performance decrease in FC between the left SFG and the left thalamus

CIS: clinically isolated syndrome, CTIP: Computerised Test of Information Processing, DMN: default mode network, FC: functional connectivity, ISD: individual standard deviation, LCT: letter comparison task, NF: not fatigued, PCT: pattern comparison task, ROI: region of interest, RRMS: relapsing-remitting multiple sclerosis, RS: resting state, RT: reaction time, SFG: superior frontal gyrus, SSRT: semantic search reaction time, vmPFC: ventro medial prefrontal cortex, WF: with fatigue.
N.R. = not reported.
[a] Mean (SD).
[b] Median (Range).

Table 4
Characteristics of studies on both structural and functional connectivity.

Study	Sample	Age (years)[a]	Duration (years)[a]	EDSS[a]	PS measure	Covariates	Analysis	Results about correlations
Rocca et al. [62]	75 MS: 33 SPMS 24 PPMS 24 HC	46.3 (24–65)[b] 47.9 (29–64)[b] 47.4 (26–65)[b]	15.5 (4–32)[b] 12.7 (3–39)[b]	6 (4–9)[c] 6 (3–8)[c]	PASAT 3″	DTI: none RS: age, head motion	DTI-ROIs: CC, CST, OR, cingulum RS-ROI: DMN	PASAT 3″ positively correlated with mean FA and MD in the CC and the cingulum globally; and positively correlated with RS activity of the ACC and of the left medial PFC
Tona et al. [63]	48 RRMS 24 HC	36.7 (8.1) 31.1 (6.5)	7.4 (6.1)	2 (1–4.5)[c]	PASAT 2″ PASAT 3″	Both: age, thalamic volume	DTI: whole brain RS-ROI: thalamus	PASAT 2″ and 3″ not correlated with DTI measures; PASAT 2″ and 3″ negatively correlated with FC between thalamus and several areas in both hemispheres
Zhou et al. [34]	24 RRMS 24 HC	39.5 (20–56)[b] 39.6 (21–56)[b]	3 (1–16)[b]	1.6 (1–2.5)[c]	PASAT 3″	None	DTI-ROIs: tracts linking DMN areas RS-ROI: DMN	PASAT 3″ positively correlated with FA in WM tract connecting the PCC/precuneus and the right IPL; and negatively correlated with increased FC between PCC/precuneus and left mTL
Shardella et al. [64]	30 RRMS 24 HC	35 (8) 32 (6.1)	10.1 (6.2)	2.5 (0–4)[c]	PASAT 3″	DTI: age, sex, PD-LV RS: age, sex, NGMV	DTI: TBSS RS-ROIs: 11 networks	PASAT 3″ correlated positively with FA and negatively with AD, MD and RD in widespread bilateral WM tracts; and negatively correlated with FC of the ECN and of the medial VN bilaterally
Zhou et al. [35]	20 RRMS 20 HC	39.3 (20–57)[b] 38.1 (22–51)[b]	2 (1–3)[b]	1.6 (0–2.5)[b]	PASAT 3″	Both: age, sex, thalamic fraction	DTI-ROIs: thalamic tracts RS-ROIs: thalamus and 7 linked areas	PASAT 3″ negatively correlated with mean AD in the whole WM tracts between thalamus and PFC; PASAT 3″ not correlated with thalamic FC

ACC: anterior cingulate cortex, CC: corpus callosum, CST: corticospinal tract, DMN: default mode network, DTI: diffusion tensor imaging, ECN: executive control network, FC: functional connectivity, IPL: inferior parietal lobule, ISD: individual standard deviation, mTL: medial temporal lobe, NF: not fatigued, NGMV: normalised grey matter volume, OR: optic radiations, PCC: posterior cingulate cortex, PD-LV: lesion volume on proton density images, ROI: region of interest, RRMS: relapsing-remitting multiple sclerosis, RS: resting state, RT: reaction time, SFG: superior frontal gyrus, VN: visual network, WF: with fatigue.

[a] Mean (Standard deviation).
[b] Mean (Range).
[c] Median (Range).

between the left superior frontal gyrus and the left thalamus [31]. However, PS function was not correlated with functional connectivity of the left superior frontal gyrus at baseline. Finally, Janssen and colleagues [59], instead, calculated a PS composite score comprehensive of both verbal and visuospatial components and including performance on the PASAT 2″, the PASAT 3″, the letter comparison and the pattern comparison tests. No associations were reported for the composite score with any of the 6 resting-state networks investigated: default-mode (DMN), executive control, left and right fronto-parietal, cerebellar, and sensorimotor networks.

3.3. Combination of structural and functional connectivity

Papers that combined DTI and resting-state analysis were characterised by greater homogeneity in sample composition: four out of five were carried out on RRMS while only one on a mixed sample of PPMS and SPMS [62]. Clinical information was in general extensively reported, apart from the presence of depression (Table 4). Moreover, all of the studies used the PASAT 3″ as test of PS functionality apart from one which investigated also the PASAT 2″ [63]. Most studies used statistical correction to account for multiple comparisons, namely Bonferroni and family wise error corrections. Apart from one [34], all studies included covariates of no interest in statistical models, especially age and sex.

Compared to DTI studies, those that investigated both structural and functional connectivity, showed significantly higher quality with two out of five reaching the maximum quality score in our criteria [35,63]. However, the hypotheses underlying the aims of the studies were not always overtly reported in these papers [34,62,64].

In RRMS DTI findings confirmed those from studies focussing exclusively on this technique, showing lower fractional anisotropy and higher mean, radial, and axial diffusivity globally [63] and in the corpus callosum, the inferior and superior longitudinal fasciculi [64], thalamic tracts [35], and tracts connecting cortical areas of the DMN [34] compared to controls. Moreover, in SPMS more severe alterations of diffusivity indices were seen in the corpus callosum and the cingulum [62].

Widespread correlations between scores on the PASAT 3″ and fractional anisotropy mainly centred on the corpus callosum were found in one study that used tract-based spatial statistics analysis [64]. Furthermore, several regions of interest were investigated and were found to be associated with PS performance: the corpus callosum and the cingulum, but not the corticospinal tract and the optic radiations [62]; tracts connecting the posterior cingulate and the precuneus with the right inferior parietal lobule [34]; and the anterior thalamic radiations [35]. One further study reported no correlations between the two PASAT versions analysed (2″ and 3″) and global fractional anisotropy and mean diffusivity [63].

Results of studies of functional connectivity in RRMS were more variable when compared to healthy controls: thalamic connectivity was increased with dorsal and lateral frontal areas, but decreased with medial frontal, medial temporal and occipito-parietal cortices [35,63]; increased connectivity was found between various pairs of areas part of the DMN [34]; and finally decreases in functional connectivity between the left fronto-parietal network and the executive control network were found [64].

When functional connectivity was found to correlate with measures of PS, PASAT 3″ score was positively associated with connectivity of the left medial prefrontal cortex and the anterior cingulate [62] and negatively with the posterior DMN on the left side [34], the ECN and the medial VN [64], and between the thalamus and distributed cortical and subcortical areas in both hemispheres [63]. Zhou et al. [35], instead, found no correlation between thalamic connectivity and performance on the PASAT 3″.

Reductions in functional connectivity were also reported in the progressive forms of MS, with slightly different patterns across

phenotypes: in the medial prefrontal cortex and the precentral gyrus for SPMS; in the anterior cingulate cortex and the precentral gyrus in PPMS [62].

Finally, no correlations were observed between measures of structural and functional connectivity by studies that focused on the thalamus [35,63]. Mean and axial diffusivity in tracts connecting the anterior and posterior portions of the DMN were, however, found to be correlated with their functional connectivity [34], and fractional anisotropy in the corpus callosum and the cingulum correlated with functional connectivity of the anterior DMN [62].

4. Conclusions and future directions

The aim of this review was to summarise current knowledge on how brain connectivity measures in MS are associated with PS function, a cognitive domain known to be particularly affected, and to provide insight for future lines of research.

The published literature shows contrasting results on correlations between PS function and measures of functional and structural connectivity. DTI studies have highlighted mainly vague and variable findings. Indeed, when voxel-wise analyses were carried out, multiple and widespread clusters of WM correlated with PS tasks [29,39,41,55,57,58] and the same was observed in the investigation of more specific regions of interest across a range of tests [42,43,50,51,54]. Lack of correlation with brain connectivity measures was noted more often for the PASAT (especially the 3″ version) than the SDMT and various explanations may account for these differences. Firstly, the sensory modality used to present stimuli differs between the two tests: auditory for the PASAT and visual for the SDMT. The latter, in fact, has been reported to be more susceptible to impairment in MS than the former and may better evaluate PS deficits associated with this disease [65]. Secondly, the way stimuli are presented during test performance differs across tests: for the PASAT stimuli are presented one at a time in sequence, while all the stimuli are presented simultaneously on the same page for the SDMT, thus increasing the demands posed on inhibition of processing of possible distractors. For the PASAT, it has also been suggested that patients may put in place different solving strategies when facing different versions of the PASAT. In fact, Snyder et al. [66] reported that patients tend not to perform the task continuously but to skip every third item, thus reducing considerably the difficulty of the test and achieving a higher, though less reliable, score. Finally, we cannot ignore that the two tests, although both used as PS measures, require the engagement of different cognitive domains: verbal auditory working memory for the PASAT and visual attention for the SDMT. These cognitive functions are long known to rely on activity of different brain areas of both hemispheres [67], suggesting these tasks may assess different aspects of the PS function.

Indeed, partially different WM tracts were observed to be related to the tests reviewed. Performance on the PASAT was more associated to the level of microstructural integrity of the left cingulum, the superior longitudinal fasciculus (especially left-lateralised), and the inferior longitudinal fasciculus. The SDMT, instead, seems to be more associated to DTI measures in bilateral tracts: the fornix, the cingulum and the posterior thalamic radiations. However, the corpus callosum emerged as the WM tract that most consistently correlated with PS performance of people with MS across cognitive tasks. This suggests the importance of multiple WM tracts to support cognitive PS performance across cognitive domains through fast integration of information processed in distributed brain networks.

Despite the variability of results, DTI indices seem to be more consistently correlated with different PS performance than measures of lesion load and parenchymal atrophy. This may result from the fact that microstructural WM damage can spread across fibre tracts [68], and can precede the detection of new macrostructural lesions [69]. Hence, diffusion indices may be more sensitive in detecting subtle MS pathology leading to decline in PS function than conventional MRI. In fact, apart from commissural fibres (i.e. the corpus callosum) associative WM tracts appear to be more critically involved in PS performance, namely the superior and inferior longitudinal fasciculi and the cingulum. Nevertheless, given the variability in PS tasks used, differential WM involvement may have been detected according to the specific measures used.

In contrast, higher quality and more consistent results were observed in RS-fMRI studies. Functional neuroplasticity seems to be the underlying mechanism supporting cognitive changes, or stability, in the early phases of MS. In fact both people with clinically isolated syndrome and RRMS showed functional connectivity changes, both increases [34,35,63] and decreases [35,59,60,63,64], within various brain networks. Furthermore, PS performance correlated with functional connectivity alterations in frontal areas, such as the prefrontal and anterior cingulate cortices, and fronto-thalamic connections [31,35,60,62,64]. It is also worth noting that, in contrast to DTI studies, almost all those studies exploring functional connectivity used exclusively the PASAT to measure PS abilities.

Even though a relationship between macrostructural damage and resting-state functional changes in MS appears likely [70], current findings are not consistent. In fact, while some studies observed correlations between total lesion volume and changes of resting-state activity [35] others reported no correlation [62,64]. The same discrepancy has been observed about the association between structural and functional connectivity measures, where significant correlations were found only in a small number of studies [34,62]. Indeed, the relationship between functional and structural brain changes may not be that straightforward in consideration of the fact that if structural connectivity between two areas predicts functional connectivity, the reverse is not necessarily the case, since functional connectivity can also depend on indirect connections to and from other brain areas [71].

Current knowledge of how MS-related damage to both structural and functional connectivity affects PS function is incomplete and preliminary. This may be due to methodological shortcomings detected in the reviewed articles. Firstly, most studies, especially those on structural connectivity, were carried out on samples of mixed MS phenotypes. Such lack of differentiation may confound results, particularly since the neuropathology in progressive forms of MS is increasingly recognised to be mainly characterised more by neurodegenerative rather than inflammatory processes [72]. Secondly, to date many studies have been carried out using a more explorative approach, often without a clearly defined hypothesis to test, and have been based on a cross-sectional design that does not allow for the assessment of PS decline over time. Finally, a lack of theoretical background on PS decline in MS has emerged from the published literature. The majority of the studies focused mainly on the most common tests of PS that are intrinsically related to various cognitive domains (i.e. working memory for the PASAT and visuospatial attention for the SDMT), neglecting alternative strategies of investigation. These could include better characterisation of the neural correlates of PS deficits in MS considering sensory, cognitive, and motor contributions [9] or clarifying any possible influence of PS decline on other cognitive domains when assessing correlations with MRI measures [41].

PS performance has been repeatedly found to be impaired in people with MS and to correlate with both structural and functional brain reorganisation, in particular degeneration of the corpus callosum

[37–39,41,46,47,49,52,56,57,62] and altered activity in frontal areas [31,60,62,64]. Nevertheless, the dynamic properties and topography of neural breakdown in MS have yet to be clarified. Recent meta-analyses have been published with the aim of advancing our understanding of brain regions mostly affected by MS. Lansley et al. [73] showed that GM appears to degenerate especially in the thalamus, a crucial hub for information distribution across the brain, the basal ganglia, precentral and postcentral gyri, and the cingulate cortex, involved in complex cognitive functions. Furthermore, Welton et al. [26]. highlighted how WM microstructural degeneration could be functionally related: physical disability was found to be mainly related to the posterior corpus callosum and right inferior fronto-occipital fasciculus, while cognitive decline was mainly linked to the anterior part of the corpus callosum, the thalamus, and the fornix.

The published literature suggests that connectivity of the frontal cortices and between hemispheres is involved in PS function in MS. Interestingly, the *cognitive efficiency* theory [74] postulates that activity of the prefrontal cortex plays a pivotal role in PS performance as do dynamic interactions with parietal cortices [75–77]. However, caution is needed when drawing conclusions based on current published evidence in light of the limitations we have identified. In fact, only a review by Lopes Costa et al. [9] has extensively explored the issue regarding a thorough definition of PS. Further theoretical discussion on MS-related cognitive impairment may aid the stimulation of a more hypothesis-driven approach to plan future investigations.

This review has some limitations: studies carried out on mixed MS phenotypes were included and all articles written not in English, though very few, were excluded. Possible selection bias was minimised during the review process by carrying out a systematic search of the literature on the topic without setting strict limitations (e.g. narrow time windows). All papers investigating at least one measure of PS were included, though the possibility of having missed eligible records cannot be completely ruled out. No specific issues regarding publication bias were detected through quality assessment, since different studies also reported negative results and most studies discussed their own limitations.

In conclusion, while the reviewed studies have shown significant promise for the use of resting-state functional MRI and DTI to explore the neural underpinning of PS in MS, results to date have not been consistent, and future investigations may benefit from considering the limitations identified in this review. Firstly, more detailed analysis of concepts related to PS function should be brought about in order to provide better theoretical frameworks to the neuroscientific investigation of this domain and its decline due to MS [9]. Secondly, the differential associations between different measures of PS ability, which may potentially capture different cognitive aspects of this function, and their neural correlates need further characterisation. Thirdly, the use of a longitudinal design, that so far has been largely neglected, is needed to clarify the interplay between neural and cognitive changes over time and potential maladaptive plasticity in MS. Indeed, Loitfelder et al. [78] observed that higher activity in the left inferior parietal lobule at 1-year follow-up was negatively correlated with SDMT performance in RRMS. Finally, considering the higher scientific quality observed in studies combining different connectivity measures we argue that the use of multimodal imaging with a focus on network and graph theory analyses [33,61] may prove to be particularly helpful in tracking PS decline in MS. Combined use of different MRI techniques might allow a more comprehensive approach to mapping connectivity that may help unravel the complexity that characterises MS symptoms. Furthermore, the integration of multimodal MRI and targeted neuropsychological assessment may provide more detailed outcome measures also in clinical trials, both for pharmacological and non-pharmacological interventions, and highlight beneficial treatment effects that may go otherwise undetected.

Conflicts of interest

None.

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Appendix A. Search strategy

Table A.1
Strings with keywords used in the literature search.

"Multiple sclerosis"	AND	DTI	AND	"Information processing"
"Multiple sclerosis"	AND	DTI	AND	"Processing speed"
"Multiple sclerosis"	AND	DTI	AND	"Speed of processing"
"Multiple sclerosis"	AND	DTI	AND	PASAT
"Multiple sclerosis"	AND	DTI	AND	SDMT
"Multiple sclerosis"	AND	"Resting state"	AND	"Information processing"
"Multiple sclerosis"	AND	"Resting state"	AND	"Processing speed"
"Multiple sclerosis"	AND	"Resting state"	AND	"Speed of processing"
"Multiple sclerosis"	AND	"Resting state"	AND	PASAT
"Multiple sclerosis"	AND	"Resting state"	AND	SDMT
"Multiple sclerosis"	AND	"Functional connectivity"		
"Multiple sclerosis"	AND	"Structural connectivity"		

Appendix B

Table B.1
Quality assessment criteria.

Area	Question	Values
Methodology	1. Were a priori hypotheses clearly stated?	No = 0; yes = 1
	2. How large was the sample size?	< 30 = 0; ≥ 30 = 1
Clinical characteristics	3. What MS phenotypes were included?	Not defined = 0; mixed = 1; one type only or distinct groups = 2
	4. Was information on history of comorbidities reported?	No = 0; yes = 1
	5. Was information on pharmacological treatments reported?	No = 0; yes = 1
	6. If RRMS was included, was information on relapses reported?	No = 0; yes = 1
MRI parameters	7. How strong was the MRI field used?	1.5 *T* = 0; ≥ 3 *T* = 1
	8. How many diffusion-weighted directions, for DTI, or slices, for resting-state fMRI, were acquired?	< 30 or missing = 0; ≥ 30 = 1
Statistical analysis	9. Was the imaging analysis coherent with the hypothesis?	No = 0; yes = 1
	10. Was correction for multiple comparisons used?	No = 0; yes = 1
	11. Were covariates of no interest included in the analysis or, if not, was their exclusion motivated?	no = 0; yes = 1
Results	12. Were limitations of the studies clearly stated?	No = 0; yes = 1

Table B.2
Quality assessment of DTI studies.

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	TOT
Lin et al. [49]	0	1	2	1	1	0	0	0	1	0	0	0	6
Roca et al. [50]	0	0	2	1	1	1	0	0	1	0	0	0	6
Bonzano et al. [51]	0	1	2	1	1	1	0	0	1	0	0	0	7
Dineen et al. [29]	0	1	2	1	1	1	1	0	1	1	1	1	11
Mesaros et al. [47]	0	1	1	1	1	1	0	0	1	1	1	1	9
Roosendaal et al. [37]	0	1	1	0	0	0	0	0	1	0	1	0	4
Warlop et al. [48]	1	0	0	0	0	0	1	0	1	0	0	1	4
Ozturk et al. [38]	1	1	1	0	0	1	1	1	1	1	0	1	9
Van Hecke et al. [39]	0	1	1	0	0	1	0	1	1	1	0	1	7
Rimkus et al. [52]	0	1	2	0	1	1	1	1	1	0	0	0	8
Llufriu et al. [53]	1	1	2	0	1	1	0	1	1	0	0	1	10
Yu et al. [55]	0	1	2	0	0	0	1	0	1	1	1	1	8
Benedict et al. [40]	0	1	1	1	1	1	1	0	1	0	1	1	9
Bester et al. [46]	1	1	1	1	1	1	1	0	1	1	1	1	11
Bozzali et al. [56]	0	1	2	0	1	1	1	1	1	1	1	1	11
Genova et al. [41]	1	1	1	0	0	0	1	0	1	1	1	1	8
Mazerolle et al. [57]	1	1	2	1	1	1	0	0	1	1	1	1	11
Sbardella et al. [58]	0	1	2	1	1	1	1	1	1	1	1	1	12
Koenig et al. [42]	0	1	1	0	0	0	1	1	1	0	0	1	6
Kern et al. [54]	1	1	2	1	1	1	1	1	1	0	0	1	11
Koenig et al. [43]	0	1	1	0	0	0	1	1	1	1	0	1	7
Meijer et al. [45]	1	1	2	1	0	1	1	0	1	1	1	1	11
Moroso et al. [44]	1	1	2	1	1	1	1	0	1	0	0	1	10

Table B.3
Quality assessment of RS-fMRI studies.

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	TOT
Janssen et al. [59]	1	1	2	1	1	0	1	1	1	1	0	1	11
Gamboa et al. [30]	1	1	1	0	0	0	1	1	1	0	0	0	6
Wojtowicz et al. [60]	0	1	2	1	1	1	0	0	1	0	1	1	9
Pravatà et al. [31]	1	1	2	1	1	1	1	1	1	0	1	1	12

Table B.4
Quality assessment of studies combining DTI and RS-fMRI.

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	TOT
Rocca et al. [62]	0	1	2	0	1	1	1	1	1	0	1	1	10
Tona et al. [63]	1	1	2	1	1	1	1	1	1	1	1	1	13
Zhou et al. [34]	0	1	2	0	1	1	1	1	1	1	0	1	10
Sbardella et al. [64]	0	1	2	1	1	1	1	1	1	1	1	1	12
Zhou et al. [35]	1	1	2	1	1	1	1	1	1	1	1	1	13

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APPENDIX B

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Sep 11, 2018

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Licensed Content Author	Riccardo Manca,Basil Sharrack,David Paling,Iain D. Wilkinson,Annalena Venneri
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APPENDIX C

Re: Permission to include work published prior to submission in PhD thesis.

I,

As one of the co-authors of the paper "*Brain connectivity and cognitive processing speed in multiple sclerosis: A systematic review*" published in 2018 in the volume 288 of the Journal of the Neurological Sciences,

Declare that I have no objections to and I grant permission for the reproduction of the abovementioned paper in the PhD thesis "**A neuroimaging approach to clarifying cognitive decline in multiple sclerosis: implications for rehabilitation**" to be submitted by Riccardo Manca, for which licence has been granted by the publisher Elsevier on 11th September 2018.

Prof Basil Sharrack

Dr David Paling

Prof Iain D. Wilkinson

Prof Annalena Venneri

APPENDIX D

Original document of the ethical approval granted by the Regional Ethics Committee of Yorkshire and Humber.

NHS
Health Research Authority
NRES Committee Yorkshire & The Humber - Sheffield
Yorkshire and the Humber REC Office
First Floor, Millside
Mill Pond Lane
Meanwood
Leeds
LS6 4RA

Telephone: 0113 3050128

28 December 2012

Professor Annalena Venneri
Professor of Clinical Translational Neuropsychology
University of Sheffield, Department of Neuroscience
Medical School, N Floor, Rm N130
Beech Hill Road
Royal Hallamshire Hospital, Sheffield
S102RX

Dear Professor Venneri

Study title:	**Assessment of age and disease related cognitive impairment in normal volunteers and people with degenerative and vascular brain disease and assessment of the potential neuroplastic effect of non pharmacological treatment**
REC reference:	**12/YH/0474**
IRAS project ID:	**84442**

Thank you for your letter of 19 December 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Rachel Bell, nrescommittee.yorkandhumber-sheffield@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		18 September 2012
GP/Consultant Information Sheets	1.0	18 September 2012
Investigator CV		
Participant Consent Form: Patient 1	2	19 December 2012
Participant Consent Form: Patient 2	2	19 December 2012
Participant Consent Form: Volunteer 1	2	19 December 2012
Participant Consent Form: Volunteer 2	2	19 December 2012
Participant Information Sheet: Patient 1	2	19 December 2012

Participant Information Sheet: Patient 2	2	19 December 2012
Participant Information Sheet: Volunteer 1	2	19 December 2012
Participant Information Sheet: Volunteer 2	2	19 December 2012
Protocol		
REC application		
Response to Request for Further Information		19 December 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/YH/0474	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Pp Professor Basil Sharrack
Chair

Email:nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Ms Ramila Patel, STH Research Department*

Amended ethical approval document: final version.

NHS
Health Research Authority

National Research Ethics Service

NRES Committee Yorkshire & The Humber - Sheffield

HRA NRES Centre Manchester
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Tel: 0161 625 7832
Fax: 0161 625 7299

20 November 2013

Ms Jodie Keyworth
Research Coordinator
Academic Directorate of Neurosciences
Sheffield Teaching Hospitals NHS Foundation Trust
N125d, N Floor
Royal Hallamshire Hospital
Glossop Road
Sheffield
S10 2JF

Dear Ms Keyworth

Study title:	**Assessment of age and disease related cognitive impairment in normal volunteers and people with degenerative and vascular brain disease and assessment of the potential neuroplastic effect of non pharmacological treatment**
REC reference:	**12/YH/0474**
Amendment number:	**Amendment 01**
Amendment date:	**03 October 2013**
IRAS project ID:	**84442**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

Approval was sought to add two additional patient groups. These would be patients with Multiple Sclerosis and Parkinson Disease. There would also be an additional three key collaborators.

The Committee specified that in paragraph four on page one of the Participant Information Sheets the sentence starting *'Your participant will be vital...'* should be revised as this wording is too persuasive.

The Committee also thought that the information given on page two about timings and visits could be clarified as it was not exactly clear how this would vary from four weeks to twelve weeks. Please could you revise the wording.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Notice of Substantial Amendment (non-CTIMPs)	Amendment 01	03 October 2013
Protocol	2.0	05 November 2013
Parkinson Disease Patient Information Sheet 2	1.0	05 November 2013
Parkinson Disease Consent Form 2	1.0	05 November 2013
Multiple Sclerosis Patient Information Sheet 2	1.0	05 November 2013
Multiple Sclerosis Consent Form 2	1.0	05 November 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

12/YH/0474:	**Please quote this number on all correspondence**

Yours sincerely

On behalf of
Mr Neil Sykes
Vice Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Professor Annalena Venneri
University of Sheffield

Dr Ramila Patel
Sheffield Teaching Hospitals NHS Foundation Trust

NRES Committee Yorkshire & The Humber - Sheffield

Attendance at Sub-Committee of the REC meeting on 20 November 2013

Name	*Profession*	*Capacity*
Mrs Yvonne Stephenson	Lead Technician in the Department of Infection and Immunity	Expert
Mr Neil Sykes	Retired Engineer/ Scientist	Lay Plus

APPENDIX E

The University Of Sheffield.

Department Of Neuroscience.

PIS_MS Version 2.0, December 2013

Patient Information Sheet 2

Invitation to participate in research on

"Improving detection and prevention of cognitive decline in Multiple Sclerosis"

Invitation
We would like to invite you to take part in the study described below. Before you decide we would like to understand why the research is being done and what it would involve for you. One of our team will go through the information with you and answer any questions you may have. We suggest this would take about 5 minutes. Please ask if there is anything that is not clear.

Purpose of study
Practicing tasks which involve the use of memory, language, attention and other cognitive skills may have some benefits to maintaining and improving brain functioning. We would like to investigate this by using cognitive training exercises aimed at improving specific aspects of functioning such as memory and reasoning. We will then measure the effect that this cognitive exercise has on the brain's performance. In order to do this, we will do an fMRI scan which is a type of brain scan which measures brain activity while you are resting in the scanner. This procedure will tell us how well areas of the brain are communicating with each other.

Your participation in this study would help us better understand how effective this kind of treatment is for people with occasional cognitive problems, such as infrequent memory loss.

If we can assess and establish the effectiveness of such treatment on a person's cognitive abilities and brain function we may be able to help reduce some of the problems patients have in their day to day lives. Your participation will be valuable to us to better understand how our brains work and can be influenced by a course of specific brain training.

Do I have to take part?
It is up to you to decide to join the study. If you agree to take part, you will be asked to sign a Consent Form. Your personal results will be anonymous and identified by a code and will be kept strictly confidential. The results will be stored in a restricted access database for approximately 5 years after the end of the study. You have, of course, the right to refuse to participate, and you may withdraw from the research at any time. Any data collected will then be destroyed.

The University Of Sheffield.

Department Of Neuroscience.

What will happen to me if I take part?
You will visit the researcher at the Royal Hallamshire Hospital on different occasions where you will take part in some cognitive training, give a saliva sample and undergo two brain scans. The entire process will take place over 4 weeks. The completion of the training and the scans marks the end of your participation.

What will I need to do?

Part 1: Cognitive Training
You will be required to attend 20 training sessions in total (1 hour per day, 5 days a week, for 4 weeks). We can schedule your training sessions at times which are most convenient for you.

Prior to any training, you will undergo initial testing to establish a baseline of your abilities including an assessment of your ability to recall information from your long-term general knowledge, your ability to learn new material and of your attention. This initial assessment will take approximately 1 hour. This will then be repeated after training is completed.

For the training, you will be seated in front of a computer screen where instructions to each exercise will be given. The exercises will consist of pictures and phrases or a combination of the two. Each training session will last approximately 1 hour and, if necessary, can be split into three 20 minute sessions.

Part 2: Brain Imaging
The fMRI scanner uses a kind of camera which can take photos of your brain but also provides information on how well the various parts of your brain are communicating with each other while you are resting with your eyes closed in the scanner. You have to lie on a bed and the part where your head rests is moved to the middle of the scanner. You will have to lie still for about 30 minutes while the brain image is acquired. You will have two scans, one at the beginning of the study and one at the end of the course of brain training.

What are the risks involved in my participation?
Part 1: This study poses minimal risk to the participant. Some persons may find the training session tiring, in which case frequent breaks may be offered and the training can be split into shorter sessions.
Part 2: An fMRI scan involves no radiation so there is no health risk involved with the scan. You have to lie on a bed which is pushed into a large tube-like structure for the scan. The

scanner is quite noisy so ear plugs are provided and it can also be a little claustrophobic for some people. There is a button to press for help and the scan can be stopped at any time if you feel uncomfortable. An MRI cannot be performed if you have a cardiac pacemaker or any metal in your body - for example a hip replacement.

In this study, the MRI scan is not being done for a medical reason but if we see anything that is considered unusual, or in our opinion warrants concern, the researchers will consult with a clinical MRI expert and if necessary your GP will be informed.

Taking part in the research

By carrying out this research, we hope to gain a better understanding of the way memory, attention, and other cognitive abilities' decline can be improved with intensive specific training and offer a viable form of treatment for persons with mild cognitive impairment, persons who have had a minor stroke or who are in the early stage of dementia.

Taking part in this research does not alter your standard care or interfere with any current treatment you are receiving.

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Sheffield Teaching Hospitals NHS Foundation Trust but you may have to pay your legal costs.

You will be reimbursed for all travel expenses incurred by taking part in this research.

Your access to the results of the study

If you would like to see your results from the cognitive training, we would be happy to provide you with a transcript of them.

Contact Details

For further information about our research, or if you have a concern about any aspect of this study, please feel free to contact Professor Venneri of the research team. Details are given below.

Prof. Annalena Venneri E-mail: a.venneri@sheffield.ac.uk Telephone: 0114 2713430

If you have a complaint, you may contact the Patient Services Team (PST) at 01142712400 or by email at PST@sth.nhs.uk

APPENDIX F

The University Of Sheffield.

Department Of Neuroscience.

ICF_MS Version 3 November 2013

CONSENT FORM 2

Title of project: Improving detection and prevention of cognitive decline in Multiple Sclerosis

Names of Researchers: *Prof A. Venneri, Dr. K. Harkness, Dr M. Randall, Dr D. Blackburn, Professor Sharrack, Dr. O Bandmann, Dr. M. Mitolo, Mr. M. De Marco, Ms. S. Wakefield, Mr. B. Malik, Ms C Carta, Mr R. Manca*

Please initial box

1. I confirm that I have read and understood the information sheet (PIS_MS Version 2.0, December 2013) which describes the reasons I have been asked to participate in a cognitive training programme, give a saliva sample and undergo an fMRI scan and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, without my medical care or legal rights being affected. ☐

3. I agree to undergo the fMRI scan and understand that it is for the purpose of scientific study. ☐

4. I agree to take part in a cognitive training programme ☐

5. I agree that my GP can be informed that I will undergo an MRI scan and that the results of the MRI scan can be shared with my GP if necessary ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature

APPENDIX G

Document of ethical approval granted by the Institutional Review Board of the IRCCS Fondazione Ospedale San Camillo (Venice) for the study including patients with SPMS.

FONDAZIONE OSPEDALE SAN CAMILLO
OSPEDALE NEURORIABILITATIVO | ISTITUTO DI RICOVERO E CURA A CARATTERE SCIENTIFICO
SEDE LEGALE: 30126 | VENEZIA-LIDO | VIA ALBERONI 70 | TEL. 041 22 07 111 | FAX 041 73 13 30
C.F. 94071440278 | P.I. 03953700279 | ISCRITTA PREFETTURA DI VENEZIA: REG. P.G. N. 409
IRCCS

Lido di Venezia, 21 dicembre 2011
Rif. CE: Protocollo 11.09

Preg.mo Signore
Prof. Leontino BATTISTIN
IRCCS S. Camillo

Oggetto: Protocollo 11/09 – vers. 2: Studio dei pattern dell'attività cerebrale di default nelle patologie neurodegenerative. Parere del Comitato etico.

Preg.mo Prof. Battistin,

il Comitato Etico per la Sperimentazione dell'IRCCS S. Camillo, nella seduta del 15 dicembre u.s., ha esaminato la versione 2 del protocollo da Lei presentato, di cui in oggetto, e ha espresso PARERE FAVOREVOLE alla sua effettuazione. Si allega alla presente estratto del Verbale della seduta.

Colgo l'occasione per salutarLa cordialmente.

Il Presidente
Don Corrado Cannizzaro

FONDATORE PROVINCIA LOMBARDO - VENETA DELL'ORDINE RELIGIOSO DEI CHIERICI REGOLARI MINISTRI DEGLI INFERMI (CAMILLIANI)
ENTE ECCL. CIVILM. RICON. - R.D. N° 682 DEL 22.05.33 | ISCRIZ. PREFETTURA DI MILANO - REG. P.G. N° 514 VOL. III PAG. 893

IRCSS Ospedale San Camillo
Comitato Etico per la Sperimentazione

Verbale della riunione del 15 dicembre 2011

---------- estratto ----------

[omissis]

Il giorno 15 dicembre 2011 alle ore 11.30, presso la sede dell'IRCCS San Camillo si riunisce il Comitato Etico per la Sperimentazione (CESC), così composto:

Don Corrado **Cannizzaro**, *Presidente*	*Esperto di bioetica*	PRESENTE
Prof. Francesco **Grigoletto**, *Vice presidente*	*Biostatistico*	PRESENTE
Prof. Leontino **Battistin**	*Direttore scientifico dell'IRCCS*	ASS. GIUST.
Prof.ssa Gabriella **Cargnelli**	*Farmacologa*	ASS. GIUST.
Dott. Mauro **Cenedese**	*Clinico*	PRESENTE
Sig.ra Antonella **Chinellato**	*Rappresentante del settore infermieristico*	PRESENTE
Dott. Tiziano **Lazzari**	*Medico di Medicina Generale*	PRESENTE
---	*Direttore sanitario dell'IRCCS*	---
Dott. Francesco **Palladin**	*Clinico*	ASS. GIUST.
Dott. Pietro Emilio **Pisani**	*Esperto in materia giuridica*	PRESENTE
Dott.ssa Olivia **Rabbaglietti**	*Rappresentante del volontariato*	PRESENTE
Dott.ssa Susanna **Zardo**	*Farmacista*	ASS. GIUST.

[omissis]

4. Esame del protocollo 11/09 – vers. 2. Studio dei pattern dell'attività cerebrale di default nelle patologie neurodegenerative

A – DATI GENERALI DEL PROTOCOLLO DI STUDIO 11/09

A.1 – Identificazione del protocollo	
Titolo	Studio dei pattern dell'attività cerebrale di default nelle patologie neurodegenerative
Versione:	2.0
Data:	07/12/2011
Codice/nome	-
Codice EudraCT	---
Tipologia:	studio sperimentale
Fase clinica:	---
A. 2 – Caratteristiche del protocollo	
Pazienti coinvolti	400
Pazienti presso IRCCS	400
Tipologia di pazienti	Pazienti affetti da patologie neurodegenerative
Copertura economica	Approvata dal Dott. Stigliano (vedi allegato)
Copertura assicurativa	Polizza n. 270269323 Assicurazioni Generali emessa in data 08/09/2011
A.2 – Sponsor e promotore	
Sponsor	---
CRO	---
A.3 – Centro coordinatore	

Centro Coordinatore	IRCCS- Fondazione ospedale San Camillo
Sperimentatore principale	*Prof. Leontino Battistin*
Parere Unico Favorevole	---
Emesso il	---
A.4 – IRCCS San Camillo – Lido di Venezia	
Responsabile IRCCS	*Prof. Leontino Battistin*
Num. Protocollo IRCCS	11.09

B – DOCUMENTAZIONE ESIBITA

Lettera di intenti e di richiesta di emissione parere al Comitato etico datata 13 giugno 2011, a cui è allegato il fascicolo del progetto contenente i seguenti documenti:

1. progetto 11/08 versione 1.0;
2. elenco dei centri coinvolti
3. test e scale di valutazione;
4. scheda di raccolta dati;
5. foglio informativo per il paziente;
6. dichiarazione di consenso del paziente;
7. modulo informativo per il medico di famiglia;
8. quantificazione delle risorse e dei costi impiegati nel progetto;
9. delibera regionale approvazione finanziamento.

C – PARERE

Il Comitato Etico dell'IRCCS San Camillo esprime PARERE FAVOREVOLE purché venga osservato quanto previsto dalla normativa vigente e regionale nonché dai regolamenti aziendali.

Contestualmente il Comitato etico ricorda che:

- la sperimentazione clinica nell'uomo deve essere seguita secondo i principi etici fissati nella dichiarazione di Helsinki e successivi emendamenti e che tutte le fasi degli studi clinici devono essere predisposte, attuate e descritte secondo i principi della buona pratica clinica;
- sussiste l'obbligo di non avviare deviazioni dal protocollo, né modifiche allo stesso, senza che il Comitato etico abbia espresso per iscritto approvazione o parere favorevole ad ogni singolo e specifico emendamento;
- deve essere garantito il diritto alla diffusione e/o pubblicazione dei risultati favorevoli o non favorevoli da parte degli sperimentatori che hanno condotto lo studio, nel rispetto delle disposizioni vigenti in tema di riservatezza dei dati sensibili e di tutela brevettuale e che non devono sussistere vincoli di diffusione e pubblicazione dei risultati da parte del Promotore;
- il Responsabile della sperimentazione è tenuto a dare comunicazione al Comitato etico dell'effettivo inizio della sperimentazione, in occasione dell'arruolamento del primo paziente;
- il Responsabile della sperimentazione è infine tenuto a far pervenire una relazione finale sull'esito della sperimentazione.

In difetto delle suddette indicazioni, l'efficacia dell'approvazione del protocollo deve intendersi sospesa a tutti gli effetti.

[omissis]

Il Segretario Verbalizzatore
Dott. Nicolò Anesa

il Presidente
Don Corrado Cannizzaro

Venezia, 15 dicembre 2011