Mind the gap: From neurons to networks to outcomes in multiple sclerosis

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Abstract

Magnetic resonance imaging studies have provided valuable insights into the structure and function of neural networks, particularly in health and in classically neurodegenerative conditions such as Alzheimer's disease. However, such work is also highly relevant in other diseases of the central nervous system, and in this review we look at multiple sclerosis. Studying multiple sclerosis is challenging as its pathology encompasses both neurodegenerative and focal inflammatory elements, both of which may disrupt neural networks. Disruption of white matter tracts is reflected in changes in network efficiency, increasingly random grey matter network topology, relative cortical disconnection, and both increases and decreases in connectivity centered around hubs like the thalamus and default-mode network. Initial longitudinal studies suggest that these changes evolve rather than simply increase over time and are linked with clinical features. Studies also highlight the potential role of treatments that functionally rather than structurally modify neural networks.

Introduction

That the brain is a connected organ was recognised long before the advent of connectomics, but only recently has technology provided us with practical tools to assess the integrity and function of brain networks in life. Despite ongoing methodological development, and known limitations, studies investigating networks have already provided fundamental insights into disease processes and how these translate into disability. To date most work has been performed in classically neurodegenerative conditions. In Alzheimer's disease the sequential involvement of brain regions can be explained by the spread of pathology through neural networks, in particular the default mode network, and patterns of network involvement can explain clinical phenotypes.[1]

Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS), in which sodium channel function, energy consumption and tissue blood perfusion are altered. It is the commonest non-traumatic cause of neurological disability in younger adults in Europe, yet its cause and the mechanisms underlying long-term disability remain uncertain. There is a well-recognised disparity between neurological - and cognitive - impairment and MS brain pathology as assessed using magnetic resonance imaging (MRI).[2] This disparity is also seen in treatment trials, for example in a recent phase II study of ibudilast a treatment effect was seen on whole brain and cortical atrophy, but not on clinical outcomes.[3] Resolving this is important as MRI measures are now the main outcome in early phase clinical trials, and is increasingly used in clinical practice to assess MS pathological progression, and so this has practical implications for development and use of disease modifying treatments.

Can network-based approaches to modelling MS pathology effectively bridge the gap between clinical outcomes and conventional MRI measures, and provide useful insights into the targeting treatments? We considered this in more detail, addressing three questions: How does MS pathology affect brain networks? How does disruption of networks translate into neurological or cognitive impairments in MS? What do such studies tell us about the targeting and effects of MS treatments? Before addressing these questions, we briefly review relevant clinical and pathological features of MS, brain networks and the methods used to model them *in vivo*, and caveats to keep in mind when interpreting clinical study findings. Future directions of research are discussed.

Clinical features of MS

MS can affect any aspect of CNS function; for example, while it is usually thought of as causing motor and sensory symptoms, a third to two-thirds of people with MS have cognitive impairments.[4] Symptoms may develop acutely (relapses) or progressively over months to years. Relapses occur when lesions form in clinically eloquent parts of the CNS, for example the optic nerves, although most lesions occur without direct clinically apparent effects.[5] The mechanisms underlying progressive MS are less well-understood. The correlations of clinical measures of disease progression with the accrual of white matter (WM) lesions are relatively modest,[6] but appear to be stronger with brain and spinal cord atrophy, albeit still insufficient to explain or predict clinical impairments in individuals with MS, and still leaving in the order of half of variability in disability scores unexplained.

In this review we use the motor and cognitive functions as our main examples, as both are affected early in the course of MS, increase in progressive MS, and are significant causes of disability and unemployment. Motor function (as measured using the expanded disability status scale [7]) is the most commonly assessed outcome in clinical trials and cognitive dysfunction is increasingly also being included. Two additional symptoms that closely relate to network functioning will also be discussed, namely visual disturbances and fatigue. The visual system is also commonly one of the first to be affected by MS, with between 30 and 40% of people with MS having an episode of optic neuritis as their first symptom, [8] but rarely symptomatically progresses once an episode of optic neuritis has resolved. However, the visual pathway is one for which we have detailed clinical and neurophysiological ways to assess function, and so we consider this as we discuss the challenges of linking structure and function. A very common symptom throughout the clinical course of MS is fatigue. Unfortunately, fatigue is particularly difficult to study as people with MS report very different symptoms as fatigue, and even in research there is no consensus definition of how to classify nor measure it. However, as previous work has noted that patients with fatigue show extensive changes in cognitive networks (see [9,10] for more in-depth reviews), we briefly consider studies of fatigue as an example of diverse symptoms being in part influenced by a common underlying cause.

MS pathology

MS pathology can impact on neuronal network function in a variety of ways (Table 1). For example, neuro-axonal loss will stop neural signal conduction across a network, while demyelination will slow and disperse transmission. Both can significantly affect neurological function, as has been demonstrated in optic neuritis.[11] WM lesions are the most studied aspect of MS pathology. Acute inflammation in WM lesions is associated with demyelination and axonal transection,[12] with trans-synaptic consequences. [13–15] In extra-lesional (normal-appearing) WM, axonal loss, demyelination and gliosis occur, [16] although it is unclear how much of this is secondary to axonal transection in lesions or occurs independently. [15] GM is often as, if not more, extensively demyelinated than WM. [17] Axonal loss is seen in GM lesions, [18] but synaptic and neuronal loss is not confined to lesions, occurring with similar intensity in lesional and extra-lesional tissues. [18,19] Deep GM structures are not spared, and the thalamus appears to be affected early (even after a single inflammatory episode [20]) and more so than other deep or cortical GM, with substantial neuronal loss seen. [21] This may be of particular relevance when considering the effect MS pathology has on network performance, as the thalamus is thought to play a pivotal role as a 'hub' in many brain networks. However, it is important to note that the thalamus is a not a homogenous structure, but instead consists of nuclei which appear to be differentially associated with clinical outcomes, for example cognition and fatigue. [22]

Less reported, but still important for brain function, are physiological alterations, such as: grey matter hypo-perfusion, with delayed arterial bolus arrival transit times; [23] sodium channel polymorphisms, for example in Nav 1.8 type channels which are ectopically expressed in cerebellar Purkinje cells in MS, and have been linked with differences in MS effects on cerebello-thalamic functional connectivity; [24] and energy deficits, associated with tissue hypoxia, have been shown to correlate with processing speed in MS. [25, 26] While neurological and cognitive deficits in people with MS are often thought of as disconnection syndromes, due to WM pathology, [27] GM changes have significant network implications as well, and are also associated with cognitive and disability progression. [28-31]

Assessing brain networks using MRI

The three main MRI methods that have been used to study brain networks are diffusion tensor imaging (DTI, assessing WM tissue microstructure), 3D T1-weighted scans (providing anatomical imaging of GM and WM), and functional MRI (fMRI) which can be resting-state [32] (i.e. looking for patterns of simultaneous activity while the brain is at rest) or task-based (i.e. looking for brain activation correlations during a task). For context, it is worth noting that a ~8 mm^3 DTI voxel contains ~200,000 neurons and that a typical fMRI cluster is ~80 mm^3 i.e. about 2 million neurons). [33, 34] Further, the temporal resolution of fMRI is ~10 seconds, while electroencephalography (EEG) and magnetoencephalography (MEG) demonstrate neural activity varying in milliseconds. [35]

Network architecture is described by connections and their layout (topology). Connectivity can be determined by tracing (anatomical) links from region to region, by looking for (functional) associations in neural activity, or by assessing the similarity in structural features, e.g. cortical thickness between them. The current main basis for describing and quantifying brain network

topology is 'graph theory', [36] where a 'graph' represents a connectivity map, with 'nodes' representing brain areas and the connection between nodes termed 'edges'. Graph theory can be applied to both structural and functional MRI, however it is worth stressing that structural and functional connectivity metrics are usually different, as they represent different properties of the brain connectome. In both cases, nodes are located in GM regions. Edges are more complicated, and they may represent WM tracts traced between GM regions, synchronisation of fMRI activity between GM regions, or co-variation in cortical structure (e.g. thickness). With the latter two definitions 'connectivity' may exist in the absence of specific WM tracts.

WM connections can be traced on DTI scans, but this may be difficult where tracts cross, or pass through WM lesions (DTI measures are often affected by MS lesions [37, 38]). However, another way of considering WM connectivity that may be particularly pertinent in MS, is from the perspective of a 'disconnectome', [39] which maps the extent of disconnection between GM regions arising from focal lesions. Functional connectivity is usually assessed by looking for direct or indirect correlations between GM regions (for example blood oxygenation level dependent (BOLD) signal variation): direct correlates are looked for between regional measures regardless of the state of the brain (resting-state fMRI); indirect assessment looks for an association with a common feature, for example brain regions that show simultaneous functional activation during a motor task (task-based fMRI). Falling between direct and indirect assessment, effective connectivity [40] relies on building models that incorporate both types of connections, and aims to define directionality in the information flow between regions. There are several methods used for assessing effective connectivity, including structural equation modelling, [41-43] psycho-physiological interactions [40] and dynamic causal modelling (DCM). [44] It is worth noting that, given the rather noisy nature of individual connections, most network-based analyses average findings at a group level, but in clinical trials or practice robust measures are needed in individual people. However, such analyses have proven possible using structural MRI. [45]

Interpreting results

Connectomics is a rapidly developing field and new measures of connectivity are still being proposed. The statistical methods underlying network-based analyses are complex, and the approach used can significantly influence apparent network structure and function. As such, links between cortical network connections and (classical) functional or anatomical connections remain controversial. [46]

Of the several topological features that can be extracted from brain networks (Table 2), it is unclear which have the greatest relevance in health and disease. In part this reflects the bespoke nature of brain networks with different topologies optimised to serve different functional outcomes, and so it is difficult to generalise. In disease states, some network features are preserved, while others are lost or emerge. It is the appearance of new features that has proven most difficult to explain, in particular whether or not this represents an unmasking of a (potentially deleterious) network feature or functionally useful neural plasticity. [47]

Structural connectivity established by tracing WM tracts clearly demonstrates a link between regions, and a decrease in connectivity can reasonably be interpreted as representing the disruption of a connection. However, some tractography based studies have shown apparent

increases in connectivity: in the adult CNS, entirely new axonal connections are not thought to occur (although neurogenesis with synapse formation is seen) [48], and loss of crossing fibres - which disrupt tractography - provides a more plausible explanation for apparently increased connectivity. Using tract templates rather than tractography to extract measures such fractional anisotropy from DTI overcomes this, but such measures then provide an assessment of tract structural integrity rather than connectivity directly. [49] In the case of structural connectivity based on GM characteristics, e.g. cortical thickness, processes that heterogeneously and randomly affect the cortex will tend to reduce correlations, while those that show a regional predilection may increase them, [50] even if the axonal connectivity between regions is unchanged. Similarly, tract-specific pathology may reduce correlations when it disrupts connections, or increase correlations where pathology simultaneously affects both the origin and target of a tract. To add to the complexity of this, multiple disease effects may simultaneously act on the same area, some network-mediated and others not. [50, 51]

In fMRI studies, while reduced correlations may be due to decreases in structural connectivity, they may also represent a reduction in synchronised activity. For example, demyelination may slow and disperse rather than stop action potential conduction, but they may all result in seemingly reduced functional co-activation. Increased fMRI connectivity may be due to increased neural communication, but determining if it actually represents an unmasking of previously hidden features (through loss of competing functional activity or disinhibition), structural reorganisation, functional adaptation or compensation is challenging. This is further complicated by the dynamic nature of functional connectivity, which can vary over seconds, i.e. during the course of an fMRI scan. [52]

Findings in MS

How does MS pathology affect brain networks?

Function

MS pathology significantly impacts on the structural and functional integrity of brain networks, and does so through a combination of effects on GM and WM. However, studies have yielded seemingly inconsistent results, and this in part is likely to be due to differences in the location and magnitude of tissue damage seen in people with different clinical subtypes of MS, or with longer-duration or more disabling MS. [53]

To date most functional connectivity studies in MS have used (mainly motor and cognitive) task-based fMRI, although more recently resting state fMRI has been used (particularly in studies on fMRI correlates of cognitive deficits). Findings have been variable (Table 3) (for a review see Pantano et al.) [54] Task based fMRI has shown a combination of increased and decreased functional brain activation (motor task examples are given in Table 3) and several factors may explain this: in addition to difference in the distribution and severity of pathology between cohorts, functional connectivity abnormalities will reflect both pathological abnormalities and compensatory mechanisms, and task based fMRI may also be confounded by variations in the design and performance of a task. The latter limitation is circumvented by using resting state fMRI, and with this some unifying themes have started to emerge, principally that functional connectivity is

increased in early MS [55,56] and decreased in people with more disabling or longer duration disease [57–59] (see Table 3 for examples of resting-state fMRI studies). Consistent with this, a recent longitudinal resting state fMRI study in RRMS has shown first increases and then a tendency to decreases in functional connectivity with disease progression, and that these changes correlate with disability progression. [60]

These changes in resting-state connectivity seem to be mostly centered around hub areas like those that comprise the DMN. Interestingly, default-mode changes in MS seem somewhat non-specific, as DMN changes have been related to several patient symptoms, including cognitive impairment [61], disability [62], and fatigue. [63] This might be explained by the notion that dysfunctions within this important hub network could influence the efficiency of the entire brain network, which in turn could relate to many symptoms in MS [64] It has also been hypothesized that fatigue is related to alterations in these cognitive and motor networks because it could be driven by a chronic mismatch between expected and measured output due to erroneous signals arising from these networks. [9] It should be noted, however, that such hypotheses remain difficult to prove experimentally given aforementioned difficulties in quantifying fatigue, warranting additional studies on the topic. Preliminary work on treating fatigue through stimulation of cortical areas showing altered fatigue-related connectivity (i.e. the default-mode and motor networks as well as the insula) seems promising, but still require validation in larger samples. [10]

Most studies have focused on cortical GM, but deep GM is also significantly affected in MS, particularly the thalamus, as well as the cerebellum. [65,66] Similar to the cortex, functional connectivity between the thalamus and other regions often appears to be altered, with both increased and decreased functional connectivity observed between the thalamus and various cortical regions. [67–71] As mentioned, it is of interest to study individual thalamic nuclei within this context. Recent data suggests that patients with fatigue [70] or cognitive impairment [22] how a combination of hyper- and hypo-connectivity, depending on the thalamic nucleus, all related with worse symptoms. As such, more work is needed to not only disentangle effects of fatigue from cognitive impairment, but also to identify why individual nuclei would behave so differently. As part of the thalamo-stiato-cortical loops, altered basal ganglia connectivity is also frequently seen, especially in the context of fatigue [72]. Interestingly, the evolution of functional connectivity appears different in the deep GM and the cortex. While functional connectivity between the deep GM structures, and with the cortex, tends to increase with disease progression, inter-cortical connectivity tends to decrease. [73] The topology of networks may also change, hubs (more highly connected nodes, for example the thalamus) usually appear to be preserved. [60,71] Few studies have investigated how cortical networks are affected by WM pathology, but local network efficiency appears to decrease as whole brain WM lesion load increases. [60]

Structure

Considering structural features, tissue atrophy affects some deep GM structures (for example the thalamus) [74] and cortical regions [75] more than others. In the cortex, recent work using source-based morphometry has revealed over-lapping patterns of atrophy, [50] raising the possibility that a combination of network and non-network mediated effects may be responsible.

However, while shared regional disease effects may increase some associations between regions, GM neural networks also appear to be less structured in people with MS, for example Tewarie et al. [53] found more random topology in people with long-standing MS compared to healthy controls, and Rimkus et al. [76] have shown that a more random network topology explains deficits in cognitive functioning in excess of that attributed to either localized atrophy or lesion measures. This may be more apparent relatively early on in the course of MS, before network mediated pathology induces structural correlations between regions, and Tur et al. [77] observed that while similarities in cortical thickness between regions decrease following first symptoms suggestive of MS, they increase in PPMS.

In WM, tractography has shown abnormalities, again variable, in global and local network efficiency: Shu et al. [78] found reductions in local and global network efficiency, while Fleischer et al. [79] found increases in efficiency along with changes in network topography (increased modularity and clustering), although cohort (despite similar median disease durations, the two populations significantly differed in term of maximum disease duration) and methodological difference may explain this apparent discord. In addition, in the context of fatigue, reductions of default-mode as well as caudate connectivity was observed, in line with aforementioned functional effects. [80]

Structure and function

Linking structural and functional studies has proven difficult, for both methodological reason and because MS pathology appears to differentially affect structural and functional networks throughout the course of the disease.

Here it is useful to consider the visual system, a discrete functional entity whose main pathways are well characterized, and, crucially, that can be assessed with different complementary modalities providing specific information about its structure (for example optic nerve MRI, DTI of the optic tracts and radiations) and its function (for example visual acuity and visual evoked potentials). This has provided a unique opportunity to disentangle the complex relationships between an acute insult in the visual pathways, and subsequent structural and functional changes. Studies have shown that the structural consequences of optic neuritis are not necessarily restricted to the optic nerve, and can extend progressively to the anterior and posterior visual pathway. [14, 81, 82] Functionally, during the acute phase of optic neuritis reduced activation is seen in the visual cortical areas, and this is associated with visual acuity. [83-86] Greater activation in extra-striate visual regions – particularly the lateral occipital complex – is associated with better visual acuity whatever the level of structural and functional damage within the anterior and posterior visual pathways [85] and, importantly, greater activation in this region predicts eventual visual recovery [87] More recently, studies using rs-fMRI have shown that there is a difference between cortical functional responses to the first and subsequent episodes of optic neuritis. Following a single episode, even with apparently structurally intact optic radiations, functional connectivity within the visual network is increased. [88] With multiple episodes, increased functional connectivity may still be seen in the lateral occipital complex but coexists with decreased functional connectivity in other part of the visual cortical network. [89] Intriguingly, stimulation of the unaffected eye in someone who has had optic neuritis has also shown abnormal functional responses. [85, 86] Considered together, this highlights that functional changes do not

simply mirror structural damage, but instead reflects potentially adaptive and non-adaptive functional changes that can extend beyond cortical regions immediately connected with the affected eye.

With cognition similarly complex relationships between structural damage and functional activation emerge. For example, Koini et al. [90] in their study on cognitive outcomes in MS found that thalamic volume and activation both - and in part independently - were associated with information processing speed and executive function. Recently, it has been shown that structural damage (composite score of lesions, atrophy and fractional anisotropy) explains information processing speed better than functional changes. [91] However, in patients with a similar level degree of structural damage, more severe functional changes resulted in worse information processing speed compared to those with only mild functional changes. Liu et al. [92] have shown that while changes in network structure (assessed using WM tractography) are apparent soon after first symptoms suggestive of MS occur, changes in functional networks only become detectable in people with clear on-going disease activity, and that correlations of decreased structural with functional connectivity are most apparent in subcortical networks. Lesions and extra-lesional changes within relevant tracts may have different effects on outcomes: Dineen et al. [93] have shown limited overlap between tracts and lesions, and so that extra-lesional damage is relevant, but Mesaros et al. [94] have shown that where lesions occur in a tract they dominate associations with clinical outcomes.

Drawing this all together is difficult at present, but it is clear that we must carefully take into account the clinical and MRI characteristics of study cohorts to build a coherent model of brain network changes over the course of MS. We must also recognise the potential that WM and GM pathology has to have opposing effects on apparent network performance at different points in the course of MS; for example Tewarie et al. [95] simulated the effects GM and WM pathology may have on function connectivity, and found a global increase in functional connectivity associated with cortical and thalamic pathology, and first an increase and then a decrease in connectivity associated with increasing WM pathology. This also suggests that a proportion of apparent changes in functional connectivity represents the direct effects of structural damage and has nothing to do with adaptive or maladaptive network changes.

How does disruption of networks translate into neurological and cognitive impairments? (Figure 1)

Studies looking for associations with disability have proven difficult to unify, with changes in network topology, [76] and increases and decreases in structural and functional connectivity, correlating with disability (for example [58, 59, 96]). However, importantly, fMRI measures correlate at least partly independently of structural measures with clinical outcomes, [58, 68, 97, 98] suggesting that functionally meaningful effects on network performance may be achieved through modulation of neuronal function.

Indeed, this has already proven relevant in clinical practice: fampridine, an agent that modifies potassium channel excitability, rather than promoting remyelination or neuronal repair, has been shown to improve walking speed in people with MS, [99] and in a small study (n=12) functional connectivity. [100] Rivastigmine has also been shown to increase functional connectivity in people

with MS undertaking cognitive tasks (n=15), and this was associated with a trend towards improvements in neuropsychological performance. [101] It is worth noting that the clinical effects of structural damage, measured as GM atrophy, may also be offset by a higher baseline cognitive reserve, measured as verbal intelligence, and this too is reflected by relative preservation of functional connectivity. [102] It has also recently been shown that functional connectivity is itself dynamic, waxing and waning over time, and this too is clinically relevant. Better memory function has been associated with less hippocampal dynamic functional connectivity, [103] higher information processing speeds with a greater increase in dynamic connectivity between resting and task-based states, [104] and executive functioning appears to be more closely linked with dynamic than static functional connectivity. [105] Chronic neuropathic pain in MS also appears to be associated with increased dynamic connectivity in the default mode network, [106] and greater connectivity between the default mode and salience networks.

Considering the vulnerability of networks to MS pathology, Llufriu et al. [107] found that for two cognitive tests commonly used in MS research, the paced auditory serial addition test [PASAT] and symbol digit modalities test [SDMT]), there were substantial differences in the number of structural connections associated with each (160 correlated with PASAT scores, while only 11 did so with SDMT). They concluded that the PASAT was more cognitively demanding and so more vulnerable than the SDMT to pathology. It has also been suggested that clinical progression represents a 'network collapse', [64] with unfolding pathological processes having ever greater effects on clinical outcomes as brain networks deteriorate, and in simulations run by Pagani et al. [108] lesions appear to have a greater or lesser impact on networks performance in different MS phenotypes. Castellazzi et al. have also considered associations between functional connectivity and lesions, and by setting them both in the context of clinical outcomes, have sought to identify which functional connectivity changes are due to lesions themselves, and which are network adaptations that either enhance or impair clinical performance. [109]

What do such studies tell us about the effects and targeting of treatments?

There are a variety of mechanisms through which treatments may affect brain network function (Table 4). For those that alter their structure it may take many months before changes become apparent, while those that affect function should have more immediate impact. Relatively few studies have looked for treatment effects on connectivity in MS but they do suggest that improvements in clinical function can be achieved without necessarily structurally altering a network. Cognitive rehabilitation is associated with significant increases in default-mode network (DMN) and variable changes in task-based fMRI activity [110, 111] but functional changes are not necessarily mirrored by structural alterations. [110] This is further supported by evidence from trans-cranial magnetic stimulation studies in MS that have shown rapid improvements in fatigue [112] and working memory and reduced spasticity with associated fMRI changes, that cannot plausibly be mediated by structural change in networks. [113, 114] Similarly, as noted earlier, treatment with fampridine is associated with increased motor-evoked fMRI activation. [100] From this, it is tempting to conclude that functional connectivity measures are more promising rather than structural connectivity measures as MRI markers of treatment efficacy in MS, but this may be an artefact of the nature of studies to date; it has yet to be determined if treatments that suppress inflammation, slow neuro-axonal loss, or promote re-myelination have significant effects on MRI-measurable network topology and connectivity, and for such effects to become

apparent longer-term studies will be required. Importantly, even transient improvements in clinical function with trans-cranial magnetic stimulation or drug treatments imply that structurally the underlying network is still sufficiently intact that there is function that can be preserved or regained, and so this may serve as a useful marker of those who may have most to gain from treatments designed to prevent neurodegeneration or promote remyelination.

Next steps

There is much to be done before brain network measures can be used, in MS or other neurological conditions, more routinely in clinical trials and in practice. To facilitate meaningful comparisons between studies, methods need to be standardised and results reproduced. There has been some work on comparative connectomics, [115] with a view to identifying common themes in networks across species, and meta-connectomics [116] seeking to identify consistent observations across studies. There remains the issue of scale, with current MRI (and EEG and MEG) techniques assessing neural networks in multi-mm to cm terms, so potentially overlooking small but highly relevant GM or WM features. This is further complicated by the issue of temporal resolution, and even with measurements made in milliseconds using EEG can prove difficult to link with a neurological or cognitive function, except where EEG changes can be linked with discrete neurological or cognitive events, *so allowing event-related potentials to be looked for*. [117] In turn, this makes the task of reconciling structure and function more difficult, without methods to infer missing elements that could explain discrepancies. Further, understanding the link between cellular architecture and physiology, and large-scale network function and structure, will require concerted interdisciplinary work. [118] Unifying network topology derived from fMRI and structural MRI may be a more tractable problem, albeit still challenging.

The pathological substrates of network changes in MS are not clear, but one *post mortem* study in MS has shown that graph theory descriptions of network topology are linked with neuronal size and axonal density. [119] However, the basis of functional network changes are unknown, for example does a decrease in network node activation in MS represent loss of neurons, their axons or arborisation, or non-structural factors such as inflammation or mitochondrial dysfunction? This is highly relevant when we consider the focus of treatments and how to assess their efficacy in early phase clinical trials. The possibility that pathological processes in MS may, in part, be mediated through neural networks and interact with other factors, such as regional vulnerabilities to pathology, has already been raised. [50, 120] In Alzheimer's disease the concept of 'nexopathies' has recently been proposed to explain how pathology may spread through networks and interact with intrinsic vulnerabilities resulting in the patterns of neurodegeneration observed, [121] and this could perhaps be pursued further in MS too.

There have been very few longitudinal functional or structural studies of brain networks, which means that we still have little insight into the dynamics of brain network degeneration, repair and plasticity. In particular, it is proving very difficult to determine which elements of structural or functional network change represent disease effects, which are adaptive or compensatory, without knowing how each relates to changes in neurological or cognitive function. In order to resolve these uncertainties, studies will have to characterise the evolution of structural and functional abnormalities simultaneously and do so reproducibly.

Attention will also need to be given to clinical outcome measures. The main measure currently used in MS clinical studies is the expanded disability status scale score, [7] which is essentially a measure of impaired mobility. However, as impaired mobility may arise from impairment of motor, cerebellar and sensory function, it is intrinsically a poor measure of any particular neurological function and so a specific underlying network, and there is a clear need for more network specific outcome measures. Cognitive outcomes are more network specific, but still imperfect as they may rely on visual function that is often also affected in MS, and may also have been developed for diagnostic rather than monitoring purposes.

Conclusions

Network-based functional and structural studies have provided useful insights into the pathogenesis of MS, and the cause of neurological and cognitive symptoms. Multiple sclerosis is associated with disruption of WM tracts reflected in changes in network efficiency, increasingly random GM network topology, relative cortical disconnection, and both increases and decreases in connectivity centered around hubs like the thalamus and default-mode network. With the caveat that longitudinal studies remain rare, these changes appear to evolve rather than simply increase over time, and are linked with clinical phenotype and disability. Network-based studies also highlight the potential role of treatments that functionally modify neural function rather than structurally change networks.

Table 1: Pathological factors potentially affecting neural network function in MS

Structural	Functional
• Demyelination • Axonal transection and degeneration • Synaptic loss • Neuronal loss • Gliosis	• Inflammation • Hypoxia • Mitochondrial dysfunction • Sodium accumulation • Neurotransmitter deficits

Table 2: Graph theory measures. Modified from Rubinov & Sporns 2010 [36].

Integration	• Characteristic path length • Global efficiency	Network motifs	• Anatomical and functional motifs • Motif z-score • Motif fingerprint
Segregation	• Clustering coefficient • Transitivity • Local efficiency • Modularity	Resilience	• Degree distribution • Average neighbour degree • Assortativity coefficient
Centrality	• Closeness centrality • Eigenvector centrality • Betweenness centrality • Within-module degree z-score • Participation coefficient	Other	• Degree distribution preserving network randomization • Measure 'of network small-worldness'. • Rich club coefficient

Table 3: Motor task and resting-state fMRI studies in people with MS compared with healthy controls

Study	Functional changes#	MS phenotype	Disease duration (years)*	Age (years)*	Number of subjects	EDSS (median, range)
Motor task associated activation						
Wegner et al. 2008 [124] Manson et al. 2008 [125] Mancini L et al. 2009 [126]	Increased activation Reduced deactivation	RRMS/SPMS	6.7 (median)	MS 35 (median) HC 30 (median)	MS 56 (RRMS/ SPMS numbers not given) HC 60 Multicentre	2.0, 0 to 7.5
Colorada et al. 2012 [127]	Increased activation	RRMS	10.2	MS 41.8 HC 38.1	MS 22 HC 23	0, 0 to 1.5
Rocca et al. 2016 [128]	Increased and decreased activation related to fatigue	RRMS, sub-grouped based on fatigue impact scale.	Non-fatigued 10.6 Fatigued 12.9	MS non-fatigued 40.0 MS fatigued 42.6 HC 39.2	MS non-fatigued 29 MS fatigued 50 HC 26	Non-fatigued 1.5, 0 to 4.0 Fatigued 2.0, 1.0 to 4.0
Resting state connectivity						
Rocca et al. 2012 [129]	Decreased and increased connectivity	RRMS	9.0	MS 41.4 HC 40.6	MS 85 HC 40	2.0, 0 to 6.0
Schoonheim et al. 2014 [67]	Decreased cortical centrality and increased	RRMS/SPMS/PPMS	7.7	MS 40.98 HC 40.38	MS 128 (RRMS 112, SPMS 9, PPMS 7)	2.0, 0.0 to 8.0

	thalamic connectivity				HC 50	
Rocca et al. 2015 [130]	Reduced functional connectivity	Relapse-onset MS	10.8	MS 37.5 HC 36.4	MS 69 HC 42	1.5, 0.0 to 6.5
Rocca et al. 2016 [131]	Loss of hubs and decrease in nodal degree.	RRMS/SPMS/BMS	13.7	MS 42.3 HC 41.7	MS 256 (RRMS 121, SPMS 80 BMS 45) HC 55	3.0, 0.0 to 9.0
Eijlers et al. 2017 [132] Meijer et al. 2017 [133]	Only in cognitively impaired MS showed increased connectivity.	RRMS/SPMS/PPMS	14.6	MS 48.1 HC 45.9	MS 332 (RRMS 243, SPMS 53, PPMS 36) MS cognitively impaired 87, mildly impaired 65 and preserved 180. HC 96	Cognitively impaired 4, 0 to 8, mildly impaired and preserved both 3, 0 to 8.
Rocca et al. 2018 [58]	Reduced connectivity, no global differences between MS phenotypes.	CIS/RRMS/SPMS/PPMS/BMS	12.1	MS 41.0 HC 42.7	MS 215 (CIS 13, RRMS 119, SPMS 41, PPMS 13, BMS 29) HC 98	2.0, 0.0 to 8.5

Hidalgo de la Cruz et al. 2018 [70]	Increased and decreased connectivity. Increased and decreased connectivity in fatigued compared with non-fatigued MS.	RRMS/progressive (PPMS or SPMS not specified) Sub-grouped based on fatigue impact scale.	Non-fatigued 10.8 Fatigued 13.4	MS non-fatigued 35.0 MS fatigued 44.3 HC 41.5	MS 122 (RRMS 100, progressive 22) MS non-fatigued 86 and fatigued 36 HC 94	Non-fatigued 1.5, 0 to 8.0, and fatigued 4.0, 0 to 6.5
Tommasin et al. 2018 [134]	Reduced connectivity only MS with EDSS >3	RRMS/SPMS Subgrouped by EDSS ≤ or > 3	8.63	MS 38.3 HC 35.6	MS 119 (RRMS 91, SPMS 28) MS EDSS ≤3 79 and >3 40 HC 42	2.0, 0 to 7.5
Meijer et al. 2018 [91]	Increased and decreased functional connectivity. Increased functional connectivity in information processing impaired compared with preserved.	RRMS/SPMS/PPMS Sub-grouped based on impaired or preserved information processing speed.	Information processing impaired 15.82 and preserved 9.80	MS 48.14 HC 45.9	MS 330 (RRMS 243, SPMS 51,PPMS 36) MS information processing impaired 130 and preserved 200 HC 96	Information processing impaired 4.0, 3.0 to 6.0. and preserved 3.0, 2.0 to 4.0.

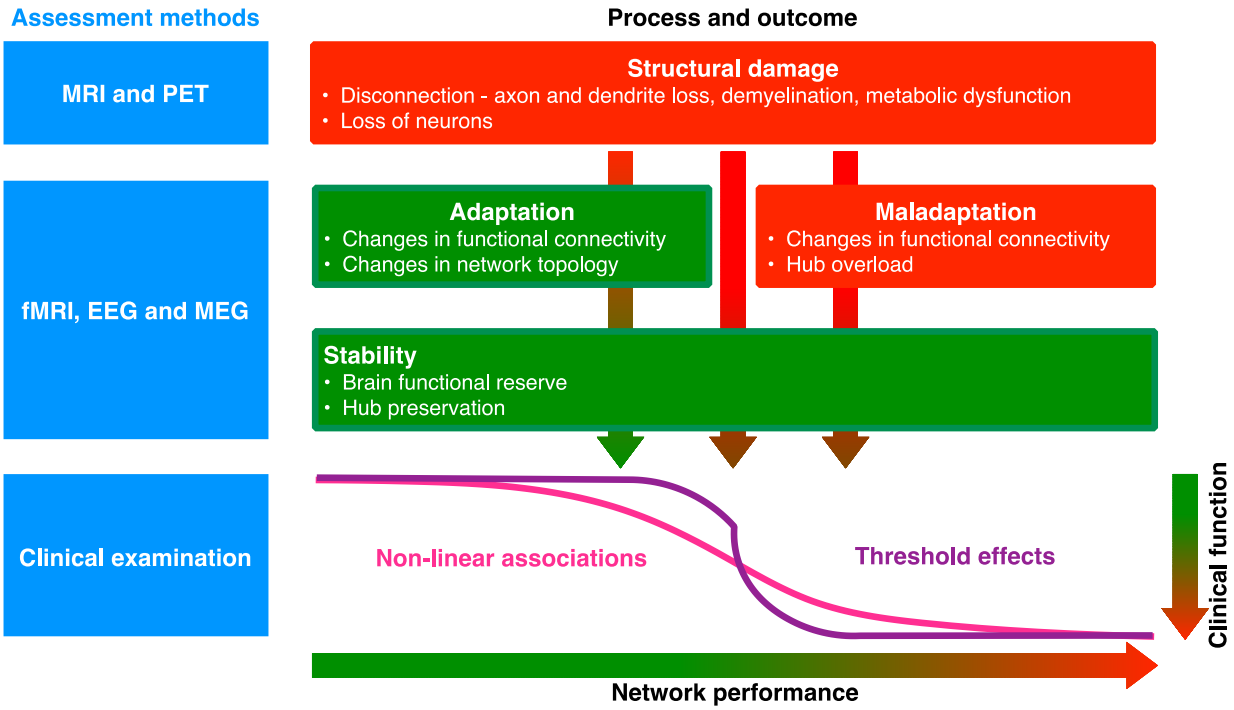
Cordani et al. 2019 [98]	Increased and decreased functional connectivity.	RRMS/progressive (PPMS or SPMS not specified)	12.6 (median)	MS 43.0 (median) HC 38.0 (median)	MS 366 (RRMS 251, progressiv 115) HC 134	2.5, 1.5 to 5.5

Examples of motor task and resting-state fMRI studies in MS. For motor task studies, based on previous work highlighting that small sample sizes may yield unreliable results (Thiron et al. 2007) [122], only studies with ≥20 participants per group are shown. For resting state studies only studies with ≥40 participants per group are shown (Chen et al. 2018) [123]. CIS = clinically isolated syndrome; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; BMS = benign multiple sclerosis; HC = healthy control. # MS compared with HC unless stated otherwise. * Mean value unless stated otherwise.

Table 4: Potential mechanisms through which treatments may sustain and promote brain network function in MS.

	Substrate	Slow/prevent	Repair/improve
Structural	Neuroaxonal loss	Neuroprotection	• No
	Synaptic loss	Neuroprotection	• Promote synaptogenesis • Slow synaptic stripping
Functional	Signal conduction	Prevent demyelination	• Promote remyelination • Improve signal conduction

Figure 1: From neurons to clinical outcomes.



The clinical outcomes we observe represent the effect of combinations of pathological processes on network performance, compensated for or augmented by a network adaptation or maladaptation, and offset by innate network stability. Each element of this can be assessed in life using different techniques, but bridging the gaps between imaging, neurophysiological and clinical measures to provide an integrated model of MS pathology and its clinical consequences has yet to be achieved. MRI = magnetic resonance imaging; PET = positron emission tomography, fMRI = functional MRI; EEG = electroencephalography; MEG = magnetoencephalography.

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