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Title:
Towards Measures for Translation

An Exploration of Neurophysiological Biomarkers of Disability in Multiple Sclerosis

'Towards Measures for Translation'

An Exploration of Neurophysiological Biomarkers of Disability in Multiple Sclerosis

BY

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A thesis submitted to the University of Bristol in accordance with the requirements of the degree of Doctor of Philosophy in the Faculty of Clinical Sciences

AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....

NOTIFICATION

Some work from this thesis has been published prior to submission (citations below) and sections from '*The Growing Potential of Neurophysiology in Multiple Sclerosis'* are directly included in both the first introductory chapter and also in the fifth section entitled '*Closing the Cognitive Gap In Multiple Sclerosis*'.

The *Multimodal neurophysiological evaluation of primary progressive multiple sclerosis – An increasingly valid biomarker, with limits* serves as the basis for the third chapter of work.

I authored the submitted manuscripts of these works with specialist editorial oversight provided by other named investigators. The contribution of others, including the nature of such contribution is duly acknowledged at the opening of each chapter throughout this thesis.

PUBLICATIONS

Canham L.J.W., Western D.G., Walsh P., Kane N., Inglis K., Cottrell D.A. (2016) ***The Growing Potential of Neurophysiology in Multiple Sclerosis.*** J Mult Scler (Foster City) 3:190. doi:10.4172/2376-0389.1000190

Canham L.J., Kane N., Oware A., Walsh P., Blake K., Inglis K., Homewood J., Witherick J., Faulkner H., White P., Lewis A., Furse-Roberts C., Cottrell D.A. ***Multimodal neurophysiological evaluation of primary progressive multiple sclerosis - An increasingly valid biomarker, with limits.*** Mult Scler Relat Disord. 2015 Nov;4(6):607-13. doi: 10.1016/j.msard.2015.07.009. Epub 2015 Aug 7.

Acknowledgements

Dr. David A. **COTTRELL** *Primary Supervisor*

Dr. Alan L. **WHONE** *Secondary Supervisor*

Dr. David G. **WESTERN** *Team Engineer*

Dr. Kirsty E.A. **INGLIS** *MS Speciality Doctor*

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Dr. Howard J. **FAULKNER** *Consultant Neurologist*

Dr. Elizabeth **COULTHARD** *Consultant Neurologist*

Prof. Adam **ZEMAN** *Consultant Neurologist*

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Mrs. Denise **OWEN** *MS Nurse Specialist*

Dr. Magaret **NEWSON** *BrACE Clinical Psychologist*

Dr. Hazel **BARKER** *BrAMS Clinical Psychologist*

Dr. Laura **HANLEY** *BrAMS Clinical Psychologist*

Dr. Vera C. **FIXTER** *BrAMS Clinical Psychologist*

Dr. Martin **BUNNAGE** *NBT Clinical Psychologist*

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Miss Laura **COLE** *MSc Psychology Student*

Mrs. Charlotte **FURSE-ROBERTS** *MSc Psychology Student*

Miss Rebecca **LEGG** *Medical Secretary*

Mr. Adam **PEARSON** *Grey Walter Department Physiologist*

Dr. Sue **WILSON** *Clinical Scientist, CRIC*

Dr. Claire **DURANT** *Clinical Scientist, CRIC*

Special Acknowledgements

i. It has been an honour and privilege to serve, work alongside and be inspired by the many patients who both came under our care and gave of their time and effort to the works described herein. It should be noted that all did so knowing the work offered no direct or immediate benefit to themselves but was hopefully a contribution to a better distant future for others.

ii. The special contribution made by Mr. Shaun McCarthy, Mr. Roger Wall and the others at the MS People's Help charity in Thornbury moved me deeply and has inspired ongoing commitment to this cause. The contributions of many others, who I will not name out of respect for confidentiality, are deeply respected and hugely appreciated.

iii. I would like to thank the wider BrAMS team for their friendship and support including Mrs. Louise Tovey, Ms. Lucy Chemanais, Mr. Clive Lambert, Mrs. Hannah Nelson and Mrs. Carole Wheaton. I am grateful for the opportunity to work alongside our MS Nursing team of Mrs. Denise Owen, Mrs. Gemma Watkins, Mrs. Hannah Lee, Mrs. Samantha Messenger and Mrs. Tracey Chapman and for the care they provided to our patients. I am also grateful to Mrs. Jane Turk, the current BrAMS Trial Coordinator and her predecessor, my friend, Mrs. Jenny Homewood for their assistance in our endeavours. A special thanks to the ever-friendly Jennifer & Allison Thornton for keeping our department so clean and spotless is also extended.

iv. I am particularly grateful to *all* members of the Grey Walter Neurophysiology Department, administrative and clinical, including Dr Sabine Klepsch in addition to Dr Agyepong Oware, Dr Ian Ormerod and Dr Nick Kane for their support and encouragement during my period of Clincal Fellowship and for their assistance during the research endeavours herein. I am sincerely grateful in particular to Mr. Peter Walsh, for his continued guidance, expertise, help with my potentials and friendship. The contributions of Mrs. Kelly Macey (nee Blake), Mr. Adam Pearson and Mrs. Abby Parks-Galton were also invaluable.

v. I feel fortunate to have encountered several fellow Consultant Neurologists who have provided insights, a spectrum of example and guidance across the field of MS and neuroinflammatory disease – in particular Dr Roswell Martin, Dr Paul Lyons, Dr Edward Fathers, Professor Gavin Giovannoni, Dr Niraj Mistry, Dr William Brown, Dr Belinda Weller, Dr Richard Nicholas, Dr David Rogg, Dr Owen Pearson, Dr Sebastian Luppe, Professor David Bates, Professor Neil Scolding, Dr Claire Rice and Dr Alastair Wilkins.

vi. I must also express gratitude 'at a distance' to Professor Guilio Tononi, Professor Paul Nunez, Professor Olaf Sporns and Professor Georgi Buszaki for sharing such wonderful ideas in their beautiful books. Their words have shaped my thoughts.

vii. Importantly, I must express gratitude to our many colleagues across the pharmaceutical industry – Mr. Frank Hagenow, Mr. Simon McKenna, Mr. Ched Hill of Biogen and Mrs. Katie Fry and Mr. Toby Kibble from Novartis who have all provided considerable encouragement, insight, educational support and excellent opportunities for professional development. The similar support and encouragement of Mr. Terry O'Regan and Mrs. Fiona Thomas from Biogen Idec similarly deserves special mention and appreciation.

viii. Ms. Jane Ibunnson and Mrs. Elizabeth Bond formerly of Southmead Hospital Charity proved extremely supportive and resourceful in helping us find means to support our continued endeavours and my gratitude for their help is considerable.

ix. My thanks are extended also to Mrs. Sheila Morrison and Mrs. Teresa Smith of the Neurology Nurse Specialist Team of Musgrove Park Hospital in Taunton for their friendship, encouragement and support.

x. Special thanks are extended also to the irreplaceable and ever helpful Ms. Jane Sweetland of the Burden Neurology Library, her colleague Mrs. Lioudmila Smirnova and to Mrs. Annette Clarke, Ms. Mary Kisanga and Mrs. Helen Lewis-White of the North Bristol Research & Innovation Department who have all been extremely supportive.

Personal Thanks

To Dr David Cottrell for inviting me on the long climb to help our patients, sharing his hard won wisdom along the way and becoming a friend who clearly cares whilst remaining a good doctor in challenging times.

To Dr David Western for his patience, for helping to make the improbable not only possible but achievable in the present; and for taking me seriously.

To Dr Kirsty Inglis and Dr Alan Whone for their friendship, time, efforts to help our journey and sage guidance.

Unquantifiable thanks is due to Sister Rachel Almond for all her loving support and that of her parents Marion and Pat, and for Rachel's understanding and gift of our wondrous children;

Qualia and Quintessence – the greatest joys in my life

…and Shadow.

I remain eternally grateful for the endless love and encouragement of my grandparents, Maisie and Jim also;

And for the support of my wider family, including my sister Lara.

And to *Shondells*, for his enduring friendship, belief and encouragement.

Dedication

This work and all my efforts are dedicated out of Love to my wonderful parents Ann and Keith, for the Love they shared and the Love they showed; and for all those many others who journey through misery like they did and yet truly deserve so very much better.

Disclosure

The author has received educational support from Biogen Idec, TEVA, Novartis, Merck, Roche and Sanofi-Genzyme pharmaceutical companies in the form of sponsored attendance at international courses and conferences. Novartis and Biogen have also provided grants to support clinical, trial and research activity at the Bristol and Avon Multiple Sclerosis Service at North Bristol NHS Trust.

The MS People's Help, Southmead Hospital Charity and The Psychology Department of University of Bristol have also given financial support for the author's research activities.

ABSTRACT OF THESIS

Multiple Sclerosis is the most common acquired cause of neurodegeneration and disability in young adults. Despite recent therapeutic advances in control of the initial inflammatory component, the progressive neurodegenerative aspect remains *almost* completely untreatable whilst conferring the greatest burden of disability, cognitive dysfunction and misery. Reliance on the current clinical measurement tools accepted by the Food & Drug Administration and European Medicines Authority has and will continue to prove prohibitive to rapid, sensitive and smaller scale clinical research in Progressive MS cohorts.

We have sought to explore the utility of neurophysiological techniques to provide surrogate biomarkers which may predict meaningful clinical endpoints in Progressive Multiple Sclerosis; and which may therefore satisfy regulatory criteria for trial outcome measures and potentially enable an acceleration of translational therapeutic research.

The central clinical outcomes of interest herein are disease related physical disability and cognitive dysfunction.

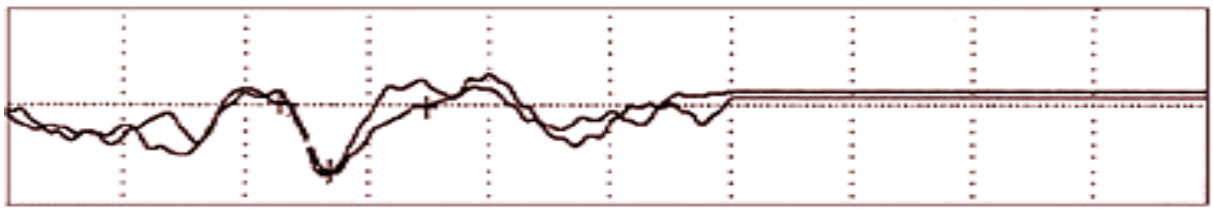
We demonstrated that Multi-modality Evoked Potential batteries have a consistently meaningful correlation with ratings of physical disability amongst patients with a primary progressive phenotype and explored the nature and utility of this relationship longitudinally over an interval of three years. The relative superiority over conventional neuroimaging metrics derived from MRI was also assessed and reasons for such considered.

The lack of association of long tract neurophysiological abnormality with Multiple Sclerosis related cognitive impairment identified herein prompted pursuit of alternate methods to index this disabling and complex problem.

A composite psychometric outcome metric to quantify disease related cognitive impairment was derived against which outcomes from cognitive evoked potential and electroencephalographic connectivity analyses from a typical Multiple Sclerosis patient cohort were compared.

The findings of such stimulated a reconsideration of the pathophysiological substrate underpinning cognitive impairment in Multiple Sclerosis and the development of a conceptual framework based on '*disintegration*' rather than the contemporary model of '*disconnection*'. Empirical support for the emerging model was pursued in a final pilot study and plans for future endeavours in pursuit of the team objectives have been developed and are outlined herein.

LJWC



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GLOSSARY OF TERMS & ABBREVIATIONS

9HPT	9 Hole Peg Test
BA	Brain Atrophy
BAC	Back Propagated Calcium Channel Activation
BDSS	Biomarker of Disability Severity Scale
BICAMS	Brief International Cognitive Assessment in Multiple Sclerosis
BIS	Bispectral Index
BSAEP	Brainstem Auditory Evoked Potential
BVMT-R	Brief Visual Memory Test - Revised Edition
CCA	Cervical Cord Cross Sectional Area
CDMS	Clinically Definite Multiple Sclerosis
CDP	Confirmed Disability Progression
CEI	Cause-Effect Information
CEN	Central Executive Network
CEP-3	Cognitive Evoked Potential 3 Score
CER	Cause-Effect Repertoire
CES	Cause-Effect Structure
CI	Cause Information
CIS	Clinically Isolated Syndrome
CMCT	Central Motor Conduction Time
Coh	Coherence
COWAT	Controllowed Oral Word Association Test
CSF	Cerebrospinal fluid
CVLT 2	California Verbal Learning Test version 2
DASS	Depression, Anxiety and Stress Scale
DEG	Degree
DKEFS	Delis Kaplan Executive Function Sorting Task
DLPFC	Dorsolateral Prefrontal Cortex
DMF	Dimethyl Fumarate
DMN	Default Mode Network
DTF	Direct Transfer Function
EDSS	Expanded Disability Severity Scale
EEG	Electroencephalograph
EI	Effect Information
EMA	European Medicines Authority
EP	Evoked Potential
FDA	Food And Drug Administration
FFT	Fast Fourier Transform
fMRI	Functional Magnetic Resonance Imaging
FSIQ	Full Scale Estimated Intelligence Quotient
γ	Clustering Coefficient
GA	Glatiramer Acetate
GE	Global Efficiency
GEPS	Global Evoked Potential Score
H_1	Shannon Entropy
HFD	Higuchi Fractal Dimension
IFCN	International Federation of Clinical Neurophysiology
IIT	Integrated Information Theory
INFB (x)	Interferon Beta (type, 1a or 1b)
JLO	Judgment of Line Orientation Test
λ	Path Length
LZ	Lempel-Ziv
MACFIMS	Minimal Assessment of Cognitive Function in Multiple Sclerosis
MEP	Motor Evoked Potential
MEP-4	4 Limb somatosensory and motor evoked potential score
MEPS	Multimodal Evoked Potential Score
MFIS	MS Fatigue Impact Scale
MI	Mutual Information
MICE	Maximally Irreducible Cause-Effect Structure
MIP	Minimal Information Partition
MMEP	Multimodal Evoked Potential Battery
MMN	Mismatch Negativity Evoked Potential
MRDSS	MRI Disease Severity Score
MRDSS-2	MRI Disease Severity Score v.2
MS	Multiple Sclerosis
MSCI	MS Related Cognitive Impairment
MSFC	Multiple Sclerosis Functional Composite
MSQ	Multiple Sclerosis Cognitive Impairment Quotient
MSQLI	MS Quality of Life Index
NFI-MS	Neurological Fatigue Index - for MS
NTZ	Natalizumab
OCB	Oligoclonal Bands
p300	Cognitive Evoked Potential (Positive at 300ms)
P300	Attentional Cognitive Evoked Potential
P3a	P300 response to Deviant
P3b	P300 response to Target
PASAT	Paced Auditory Serial Addition Test
PASAT 2'	Paced Auditory Serial Addition Test (2 second intervals)
PASAT 3'	Paced Auditory Serial Addition Test (3 second intervals),
PCA	Principal Component Analysis
PDQ	Perceived Cognitive Deficits Questionnaire
PING	Pyramidal Interneuron Gamma
PMS	Progressive Multiple Sclerosis
PPMS	Primary Progressive Multiple Sclerosis
qEEG	Quantitative EEG
RCT	Randomised Controlled Trial
RIS	Radiologically Isolated Syndrome
σ	Small World Index
SAN	Salience Network
s-EP-Q	s-EP-Q Summed EP Latency Z Score Quotient
SL	Synchronisation Liklihood
SLF	Superior Longitudinal Fasiculus
SMDT	Symbol Digit Modalities Test
SOC	Self Organised Criticality
SPMS	Secondary Progressive Multiple Sclerosis
SSEP	Somatosensory Evoked Potential
T25ft	Timed 25ft Walk
TMS	Transcranial Magnetic Stimulation
TMS-MEP	Transcranial Stimulation induced Motor Evoked Potential
TOPF	Test of Premorbid Functioning
VEP	Visual Evoked Potential

STRUCTURE OF THESIS

This body of work consists of six sequentially related components of investigation described after the initial introduction to the field of interest and an outline of general methods, which where necessary are expanded upon within respective chapters.

The **first** line of inquiry was a cross-sectional examination of the relationship between physical disability and multi-modality evoked potential batteries in the setting of Primary Progressive Multiple Sclerosis. Within the examined cohort of patients (n=33) this identified a meaningful degree of association with physical impairments but not cognitive difficulty; seeking to close this '*cognitive gap*' served as the basis for the fourth, fifth and sixth pursuits.

The **second** line of inquiry sought to further evaluate the degree of cross sectional association of Multi-modality evoked potential batteries with physical disability and compare this with findings from structural assessment by MRI and then subsequently consider how further benefit may arise from integrating the information from both modalities.

The **third** line of inquiry was to evaluate the longitudinal changes of Multimodality evoked potential findings in relation to physical disability in a cohort of Primary Progressive Multiple Sclerosis patients. This was performed over an interval of three years to assess their candidacy as putative surrogate biomarkers over a period slightly longer than the average duration of typical clinical therapeutic trials in such a context.

With a view to begin closure of the cognitive gap identified in the first study, consideration of the nature of MS cognitive impairment and approaches to addressing the problem identified a primary need for an applicable clinical cognitive outcome measure derived as a composite of results from psychometric tests sensitive to the MS '*cognitive footprint*'. The **fourth** line of inquiry featured a principle component analysis of a large retrospectively acquired cognitive dataset (n=100) from a real-world cohort which enabled synthesis of a singular 'MSQ' index to serve as a clinical outcome against which subsequent putative neurophysiological biomarkers of cognitive dysfunction could be compared.

The initial series of possible electrophysiological biomarkers we explored examined properties related to functional connectivity, spectral indices, phase dynamics and in line with the earlier inquiries, cognitive evoked potentials. This **fifth** line of inquiry took the form of an exploratory prospective cross-sectional pilot study of a new cohort (n=30) of phenotypically mixed MS patients. Some initially promising findings identified important methodological considerations to be solved as part of future work and stimulated a necessary re-evaluation of the nature of MS cognitive impairment.

The final, **sixth** line of inquiry began with an attempt to re-formulate the problem of MSCI on its own terms; reconsidering it not simply as a consequence of '*disconnection*' as has been conventionally advocated but more so as a problem of '*disintegration*' in a complex adaptive system undergoing threatened collapse with a loss of emergent phenomena. This prompted an initial attempt to apply the framework of Integrated Information Theory and generate a system of brain functional measurement calibrated against a reasonably broad range of electroencephalographic samples from varying clinical states (n=210) to offer a metric of the integrative capacity lost as a result of Multiple Sclerosis.

Future plans stimulated by the outcomes of these endeavours are outlined prior to a final conclusion. The contributions of others where made are specified at the beginning of each section and a full bibliography of references is included at the rear.

LJWC

PLAIN LANGUAGE SUMMARY

Multiple Sclerosis is a common, disabling and in some cases fatal neurological disease characterised by disseminated injury wrought by a person's own immune system to their brain and spinal cord, which along with subsequent neurodegeneration leads to dysfunction. The translation of possible therapeutic agents identified by pre-clinical research into licensed treatments which may ameliorate or delay disability in the later progressive phase of this disease could be accelerated by the availability of a sensitive and reliable measurement system. This would ideally identify beneficial effects in small groups of patients over relatively short intervals and thereby facilitate research.

We have explored the ability of various electrophysiological techniques to provide such biological measures of both physical and cognitive disability.

Small electrical responses produced by transmission of stimulated signals along the neural pathways in the spinal cord and brain (so termed evoked potentials) were recorded in a group of patients with Multiple Sclerosis whose disability had been progressively worsening since onset . We demonstrated that the amount of abnormality in the evoked potentials consistently related to the severity of their physical disability. The extent of association appeared tighter than that seen with measures of their brain and cord structure achieved by detailed scanning techniques. In following up the same group over three years the evoked potentials attained from the spinal cord not only worsened in a manner which matched the increasing physical disability but those individuals with more abnormal responses at the start were seen to progress faster. This suggests evoked potentials could be used to detect beneficial drug effects and enrich recruitment for those participants most likely to demonstrate worsening without the benefit of intervention.

Patterns within the electrical activity produced on the scalp by the underlying function of the brain were explored using a variety of means in pursuit of a measure of cognitive disability. A system for gauging the clinical severity of thinking and memory difficulties in patients with Multiple Sclerosis was also developed to aid this exploration. The findings of this investigation ultimately prompted a reconsideration of why such difficulties arise and a new approach to analysing the electrical activity from the brain based on its complexity and how it dynamically changes over time, which we tested in a preliminary pilot investigation. Ideas for how to go forward from the works undertaken and lessons learned were finally formulated.

I

INTRODUCTION

'MULTIPLE SCLEROSIS IS SO VERY CRUEL'

ANON.

Multiple Sclerosis is a common neurological disease with inflammatory and neurodegenerative components affecting the Central Nervous System(1). It is currently incurable and remains a leading cause of acquired disability in young adults (2).

Amongst the 100,000 estimated sufferers in the United Kingdom and 2.3 million globally(3, 4), the personal impact of the condition is simply immeasurable. The quantified economic burden upon society a decade ago was 12.5 billion Euros per year across Europe, rising to 23 billion on inclusion of intangible costs (5, 6). Notably a minority of this expenditure arises from therapeutics.

The defining feature of Multiple Sclerosis is the dissemination of inflammation in time and space throughout the Central Nervous System(7, 8). This cardinal requisite has been central to all the diagnostic criteria (including those of Schumacher 1965, Poser 1983, McDonald 2001, McDonald 2005, McDonald 2010, McDonald 2017) which have evolved over the past half-century to include meaningful findings from paraclinical tests such as neurophysiology, neuroimaging and cerebrospinal fluid analysis (7-9). **The contemporary 2010 diagnostic criteria that were employed in clinical practice and throughout these works are on the next page (table 1)** with inclusion of the recent 2017 modification (11) additionally outlined.

Table 1 Diagnostic Criteria for Multiple Sclerosis: McDonald 2010 Revised Edition

CLINICAL PRESENTATION	Additional data required
≥ 2 attacks; objective evidence of at least 2 lesions or objective clinical evidence of one lesion with reasonable historical evidence of a prior attack	None.
≥ 2 attacks; objective clinical evidence of 1 lesion	**DIS** demonstrated by ≥1 T2 lesion in ≥2/4 MS-typical regions of CNS (Periventricular/Juxtacortical/Infratentorial/Cord) ; or await a further clinical attack implicating a different CNS site
1 attack, objective evidence of ≥2 lesions	**DIT** demonstrated by simultaneous presence of asymptomatic gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or await a second clinical attack.
1 attack; objective evidence of 1 lesion - 'Clinically Isolated Syndrome'	**DIS** and **DIT** demonstrated by same criteria for each as above.
Insidious neurological progression suggestive of multiple sclerosis – <u>Primary Progressive Multiple Sclerosis</u>	1 year of disease progression (retrospectively or prospectively determined) along with 2/3 criteria of: i) Evidence of DIS with at least one T2 lesion in brain (Periventricular, juxtacortical, infratentorial regions) ii) And ≥2 T2 lesions in the cord. iii) Positive CSF (Oligoclonal Bands or elevated immunoglobulin G index)

DIS- Dissemination in Space

DIT- Dissemination in Time; Of note the 2017 modification (11) now allows the demonstration of unmatched OCBs to constitute evidence of dissemination in time; this now facilitates an even earlier diagnosis and treatment opportunity.

'Attack' (Relapse or Exacerbation) = is defined as '*patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the central nervous system, current or historical with a duration of at least 24 hours in the absence of fever or infection'*. Such events are ideally corroborated by neurological examination.

NB – all the above is predicated on the absence of a better alternate explanation or diagnosis.

10. Katz Sand I. Classification, diagnosis, and differential diagnosis of multiple sclerosis. Curr Opin Neurol. 2015;28(3):193-205.

The clinical features seen are extremely variable both with regard to their manifestations and temporal course(12-20).

After onset typically between the ages of 20-40 years, most (85% of) patients initially exhibit a relapse-remitting phenotype, characterised by discrete subacute attacks of neurological symptoms (motor, sensory, special sense, sphincter, coordination or psychomotor disturbance) referable to CNS inflammation which then resolve either completely or less so over weeks to months(12-14, 18, 19, 21). The average reported annualised relapse-rate is approximately one per year albeit this is variable(12, 19, 22) and the likelihood of relapse typically but not always decreases over time (19).

Typical clinical features of monocular visual obscuration related to optic neuritis, or sensorimotor dysfunction of the limbs referable to myelitic cord injury of the efferent corticospinal pyramidal tracts or their afferent sensory partners in the dorsal and spinothalamic tracts or intracranial connections are common(23, 24). The longest pathways running between the brain and terminal cord which mediate sphincteric control and sexual function are also common targets(25) and this is reflected in the very high prevalence of symptoms in these domains(23, 26). Infratentorial structures within and coupled to the brainstem, in particular the cerebellum and its associated pathways are similarly common foci for pathology(23, 25, 27) producing the frequently observed ataxic incoordination, dysarthria, dysphagia, diplopia and more complex disorders of voluntary gaze (such as various forms of interrupted pursuit, saccadic intrusion and nystagmus) not uncommonly encountered within MS patients(23, 26, 27). With pyramidal tract injury comes associated spasticity of the limbs and trunk which can range in severity from that which mildly impairs motion to that which is disabling through mechanical restriction and profound discomfort(26). Insult anywhere along the human sensory pathways brings with it opportunities for varying types of neurogenic pain, dysaesthesiae and functionally limiting loss of somatosensory and visceral sensory feedback(26). All of these symptoms are only modestly ameliorated by currently licensed therapies(23, 28) and two further consequences of the disease which are both almost as ubiquitous as they are disabling, namely cognitive impairment(29) and fatigue(30) have, with the exception of modest benefits for some patients(31-38), remained without effective symptomatic alleviation for most individuals affected by the disease.

Whilst symptoms across all domains may deteriorate with advancement into the progressive phase, failing mobility, deteriorating sphincteric control and worsening cognitive impairment are the three leading functional impairments(25).

Although readily acknowledged to remain incomplete(25) the model of the cellular pathology underlying multiple sclerosis has continued to evolve over the timespan of the works described herein(1, 39-47). The current leading position is that Multiple Sclerosis sits within a family of conditions characterised by a polygenic

autoimmune disease (48-51), featuring damage wrought by components of both the adaptive (T and B lymphocytes) and innate (complement, microglial and macrophage) immunological systems(25, 52, 53) against targets within the CNS parenchyma, particularly but not solely the oligodendrocyte population which affords central myelination(39, 40, 42, 46, 54).

The disseminated lesions which characterise the disease display a degree of heterogeneity with respect to the exact dominant immunological effector process at work within each region of inflammation and features related to the chronicity of their establishment (25, 39, 41, 43, 45). Nonetheless, their predilection for perivenular and periventricular tissue and the accompanying breakdown of the local blood-brain barrier (as demonstrated by gadolinium permeability) allowing egress of immunological culprits into the CNS substance is a general commonality, at least early on(23, 27).

The appearance of early active inflammatory lesions may arise over months(25, 55) and notably the association between focal inflammation and the arrival of clinical symptoms or manifestation of signs is incomplete(56, 57), with the latter only apparent both when a threshold of dysfunction is exceeded and then identified by the patient or clinician respectively(58-61).

Early active inflammatory lesions typically fall into three categories, the first type being a classical T lymphocytic infiltration which drives local monocytic phagocytic activities leading to direct attack of myelin bearing cells and denudation of their ensheathed axons(25, 41). A second type features a marked additional prevalence of immunoglobulin and complement alongside these cellular culprits and it is particularly noteworthy that such lesions are seemingly dramatically responsive to systemic immunoglobulin depletion by plasma exchange(62). The co-occurrence of positive Anti-MOG serology in some clinical cases demonstrating such a response(63) has continued to fuel concern that MS may still represent a collection of disparate neuro-immunopathies with distinct antigenic targets which are united by a similar final common pathway.

A third type of early active lesion additionally features a ‘dying back’ oligodendrogliopathy within them in addition to features of oligodendrocyte apoptosis; it is notable that this feature is often a hallmark of non-immunological disease; being evident in settings of toxic, viral and metabolic insults(25, 41).

After the initiation of active inflammatory attack each lesion may then follow three possible paths; either there is resolution, with glial mediated debridement and subsequent repair with remyelination from the many resident oligodendrocyte precursor cells(64-69), or the cessation of active immunological attack is met with the formation of organised astrocytic scarring of the kind which typifies the characteristic plaques of the disease seen macroscopically(23, 27). The molecular signalling which appears to block effective remyelination despite the prevalence of cells effectively resident and waiting for this purpose(70, 71) is being

increasingly delineated(64, 66, 72, 73) and has served as a promising target for monoclonal modulation (by anti-Lingo-1)(74) with phase III studies underway.

All too commonly however the nature of the early active inflammatory processes may evolve into a chronic '*smoldering*' lesion featuring slow continued tissue destruction and failed repair(1, 39-41, 43, 45-47, 72); it is these lesions which are a dominant feature with advancing disease(1, 46) in addition to subpial inflammation which appears to migrate in from the leptomeningeal coverings and feature the establishment of *self-sustaining* ectopic lymphoid follicles(75-78). Despite the perivascular association of cortical disease, the lesser extent of blood-brain barrier breakdown and more horizontally confluent and distributed nature of disease along the cortical expanse renders it far less amenable to gadolinium enhancement or conventional cortical imaging(25, 79). This is regrettable particularly given the predominant association of such mechanisms with more advanced disease(76, 80-83).

Indeed, the tendency and capacity to mount effective remyelinative repair also appears to diminish with age(66, 67, 84).

Moreover in addition to the axons which may have been lost by direct attack and transection(79, 85, 86) through the misfortune of traversing an active lesion, those which initially survive denudation may ultimately succumb to the chronic effects of mitochondrial dysfunction related to insult by radical species and iron deposition, impaired axonal transport and the increased energy demand accompanying the necessary increase in sodium channel upregulation arising from loss of capacity for saltatory conduction (previously bestowed by the now lost myelin)(44, 46, 87, 88).

The innumerable resident microglia within the CNS also appear to play a diverse array of roles with respect to the chronic inflammatory component; some benefical with respect to removal of debris and some not(89), such as the directly observed pruning of synapses on otherwise surviving neuronal elements(90).

The growing list of relevant inflammatory cellular culprits is further matched by an entire gamut of humoral and cytokine messengers between them which collectively interact to induce tropism of adaptive and innate immune cells into lesions, provoke demyelination and thereafter either restore immunological tolerance or sustain the pathological cascade(23, 27). Indeed attempts to therapeutically '*nudge*' the balance of the cytokine profile away from a proinflammatory to an anti-inflammatory pattern led directly to the first successful licensed therapies in the form of interferon beta(27, 91).

Despite the fact that an unmatched pattern of oligoclonal bands within the cerebrospinal fluid has offered paraclinical support of the diagnosis for decades(2, 23) and they in turn represent intrathecal immunoglobulin antibody synthesis(25), it is only within the past years that the B cell lymphocyte population has received

the same attention(52, 53, 92) as the T cell subsets of lymphocytes more classically associated with MS pathology(23).

Indeed, the efficacy of the anti-CD20 monoclonal agent rituximab in the setting of relapse remitting multiple sclerosis (93-95) and subsequently demonstrated effectiveness of the similar anti-CD20 Ocrelizumab against both relapse-remitting and more recently (and more modestly) *primary progressive* disease(96-99) is a strong testament to the dominant relevance of these cells. The initial supposition that this benefit is 'simply' mediated by a reduction in circulating immunoglobulin in the same fashion as that offered by plasma exchange(62) is not wholly congruent with the appearance of clinico-radiological disease modulation anteceding a drop in circulating immunoglobulin provoked by the anti-CD20 agents(25). As such, additional mechanisms including attenuation of B-cell mediated cytokine production and their role in antigen presentation to T cell populations (and the pathological cascades which would otherwise follow) are considered equally central(25).

From an aetiological perspective, that *all* of the demonstrably effective and licensed therapies for multiple sclerosis (table 2) are either immunomodulatory or immunosuppressant in nature(100) speaks to the clear centrality of auto-immunity in this disease; as does the unfortunate demonstration of inadvertent exacerbation by alternate immunomodulators which consequently failed translational evaluation(91, 101). Furthermore the overwhelming predominance of immunological genes amongst the several hundred (and growing) number of risk alleles identified in the Genome Wide Association Study(102-105) examining thousands of MS patients against matched controls futher underpins this dominance as does the increasingly appreciated efficacy of immune ablation and reconstitution afforded by haematopoietic stem cell transplantation following chemotherapy, particularly earlier in the condition(106).

Agent	Nature	Mechanism of Action
Interferon Beta	Cytokine	Encourages a favourable shift toward anti-inflammatory cytokine profile, reduce lymphocytic transmigration into CNS(107)
Glatiramer Acetate	Mixture of 4 Synthetic Analogues of Myelin Basic Protein	Competitively Inhibit Presentation of endogenous myelin antigen to host T cells(108)
Natalizumab	Monoclonal Antibody	Selective Inhibition of α4-integrin adhesion molecule limiting transmigration of lymphocytes into CNS parenchyma(109)
Alemtuzumab	Monoclonal Antibody	Selective Depletion of CD52 bearing T and B Cell populations with subsequent restoration of self-tolerance(110)
Ocrelizumab	Monoclonal Antibody	Depletion of CD20 bearing B cells; reduced immunoglobulin production and antigen presenting effects(111)
Fingolimod	Small Molecule	Agonist of Sphingosine-1-phosphate receptor agonist which promotes downregulation and limitation of lymphocyte egress from lymphoid tissues(112)
Teriflunomide	Small Molecule	Selective inhibition of mitochondrial dihydro-orotate dehydrogenase mediated synthesis of pyridimines required for lymphocyte proliferation(113)
Dimethyl Fumarate	Small Molecule	Multiple; Appears to Shift balance of lymphocyte population toward anti-inflammatory composition, via Nrf-2 pathway may also mediate central protection against oxidative stress(114)
Cladribine	Small Molecule	Synthetic Adenosine Analogue which accumulates within select T and B populations and provokes depletion by interfering with DNA synthesis(115)

Table 2 Currently Utilised Disease Modifying Therapeutics for the Treatment Of Multiple Sclerosis

Of note, the understanding of the beneficial mechanism(s) of action accompanying each of these licensed agents continues to evolve beyond(114, 116) the putative influences outlined here.

That the concordance of MS between monozygotic twins is only on the order of 30% and that between first degree relatives 2-4%(23, 25, 27), despite the number of identified risk alleles (most of which bestow a modest increase in relative risk(105)) speaks to the dominant contribution of non-heritable factors which conspire in an as yet undiscerned manner to *ignite* the aforementioned autoimmune inflammatory cascade.

The capacity to arrest decline in some if not all patients(117), the appearance of 'burnt out' multiple sclerosis on histopathology(1, 46, 47) and the genetic profile

being barely distinguishable from other non-neurological autoimmune diseases(50, 103, 105) whilst possessing minimal similarity to any of the other neurodegenerative diseases strongly speaks against the alternate position of this being a 'primary' neurodegenerative disease with inflammatory epiphenomena(118). Epidemiologically significant associations with obesity(119, 120), smoking(121, 122) and vitamin D(123-126) (and *independently)* cumulative ultraviolet light(127) exposure have all been identified to modify risk of developing the disease and subsequent inflammation once the diagnosis is established. The influence of hormones, the predominance amongst women (in a manner common to autoimmune disease generally) and the protective influence of pregnancy is also long recognised(2, 23).

However, the particularly strong association of the condition with exposure to the ubiquitous Epstein Barr Virus is a fascinating narrative which continues to evolve(92, 128, 129) with proponents making strong arguments both for role of this agent or similar either initiating an autoimmune cascade by molecular mimicry or provoking cellular injury which then evokes antigen presentation and a subsequently excessive and unquenched immunological cascade in those individuals carrying a genetic predisposition for doing so. Notably, other factors such as low vitamin D (receptors for which are found in control elements for most of the culprit cellular players of MS)(130), ultraviolet light exposure(131) and the generally pro-inflammatory cytokine profile associated with excess adiposity(132, 133) are all recognised adverse modifiers of adaptive immune mediated inflammation once it has initiated. The effects of smoking are likely manifold(121, 122), with direct endothelial injury, blood brain barrier permeability and oxidative injury to an already challenged system probably being particularly relevant.

The very recent recognition of anti-NMDA autoimmune limbic encephalitis following as a short successor to confirmed and adequately treated herpes simplex encephalitis(134) is a real-world demonstration that viral insults may trigger subsequent B-cell driven autoimmunity within humans. Furthermore, the absence of a naturally occurring form of multiple sclerosis outwith *Homo sapiens*(27) is a particularly striking feature despite the marked shared genetic, immunological and neurological similarity with other primate cousins(135, 136). This can be reconciled either by the presence of a unique toxic element to which only humans with a predisposition to MS-type autoimmunity are exposed or species-specific tropism which accompanies viral species.

The lower observed prevalence of multiple sclerosis in subjects with HIV on Highly active antiretroviral therapy(137) and the *absolute absence* of EBV-negative serology in MS subjects(138) has led many to suspect a viral trigger, whether EBV or HSV (exposure to which also raises relative risk of MS development(139)) or a more primitive paleovirus or human endogenous retrovirus(140) may be the salient index event in predisposed individuals. Whether the relapse-remitting pattern observed initially in most patients is reflective of either subacute loss of

host-suppression of latent viral agents (in a manner familiar to cold sore sufferers) or a manifestation of epitopic spread(141), where the immune response to the first myelin based antigen grows to include a collection of different targets remains to be seen. It is noteworthy that epitope spread is both a demonstrated phenomenon in the MS field(141) and its role as a target for therapeutic intervention is still under test(142). Attempts to limit viral activation by vaccination are under consideration and trials of antiretroviral therapy are underway, albeit the phase 2a INSPIRE study of the antiretroviral agent raltegavir in RRMS patients using clinical and imaging metrics has recently been reported as negative(140).

It is finally noteworthy that the very animal disease model (experimental allergic encephalitis) used for pre-clinical MS basic and translational research(143) does not recapitulate the initial relapsing and subsequent secondary progressive phase of the disease observed in humans in the same manner as the two-stage disease model that is provoked by exposing murine subjects to Theiler's Encephalomyelitis Virus (TMEV) from the picornaviridae family of RNA viruses(144). This notably demonstrates a tropism for and persistence within glial consitutents and provokes early activation of the innate microglia which subsequently elicit activity from the adaptive community of T and B cell components(144). A novel human analogue in the same family, the Saffold virus discovered in 2007 demonstrates ubiquitous seropositivity and capacity for latent persistence amongst humans and derived cell lines (145, 146)however its relationship to MS remains wholly uncertain.

Thus, whilst the final details remain unclarified at this stage it is reasonable to at the least assert that MS is primarily an autoimmune condition featuring numerous elements and mechanisms comprising inflammatory attack and secondary neurodegeneration which run concurrently contributing to the hallmark temporal patterning and spatial dissemination of disease in individuals endowed with an unfavourable balance of different risk modifiers and potentially exposed to one (or one of many) triggering factors. The interplay between all such variables and the *non-linear* nature of interactions therein coupled with varying capacity to tolerate, adapt and recover from such insults all collectively contributes to the marked clinical heterogeneity seen in the natural history observational studies of the disease(13, 14, 16, 17, 147-151); which is only further compounded by the fortunate advent of effective disease modification.

The persistence of a background genetic predisposition, coupled with an uncertain series of triggers and the liability to cascading failure of immune self-tolerance by an assortment of means could lead one to conclude that the *ideal* of 'personalised medicine' in matching patients perfectly to singular definitive agents for their 'form' of the disease and offering prognostic decision making from the *outset* to guide decisions(152, 153) is fundamentally *unattainable* at least in a complete sense; for exactly the same reason that despite quantum leaps in data modelling and precision metrological data collection, the accuracy of weather forecasting still breaks down after 7 days – owing to the complexity and chaotic nature of the

non-linear interactions involved(154, 155). In the right adverse circumstances a system predisposed to muster the autoimmune inflammatory attack will still do so; even if natural history data, age and distant immunomodulatory therapy suggest relapses later in the disease should be unlikely, they nonetheless are still observed to occur(13, 19, 22) in a manner characteristic of complex systems(156). One acknowledges that certain clinical and radiological features do reliably portend a more adverse outcome (157-160) yet, as clinicians we are still lacking a capacity to predict an outcome which is *good*, which is itself perhaps far rarer(161-163) than has been suggested particularly with respect to cognitive considerations(164) and clinical function over the entire normal working life of adults, in a disease which often strikes in their prime(23, 24, 27) or even more heartbreakingly in childhood(165).

Unfortunately with time also comes the accrual of an increasing burden of damage resultant from inflammation and attendant neurodegeneration which produces escalating physical disability, cognitive impairment and falling quality of life (17, 22, 29, 166-168). These features deteriorate in a more insidious and relentless manner characteristic of the progressive phase which is seen to occur secondarily within 80% of the initially relapse-remitting patients after 20 years, and indeed primarily from onset in 15% of all patients(2). There is much to suggest these two phenotypic entities are 'sufficiently similar'(151, 169, 170) to be considered the same, as will be discussed later. Understanding of the actual pathology at work in multiple sclerosis has presented an increasingly complex disease model which continues to rapidly evolve (1, 46, 85, 171-176), it is beyond the scope of the current discussion and reviewed more thoroughly elsewhere (1, 46).

It is a striking observation and key motivator for this work that despite the fact the past two decades have seen the advent, evolution and deployment of increasingly impressive and effective immunomodulatory therapies for reducing relapse activity in Multiple Sclerosis(177), with 12 licensed interventions made available to date internationally, as clinicians we still have nothing of any proven benefit to offer patients with progressive disease(178).

When one considers the vast majority of disability, misery and economic cost arises from the progressive phase the paucity of clinical treatment trials in Progressive Multiple Sclerosis to date appears unjustly disproportionate(179).

Furthermore, given that 1) epidemiological natural history studies suggest the vast majority of patients will manifest progressive deterioration over time(12, 21, 22), 2) insights from neuroimaging and neuropathology suggest the cellular correlates and substrates of progression are present from the very earliest phases of the disease (180), 3) the incidence and prevalence of multiple sclerosis appear to be generally increasing (3, 4) and finally 4)it has not been incontrovertibly demonstrated that any of the currently licensed 'disease modifying therapies' actually have a truly meaningful or satisfying impact on the downstream later

progressive phase(178), accordingly there has never been greater urgency or demand for 'anti-progressive' therapies.

An accurate but overly-simple answer to the question of '*why is this situation the case?*' might be '*because an effective treatment has not been found'*. The more useful question is '*why is that the case?*' and the answer here is less intuitive, more subtle but nonetheless perhaps represents the single greatest challenge in contemporary Multiple Sclerosis research.

If we begin from the standpoint that Progressive Multiple Sclerosis in itself is not inherently incurable or untreatable because of some fundamental unassailable biological principle then we must accept that finding a solution will just be a matter of time, knowledge and thought – much as it was for developing agents for relapse control.

The most difficult hurdle to overcome in translating similar solutions to progressive disease from 'bench to bedside' after the point of inception will be demonstrating their benefit in clinical trials and herein lies the central problem with which we and many others(162, 181-197) in the field are profoundly concerned and likely accounts for much if not most of the delay in our arrival at a time of having effective anti-progressive therapies for Multiple Sclerosis.

Whilst in the setting of relapse control because such events are generally discrete, objectively quantifiable, almost directly disease-related and occur with a variable but anticipatory frequency, the accompanying study design and analysis with their use as a primary outcome measure is relatively straightforward and offers a relatively robust opportunity for interpretation. A significant reduction in relapse frequency in patients on a putative Disease Modifying Therapy compared to placebo can (with due caution) be readily taken as an ability to do exactly that(198-200).

Conversely the situation in evaluating ability to ameliorate progression is far more complex(201-203).

Even putting to one side the important consideration that the sustained progressive worsening of physical disability seen in MS is a joint consequence of inflammation with incomplete repair and distinct neurodegenerative loss of neuronal matter (1, 46) which are typically combined in a manner which confounds analysis of therapeutic effect and the more fundamental recognition that there is a gulf of individual variation in how pathological disease burden translates into functional disability consequent of numerous biopsychosocial factors(204, 205) – we are still left with the difficulty that arises from how progression is judged and disability is 'measured'(202, 203, 206).

Both the EMA and FDA require putative treatments to demonstrate a capacity for improving meaningful real-world clinical outcomes for patients with respective conditions (207-209). The international 'Gold Standard' rating scale used to grade

severity of Multiple Sclerosis, the Expanded Disability Status Scale (EDSS) developed by Kurtzke (1983) (210, 211) importantly features such outcomes with particular emphasis, including walking difficulty, requirement for mobility aid, immobility and death. Its ease of administration and interpretation by clinicians coupled with minimal logistical demands enabled a wealth of objective international data on the natural history of the disease (12-14, 17, 20-22, 148, 151, 212-215) and its phenotypes to be collected with longitudinal acquisitions over decades. Re-examining the unaltered natural history of the disease with newer albeit potentially superior rating scales would be unethical in the modern age of available therapeutics, as such the EDSS is unlikely to become unseated as the primary means of measuring disability and clinical outcome in studies despite a number of significant weaknesses which confer serious and often prohibitive limitations on clinical trial design (202, 213).

The EDSS itself is a 20 point ordinal scale which is derived from a mixed qualitative and quantitative composite grading of 8 ordinal observer-rated subscales reflecting dysfunction in 8 relevant Functional Subsystems of Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Cerebral, Sphincter and Ambulatory domains(210, 211). **It is displayed on the next page (table 3 & figure 1)**. Despite an intuitive scaling of landmarks familiar to clinical practice the steps of worsening disability are not of equal intervals, the time naturally spent at each step is generally not homogenous(22, 148, 213) and unfortunately the outcomes are not readily linearised by means of Rasch analysis or similar(203). The limited inter- and intrarater agreement achieved with the instrument has long been recognised (209) and attributed to inadequate definition of the multidimensional measurements of the functional systems and the means of their composite reduction (216). Experience from the UK Risk Sharing Scheme programme (constructed to assess cost-effectiveness of the original first line disease modifying therapies in the NHS) also demonstrated considerable general fluctuation of EDSS rating and neurological status of individuals within the typical timeframe of a clinical trial (217).

The implications of using this measure applied to trial design in the setting of Primary Progressive Multiple Sclerosis (the phenotype with a trajectory least confounded by the effects of relapse compared to other forms(169, 213) have been explored by investigators from extensive long term natural history cohort studies in both London, Ontario (151) and more recently South Wales (148) with similar findings.

Table 3 The Expanded Disability Severity Scale (210, 211)

0.0: Normal Neurological Exam
1.0: No disability, minimal signs on 1 FS
1.5: No disability, minimal signs on 2 of 7 FS
2.0: Minimal disability in 1 of 7 FS
2.5: Minimal disability in 2 FS
3.0: Moderate disability in 1 FS; or mild disability in 3 - 4 FS, though fully ambulatory
3.5: Fully ambulatory but with moderate disability in 1 FS and mild disability in 1 or 2 FS; or moderate disability in 2 FS; or mild disability in 5 FS
4.0: Fully ambulatory without aid, up and about 12hrs a day despite relatively severe disability. Able to walk without aid 500 meters
4.5: Fully ambulatory without aid, up and about much of day, able to work a full day, may otherwise have some limitations of full activity or require minimal assistance. Relatively severe disability. Able to walk without aid 300 meters
5.0: Ambulatory without aid for about 200 meters. Disability impairs full daily activities
5.5: Ambulatory for 100 meters, disability precludes full daily activities
6.0: Intermittent or unilateral constant assistance (cane, crutch or brace) required to walk 100 meters with or without resting
6.5: Constant bilateral support (cane, crutch or braces) required to walk 20 meters without resting
7.0: Unable to walk beyond 5 meters even with aid, essentially restricted to wheelchair, wheels self, transfers alone; active in wheelchair about 12 hours a day
7.5: Unable to take more than a few steps, restricted to wheelchair, may need aid to transfer; wheels self, but may require motorized chair for full day's activities
8.0: Essentially restricted to bed, chair, or wheelchair, but may be out of bed much of day; retains self care functions, generally effective use of arms
8.5: Essentially restricted to bed much of day, some effective use of arms, retains some self care functions
9.0: Helpless bed patient, can communicate and eat
9.5: Unable to communicate effectively or eat/swallow
10.0: Death due to MS

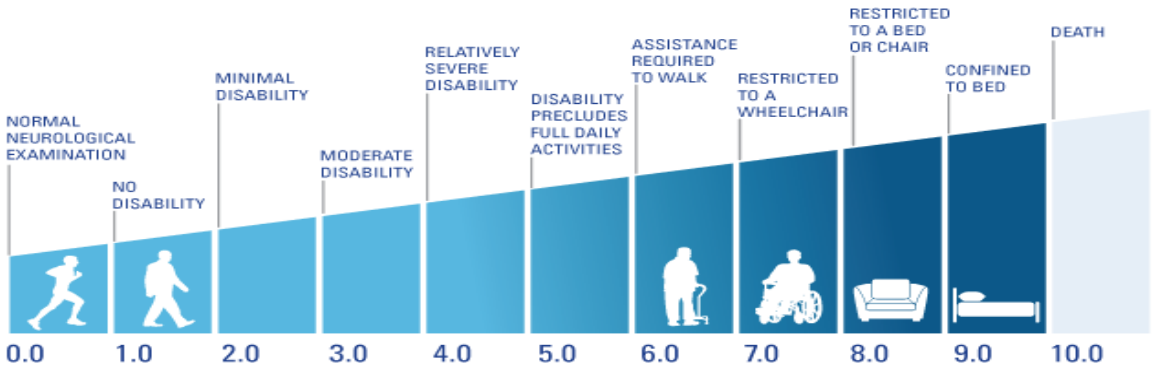


Figure 1 Clinical Milestones of The EDSS

From http://multiple-sclerosis-research.blogspot.com/2013/08/clinic-speak-what-is-edss.html Barts & The London MS Research Website.

With the EDSS as the primary outcome measure, given its limited sensitivity and the anticipated rate of decline of a recruited cohort and their intrinsic natural variability over time, a randomised control trial of an agent of at least modest efficacy requires several hundred patients over several (2-4) years to yield any reasonable power of detecting a statistically significant effect (148, 213).

Not only does this immediately confer substantial trial costs which will be finally transferred to treatment service providers in a sustained fashion but also potentially prohibitive logistical issues such as attaining sustained recruitment and selection of patients without confounding co-morbidities which are significantly more frequent in the typically older progressive patients (169).

Taking into account these issues, it becomes apparent that of all the many patients with Multiple Sclerosis, the actual pool of suitable candidates with the Primary Progressive phenotype (free of confounding factors and willing to enter a randomised trial for several years) is actually *small* in comparison to the much larger population of candidates able to participate in trials of anti-relapsing therapies and therefore represent a precious resource.

With growing saturation of the pharmaceutical market by a broad array of anti-inflammatory disease modifying therapies aimed at relapse suppression, which vary in mode of delivery, can be increasingly tailored to suit patients and generally appear to offer a reasonable balance between efficacy and risk (177), there is growing financial impetus within Industry to capture the progressive field. To date the approach of trialling drugs effective in relapse-suppression has not been matched by any significant amelioration of progression(179). From this it is clear that progression is more than just sustained inflammation and will require intervention targeting mechanisms of neurodegenerative decline. Whilst at the time of writing data is emerging suggestive of a 'delayed' benefit of immunosuppression being evident after the cessation of a standard length anti-progressive trial that had not been seen in the original primary outcome measures of sustained disability progression on EDSS (218), such effects are modest and do not counter the importance of neurodegenerative mechanisms highlighted by direct pathological (1, 46) and indirect neuroimaging studies(170, 176, 194, 195, 219-229) in the context of progression.

Therefore, in anticipation of more anti-progressive trials finally appearing on the horizon and a very finite pool of candidates being available, losing several hundred into each trial for half a decade before it becomes informative will be generally limiting.

Whilst for reasons discussed above the FDA/EMA demand evidence of objective benefit to real-world clinical outcomes in the setting of a large scale phase III trial and use of the EDSS would be required in this setting despite conferring the described limitations(209), the scale and duration of phase II could be reduced to

a more practicable level by use of a surrogate biomarker which not only associated well with the gold standard rating scales of disability but also demonstrated a capacity to predict a successful outcome at the phase III stage; a feature which does *not* automatically follow from manifesting a drug-related response *per se* (230). The recent example of the unsuccessful INFORMS study (Fingolimod in PPMS, announced AAN 2015 F. Lublin) is unfortunately testament to this, given the positive effects on progression-related biometric changes (brain atrophy) seen elsewhere (200) and similarly for the earlier and equally negative PROMISE study of Copaxone (231), an agent which is also believed to possibly attenuate cerebral volume loss (232).

Given the commitment involved in the conduct of a phase III trial in Progressive disease, every opportunity must be taken to ensure such endeavours are only conducted when there is the greatest antecedent probability of success.

IN SEARCH OF A USEFUL SURROGATE MEASURE OF DISEASE RELATED DISABILITY

A biomarker ('biological marker') was defined by the National Institutes of Health in 1998 as "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention*" (233, 234).

To date no biomarker has been found to reliably have these properties in the context of progressive MS to the degree required for acceptance by the regulatory authorities as an acceptable clinical trial outcome measure(159, 235-237).

However many putative candidate biomarkers are emerging and continue to be examined across a broad variety of modalities, including genetics (49, 51, 102, 103, 238, 239), haematology(236, 240), serology(241-243), neuroimaging(159, 223, 224, 244-250) and neurophysiology(183, 188, 190, 191, 251-254).

Given the major advances brought about by the advent of MRI both with respect to understanding and diagnosis of Multiple Sclerosis over the past three decades(186, 219, 226, 255-267), it is understandable that investigators have spent almost as long attempting to identify and validate useful biomarkers of disease activity and disability related to the condition (230, 263, 268).

Whilst use of more established and conventional MRI acquisition sequences of the kind used in routine clinical practice provided direct correlates of inflammatory relapse activity which then mirrored to a significant degree the reduction brought about by immunomodulatory therapy and were therefore useful outcome measures in phase II trials and predictive of success at phase III (230, 269, 270), none of the conventional cranial measures or even early composite combinations thereof have borne meaningful reflection of how patients are clinically between relapses or how they are likely to have progressed in the near to medium term (230).

The term ‘Clinico-Radiological Paradox’ was coined (245) to describe this situation, yet it is somewhat of a misnomer and the situation is in itself not so far removed from the very evident discrepancy between the clinical status of MS patients and their findings on Computed Tomography of the brain, itself the ‘gold standard’ of neuroimaging pre-MRI. With increasingly detailed histopathological-radiological cross-correlation it has become apparent that even within ‘Normal Appearing White Matter’ and ‘Normal Appearing Grey Matter’ as judged by conventional sequences there lay a significant burden of MS related pathology (82, 193). Newer sequences of MRI acquisition (193, 271, 272) have demonstrated some additional sensitivity to this damage revealing that which previously went unseen but their use to reflect disability and predict clinical outcome as trial biomarkers has not yet matched their apparent pathological sensitivity and specificity.

The past decade has additionally witnessed the widespread deployment of laboratory based proteomic techniques applied to a variety of body fluid and tissue types of patients with (and animal models of) multiple sclerosis (236, 241). This is with a view to identifying molecular signatures of the disease which may aid diagnostic specificity, improve pathological understanding and enable prognostication both with respect to the course of the disease and possible response to treatment enabling more guided management decisions (240, 241, 273, 274).

Unfortunately no molecular biomarkers are currently established for Multiple Sclerosis (236, 241). The presence of oligoclonal bands (immunoglobulins synthesised within the thecal sac) in cerebrospinal fluid with no matching presence in serum has for many years been a sensitive diagnostic aid (242, 275, 276) despite a lack of specificity for multiple sclerosis alone (2), being found in 90-98% of patients with the disease(2). They are taken to reflect sustained immunological activity within the CNS (242, 276). It is particularly noteworthy that with the exception of a single small case series of 6 patients treated with Nataluzimab, of whom 4 had undetectable OCBs at a second sampling after 8-14 months of therapy having initially been positive for them (273), no other therapeutic intervention currently available, including complete immune ablation (with subsequent autologous haemopoietic bone marrow transplantation) has profoundly attenuated their synthesis (277-279).

One could argue this perhaps indicates more about our available therapies (i.e. a lack of complete effect within the CNS) than the use of OCBs as a biomarker.

Indeed, a recently published subtype analysis of OCBs found in the CSF of patients who participated in the unsuccessful large placebo controlled study of Rituximab (OLYMPUS 2) in PPMS, demonstrated a significant capacity to distinguish those who manifested a positive response to immunotherapy as a consequence of having a higher inflammatory burden compared to the majority who did not (280). The specific presence of IgM OCBs appear to highlight PPMS patients with a more

substantial inflammatory component as supported by MRI markers and a tendency to more aggressive decline pre-randomisation (280). Such work was prompted by post-hoc identification of a small subset of patients within the OLYMPUS trial of Rituximab (281) and earlier trial of Glatiramer in PPMS (PROMiSe) who appeared to manifest a beneficial response (231).

Such a finding is consistent with observations of a shorter latency to second attack after the index presentation of a Clinically Isolated Syndrome in the 40% of patients on average with OCB IgM (282) and the greater than average burden of inflammatory indices on MRI and clinical relapse rates seen in this subset (283-285). Earlier arrival into the secondary progressive phase (283-285) with more rapid disability accrual and brain atrophy (286) on imaging are also associated with presence of IgM OCBS.

CSF OCB subtyping within a PPMS cohort may indeed allow categorisation and perhaps even exclusion of those with a more inflammatory component from entry into anti-degenerative trials but its subsequent use as a biomarker correlate of clinical status after entry into the progressive phase is questionable.

Other proteomic immunoglobulin candidates seen within the CSF and serum are auto-reactive antibodies to CNS constituents believed to possibly be the target of the initial immunological attack, with part of the of clinical heterogeneity of the disease explained by the spatial variation with which their respective epitopes are distributed(141, 287). Whilst such auto-antibodies including anti-MBP, anti-MOG, anti-PLP, anti-MAG, anti-HSP60, anti-HSP70, anti-CNP and anti-MOBP (243, 287) have offered insight into pathogenesis (141) and stimulated fruitful interference of antibody-epitope binding as a therapeutic route to limit and calm the cascade of immunological attack (288-290) – their biomarker utility in progressive disease is similarly unexplored and unproven.

Classic humoral agents such as cytokines, immunoglobulins, complement, prostaglandins, MHC constituents, apolipoprotein isoforms which mediate immunomodulation and the phagocytosis mediators annexin 1-5 and coronin-1a (159, 236, 240, 241) have all been examined and found to be probably relevant to the inflammatory facet of MS but nonetheless are relatively nonspecific and fluctuant. Focus on the differentially expressed proteins related to the neurodegenerative aspect of interest here has brought particular attention to neurofilaments(274, 291). The pattern of rising neurofilament levels in CSF with a corresponding fall in brain parenchymal volume is considered a hallmark of neurodegeneration(236, 291). A popular candidate is Glial Fibrillary Acid Protein, GFAP, a structural filament component of astrocytic glial cells considered to reflect their contribution to scarring within brain tissue induced by MS pathology (241). Changes in GFAP and other similar structural proteins are seen to be paralleled by changes in the expression of proteins with metabolic and enzymatic functions including the metalloproteinases, alpha-enolase, proteinase activator complex and calpain- (291). Such molecules are recognised to have significant

involvement in the neurodegeneration of other diseases, including Alzheimer's but their role in the setting of Multiple Sclerosis is unclear and again utility as a biomarker extending into the clinical trial setting far from established.

By 2014, various approaches to proteomic analysis had identified over 200 distinct protein molecules with expression differentially altered in the setting of multiple sclerosis or the murine model equivalent Experimental Allergic Encephalitis (EAE) which were detectable in brain, blood or cerebrospinal fluid (241). Whilst collectively their significance as biomarker candidates remains untested, there are important practical limitations which may also limit their deployment. As much as the routine acquisition of brain tissue itself would never be ethically permissible in the setting of a clinical trial, it is equally hard to envisage avid large scale recruitment to studies requiring repeated lumbar punctures even if 'ideal' putative markers could be identified and assays calibrated accordingly.

A 'blood test for multiple sclerosis' is a proposition as attractive as it has been elusive. Even after acknowledging the low fractional contribution many putative serological markers make to the total protein content of sera (<1%) and the technical challenges surrounding the required selective depletion of more abundant constituents one is still faced with uncertain and unreliable degrees of blood brain barrier permeability which is one of several factors contributing to the relative paucity of CNS molecules being detectable in blood (241). Despite some recent success with microRNA analysis (non-coding single stranded molecules which control gene expression and protein synthesis) being able to demonstrate some modest differentiation between phenotype and gross disability severity (292), the candidates appear mainly immune-related and exploration in the setting of PPMS is again outstanding.

A Timely Return to Neurophysiology for Answers

Prior to the advent of neuroimaging and its subsequent incorporation into modern diagnostic criteria(293), the ground-breaking work of Halliday, McDonald and Mushin (294, 295) in demonstrating increased latency of visual evoked potentials (VEP) in the setting of optic neuritis offered a valid technique for confirming second-site involvement in this condition.

The principle of demyelination causing slowed transmission and axonal loss leading to relatively reduced amplitudes of potentials evoked in a response time-locked to standardised stimuli (296) underpins the application of these techniques across a range of modalities.

This scientific rationale is directly supported by the established linear augmentation of saltatory axonal conduction velocity conferred by myelination observed *in vitro* and *in vivo* (297). The large scale perturbation and slowing of

peripheral nerve conduction in neuropathies similarly characterised by pathology of demyelination is also broadly familiar and frequently present with a diagnostic specificity(296).

Although human post-mortem studies (1, 46, 172, 266, 298, 299) have yielded invaluable insight into the pathological cascades producing MS the direct nature of their relationship to electrophysiological disturbance in such tissue is less well established. However, application of near identical evoked potential paradigms to animal models of certain MS components, namely variants of Experimental Allergic Encephalitis in mice, have unequivocally demonstrated the tripartite interaction between morphology, electrophysiology and function (300-302). Furthermore such models have elegantly and directly demonstrated the consequences of typical MS pathology on these properties and most recently, not only the neuro-preservant effects of some interventions (303, 304) but the directly reparative and remyelinating effects of others (301, 305). Putting acknowledged caveats regarding the generalizability of animal models to the singularly human condition(306) of MS temporarily aside (including the undeniable difference in morphological scale between human and murine neural pathways(307)) – the tight causal association between neurophysiology and clinical function seen with both disease and its positive response to intervention in the animal setting at the very least suggests promise for human investigations.

Although axonal loss is considered both a driver and determinant of disability in the progressive phase (1, 46, 85, 118, 173, 308) of Multiple Sclerosis comparatively less focus has fallen on evoked potential amplitudes which are effectively a function of synchronous induced neural activity and hence neuronal numbers(296, 309). The dominant interest in latency, as a function principally of myelination both for clinical and research purposes is not however inappropriate.

Myelination is a reasonable principle target of investigation given a) demyelination is the hallmark feature of the disease pathologically (1, 24, 46), b) the primacy of demyelination in leading to secondary axonal degeneration(85, 176), c) the appreciation that myelin contributes not only to conduction but also to invaluable trophic support of axons and their survival (310) and d) the amount, extent and pattern of myelination is exquisitely 'tuned' to provide optimal function (307). Far from being a binary, categorical or even ordinal property, where elicited EP latency offers a relatively continuous interval scaled system of quantification which offers classically accepted measurement attributes (311). A discontinuity arises for both amplitude and latency when no response is provoked (312). Such behaviour is not uncommonly encountered in neurophysiological practice in MS patients(313) and undeniably contributes to a ceiling effect but nonetheless does not preclude such findings from being properly informative.

This is supported by the use of a range of quantitative and semi-qualitative EP rating systems which have been applied to recordings from MS patients (181, 183, 184, 189, 314-316). The fine granularity of EP measurement is a major advantage

over traditional clinical rating systems wherein despite a steady rate of decline compatible with other neurodegenerative conditions (13-15, 148, 149, 151, 213, 214) individuals spend unequal periods at disability steps (148, 213) owing to very arbitrary placement of albeit clinically relevant milestones.

This latter factor not only confers a statistically under-powering effect of recruiting individuals at certain levels of disability (148, 213) (with a resultant bias against their participation) it also creates difficulty in attempting to validate candidate biomarkers with possibly superior measurement properties against ill-constructed and misapplied ordinal scales whose persistence as the regulatory 'gold standard' owes as much to simple familiarity as it does accepted tradition(199). Moving toward more useful systems of measurement using the EDSS as a foundation is therefore calibration through 'bootstrapping' and inherently less ideal in contrast to the more scientifically familiar definition of metrics and units with the highest attainable precision as their internationally standardised basis.

In judging the utility of neurophysiological techniques to biologically mark functionally relevant disease related decline in Multiple Sclerosis we should not therefore expect perfect correlation with accepted ordinal outcome ratings even though moderate correlation should at least be present.

We might also anticipate a similar pattern of dynamic changes over time between clinical and neurophysiological status; whether contemporaneously or with the latter antecedent to the former, particularly if there is a genuine causal relationship between the two.

A final reasonable expectation would be that interventions able to prevent, attenuate or even reverse neurophysiological decline should have a much greater than chance effect at ameliorating clinical decline or improving physical status also.

Several key issues could however affect the translation of a positive neurophysiological response at or before phase II into success at phase III using a clinical outcome accepted by regulatory bodies, which currently remains the EDSS (235).

A positive neurophysiological response simply may not be enough to produce a detectable clinical benefit; either because of the small magnitude of the former, the coarse measurement of the latter or lack of relation to the property measured on neurophysiology to physical disability itself. This pitfall is well highlighted by the recent failure of agents considered putatively neuroprotective on the basis of a manifest ability to generally attenuate MRI measured brain volume loss at phase II (269, 317) to translate into any semblance of positive impact on hard clinical disability outcomes in progressive MS at phase III (231, 318), and is readily understood in light of the recent volumetric work by Daams *et al*., (2015)(319).

Therein it is evident that corticospinal tract integrity accounts for much variance in EDSS and global brain atrophy measures, in cross-section simply do not (319). Therefore whilst both may deteriorate with disease, the former is causally associated with the clinical outcome in question whereas the latter is simply co-variant (albeit outwith the duration of a standard clinical trial nonetheless may have longer term predictive value (262)). Therefore any selection of EP metrics to act as surrogates of disability should focus on those modalities with a deterministic relationship. It is also notable that certain agents currently licensed for use in MS (e.g. Fampridine) exert their beneficial effects directly by improving conduction (320), which is evident on neurophysiology and yet without any attendant benefit on myelination or other proven disease modifying effect. Without clear pre-existing appreciation of such an agent's biological effect, on the basis of neurophysiology alone it could be misconstrued as having reparative action. Conversely it will be necessary to identify any directly pro-conductive effects in putative reparative therapies to similarly avoid misinterpretation of positive biomarker responses. The lack of pseudoatrophic responses during the initiation phases of some conventional disease modifying therapies and not others (321, 322) does suggest their impact on volumetric surrogates is not solely mediated by a simple homogeneous reduction of inflammation. This contemporary observation further highlights the need for caution when inferring meaning from biomarker outcomes and going forward underscores the need for great rigour in exploring the validity of neurophysiological surrogates and the effects of candidate therapeutics upon them.

The trophic support effect of myelin is also well recognised (310, 323-326) and may dominate in relative importance over conduction benefits *per se* and persist, enabling tract survival long after evoked responses are no longer reliably clinically detectable. In our own experience(314), although higher neurophysiological burden is unquestionably associated with greater physical disability, rarely some patients may nonetheless entirely lose recordable sensorimotor long tract EPs yet still manifest reasonable function and very slow decline.

That ultimate axonal loss perhaps bears a tighter cross-sectional association with disability than burden of demyelination (230, 245) *per se* is perhaps congruent with such observations and an important consideration. However the absence of significant axonal loss without pre-existing myelin injury (1, 176) as the index event reinforces the standing of EPs as both indicators of disease presence and ensuing downstream severity.

A further reflection is that to date exploration of combining different EP types into Multi-modal composite batteries has through various means of scoring and summation generally considered their contributions equally (162, 181, 183, 184, 188-191, 316). Given the variable eloquence and prognostic impact of identical MS pathology arising in different sites(151, 327) such composites will perhaps not offer their optimal real world ecological value unless their components are tuned, for instance by regression, against desired clinical outcomes.

Finally, there is the challenge conferred by the millimetre or smaller scale of pathological change(172, 266, 299, 328) and impressive sensitivity of neurophysiological techniques to it (329), which frequently exceeds the resolution of even contemporary imaging techniques and certainly clinical detection. As MS moves possibly into an era of simultaneously applied therapeutics used for the synergistic benefit of multiple actions – in a manner familiar to many other chronic diseases where real difference has been achieved, only the availability of such sensitive measures will enable accurate estimation of individual effect sizes. In isolation the magnitude of effect may be less than the standard and large-trial demanding 0.3 but nonetheless substantial when applied synergistically (*c.f.* combination therapy in cerebrovascular secondary prevention (330)). It is therefore a real concern that agents provoking neurophysiological improvements may be disregarded entirely from further consideration if they do not lead to accompanying clinical and radiological benefits as monotherapy, despite objective findings which point to promise as part of polytherapy.

The phenomenon of clinical deterioration in the absence of contemporaneous radiological alteration and *vice versa* is already familiar to the MS clinician (230, 245). Whilst neurophysiology may ultimately offer a route to understand such apparent structure-function dissociations (331-334), its cross-validation against such modalities is likely to generate similar challenges for contemplation.

The purpose of performing clinical disability ratings or biomarker quantification in the context of clinical investigation is to ascertain the impact of disease upon individuals, the effect of any candidate intervention on such a condition and the relationship of both disease and therapy to ultimate outcome (203). Discerning such truth with reasonable statistical confidence demands precision and both the recognition and minimization of error.

It is broadly appreciated that a range of concrete and abstract factors hugely modify the translation of disease related damage into functional impairment and this subsequently into disability. In the setting of Multiple Sclerosis body weight, age and physical fitness are examples of the former, with mood, motivation and placebo effects typifying the latter.

Various subjective biases from clinician and patient alike also couple with the natural fluctuation (216, 335, 336) of clinical status to increase error and reduce precision. Such issues coupled with ordinality and not-insignificant phenotypic heterogeneity demand a prohibitive scale of investigation when using the EDSS outcome system (148, 169, 213).

Although it is unlikely to be imminently supplanted as the instrument of investigation in pivotal phase III studies supporting regulatory approval, the use of evoked potential analysis at phase II could offer metrics relatively invulnerable to many if not all of these confounding factors and facilitate more feasible enquiry(314).

Most EP paradigms involve the extraction of a summed average waveform from background physiological noise following the presentation of several hundred stimuli (313, 329). Such an approach therefore offers an objective, minimally variant and highly confident estimation of mean latency and amplitude of the underlying neurophysiological process. The rapidity of these processes which occur on the millisecond scale makes their acquisition either in isolation or as part of a wider battery logistically very feasible, non-labour intensive and ultimately affordable. In our experience they are also non-invasive, easy to perform and very well tolerated (314, 329, 337).

The standard MS clinical trial duration, whether interventional or observational is typically in the order of years and rarely more than 24 months (321). Whilst evoked potential studies have demonstrated a relationship at baseline with the clinical change that ensues over such a period (181, 183, 188, 315) and a natural deterioration over such an interval themselves, if they are to be a useful instrument in short (12 month) and small scale (n<50) 'signal studies' of putative reparative therapies it will be essential to quantify the normal near and medium term variability of such metrics. This is particularly pertinent in a condition characterised for many by almost daily fluctuation (216, 335) in their real-world functional capacity.

Application of Evoked Potentials to MS

With respect to those EP techniques which have already gained widespread clinical application Visual, Brainstem, Somatosensory and Motor are the most established, with international consensus based standards of acquisition (309) and comparative normative data from healthy controls available to enable standardisation.

The original visual stimulus and recording paradigm of Halliday, McDonald and Mushin (294) has evolved considerably with the development of multi-focal recording techniques (338, 339) allowing finer localisation of abnormalities within the optic pathway and addition of electro-retinography has offered further insights and precision (340). The development of fine structural imaging with MRI and optical coherence tomography (OCT) in particular has given support to the localisation and interpretation of VEP findings and yet not significantly surpassed their relationship with objective ratings of ocular function (341) whilst also appearing less sensitive to MS damage than VEP measurement (342). A strong positive correlation between lesion size on MRI of the optic nerves and VEP latency has been observed in neuritis acutely (343, 344), with follow up over several years (344) and in the progressive phase. Congruent with its manifestation of axonal integrity VEP amplitudes are proportional to both cross-sectional optic nerve area on MRI and also Retinal Nerve Fibre Layer (RNFL) thickness on OCT (345).

Local experience in the Grey Walter Neurophysiology Unit found that of 273 patients referred on clinical suspicion of MS, 92.5% demonstrated characteristic abnormality in those with eventual clinically definite diagnoses (329). Typically 80% of patients will have an abnormality even without a history of optic neuritis(329), with this rising to over 90% in those who do (346). A particular pitfall relating to use of newer digital rather than older and more precise optomechanical checkerboard stimuli presentation is thought to underlie variability in estimates of abnormality prevalence in the wider literature (329) and is therefore relevant during consideration of trial design.

Although the broad transverse course of the optic radiation fibres suggest VEP may offer a useful index of intracranial demyelination burden, extrapolation to serving as a cognitive biomarker would not be wholly valid as any relationship would be inherently non-deterministic.

Interrogation of afferent responses to auditory stimuli has offered indicators of demyelination of the respective pathways at the level of the brainstem (309, 347, 348). Natural history imaging studies have demonstrated the adverse long term prognosis of brain stem damage which is indeed common (349), suggesting utility of incorporating Brainstem Auditory Evoked Potentials (BSAEP) into larger multimodality batteries. However, the correlation between BSAEP and Brainstem FSS score is only modest (r=.36 p=.0008) (350) likely consequent of exquisite pathway specificity and FSS ordinality. Nonetheless sensitivity to subclinical damage is substantial with BSAEP abnormality being evident in 40% of MS patients without clinically evident deficit (329). Examination of the efferent component of the auditory pathway by measurement of Transient Evoked Otoacoustic Emissions from medial olivo-cochlear bundle mediated hair cell tuning is a newer technique which although simple and sensitive to MS damage (351, 352) has enjoyed limited exploration to date(351-353).

Alternate brainstem modalities of facial sensation and vestibular function have been explored (again to a much lesser degree than BSAEP) with elicitation of Brainstem Trigeminal EP (BTEP) (347) and Vestibulo-Ocular, Vestibulo-Massetteric and Auditory-Massetteric responses respectively(332, 354). Their performance in detection of subclinical abnormalities and relationship to Brainstem FSS is similar to conventional BSAEP; however the combination of BSAEP and BTEP appears to offer a synergistic gain in sensitivity to brainstem functional abnormality and lesion burden (347).

Exploration of long tract functional integrity with evoked potentials has a much longer history dating back to the original demonstration of ulnar nerve stimulation somatosensory EP by time-locked cathode ray tube photography in 1947 by George Dawson(296) and the temporal intervals and morphology of peripherally elicited waveforms on route to the primary sensory cortex from all limbs have subsequently been well characterised (309). Importantly, meaningful linear relationships between SSEP abnormality and quantitative sensory

thresholds to vibratory and thermal stimuli in all limbs have been demonstrated (355-357). Such abnormalities are also evident in 80% of MS patients without referable signs or symptoms suggesting impressive subclinical sensitivity (329, 358). The established paradigm relies on induction and subsequent conduction within the predominantly large-fibre dorsal column pathway(309); although peripheral laser stimulation has yielded some specific insights as to spinothalamic integrity in MS patients(359), the residual tissue effects of the technique suggest further exploration at this time is likely to be limited.

The extensive anatomical course of the long tracts, which are typically over a metre in length, is considered a principle determinant of their eloquence from a neurophysiological perspective (182, 312, 360). Their marked sensitivity, superior to both clinical and radiological detection (182), stands in contrast to the poor spatial resolution of the technique. Nonetheless, and unsurprisingly, in keeping with the physical disability of MS being principally driven by myelopathy(186, 219, 226, 264, 314, 319, 361, 362) - at least as captured by the EDSS, the SSEP from lower limb stimulation bear the closest direct association with Global EDSS (357, 360).

Examination of the efferent long tract integrity by the alternate means of non-invasive cortical stimulation with Transcranial Magnetic Stimulation (TMS) to provoke downstream electromyographic responses (MEP – Motor Evoked Potential)(309) has generated parallel findings(363). Central Motor Conduction Latency correlates with voluntary phasic motor strength (364), limb motor function (365, 366) and general walking ability (367). As anticipated the burden of Central Conduction abnormality is also markedly greater in those patients with progressive disease compared those at the earlier relapse-remitting phase (368).

Application of TMS-MEP is not without two important caveats; firstly although broadly safe they are relatively contra-indicated in subjects with a liability to seizure disorder (369) – itself more common amongst MS patients (370). Secondly, the actual navigation of stimulus delivery by clinical means, although standard and logistically simple has generated concern about the differential elicitation of *direct* and *indirect* stimulation of the corticospinal fibres which in turn produces a variation in resultant MEP latency and ultimate interpretation (371). Stereotactic navigation systems of TMS delivery, co-registered with subject neuroimaging have overcome this challenge and offered insight into the aberrant motor unit recruitment patterns within the motor cortices of MS patients (372). However the current cost and limited availability of these systems is likely to prohibit their candidacy of becoming a widely employed translational biomarker. Even so, clinically navigated TMS MEP paradigms have offered not only meaningful quantification of corticospinal damage but repetitive stimulation with variation of interstimulus interval to elicit phenomena such as Intra-Cortical Inhibition and Intra-Cortical Facilitation, Cortical Silent Periods and particularly premotor facilitation mediated by cortical-cortical connectivity in the wider motor hierarchy have recently illustrated the relationship of damage therein to the previously

poorly understood pathophysiology of fatigue (373-375) which is ubiquitous amongst MS patients.

Application of Multi-Modality Evoked Potentials

In respect of the multi-domain impairments encountered with MS the collection of quantified output from various modalities of EP into composite MMEP (Multi-Modal Evoked Potential) batteries is intuitive.

Numerous studies in both relapsing and progressive phenotypes have demonstrated a significant correlation between MMEP burden and physical disability rated by EDSS and the Multiple Sclerosis Functional Composite (MSFC) (162, 181, 183, 188, 314).

Several methods of abnormality quantification have been described(314), initially offering a binary categorisation of normal vs. abnormal waveforms and yielding a quotient of the number of abnormalities from the number actually acquired (The PATH-Q)(191, 316).

A lower-resolution qualitative ordinal rating influenced by not only latency but also morphological characteristics including amplitude and asymmetry between contralateral recordings, termed the GEPS (Global Evoked Potential Score) (181, 376) was subsequently deployed offering 0-3 points for every EP undertaken. A similar but higher resolution 0-5 point scale termed the MEP (Multimodal Evoked Potential Score) (162, 183) was subsequently developed and included semi-quantitative rating of the EP based on its pathological prolongation against the established normal range(309) for the modality in question. Both techniques handled the discontinuity arising from absent response simply by awarding maximal points.

Fuhr's group (188-191, 315) have deployed a method of quantification focussed principally on the recorded latency of individual EP responses by offering summed Z scores from latencies in reference to published normative data. Alterations in morphology and amplitude are not included, this averts difficulties associated with inter-rater variability in making qualitative judgements of morphology and also the risk of erroneous interpretation of amplitude disparity between individuals due to non-disease related factors. Ultimately it offers a precise, unbiased and purely quantitative system, of fine granularity and standardised units. The problem of evaluating absent responses in this case however is met by the imperfect solution of using either the maximally recorded latency as a surrogate prior to normalisation or as we did by taking this value and simply adding 1 to offer Zmax+1(314). Nonetheless all such techniques have offered meaningful cross sectional and longitudinal findings and our own recent work demonstrates that the strength of relationship is perhaps largely independent of quantification scale selected (314). This feature of relative scale-independence

likely reflects genuine measurement of functional properties deterministically related to disability outcomes.

The positive association between EDSS and global EP burden on all scales is consistent amongst all MS phenotypes (181, 183, 191) but notably increases in strength with advancing disease severity (162).

A reasonable interpretation is that EP are sensitive to early damage, namely the index event of demyelination which is initially often subclinical (329) and masked by various forms of functional adaptation and a limited degree of repair. Over time such events have translated into downstream cascades of axonal loss and a growing disease burden has exhausted limits of adaptation and with such decompensation progressive accrual of disability arises (377).

Not only are recent imaging findings increasingly supportive of the view that initially non-eloquent lesions have profound longer term consequences (159, 378), but the relatively consistent finding of higher baseline EP abnormality burden predicting worse disability outcomes over short (183, 379)and longer term(188, 380) intervals is also congruent with such a model of 'delayed effect'.

Longitudinal follow up of 245 patients with initial diagnoses of CIS demonstrated a substantially increased risk of moderate disability developing in those with a significant burden of abnormality on VEP, SSEP and MEP compared to those without (331). Although conversion to CDMS risk was not related to EP burden in that investigation, this was found in a smaller cohort of 27 patients with solely SSEP and MEP considered, and to a degree greater than that predicted by MRI lesion burden in the same individuals at baseline (381).

Retrospective analysis of 94 subjects (380) with MMEP at baseline and followed up at 5 and 10 years subsequently demonstrated unequivocally increased risk and severity of disability progression in those with abnormalities on MEP and SSEP particularly. Giffroy et al. (382) have also recently published a 6 year retrospective analysis of a further 100 mixed-phenotype patients with impressively congruent findings demonstrating the independent adverse prognostic effect of a higher MMEP abnormality burden.

A smaller investigation of MEP in 15 RRMS patients has also highlighted the increased likelihood of worsening disability even over an interval as short as 6 months with a greater MEP abnormality burden (379). Increased risk of disability progression with greater MMEP burden has been observed in both RR and PMS phenotypes over the typical 1-2 year time periods of clinical trials in several investigations (184, 189, 383).

In the context of RRMS this was demonstrated by Schlaeger et al (2012) in 50 patients prospectively evaluated at 6 monthly intervals over 3 years. The baseline MMEP burden on VEP, SSEP and MEP correlated strongly (r>.7 p<.001) with final disability outcome (188). The strength of association observed therein with the

quantitative system of rating was greater than that observed in a study of 37 RRMS patients over a slightly shorter 2 year interval when graded by the qualitative ordinal scaling systems (r=.39 p<.02) (183). This may highlight a superiority of purely quantitative MMEP evaluation. This said, in our own direct comparison of such methods (314) they all performed well with only a trend to superiority in association with disability measures from the higher-resolution qualitative semi-quantitative MEPS system.

Although clinical and paradigm heterogeneity may also account for the discrepancy in the above longitudinal studies, that no significant association was evident on cross-sectional evaluation at baseline but nonetheless emerged over the course of the investigation is in further support of the 'delayed effect' model. Indeed in a large scale retrospective analysis of 143 patients with Clinically Definite MS with a relapse-remitting phenotype and less than moderate disability there was observed to be no association with MMEP below an EDSS of 1.5 and between 1.5 and 3.5 only a weak *rho* of .39 (p=.0114) (162). Within the same cohort a similar trend to increasing strength and significance of positive association was also observed between MMEP and temporal interval between MS onset and time of baseline evaluation (162). Furthermore regression based modelling using such data has begun to offer impressively accurate probability estimation for risk of disability progression over several years from the early stages of clinical dysfunction and may even offer a way to prospectively identify those with so-termed 'Benign MS' who would presumptively benefit from avoidance of the risks associated with current disease modifying therapies (162).

The longest interval study of baseline combined EP abnormalities (VEP and MEP in this case) and ultimate disability outcome is from 20 year follow up data from 28 initially relapsing patients published by Schlaeger *et* al.,(188). Association between MMEP and EDSS at 20 years was strong (r=.72 p<.0001) and following inclusion into a regression model provided predictive ability unsurpassed by either consideration of the baseline clinical status or inflammatory indices on baseline MR imaging (188). Such modelling conducted on prospectively collected data in a cohort of 22 patients with the purely progressive phenotype of PPMS over a 3 year period suggested an ability to predict final EDSS from baseline VEP, SSEP and MEP with 92% accuracy (191) and most intriguingly the dynamics of EP change preceded EDSS declines by an average of 6 months, again congruent with the model outlined above.

The large scale natural history studies of disability progression in MS (12, 13, 148, 151, 384, 385) with anticipated variation are nonetheless remarkably consistent in the estimation of median time to salient clinical milestones. Importantly such averages are accompanied by an exceptionally broad range of clinical trajectories unfamiliar to most other neurodegenerative contexts. With both natural history and MMEP studies also supporting the adverse prognostic outcome particularly of myelopathic damage (189, 384-386), with the former evidently being foreshadowed by the latter (229) and with the challenge to date of determining

disease duration with any reasonable accuracy, it is perhaps not unreasonable to consider using MMEP to provide some form of disease staging, as so effectively applied in oncological and other medical disciplines featuring disease processes manifesting disseminated biological attack. The particular utility in translational research may in fact be to enrich recruitment for only those individuals likely to manifest confirmed disability progression within the forthcoming trial period – or alternately select for those of early chrono-biological stage to assess for the genuinely prophylactic effects of agents intended to prevent the index event of demyelination. Such an approach may offer a route to ultimately reduce trial size by favourably altering anticipated progression probabilities and partially disentangle the overlapping phenomena of inflammation and neurodegeneration in patient groups, which are not sufficiently segregated by the purely clinical criteria currently employed (169).

To date there have already been several historical and contemporary translational investigations using MMEP as outcome measures of intervention effect in the setting of MS, in addition to their aforementioned and increasingly accepted use in pre-clinical testing in animal models of the condition.

It is now 3 decades since the utilisation of MMEP (featuring VEP, BSAEP and upper limb SSEP) in the double blind placebo-controlled Azathioprine and Methylprednisolone Study involving participation of 101 patients over 3 years (387). In the investigation deterioration in VEP and SSEP were reported to parallel and precede clinical decline by an average of 1 year in the Chronic Progressive cohort (387). The EP outcomes in even earlier studies of the effects of plasmapheresis were less conclusive but nonetheless were again seen to mirror clinical trajectory (388, 389). In subsequent studies of methylprednisolone treatment for acute relapse improvements in clinical status have been accompanied by positive changes in global EP scores and such findings extend to benefit within individual components, with MEP CMCT showing responses which match clinical motor resolution (371, 390).

Contemporary disease modifying therapies including Interferon (391) and Nataluzimab (392) have been associated with beneficial effects on EP outcomes and positive effects have also been observed in phase I investigation of mesenchymal stem cells in the setting of PMS (337). Such a finding has prompted the selection of MMEP as the primary outcome measure in the subsequent phase II investigation of the technique in that context (393). Although such pioneering use remains unaccompanied amongst the increasingly numerous PMS studies currently underway (179), it is likely to represent the start of a growing trend particularly if successful and a relationship to improvements in physical disability is evident alongside a degree of neurophysiological rescue.

For the reasons discussed above, this is clearly not guaranteed. As the first successful pro-remyelination clinical trial (Anti-Lingo1, Biogen 2015 (74)) demonstrated- it is possible to successfully induce seemingly beneficial change in

VEP over placebo but this may not be accompanied by contemporaneous benefits to structural metrics on OCT or more importantly objective ocular function. Similarly longitudinal observational studies of VEP over 3 and 5 years (65, 394-396) following neuritis have shown spontaneous improvement to be not-uncommon and yet after the familiar clinical interval of 3-6 months such changes are not accompanied by ocular functional improvements. Electrophysiological improvements are considered a consequence of natural partial remyelination (68) and ion channel reorganisation in demyelinated regions (251).

Therefore, on one hand EP clearly represent a technique enabling detection of improvement and by inference some degree of often very subtle repair, and thereby stand in contrast to numerous other metrics and outcomes in use which focus on retarding decline or tissue loss. On the other hand it remains uncertain how much EP benefit (for a given modality) is required to translate into a meaningful clinical outcome and given the discussion of delayed effects how long it may subsequently take to become apparent. It is not unreasonable to presume that any anti-progressive benefits of partial remyelination or similar may only be readily evident clinically several years post-intervention, in much the same way that the benefit of DMT on Disability Progression in the UK NHS Risk Sharing Scheme only became grossly evident after 5-6 years(218), having been not overtly apparent at earlier intervals (217).

Therefore again interpreting EP benefits, as with any finding, should be done with caution particularly if serving as a basis to inform subsequent phase III studies with clinical outcomes.

Robust power calculations to inform study design using EP outcomes are outstanding and will clearly vary according to quantification technique, composite battery, cohort demographics and clinical phenotype. Given the growing pace of investigation underway and planned in all groups featuring accepted clinical and radiological outcomes it would be both extremely useful and logistically readily achievable to incorporate EP batteries alongside such instruments. This would offer cross-validation with other modalities and enable rapid acquisition of normative data for EP behaviour within this disease.

Larger more extensive cohorts would also further illuminate the dynamics of EP change with increasing pathological burden. Is accrual of neurophysiological decline generally linear over time? or 'front-loaded' being greatest after index insults as is the case seen with structural metrics such as spinal cord atrophy (196, 226) and Retinal Nerve Fibre Layer thinning following acute myelitis and neuritis respectively (397).

On the basis of the above discussion it is reasonable to infer a solid conceptual basis supports the consideration of EPs as a candidate biomarker of disease-related disability. Reflection on work by other groups thus far also suggests that although there is consensus on recording techniques, and a body of

normative data exists for comparison, a single accepted standard of MMEP composite generation and abnormality quantification remains outstanding.

Several published methods have yielded interesting findings with each having various advantages and disadvantages. These are largely with respect to their implementation and handling of censored data which arises from the discontinuous nature of progressively worsening EP recordings which become unrecordable after a variable degree of increased latency (182) Direct comparisons of the output of the various methods in the same cohort with the same phenotype are scarce.

On proceeding to the next step of biomarker instrument evaluation it would be appropriate to perform a comparison of the various MMEP scoring techniques in an observational cross-sectional study to assess their individual correlations with disability and thereafter their performance relative to conventional metrics from modern neuroimaging.

These goals are the principle focus of chapters 3 and 4 respectively.

The necessary exploration of longitudinal behaviour of MMEP in relation to physical disability and implications for future translational enquiry is then explored in chapter 5.

II

GENERAL METHODS

This section outlines a general overview of the various methods employed in the lines of enquiry explored in these works; more specific details are expanded upon where necessary within their respective individual sections.

The work features a general examination of the association between electrophysiological and clinical indices of various aspects of the disability accrued in Multiple Sclerosis; one section features comparison to Magnetic Resonance Imaging derived metrics and details of that acquisition and processing are described wholly within that section. All clinical and para-clinical evaluations were conducted on site at North Bristol NHS Trust, initially at the Frenchay Hospital site and then subsequently at the new Southmead Hospital after the transition in April 2014. All paper records have been stored confidentially within the BrAMS service as per the requirements accompanying ethics approval and digital recordings have similarly been maintained in keeping with the regulations of the Data Protection Act in the neurophysiology and radiology departments of North Bristol NHS Trust.

Details of specific electroencephalographic acquisition and post-processing are similarly included within the respective sections.

i. **Ethics and Research** approval was attained in line with contemporaneous regulatory requirements prior to the conduct of each investigation and the Research & Innovation Department of North Bristol NHS Trust served as sponsor of these works.

ii. **Funding** was supported from the Bristol & Avon Multiple Sclerosis charitable fund, part of Southmead Hospital Charity. The work on Multimodal Evoked Potentials and Progressive Multiple Sclerosis was also supported by a small charitable RD6 grant from North Bristol NHS Trust. The subsequent EEG based studies were partly supported by funds from the experimental psychology department of the University of Bristol. The author's role as a research registrar at the BrAMS unit was in part funded by a grant from Novartis pharmaceuticals to support the conduct of industrial clinical therapeutic trials.

iii. **Recruitment** for all studies was undertaken prospectively from the BrAMS service of patients attending the unit as part of their routine clinical care who additionally met the required participation criteria. All patients were under the care of attending consultant neurologists based at the North Bristol NHS Trust and their clinical care was unaffected by participation in any of the described works. Consent was attained in line with the requirements outlined by contemporaneous Good Clinical Practice standards and studies were open to monitoring oversight throughout their conduct. All retrospective data analysis was conducted with necessary regulatory permissions in the requisite manner of being irreversibly anonymised.

iv. **No harm** came to any participating individuals in the conduct of these works.

v. **Clinical Metrics of MS Related Disability**

a) **Physical disability** of participants was evaluated by joint means of both the ordinal Expanded Disability Severity Scale (EDSS) (210, 211) which is the most widely accepted clinical outcome measure used in the MS field(198, 199, 202, 203) and the Multiple Sclerosis Functional Composite (MSFC)(398) which similarly has wide deployment in translational endeavour and offers a scaled z-based aggregate rating of physical impairment.

b) The EDSS rating is derived from a structured clinical evaluation of several different functional subsystems (FSS) of vision, brainstem, cerebellar, somatosensory, sphincteric, pyramidal and cognitive function in addition to ambulatory capacity. The rating of each FSS is a further ordinal rating based on a combination of features present at that time identified either by direct specialist neurological examination (for which the rater had received formal accreditation via the *neurostatus* organisation) or volunteered on historical enquiry. The ambulatory capacity is determined by a directly observed attempt to walk (with or without aid) a distance of up to 500m if possible. The fusion of such subsystem scores into a final composite metric follows a staged algorithm based on the number of impaired FSS and the severity of disability therein. The cognitive FSS component of the EDSS features only a question capturing the subject's own perspective and do not feature a quantitative examination. Fatigue and affect are featured within the cognitive FSS rating. A copy of the EDSS rating system used in these investigations is included within the appendix.

c) The MSFC is a purely objective rating system featuring evaluation of performance on three tasks employed to interrogate sensorimotor function of the upper and lower limbs and information processing speed and working memory impairments in the cognitive domain. The three tests administered

by a trained neurologist were the Timed 25 Foot Walk (T25FW), the 9-Hole Peg Test (9HPT) and the Paced Auditory Serial Addition Test (PASAT). Examples of testing forms are also included in the appendix.

In The T25FW individuals must ambulate as quickly as possible without rest and minimal aid a distance of 25 feet. They have two attempts, with an average time (in seconds) providing the result taken.

The 9HPT requires individuals to sequentially place and subsequently remove a series of pegs into a testing board as quickly and accurately as possible. Each upper limb, the dominant and non-dominant is tested twice, again with average time (in seconds) for each taken as the result.

The PASAT test is described below, for generating the MSFC scores only the 3-second interval form was used. Alternate testing forms were deployed on consecutive visits during the longitudinal study.

In line with the MSFC administration guidelines the performance on each component test was converted to a *z* score based on the published normative data(399), to yield a final composite value for disability;

$$MSFC = \frac{\left(\frac{\frac{1}{9HPT} - 0.00439}{0.0101}\right) - \left(\frac{t25fw - 9.5353}{11.4058}\right) + \left(\frac{PASAT3 - 45.0311}{12.0771}\right)}{3}$$

d) **Cognitive Disability** was interrogated by means of a neuropsychometric battery described as the Minimal Assessment of Cognitive Functional Impairment in Multiple Sclerosis (MACFIMS)(400) (table 4). This features a collection of several tests which are outlined in the accompanying table; three of its constituent tests (the SDMT, CVLT2 and BVMTR) also collectively form the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS)(401, 402). Specialist training in test administration was provided by resident Clinical Psychologists of the BrAMS centre (Dr. Laura Hanley and Dr. Vera Fixter) and Dr. Margaret Newson of the Remember Dementia Research Group based at North Bristol NHS Trust.

e) Normative data for all were attained from a published reference group(400) and estimations of premorbid intelligence were based both on the demographic-occupation method described by Crawford *et al.*,(403, 404) to yield Estimated IQ (Est. IQ) and by use of the validated Test of Premorbid Functioning (TOPF) instrument from Weschler(405) to attain a value for Full Scale IQ (FSIQ). The Demographic-Occupation formulation offers an estimate of intelligence which accounts for more than half of the observed variance on the more extensive Weschler Adult Intelligence Scale derivation

of Full Scale Intelligence Quotient (403, 404) and both approaches were considered to offer a satisfactory estimation for the purposes of these initial enquiries.

For Years of Education (YOE) this is quantified as 1 for each year of full time study and 0.5 for part time commitments. Occupation is graded on a five point scale based on appreciation of the most cognitively challenging role they are known to have undertaken. Professionals were awarded 1 point, Non-professional cognitively demanding roles 2 points, skilled occupations (requiring considerable training) 3 points, semi-skilled workers 4 points and unskilled labourers 5 points with decisions made with reference to examples in the original literature(403).

$$Estimated\ IQ = 87.14 + (-5.21 * Occupation) + (1.78 * YOE) + (.18 * Age\ )$$

Conversely the Full Scale IQ estimate derived from use of the TOPF utilises this formulation:

$$FSIQ = \sum 29.991 + (2.09426 * TOPF) - (0.0404559 * TOPF^2) + (0.000340705 * TOPF^3) + (1.4617126 * YOE) + (4.925 * Gender)$$

Wherein for gender, female is taken as 1 and male as 2. An exemplar of the TOPF testing sheet is included within the appendix.

e) **Subjective ratings** of key symptomatic domains relevant to MS related inquiry were attained by the use of validated patient reported outcome instruments, for the domains of perceived cognitive impairment, fatigue severity, sleep disturbance and affective difficulties. The nature of these questionnaire based tools is outlined in table 5. They were administered at the time of clinical evaluation.

vi. **Electrophysiological Evaluations** featured three distinct approaches, firstly Multimodal Evoked Potentials (MMEP) which comprised visual, somatosensory, transcranial motor and brainstem auditory evoked potentials were conducted according to IFCN guidelines(313) utilising a Medelec Synergy device and a Magstim TMS device utilising single induction coil targeted by clinical navigation. These were performed at the Grey Walter Neurophysiology Department of North Bristol NHS Trust by trained specialist neurophysiology practitioners. Stimulus and recording characteristics are outlined in detail in table 6.

Recording positions for all evoked potentials and the cognitive event related potentials and resting state electroencephalography which followed were all in accordance with the international ten-twenty system for scalp based recording(406). Details of the EEG and cognitive potential acquisition methods are described in the respective chapters, these recordings were performed at the Bristol Brain Centre using an R40 Lifelines EEG recorder coupled with a programmable Arduino tone producer and Sony Headphones.

vii. **Blinding.** Herein to minimize influence of confirmation bias findings from clinical ratings, neurophysiological signal abstraction and neuroimaging processing were attained by investigators who were deliberately kept blinded to outcomes in different domains. This applied both to the study of multimodality evoked potentials and the evaluation of cognitive evoked potentials with EEG.

Vii. **Statistical Analysis** – all statistical analysis in these works was performed by the author utilising the IBM SPSS package, in sequential versions 21-25 inclusive. Where a normal distribution of any given variable was not apparent bootstrapping with 1000 iterations was applied unless otherwise stated. Oversight of statistical methods employed within the first four lines of enquiry was afforded by an independent statistician (Dr. Paul White) from the University of the West the England.

Table 4 Neuropsychometric Instruments Applied M=Component of MACFIMS, B=Component of BICAMS, T=minutes taken

Cognitive Domain	Psychometric Test		Nature	M	B	T
Auditory Processing Speed & Working Memory	**PASAT**	Paced Auditory Serial Addition Test (399)	Patients listen to a series of 60 numbers between 1-9 issued from a CD player at three second intervals for 3 minutes. They are asked to verbally provide the sum of the last two numbers which they have heard (not a running total). A trial of ten numbers at 3 second intervals is provided as a practice run. The test is then repeated in the same manner providing only 2 second intervals between numbers. It is internationally utilized in MS research as sensitive gauge of working memory capacity. It provides a numeric raw score (0-60) for both the 3 second and 2 second forms; alternating use of Form A and B were used during alternating visits where necessary in the studies described herein.	*		6
Visual Processing Speed & Working Memory	**SDMT**	Symbol Digit Modalities Test (401)	Patients are provided with a legend of nine symbols which represent the numbers 1-9. They are provided with a sheet with a series of the symbols and asked to verbally report which number should go in the adjacent associated empty box provided. The legend is visible throughout the test. After an untimed trial run of ten symbols they then have 90 seconds to 'decode' as many as possible. For maximum patient comfort and minimum burden (and to overcome challenges of ataxic-paresis of the dominant hand) only verbal report was used herein. This is a trial of working memory and executive function (problem solving). Output is the raw number of correctly decoded symbols in the given interval; this can be subsequently scaled.	*	*	5
Auditory/Verbal Memory	**CVLT-2**	California Verbal Learning Test 2nd Edition (407)	In this trial patients are provided with a list of 16 words presented at 1 second intervals. After each presentation of the list they are asked to freely recall as many as possible in their own time. The same list is used for five successive trials. Subsequently a single trial of a different list is used. After a delay of several minutes (up to 25) they are asked to freely recall the original list without cue. This is a test of verbal memory. Output is the number of correctly recalled items in each instance and this is subsequently scalable.	*	*	10+5
Visuospatial Memory	**BVMT-R**	Brief Visual Memory Test – Revised (407)	In this test patients are shown a single A4 page featuring six different line figures. They are shown the diagrams for ten seconds at a distance of 45cm and then asked to draw from memory what they saw in their own time. Points are awarded for accuracy of both shape and location. This process is repeated for two further trials with the same figures so they have the chance to learn them. A delayed free recall is tested at 25 minutes after the last presentation of the drawings. It is a validated test of visual memory and provides a scalable numeric raw score of cumulatively correct submissions.	*	*	10+5
Language	**COWAT**	Controlled Oral Word Association Test(400)	Patients are asked to provide series of words belonging to specific categories or possessing particular features, such as beginning with a certain letter but not being nouns for example. They are given 1 minute to give as many examples by verbal report as possible. Three trials with different letters are typically used (herein the letters F,A and S were used and the subsequent category of 'animals'). This is a commonly used screen for cognitive fluency and executive function. Output was a cumulative raw score of the different correct words offered over the given time intervals; this is similarly scalable subsequently.	*		6
Spatial Processing	**JLO**	Judgement of Line Orientation (400)	Patients are shown pairs of lines at various orientations to one another. The image is a small post-card size picture presented at approximately 45cm from the patient. They are asked to give verbal report as to how they would rate the relative angular positions of each pair of lines during 30 trials after a series of practice exposures. This measures visuospatial processing and provides a raw numeric score which can then be subsequently scaled. Form V was employed in the studies herein.	*		6
Higher Executive Function	**DKEFS Sorting**	Delis-Kaplan Executive Sorting Task(400)	Patients are provided with groups of 8 cards carrying labels and features which can belong to differing categories. They are asked to sort out the small pile of cards into different sets (e.g. those labelled with fruits or of a particular colour or shape). There are multiple potentially employed sorting rules for each of two sets of cards; subjects are also presented with different groupings and asked to identify the categorisation system if possible. It is a measure of executive function and provides a numerical raw score from the cumulatively correct judgements made by the patients; this is similarly subsequently scaled.	*		15-20
Estimating Premorbid IQ	**TOPF**	Test of Premorbid Function (405)	Patients are presented with 70 irregular words in English and asked to pronounce them verbally; correct responses depend on pre-exposure to the words and are taken as an accepted indicator both of education and premorbid intelligence given the relative resistance of literacy to MS related cognitive impairment. The raw output is used in the calculation of an estimated Full Scale IQ along with demographic variables for gender, age and years of education.			2

Table 5 Subjective Rating Instruments Utilised

Symptom Domain	Rating Scale	Full Name	Nature	Time Taken (mins)
Affective Disturbance	**DASS**	**Depression, Anxiety and Stress Scale(408, 409)**	Likert based questionnaire featuring 21 items rated on scales of 0-3 cumulatively added in a structured manner to provide ratings of the overlapping constructs of depression, anxiety and stress; scoring features cut-off thresholds for gauging symptom severity.	5-10
Fatigue	**MFIS (MFIS-5)**	**Modified Fatigue Impact Scale(410)**	A 21 Item questionnaire with subjects rating level of symptom severity across domains of physical, cognitive and psychosocial fatigue. A more concise 5 item version provides a more rapidly attainable combined index	5-10
Fatigue	**NFI-MS**	**Neurological Fatigue Index – Multiple Sclerosis(411)**	A 23-item Likert based questionnaire converting level-of-agreement into numeric scores to gauge severity of four constructs (cognitive, physical, sleep related and total fatigue related to MS). Output converted to a scaled score to yield Rasch-adjusted near-interval ratio scores.	5-10
Cognition	**PDQ (PDQ-5)**	**Perceived Cognitive Deficits Scale(410)**	A 20 item questionnaire wherein patients grade severity of difficulties on scales of 0-5 to provide ratings on constructs of planning, memory and attention, which are also combined to yield a total score. A more concise 5 item version provides a more rapidly attainable combined index	5-10
Sleep Disturbance	**ESS**	**Epworth Sleepiness Scale(412)**	An 8 item questionnaire wherein patients are asked to grade (0-3) likelihood of falling asleep in different real-world situations; used for sensitivity to excessive daytime somnolence.	5-10

Table 6 Multimodal Evoked Potential Battery Acquisition Characteristics

	Visual Evoked Potentials (VEP)	Brainstem Auditory Evoked Potentials (BSAEP)	Somatosensory Potentials – Upper Limbs (SSEP-UL)	Somatosensory Potentials – Lower Limbs (SSEP –LL)	Motor Evoked Potentials – Upper Limbs (MEP-UL)	Motor Evoked Potentials – Lower Limbs (MEP-LL)
Low Frequency Filter (LFF)	1Hz	100Hz	20Hz	20Hz	10Hz	10Hz
High Frequency Filter (HFF)	100Hz	3kHz	3KHz	3kHz	3kHz	3kHz
Time Base	250msec	10msec	50msec	100msec	50msec	100msec
Stimulation Rate	2Hz	10Hz	3Hz	3Hz	Singlets	Singlets
Number of Sweeps Averaged to form waveforms	100	2000	500	1000	20	20
Stimulation	Checkerboard (30 degree of retinal field), 2Hz flicker, 1 degree of arc check size, white brightness of 150cdm^{-2}, contrast 87.5%	Monaural Stimulation via earphones with rarefaction click stimuli of 0.1ms duration and intensity of 75 dB above subjective hearing threshold. The contralateral ear was masked with white noise.	Supramaximal stimulation applied to Median Nerve at Wrist; 200msec square-wave pulse	Supramaximal stimulation applied to Posterior Tibial Nerve at Ankle; 200msec square-wave pulse	9-cm circular Magstim Coil over vertex, 140% threshold stimulation	9-cm circular Magstim Coil over vertex, 140% threshold stimulation
Recording Points	N75-P100 recorded at Oz (Fz reference)	Wave components I,III & V recorded between Mastoids of each side and Cz with the ground placed frontally.	N9 at Erb's Point, N13-20 at c5 Spinous Process, N20-p37 over scalp; CP3 and CP4 points are 2cm posterior to C3 and C4 respectively, thus contralateral SSEP recorded at CP4-Fz for left upper limb and CP3-Fz for right upper limb	LP at T12 spinal process, N34 at the C5 Spinous Process and P37-N45 over scalp from same positions as for Upper Limb SSEP. *ULN taken as 25.7ms x height in meters as per (183)	Surface EMG recordings made over Abductor pollicis brevis; Central Motor Conduction Time =1/2(M+F+1); F as minimum F wave latency.	Surface EMG Abductor hallucis in the foot. Lower limb CMCT upper limit of normal taken as: =(0.08ms/cm * height)+3.7ms As per Claus et al.(413),

III

Multimodal Neurophysiological Evaluation of Primary Progressive Multiple Sclerosis – An Increasingly Valid Biomarker, With Limits

Background The promising utility of Multi-modality Evoked Potential Batteries to objectively measure multi-tract dysfunction has been evaluated by several groups using different methods.

Objective To independently evaluate the use of Multi-modality evoked potential batteries as surrogate biomarkers for both physical and cognitive status in a cohort of Primary Progressive Multiple Sclerosis patients and identify the most potentially useful scoring method of those described.

Methods 28 patients with Primary Progressive Multiple Sclerosis underwent clinical evaluation with Kurtzke's Modified EDSS and the Multiple Sclerosis Functional Composite (MSFC). 19 participants also underwent the Minimal Assessment of Cognitive Function in Multiple Sclerosis. Visual, Brainstem Auditory, Somatosensory and Motor Evoked Potentials were recorded on all. Results were graded by variants of the Global Evoked Potential Score, Multiple Evoked Potential Score and Summation of Z transformed Evoked Potential Latencies for correlation against the clinical scores.

Result & Conclusions Multi-modal evoked potential batteries generally show moderate and useful correlation with clinical status as measured by the regulatory standard of EDSS (r_s= .65 vs. mEPS $p<0.005$) and MSFC (r_s= .39 vs. mEPS $p<0.05$). The graded qualitative mEPS scoring system displayed the strongest relationship although the influence of scoring system applied appeared reassuringly minimal. Non-association with cognitive impairment is an important limitation however.

Plain Language Summary All of the difficulties patients with Multiple Sclerosis experience ultimately arise from either a failure to generate or to transmit electro-chemical signals within their brains or spinal cord. Attack of the insulating material around the long neural pathways in these structures by the individual's own immune system produces a slowing and ultimate loss of transmission which can be directly quantified by non-invasive recording from the skin overlying where such signals would normally ultimately arrive. We explored the relationship between physical disability experienced by patients and the cumulative amount of transmission abnormality within the pathways which support their vision, hearing and both sensation and movement of all the limbs. The recruited patient group had displayed apparent relentless progression of physical disability from onset and irrespective of how one quantified the aforementioned amount of abnormality in signal transmission it was directly related to the overall severity of their mobility difficulties. Importantly the scale of thinking and memory dysfunction the patients displayed was not related to problems with conduction on the pathways tested suggesting the need to pursue other ways to find a biological measure of such problems.

Team & Contributions

Dr Luke Canham	Data Collection, Clinical Ratings & Analysis
Dr David Cottrell	Project Design, Funding, Ethics & Recruitment
Dr Nick Kane & Dr Stuart Butler	Project Design, Conduct of TMS-MEPs (Dr Kane)
Mrs Jenny Homewood	Study Administration
Dr Paul White	Statistical Oversight
Dr K Inglis, Dr H Faulkner & Dr J Witherick	Clinical Ratings
Dr Agyepong Oware	Conduct of TMS-MEPs
Mr Peter Walsh & Miss Kelly Blake	Conduct of SSEP, VEP and BSAEP
Ms Charlotte Furse-Roberts, Ms Amy Lewis	Psychometric Testing

I N T R O D U C T I O N

The progressive aspect of Multiple Sclerosis remains disproportionately underrepresented in current treatment trials and completely so in the widening therapeutic arsenal of disease modification.

Given the limitations of conventional clinical markers in this setting, we were motivated to evaluate multi-modal evoked potentials (**MMEP**) as a candidate biomarker within a Primary Progressive (PPMS) cohort to hopefully facilitate smaller scale and more practicable research. Those patients who exhibit a primary progressive course offer perhaps the most fertile ground for clinical trials of anti-degenerative therapies. Their trajectory is largely un-confounded by the inflammatory relapses seen in the larger secondary progressive (SPMS) population. Furthermore aside from some subtle phenotypic differences (169, 414) PPMS is pathologically (1, 176), genetically(50), radiologically and serologically (169) currently indistinguishable from SPMS. That after adjustment for age and clinical landmarks the natural histories of PPMS and SPMS are also near identical (148) underscores the utility of investigation within this group.

Kurtzke's Modified Expanded Disease Severity Scale (**EDSS**)(210) remains the primary clinical outcome measure accepted by the European Medicines Authority and the Food and Drug Administration for use in clinical trials despite proving somewhat prohibitive to research in progressive disease(169). Even acknowledging the inherent methodological problems of using a multi-domain non-linear ordinal scale (202), its use in therapeutic trials in the context of progressive disease requires recruitment of hundreds of patients over several years to yield any reasonable statistical power and sensitivity for agents that may only partially slow the rate of progression (148, 213). Its utility is further undermined if recruited cohorts fail to behave in a manner anticipated from the natural history studies (12, 151, 214, 215). The EDSS remains the only clinical rating scale to have been utilised in longitudinal natural history studies (148, 151) spanning two decades or more of disease. Future such natural history studies are now practically and ethically impossible with the advent of multiple potentially disease modifying agents and clinical trials now available to most patients with MS.

A future objective biomarker of disability for use in clinical trials therefore ideally needs to reliably demonstrate some meaningful correlation with the EDSS. Despite the widespread criticism of this scale without such correlation the validity of a candidate biomarker over the long term evolution of this disease would be questionable.

Whilst the Multiple Sclerosis Functional Composite (MSFC) has been developed to address some concerns it is nonetheless a functional measure and not without limits (415). There is also growing appreciation that from the outset of disease

increasing amounts of functional adaptation are mustered within the CNS in the face of a growing burden of damage and the near relentless stereotypical slide of clinical progression occurs when the limit of 'functional adaptation' is exhausted (377). This masking effect is an important consideration in use of clinical indices as proxy measures for neuronal preservation and clinical measures are further confounded by behavioural issues of motivation, mood and placebo.

Importantly, the EDSS and MSFC are also relatively insensitive to the burden of cognitive impairment which affects 60-70% of MS patients (401, 416) and contributes equally with mobility difficulties to employment loss (29, 417) and reduced quality of life (418).

EVOKED POTENTIALS have long provided para-clinical diagnostic support for MS. That detection of clinically unsuspected lesions is approximately 30% in all major modalities - Visual (**VEP**), Brainstem (**BSAEP**), Sensory (**SSEP)** and Motor (**Motor EP**) in patients with confirmed disease (419) is a testament to their high sensitivity. Measures of MMEP abnormality have also demonstrated impressive ability to predict extent of progression within cohorts of PPMS (191) and RRMS/SPMS (162, 181, 183, 184, 315, 380) and even evolution from Clinically Isolated Syndrome to Clinically Definite MS (331).

Although EPs are finding a role as outcome measures in clinical trials of conventional disease modifying therapy (392), experimental neuro-restorative approaches (337) and neuroprotectants (420) it remains far from clear which is the most sensitive and longitudinally useful multi-modality combination or which means of abnormality quantification is superior - hence the purpose of this independent validation study.

Several methods have been used successfully, taking various combinations of VEP, BSAEP, Motor EP and SSEP and either deriving 'Global Evoked Potential Scores' (**GEPS**) (181, 184) or alternately 'Multimodal Evoked Potential Scores' (**mEPS**) (162, 183) by examiners scoring individual potentials qualitatively against 4-6 point graded scales evaluating asymmetric morphologies, amplitudes, latencies and absent responses and then summating the results for each patient. Other investigators have either counted the number of pathological responses (316) or produced a quotient of this number relative to the total number of recordings (**PATH-Q**), or gone further still and produced a quotient of the summation of *z*-transformed latencies for all recorded potentials, combined from all modalities available (**s-EPL-Q**) (191).

We compared the performance of several described methods for quantifying abnormalities within MMEP recordings which were prospectively acquired in a cohort of primary progressive patients. We explored their correlation with conventional clinical measures of EDSS and MSFC. We examined the association of MMEP results with objective cognitive impairments measured by disease-specific tools.

METHOD

Following the award of ethics approval by the NHS Frenchay Research Ethics Committee in the United Kingdom (ref. 06/Q2007/7) we attained informed consent for recruitment from 28 patients with established diagnoses of Primary Progressive Multiple Sclerosis concordant with the revised MacDonald criteria (8, 9) from our local population. Patients with significant co-morbidity were excluded. At commencement the mean duration of disease (estimated from first noticed symptom) was 10.98 years (range 1.15-30.47) and the mean EDSS 5.76 (3.5-8.0). Patients had a mean age of 51.92 years (range 34.85-70.01) and the gender composition was balanced with 14 females and 14 males. The study was a descriptive exploratory hypothesis generating study and for our sample size of n = 28 had 80% power for detecting absolute correlation of 0.5 or higher using contemporary levels of significance, alpha = 0.05, in a two-sided test.

All patients provided informed consent and were invited to undergo an MMEP battery; comprising bilateral VEP, bilateral BSAEP and SSEP of all four limbs. Where no contraindication to Transcranial Magnetic Stimulation existed Motor EPs were recorded from all four limbs also.

Acquisition followed the Guidelines of the International Federation of Clinical Neurophysiology (309) with the protocol as described by Rice et al. (41).

Upper limits of normal (ULN) were taken at 2.5 standard deviations from the mean and normal values were derived from published international (309) and local departmental normal values (for VEPs) .

For VEP N75-P100 wave presence, amplitude, morphology and P100 peak latency were considered. BSAEP interval latencies for components I, III and V were recorded in addition to the SSEP N13 and N20 intervals for upper limb and central components up to P37 for lower limb. The ULN for lower limb SSEP latency was adjusted for patient height according to the formula 25.7 ms × height in meters (183).

Motor EPs were attained using a 9-cm circular MAGSTIM coil held over the vertex. The central motor conduction time (CMCT) was calculated by subtracting ½(M + F + 1) from the motor EP latency, where M is the distal motor latency and F is the minimum F wave latency. The ULN for Lower limb CMCT was adjusted for height as described in Jung *et al.* (183).

MMEP Scoring Methods

Two neurophysiologists independently performed qualitative scoring of each summated evoked potential waveform recorded on each patient according to the 4-point graded ordinal scale used by Leocani et al., (181), wherein normal=0,

conduction delay=1, increased latency with morphological abnormality of a major component=2 and absence of a major waveform component is awarded 3 points (181, 184). These were summated to yield a Global Evoked Potential Score (**GEPS**). To accommodate for patients in whom TMS was contraindicated a quotient of the GEPS divided by the number of potentials attained, the **GEPS-Q** was derived. We also evaluated the multimodal battery scores excluding the upper limb SSEP as per Invernizzi et al. (184) but utilised height adjusted values for the ULN of lower limb CMCT and produced a quotient (**GEPS-2-Q**) based on the number of sampled potentials.

We also derived Multimodal EP Scores (**mEPS**) based on the 6-point ordinal graded scale described by Jung et al. (183). Here each summated potential is scored as 0 if normal, 1 if there is pathological asymmetry of latency, 2 if latency falls between ULN and 1.1x ULN or a >50% side difference of amplitude, 3 if latency falls 1.1-1.3x ULN, 4 if latency is >1.3xULN and 5 if there is an absent EP component (162, 183). As per Jung et al., if both latency and amplitude were abnormal, the highest scoring feature was considered. We did not include BSAEP or the ipsilateral silent period latency from Motor EP production in the analysis as we had not recorded the latter and both were felt to be reasonable to omit given the minimal contribution to final statistical analysis by the original authors (183). Margaritella et al's., (162) group had used the same scoring regimen alternatively applied to a combination of 30' VEP, 15' VEP, BAER , and SSEP from the upper and lower limbs. Although we did not perform 15' VEP we otherwise used their approach to generate another variant multimodal EP Score (the **mEPS-2**). Quotients of the mEPS scores were provided to allow for Motor EP contraindications (**mEPS-Q**); this was not required for Margaritella et al's method. A linearly scaled interval measure of evoked potential abnormality burden was also provided by summation of *Z*-transformed evoked potential latencies in the manner initially described by Schlaeger et al (191). Latencies of VEP P100, SSEP UL N13-N20, SSEP LL P40, CMCT UL and CMCT LL provided *Z* values after normalisation against international and departmental normal values from healthy controls with height adjustments made for lower limb sensorimotor potentials as detailed above. A quotient, the **S-EPL-Q**, was provided by dividing the sum total *Z* score by the number of potentials recorded per patient.

Also, as described by Schlaeger (191) a **Path-Q** score was derived by dividing the actual number of abnormal potentials by the total number recorded. We also produced a novel **B-Score** by taking the square root of the sum of all the individual *Z* scores squared for all potentials recorded. Akin to the approaches taken by Schlaeger et al.,(191) and the MSFC developers (421) in the event of no latency value being available due to absent evoked potential response the maximum *Z* value for our cohort was used, plus one, to partially allow for extra qualitative information inferred by a response more severe than even the most marked prolongation. An overview of the MMEP scoring techniques used is displayed in table 7.

Table 7 Multi-Modality Evoked Potential Scoring Systems

Qualitative Methods

GLOBAL EVOKED POTENTIAL SCORE GEPS		MULTIMODAL EVOKED POTENTIAL SCORE mEPS	
Normal	0	*Normal*	0
Conduction Delay	1	Pathological Assymetry	1
Increased Latency with Morphological abnormality of a major component	2	Latency between ULN to 1.1xULN *or* >50% difference in bilateral amplitudes	2
Absence of a component	3	Latency 1.1x to 1.3x ULN	3
		Absent Component	4
			5
$GEPS - Q = \frac{\Sigma(GEPS\ for\ all\ modalities)}{Number\ of\ EPs\ performed}$		$mEPS - Q = \frac{\Sigma(mEPS\ of\ VEP, SSEP\ UL\ \&\ LL, Motor\ EP\ UL\&LL)}{Number\ of\ EPs\ Performed}$	
$GEPS - 2 = \Sigma\ GEP\ for\ each\ modality$ **but** $excluding\ SSEP\ of\ Upper\ Limbs$		$mEPS - 2 = \Sigma\ (mEPS\ of\ VEP, BSAEP\ and\ SSEP\ of\ UL\ \&\ LL)$	

Quantitative Methods

Pathological Quotient	Summed EP Latency Z Quotient Score	BrAMS Score
$PATH - Q = \frac{Number\ of\ Abnormal\ EPs}{Number\ of\ EPs\ recorded}$	$S - EPL - Q = \frac{\Sigma(Z\ Transformed\ EP\ Latencies)}{Number\ of\ EPs\ performed}$	$B\ Score = \sqrt{\Sigma(Z\ transformed\ EP\ Latencies)^2}$

Several techniques have been described and herein employed to grade disease-related features of electrophysiological dysfunction within the eloquent tracts functionally interrogated by multi-modal evoked potential (**MMEP**) batteries. These can be categorised into those which are ordinal qualitative scales, namely the lower resolution 4 point Global Evoked Potential Score (**GEPS**) top-left and the higher resolution 6 point Multimodal Evoked Potential Score (**mEPS**) top-right. Quantitative scales were also employed. The Pathological Quotient (**PATH-Q)** bottom-left is a simple quotient of the number of abnormal potentials in the battery divided by actual number performed. Z-score transformation of waveform latencies using published international and departmental norms enabled derivation of a quotient of the summed Z score for all recorded modalities divided the number recorded to yield the Summed EP Latency Quotient (**S-EPL-Q**) (bottom-middle) and a root sum of such Z transformed latencies squared provided a locally established BrAMS Score (**B-Score**) (bottom-right). As variation in the use of the GEPS and MEPS has been published featuring modified batteries these were similarly examined as the GEPS-2 and MEPS-2 respectively. Bilateral Visual (**VEP**) and Brainstem Auditory (**BSAEP**) evoked potentials were recorded in addition to four limb somatosensory (**SSEP**) and TMS-induced Motor evoked potentials (**Motor EP**).

Participants had EDSS and MSFC assessments conducted by a neurologist directly after MMEP evaluation. *Z* scores for the MSFC and its components were derived from appropriate published normative data (422). A subset of 19 patients gave further consent to undergo a full MACFIMS (Minimal Assessment of Cognitive Function in MS) (416) battery. Assessments comprised the Symbol Digit Modalities Test (**SDMT**), Controlled Oral Word Association Test (**COWAT**), Judgement of Line Orientation (**JLO**), California Verbal Learning Test (**CVLT2**) total and delayed, Brief Visual Memory Test- Revised (**BVMT-R**), Paced Auditory Serial Addition Tests (**PASAT 2** second, **PASAT 3** second) and the Delis Kaplan Executive Function Sorting Tests (**DKEFS**). Assessments were conducted by trained psychologists. Raw scores were converted to *Z* values and scaled T scores after regression-based normalisation against age, education and premorbid intelligence as outlined by Parmenter et al., (400). The Beck Depression Inventory (BDI) and Depression, Anxiety and Stress Scale (DASS) were performed to assess for any significant confounding affective disturbance. The values for each test, the summated total (using both Z and T scores) for the MACFIMS battery and the similarly summated total for the BICAMS (Brief International Cognitive Assessment in MS (401) elements (**BVMT-R, CVLT-2 and SDMT)** were also correlated with results from a contemporaneous MMEP assessment taken for subsequent comparison.

Statistical Approach

Spearman's rank correlation coefficient (*r*) is a well-established index for the strength of the monotone association between two ranked variables and is known to be robust to skewness, the presence of outliers and is appropriate for ordinal data where relationships might not necessarily be linear. This measure of correlation has been used throughout this study. 95% confidence intervals have been derived based on 2000 bootstrap samples so as to otherwise avoid any reliance upon normality or large sample asymptotic theory. All calculations have been performed in SPSS v 21. The Null Hypothesis in each case was of no correlation between tested parameters and the critical value of alpha was set at $p=0.05$ for statistical significance and rejection of H_0. A full correlation matrix between clinical variables and MMEP scores is featured in table 1.

RESULTS

We observed no statistically significant relationship between age at testing, age at onset or estimated duration with clinical severity measured by EDSS. Performance on MSFC was negatively correlated with both age at testing and duration of disease *(r* =-.458 p= 0.014 and *r* =-.380 p=0.046 respectively). All scoring methods, with exception of the mEPS-2 yielded significant positive correlations with both age at testing and age of onset *(r* between -0.380 to -0.513, $p<0.05$) but no correlation with duration was evident. See Table 8 and accompanying figure 2 on next pages.

Table 8 Correlation Matrix of Evoked Potential Scores vs. Clinical Parameters & Cognitive Scores

All values Spearman's *r*

MULTI-MODAL EVOKED POTENTIAL ABNORMALITY SCORE

	GEPS-Q	GEPS-2-Q	mEPS-Q	mEPS-2	s-EPL-Q	PATH-Q	B-Score
Age	-0.429*	-0.421*	-0.513***	-0.324*	-0.425*	-0.411*	-0.426*
Age of Onset	-0.513***	-0.406*	-0.496***	-0.24	-0.47**	-0.38*	-0.411*
Duration	0.153	0.03	0.011	0.005	0.105	-0.082	0.025
DISABILITY							(n=28)
EDSS	0.513***	0.534***	0.514***	0.647***	0.544***	0.399*	0.492***
MSFC	0.217	0.367*	0.375*	0.39*	0.319*	0.341*	0.343*
Ambulation	-0.526***	-0.49***	-0.511***	-0.689***	-0.572***	-0.395*	-0.524***
MSFC ***subcomponents***							(n=28)
25FW	-0.521***	-0.482***	-0.5***	-0.694***	-0.6***	-0.37*	-0.601***
9HPT	-0.227	-0.075	-0.073	-0.374*	-0.308	0.045	-0.326*
PASAT 3'	0.018	0.149	0.137	0.091	0.057	0.192	0.058
							(n=28)
MACFIMS ***(BICAMS in Dark Grey)***							
CVLT2 Total	-0.507*	-0.424*	-0.305	-0.306	-0.204	-0.304	-0.133
SDMT	-0.36	-0.365	-0.297	-0.245	-0.296	-0.28	-0.177
BVMT-R total learn	-0.281	-0.352	-0.1	-0.123	0.053	-0.218	0.007
COWAT	-0.279	-0.155	-0.277	-0.138	-0.221	-0.275	0.053
JLO	-0.294	-0.288	-0.271	0.202	-0.105	-0.314	-0.088
CVLT2 delayed recall	-0.515*	-0.527*	-0.239	-0.288	-0.084	-0.308	-0.218
PASAT 3'	0.031	0.126	0.059	0.082	0.13	-0.014	0.104
PASAT 2'	0.08	0.1	0.056	0.184	0.153	0.058	0.33
DKEFS category sorting	-0.296	-0.221	-0.255	-0.266	-0.151	-0.323	-0.359
DKEFS sort description	0.097	-0.057	0.013	-0.03	0.193	-0.094	-0.202
BVMT-R delayed recall	-0.215	-0.22	0.102	0.067	0.151	-0.04	0.156
MACFIMS sum Z	**-0.415***	**-0.374**	**-0.248**	**-0.217**	**-0.081**	**-0.333**	**-0.093**
BICAMS sum Z	**-0.469***	**-0.463***	**-0.262**	**-0.249**	**-0.135**	**-0.318**	**-0.132**

(Cohort Size n=28, Cognitive subset n=19)

* P<0.05

** p<0.01

*** p <0.005

Correlation Matrix of Evoked Potential Scores vs. Clinical Parameters & Cognitive Scores

Disability was measured according to the EDSS – Expanded Disability Status Scale and MSFC- Multiple Sclerosis Functional Composite (featuring 9 Hole Peg Test 9HPT, 3 second Paced Auditory Serial Addition Test PASAT 3' and 25 foot walk, 25FW). Ambulation was graded in metres. The Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) comprised the California Verbal Learning Test 2nd edition (CVLT-2), Symbol Digit Modalities Test (SDMT) and Brief Visual Memory Test – Revised Edition (BVMT-R), Controlled Oral Word Association Test (COWAT), Judgement of Line Orientation test (JLO), 2 and 3 second interval Paced Auditory Serial Addition Tests (PASAT 2' and 3' respectively) and Delis-Kaplan Functional Executive Sorting tests comprising trials of categorical sorting ability and abstraction of categorical sort description (DKEFS sorting and description respectively). The BVMT-R and CVLT-2 feature fixed interval delayed recall elements of visual and verbal memory which are not a part of the newer Brief International Cognitive Assessment in Multiple Sclerosis (BICAMS) – which comprises only the initial learning trials of the BVMT-R and CVLT-2 in addition to the SDMT; these are highlighted in darker grey in table 2. **MMEP demonstrate consistently significant correlation with standard measures of physical disability (EDSS and MSFC). This appears due to a shared weighting toward determinants of mobility and was largely independent of the actual MMEP abnormality scoring system employed, however the higher resolution qualitative scale of the mEPS (Multimodal EP Score) displayed the strongest correlation with disability in this cohort. The evident lack of consistent association between MMEP and severity of cognitive impairment highlights an important limitation of an otherwise promising disability biomarker candidate.**

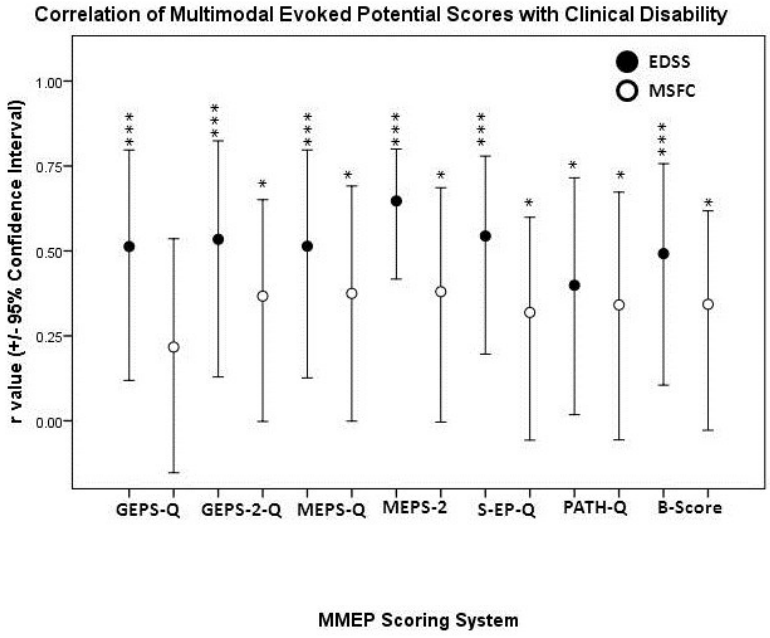


Figure 2 Correlation (Spearman's r) of Evoked Potential Scores with EDSS and MSFC

Several methods of evaluating and grading the abnormalities elicited from multimodality evoked potentials were compared for their ability to reflect disability measured by the standard Expanded Disability Severity Scale (**EDSS**) and Multiple Sclerosis Functional Composite (**MSFC**). Both primarily quantitative and qualitative scales were employed. All displayed similar and significant associations with clinical disability. In this cohort the 6-point Multimodal Evoked Potential Scale (**mEPS**) scale described by Jung *et al.,* (36) which employs a combination of quantitative and qualitative components performed marginally superiorly.

P <0.05 = *

P<0.01 = **

P<0.005 = ***

i. MMEP Scores Correlate well with EDSS & MSFC

All EP scoring methods displayed significant correlations against EDSS *(r* = 0.399 to 0.647, all p<0.05). The PATH-Q demonstrated the weakest association and the mEPS-2 method emerged strongest. A similar but weaker pattern of significant association was evident with the summated MSFC scores. The ability of the EP raw data to be either qualitatively or quantitatively graded and then composited into single scores by various means and still yield meaningful correlations with established clinical measures is striking (see figure 1 below).

ii. Correlation is weighted toward Indices of Mobility

Very much like the EDSS itself, the association with EP scores appeared heavily weighted toward indices of mobility such as ambulatory capacity and walking speed. This was also evident with MSFC which is a less walking-weighted scale and its lesser association than EDSS with MMEP is likely attributable to this. Indeed, only the 25 foot walk element of the MSFC was seen to show any meaningful relationship with all the EP scores. The mEPS-2 and B-Score were weakly correlated with upper limb function in the 9-Hole Peg Test Performance which otherwise with the PASAT 3' scores bore no statistically significant correlation with any EP scoring technique.

iii. MMEPs do not capture Cognitive Impairment

In our cohort of 19 patients, 3 (16%) displayed no abnormality in any domain of fluency, processing speed, verbal memory, visual memory or visuospatial orientation. 8 (42%) had mono-domain impairments and 8 (42%) had significant embarrassment of >1 domain, defined by performance below 1.5 standard deviations from the mean with identical outcomes for both Z based and regression-based normalisation. The prevalence is broadly in keeping with wider literature and the marked predominance of difficulties in processing speed, visual memory and verbal recall was also highly typical of MS generally(423).

However, only performance on the CVLT-2 component of the MACFIMS exhibited any association with MMEP abnormality and then only as graded by the GEPS system. The extensive remainder of the typical MS related 'cognitive footprint' evident in our cohort was generally not reflected in their MMEP scores by any method of quantification. There was no difference in the pattern of association between Z-score and regression-based T score normalised cognitive scores. It is not clear if the association of summed BICAMS and MACFIMS z-scores or T-score with GEPS in itself is meaningful or independently goes beyond a general and already established correlation with covariant clinical disease severity.

DISCUSSION

We have sought to further evaluate the potential utility of MMEPs as a biomarker of disease-related functional status in the context of PPMS. By performing an independent cross-sectional comparative analysis of several previously described methods for gauging neurophysiological dysfunction within a cohort of PPMS patients we have demonstrated that in contrast to conventional MRI measures (184, 188, 189) almost no matter how such MMEP data is analysed there remains a useful correlation with the widely accepted, validated and recognised regulatory standard of clinical EDSS in these patients. The influence of MMEP scoring method chosen appears reassuringly small.

As to whether or not such MMEP batteries can reliably show objective deterioration over time in progressive MS patients remains to be seen although recent work is promising (191). Future longitudinal studies in cohorts of such patients are required to assess both the sensitivity and specificity of changes in MMEP scores. Such studies will determine whether MMEP scores may in future allow for smaller and more achievable proof-of-concept clinical trials than the current regulatory accepted outcome measure of EDSS. Longitudinal investigation will also identify the inherent variability typically seen with markers of functional integrity and the investigative timescales required to demonstrate definitive disease-related change.

Whilst it is acknowledged that a poor correlate in cross sectional analysis may nonetheless have better longitudinal sensitivity we feel that of those tested the higher resolution qualitative **mEPS** scoring system offers possibly the strongest correlation with currently accepted measures of clinical functional status in MS and certainly warrants further evaluation.

Although the mEPS-2 grading in particular makes no use of Motor EP data and therefore in turn may offer the largest sustained recruitment within clinical trials, obviating a potential need to exclude those in whom TMS is or becomes contraindicated, the Motor EP data may in itself provide useful and well correlated findings when examined (183, 315). Furthermore, whilst certain modalities do not necessarily contribute much to the overall correlation with our clinical scales they do nonetheless provide an informative objective measure of additional pathway damage or integrity. Whilst the use of quotients also seems appropriate for values of summed *Z*-EP latencies and numbers of pathological potentials recorded, it is not clear that this is an appropriate way of combining qualitatively graded scores from those who have had full batteries including Motor EPs and those who have not into single composites, although it similarly prevents exclusion of patients with non-MS related visual, auditory or somatosensory impairments. Nonetheless we have demonstrated here that such derived scores still exhibit highly significant degrees of correlation with key clinical parameters when quotients are applied.

MMEP batteries display great promise for evaluating dysfunction secondary to demyelination and axonal loss at a 'population' level within white matter tracts. It is very encouraging that all the various MMEP scoring systems described to date and tested herein provided composite scores which significantly correlated to varying degrees with the clinical scores from widely employed disability measures. Much of this marked association is likely a consequence of both approaches being disproportionately weighted towards determinants and indices of mobility. Indeed correlation with non-motor domains on objective measures generally fared less well.

The small scale of voluntary participant engagement with the MACFIMS psychometric bundle within this study, likely as a consequence of the prolonged testing time required (~90 minutes) and perceived potential for psychological discomfort, is an acknowledged limitation of our ability to draw meaningful inference from the resultant dataset. However we have recognised a limited association of MMEP scores with objective cognitive impairments, both within those undertaking the MACFIMS but also the larger number who undertook the PASAT for the MSFC. This particular limitation of an otherwise strong biomarker candidate parallels a similar criticism levelled at the EDSS itself. Such a 'cognitive gap' is nonetheless perhaps surmountable. Other neurophysiological techniques including the cognitive event related potentials of Mismatch Negativity(253) and in particular the P3a and P3b subcomponents of the P300 have already demonstrated impressive association with MS-related cognitive impairments (424), are readily practicable and would likely sit well within an expanded MMEP paradigm.

The logistical considerations are also an important determinant affecting wide scale deployment of any biomarker candidate. Actual fiscal details will vary by region and centre but generally evoked potentials are already widely available and less technologically demanding than neuroimaging. However given the absence of contemporaneous neuroimaging which has an admittedly greater degree of histopathological specificity than EP measures in the setting of Multiple Sclerosis, it is difficult to deduce the key disease related changes which underlie EP deterioration. Given the centrality of MRI to clinical Multiple Sclerosis research, a cross-sectional comparative investigation featuring both MMEP and contemporary MRI modalities in a similar PPMS paradigm would be appropriate in the next instance.

CONCLUSION

Through inherent sensitivity to overt and subclinical disease neurophysiological approaches hold great promise in enabling future MS researchers to see a clearer picture less clouded by functional adaptation and clinical variation. Head-to-head comparison with neuroimaging modalities and longitudinal evaluation is warranted in addition to pursuit of a designated cognitive biomarker which could complement the impressive ability of MMEP to serve as a biomarker of physical disability in PPMS.

IV

Cross-Modality Integration May Be Most Informative In Progressive Multiple Sclerosis

Objective A biomarker of physical disability in Progressive Multiple Sclerosis is needed. We explored the utility of combining information from established techniques of neuroimaging and multimodality evoked potential analysis into single composite measures and their relationship with disability.

Method 27 patients with PPMS were rated by EDSS and MSFC. Each underwent a battery of multimodal evoked potentials comprising bilateral Visual, Brainstem, Somatosensory and TMS-evoked Motor of all limbs. Electrophysiological abnormalities were quantified into global composites. 3T MR imaging of the brain and spinal cord yielded T1, T2 and FLAIR sequences. Volumetric analysis was performed in addition to generation of the MDSS2 imaging composite. Metrics from both modalities were included in a novel preliminary cross-modality composite, the Biomarker Disability Severity Scale (BDSS).

Results MMEP scores demonstrated moderate/strong correlation with EDSS (r=.748 p<.005) and MSFC (r=-.57 p<.005). On imaging only lower cervical cord volume displayed a significant relationship with physical disability (r=-.634 p<.01 for EDSS and r=.71 p<.005 for MSFC) and the relationship with MDSS2 seen here was modest r=-.279 p>.05 for EDSS and r=.564 p<.05 for MSFC) Combination of MDSS2 components with MMEP parameters collectively into the novel BDSS yielded the most superior patterns of association with disability (r=.808 p<.005 for EDSS and r=-.58 p<.05 for MSFC). Regression analysis suggested most disability is determined by loss of spinal functional integrity and future composites could be weighted accordingly.

Conclusions Cross-modality composites may better capture determinants of clinical disability required for regulatory body approval and offer useful biomarker outcomes measures.

Plain Language Summary Scanning of the brain and spinal cord has revealed evidence of the disseminated immune attack which characterises Multiple Sclerosis in high detail and has an important place in the routine clinical care of patients. We sought to compare how closely measures of brain and spinal cord structure from such imaging relate to the severity of physical disability in a group of patients who had exhibited a purely progressive worsening of clinical function since onset and then further examine how such measures fared against a similar comparison of abnormal signal transmission along the main pathways previously tested. We identified that not only was it more challenging to acquire all the necessary information from scanning in such patients due to a range of technical factors but that the relationship to clinical disability was stronger with functional assessment by examination of pathway transmission than structural measurements from imaging. There was also a suggestion that perhaps the best way to generate a proxy measure of disability for use in early clinical trials of possible therapeutics may be to use a combined measurement system using information from both structural imaging and functional assessment by analysis of signal transmission along the key pathways in the brain and spinal cord, particularly those involved in limb function.

Team & Contributions

Dr Luke Canham	Data Collection, Clinical Ratings & Analysis
Dr David Cottrell	Project Design, Funding, Ethics & Recruitment
Dr Nick Kane & Dr Stuart Butler	Project Design, Conduct of TMS-MEPs (Dr Kane)
Mrs Jenny Homewood	Study Administration
Dr Paul White	Statistical Oversight
Dr K Inglis, Dr H Faulkner & Dr J Witherick	Clinical Ratings
Dr Agyepong Oware	Conduct of TMS-MEPs
Mr Peter Walsh & Miss Kelly Blake	Conduct of SSEP, VEP and BSAEP
Mrs Ann Case	Lead Neuroradiographer
Dr Nikos Evangelou & Prof Paul Morgan	MRI Acquisition Protocol Design (Nottingham Team)
Dr R Dineen, Dr D Kent & Dr E Powell	Imaging Post-Processing (Nottingham Team)

INTRODUCTION

In the setting of Progressive Multiple Sclerosis (PMS) the instrument by which benefit must be demonstrated to achieve regulatory approval remains the EDSS (179, 425). Use of this outcome is unlikely to be imminently shifted(235) and the statistical properties conferred by using EDSS criteria (213) demand a prohibitive scale of investigation.

A biomarker system with three key properties is required to partly overcome such limitations and through facilitation of enquiry accelerate the pace of translational research in PMS. Firstly, it should demonstrate strong association with disease-related physical disability. Secondly, through greater sensitivity and finer granularity of interval measurement it should permit investigation of more readily achievable scale. Thirdly and most importantly behaviour of such a metric in response to an intervention at phase II should predict with high accuracy the final outcome when judged by the clinical scales recognised by regulatory authorities.

Whilst the first two criteria are intuitive the last is well illustrated by the recent lack of success seen at phase III with Fingolimod against PPMS (INFORMS) (318), despite having a putative neuroprotective effect inferred from amelioration of brain atrophy in preceding studies (269). A similar pattern of dissociation was also seen previously with Glatiramer Acetate in the PROMiSE (231) study and preceding investigations (317).

Although MRI volumetrics undoubtedly correlate well with cognitive difficulties (249, 426, 427), predict accrual of longer term physical disability (157, 250) and offer the statistical properties necessary to perform small trials (230, 428) their actual association with clinical disability is modest (230, 245) and either non-causal or largely independent. The relative lack of physical disability in conditions characterised by profound cerebral volume loss (such as Alzheimer's Disease(260)) supports this view. Notably the recent regression analysis of Daams et al. (2015)(319) confirms the minimal contribution cranial volumetrics offer in explaining variance of physical disability in MS patients and suggest multi-parametric markers are necessary to explain motor dysfunction in MS.

Such findings may explain the failure of short term brain volume preservation to translate directly into slowed accrual of physical disability in a similar time frame to that typically employed in clinical trials. Such findings should perhaps also discourage reliance on single domain mono-parametric biomarkers in this especially complex neurological disease.

Neuroimaging is a fundamental component of MS research and generation of composite measures has been explored as one solution to the *'Clinicoradiological Paradox'*(245). Earlier attempts such as that applied in the Linomide trial (429) and the subsequent first version of the MR Disability Scale (MRDSS) (187) were both based solely on cranial parameters and met with limited success. The recently published MRDSS-2(186) features cervical cord parameters and bears closer albeit still modest association with clinical disability (r=.33 vs EDSS). However generally, with respect to the EDSS and other measures of mobility and limb function similar structural parameters even when optimally weighted and combined into single regression models appear able to account for no more than 40% of the observed variance in disability (319).

A biomarker system which both respects the structural and functional consequences of the disease in addition to its multidimensional impact would probably be most informative and ultimately useful. Evoked Potential studies have repeatedly displayed reliable association with clinical dysfunction in various commonly interrogated individual modalities (36) and a consistent correlation with physical disability when collated into multimodality EP composite systems (37-41). Notably such association is seen to be largely independent of the rating system employed (314) .

Many studies have also now emerged demonstrating the ability of MMEP to both significantly mirror and precede change in all phenotypes of multiple sclerosis even within the time frame of standard clinical trials (181, 191, 387). These inexpensive measurements have also found some limited but growing use as outcome measures in therapeutic trials (337, 387). However, the superior sensitivity and functional association of EPs stands in contrast to the higher spatial resolution and histopathological specificity of MRI.

We sought to explore the possible utility of combining contemporary structural imaging metrics from brain and cord with functional information offered by MMEP into a novel singular *cross-modality* composite surrogate biomarker of physical disability. A cohort of Primary Progressive MS patients was selected for investigation given their phenotypic homogeneity, absence of confounding relapse effects and general similarity to samples considered eligible for contemporary trials in PPMS.

METHODS

Patients. 27 people with confirmed PPMS meeting diagnostic criteria (9) were recruited and provided informed consent. Gender was balanced with 14 females and 13 males, with an average age of 49.5 (31-66) years and Mean EDSS of 5.9(3.5-8) following a mean Disease Duration of 9.75(1-23) years. Participants underwent clinical disability evaluation, multimodal evoked potential batteries and contemporaneous neuroimaging metric acquisition. Practitioners involved in derivation of results from each modality were blinded to performance in other domains to avoid perceptual bias.

Clinical Evaluation. Participants were rated according to Kurtzke's Modified EDSS (211) They also performed the 9 Hole Peg Test, Timed 25 foot Walk and 3 Second Paced Auditory Serial Addition Test comprising the MSFC (399).

Neurophysiological Evaluation. All patients were invited to undergo an MMEP battery; comprising bilateral VEP, bilateral BSAEP and SSEP of all four limbs. Transcranial Magnetic Stimulation elicited MEPs were recorded from all four limbs.Acquisition followed the Guidelines of the International Federation of Clinical Neurophysiology (309) with the protocol as described by Rice et al. (337).

Multimodal Evoked Potential Analysis. A variety of established quantitative and qualitative evoked potential scoring methods were used to derive composite scores for each battery undertaken and are summarised in table 1 below. Two blinded neurophysiologists independently provided the required qualitative ratings according to published methods. Where utilised, Z score transformation was performed using international normative data (309). In the event of no latency value being available due to absent evoked potential response the maximum Z value for our cohort was used, plus one, to partially allow for extra qualitative information inferred by a response more severe than even the most marked prolongation. See table 9.

GEPS	Global Evoked Potential Score	MEPS	Multimodal Evoked Potential Score
0	Normal	0	Normal
1	Conduction Delay	1	Pathological Asymmetry of Latency
2	Increased Latency with Morphological abnormality of a major component	2	Latency between ULN and 1.1xULN *or* >50% *difference in bilateral amplitudes*
3	Absence of a Major Waveform Component	3	Latency 1.1xULN to 1.3xULN
		4	Latency >1.3xULN
		5	Absent Component
GEPS	=Σ GEPS for each modality	**MEPSQ**	=Σ all MEPS /number (bilateral Visual and Motor and Sensory from all limbs. No BSAEP)
GEPS2	=Σ for each modality *excluding* SSEP of Arms	**MEPS2**	=ΣMEPS for both Visual, BSAEP and 4 limb SSEP; no MOTOR
S-EPL-Q	Summed EP Latency Quotient $\frac{\Sigma\ all\ z\ EP\ latencies}{Number\ performed}$		

Table 9 MMEP Battery & Scoring Systems.

Each MMEP quantification technique is based on the scoring system and permutation of evoked potentials described in the original description of the GEPS, MEPS and SEPLQ respectively. Upper limits of normal (ULN) were taken at 2.5 standard deviations from the mean and normal values were derived from published international (178) and local departmental normal values (for VEPs). For VEP N75-P100 wave presence, amplitude, morphology and P100 peak latency were considered. BSAEP interval latencies for components I, III and V were recorded in addition to the SSEP N13 and N20 intervals for upper limb and central components up to P37 for lower limb. The ULN for lower limb SSEP latency was adjusted for patient height according to the formula 25.7 ms × height in meters (293). MEPs were attained using a 9-cm circular MAGSTIM coil held over the vertex. The central motor conduction time (CMCT) was calculated by subtracting ½(M + F + 1) from the motor EP latency, where M is the distal motor latency and F is the minimum F wave latency. The ULN for Lower limb CMCT was also adjusted for height (294).

The following concise description of MRI acquisition and analysis was provided by the Nottingham team:

MRI Acquisition. Patients were scanned on a 3 Tesla Achieva MR scanner (Philips Medical, Best, The Netherlands) at Frenchay Hospital, North Bristol NHS Trust according to a pre-planned T1, T2 and FLAIR acquisition protocol devised by the Nottingham team. Sequences of brain and cord (of the cervical c2/3 and c5/6 and thoracic t4/5 t9/10 regions) were acquired using a 32 channel head coil and spine coil respectively. The brain MR protocol included a 3D T1-weighted rapid gradient echo acquisition (180 sagittal slices, 256x256 matrix, 1x1x1 mm voxels, TE 3 ms, TR 7 ms, effective TI 820 ms, 8 degree flip angle, parallel imaging factor 2, acquisition duration 390 s); 3D T2-weighted FLAIR (180 sagittal slices, 256x256 matrix, 1x1x1 mm voxels, effective TE 290 ms, TR 4800 ms, TI 1650 ms, parallel imaging factors 2.6 in-plane, 2 through-plane, acquisition duration 370 s). The MR spinal cord acquisition consisted of four stations centred at vertebra junctions C2-3, C-5-6, T4-5, and T9-10 with axial slices centred on the cord and oriented by eye to be perpendicular to the cord. Each station utilised an identical MR acquisition of a 3D balanced gradient echo (also known as a True FISP) scan (256x256x16 matrix, 0.5x0.5x1.5 mm voxels, TE 2.5 ms, TR 6.2 ms, 45 degree flip angle, 6 averages, acquisition duration per station 300 s).

MRI Analysis. Volumetric assessment was performed using FSL (430) and SPM (http://www.fil.ion.ucl.ac.uk/spm/). Briefly, the DICOM images were converted to NIFTI format using dcm2nii (431). MS lesions were semi-automatically segmented on the FLAIR images using the 3D MS Lesion Finder function in Java Image version 7 (http://www.xinapse.com/) and saved as binary masks as well as calculating lesion volume. Skull stripping was performed using FSL and the white matter lesions replaced using the lesion masks. Brain segmentation into white matter, grey matter, and CSF components ws performed using the standard VBM pre-processing in SPM8. Volumes of these components were calculated by summing their probabilities. Total Intracranial Volume (TIV) was calculated as the sum of GM, WM, and CSF volumes, whereas Brain Volume (BV) was the sum of GM and WM. Spinal cord analysis was performed using the NeuROI software (432) which performs partial volume edge detection of the cord on several slices as well as correcting for the long axis of the cord not being exactly perpendicular to the imaging plane, resulting in a corrected cross-sectional cord area at each of the four imaging stations. Normative data were based on those recently reported by Bakshi et al., (186) and where possible the MRI composite (MRDSS-2) described by that group was derived from z transformed summated average of Mean Upper Cervical Cord Area (MUCCA) taken here as the average of the c2/3 and c5/6 values, the Grey Matter volume in litres and the lesion load per brain volume.

BDSS Cross-Modality Composite. The Biomarker Disability Severity Score was generated by averaging after summation of the normalised scores of each component from the imaging MRDSS-2 score and the neurophysiological S-EPL-Q score using the formula:

$$BDSS = \frac{(MRDSS2) + (SEPLQ)}{2}$$

Where:

$$MRDSS2 = \frac{zGM + zMUCCA - zLesion\ Load}{3}$$

And:

$$SEPLQ = \frac{\Sigma\ (zVEP\ both + zBSAEP\ both\ + zCMCTlimbs + \ zSSEPlimbs)}{12}$$

Statistical Analysis. Spearman's rank correlation coefficient (*r*) has been used to gauge association where assessed throughout this study. These analyses and the Multiple Linear Regression modelling which followed used 95% confidence intervals derived based on 2000 iterative bootstrap samples so as to otherwise avoid any reliance upon normality or large sample asymptotic theory. All calculations were performed in SPSS v 21. The Null Hypothesis in each case was of no correlation between tested parameters and the critical value of alpha was set at p=0.05 for statistical significance and rejection of H_0.

Standard Protocol Approvals, Registrations and Patient Consents. Ethics approval for the study protocol was awarded by the NHS Frenchay Research Ethics Committee ref. 06/Q2007/7. Participants were recruited from the Bristol and Avon Multiple Sclerosis Service patient cohort and informed consent acquired prior to entry.

RESULTS

Correlation between individual MRI metrics and MMEP batteries with clinical disability is displayed in table 10. MMEP were successfully attained on the entire cohort and irrespective of method of abnormality rating demonstrated highly significant and consistent association with disability (*r=.634 to .808)*. The pattern of association with MSFC subcomponents (Timed Walk and 9 Hole Peg Test) also seen in table 2 demonstrates the MMEP relationship with disability is largely dependent on spinal functional integrity. Use of the individual EP modality components (graded by each system used) as variables in multiple linear regression models with EDSS as the dependent variable also support the dominant contribution of both afferent and efferent long tract EP abnormalities in driving association with clinical disability (table 11a). Similar modelling against EDSS using only sensory and motor components from upper and lower limbs further supports the paramount importance of the longest afferent and efferent pathways in determination of disability and its broad association with MMEP batteries (table 11b).

	EDSS	MSFC	Z-25ft Walk	z-9HPT	Z-PASAT 3'	N	% of set
EDSS	1	-.763***	-.842***	-.640***	-.231	27	100
GM	.054	.163	.058	-.107	.527*	19	70.4
WM	-.006	.170	.118	-.181	.578**	19	70.4
CSF	.034	.021	.081	-.107	.255	19	70.4
BV	.047	.137	.061	-.151	.541*	19	70.4
TIV	.068	.101	.053	-.17	.514*	19	70.4
BV/TIV	-.115	.255	.093	-.047	.353	19	70.4
Lesion Load	.276	-.413	-.204	.537*	-.138	19	70.4
Lesion %BV	.257	-.457*	-.186	-.537*	-.253	19	70.4
c23mm2	-.413	.436	.512	.297	.319	14	51.9
c23mm2 norm	-.413	.436	.512	.297	.319	14	51.9
c56mm2	-.544*	.710***	.456	.446	.524*	17	63
c56mm2 norm	-.634**	.381	.458	.422	-.240	17	63
t45mm2	-.348	.377	.412	.063	.500*	18	66.7
t45mm2 norm	-.348	.377	.412	.063	.500*	18	66.7
t910mm2	-.288	.344	.321	.063	.580*	18	66.7
t910mm2 norm	-.419	.219	.267	.276	-.051	18	66.7
Bscore	.684***	-.551***	-.577***	-.453*	-.223	27	100
SEPLQ	.748***	-.574***	-.639***	-.48*	-.210	27	**100**
MEPSQ	.773***	-.504**	-.666***	-.486*	-.080	27	100
MEPS2	.634***	-.506**	-.643***	-.472*	-.146	27	100
GEPS	.788***	-.583***	-.683***	-.498**	-.219	27	100
GEPS-Q	.788***	-.583***	-.683***	-.498**	-.219		
GEPS-2	.808***	-.463*	-.609***	-.339	-.142	27	100
GEPS-motor	.712***	-.429*	-.572***	-.321	-.159	27	100
GEPS-Sensory	.577***	-.510**	-.554**	-.481*	-.193	27	100
Derived Scores							
MUCCA	-.631*	.715**	.451	.527	.582*	13	48.1
zMUCCA	-.631*	.715**	.451	.527	.582*	13	48.1
MRDDS2	-0.279	0.564*	0.148	0.467	.324	13	48.1

NEUROIMAGING

NEUROPHYSIOLOGY

P<0.05 *

P<0.01 **

P<0.005 ***

Table 10 Correlation Matrix of Individual Multimodality subcomponents vs.Clinical Disability

(by EDSS and MSFC) and correlation against individual Multimodality subcomponents vs. MSFC Subcomponents (9-Hole Peg Test, 25ft Walk and PASAT 3 second).

Assessment of relations between MRI metrics and disability rating was hindered by substantial attrition of the imaging dataset, most likely due to the long overall MR acquisition duration, which only became evident at the stage of post-processing. The unavailability of individual metrics was heterogeneously spread across subjects with only a half of the original cohort ultimately yielding all the MRI metrics required to generate the described composite scores. Nonetheless a consistent association between spinal volumetrics and physical disability was observed, achieving statistical significance mainly in the cervical cord at the c5/6 level. Whilst the cranial metrics related to cognitive performance (as measured by the PASAT), their wider association with disability was consistently poor. The MRDSS-2 score displayed a weak non-significant correlation with EDSS (r=-.279) however this was of a similar magnitude to that described by the original authors in a larger cohort (186). The MRDSS-2 correlation with MSFC was superior at (r=.567 p<.05). The BDSS, in combining MRI and MMEP information offered significantly strong associations with disability measured by EDSS (r=.808 p<.005) and moderately with MSFC (r=-.58 p<.05) (figure 4). Examination of the R^2 (table 11c) would also suggest that the BDSS was more informative and able to account for more observed variance in disability (as measured by either rating scale) when compared with composites based on single modality investigations. The limited

sample size (secondary to MRI attrition) prohibited weighting the individual parameter contributions to strengthen association with disability. An attempt to gauge the respective contribution of the MDSS2 and SEPLQ to the association between BDSS and EDSS highlighted a dominant influence of functional indices from the MMEP battery, again this was irrespective of scoring technique used (table 11d and figures 3 and 4 below).

Table 11 Regression Based Modelling of The Underpinnings Of Association Between Structural and Functional Indices with Disability

	Visual	Brainstem	Sensory	Motor	*R*	p
GEPS	-.002	.304	.315	.340	.700***	.004
mEPS	.077	.099	.476	.319	.735***	.001
SEPLQ	.094	.287	.289	.266	.713***	.003

A) *Standardised Coefficients of all MMEP subcomponents (summated by modality) following linear regression based modelling against dependent variable of EDSS.*

	Sensory ARMS	Sensory LEGS	Motor ARMS	Motor LEGS	*R*	p
mEPS	.082	.504	-.227	.600	.837***	<.005
SEPLQ	.171	.045	-.047	.609	.680***	.002

B) *Standardised Coefficients of MMEP subcomponents, relating solely to sensory and motor EP from upper and lower limbs, following linear regression based modelling against dependent variable of EDSS.*

	EDSS	MSFC
SEPLQ	.372	.264
MDSS2	.020	.220
BDSS	.483	.313

C) **Linear R^2 of each modality composite against disability rating (by scale).*

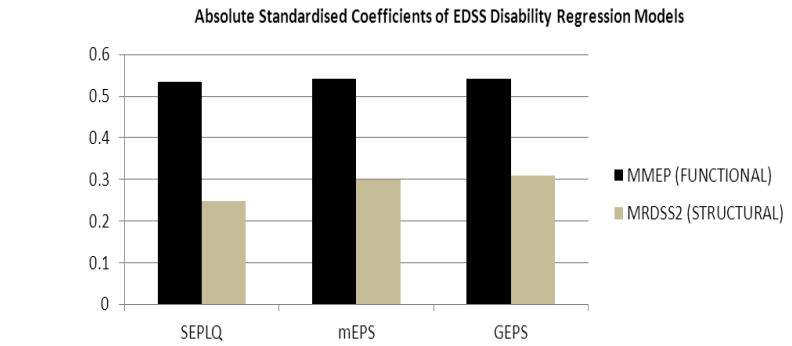
	MMEP	MRDSS2	*R*	P
SEPLQ	.534	-.248	.710**	.011
mEPS	.542	-.300	.737**	.006
GEPS	.541	-.309	.723**	.006

D) *Standardised Coefficients of BDSS components (Structural MDSS2 and Functional MMEP) following linear regression onto dependent variable of EDSS; similar findings emerged despite using different MMEP composite methods.*

*Multiple Imputation is an established technique for reducing dataset attrition consequent of parameter censorship. 20 rounds of Imputation were performed with 2000 bootstrapping iterations in SPSS v21. MRI parameters were reconstructed from demographic information, existing MRI parameters and spinal neurophysiology scores of long tract modalities. The resulting values were used to compile MRDSS-2 and subsequently BDSS scores, for each round of imputation. These latter values were seen to correlate significantly with EDSS and MSFC and the R^2 was on a par with that observed in the smaller dataset which offered full acquisition, as seen here.

Figure 3 Standardised Coefficients of BDSS components (Structural MDSS2 and Functional MMEP) following linear regression onto dependent variable of EDSS

Dominant contribution from functional investigations was evident and yielded similar findings irrespective of MMEP quantification technique used**.**



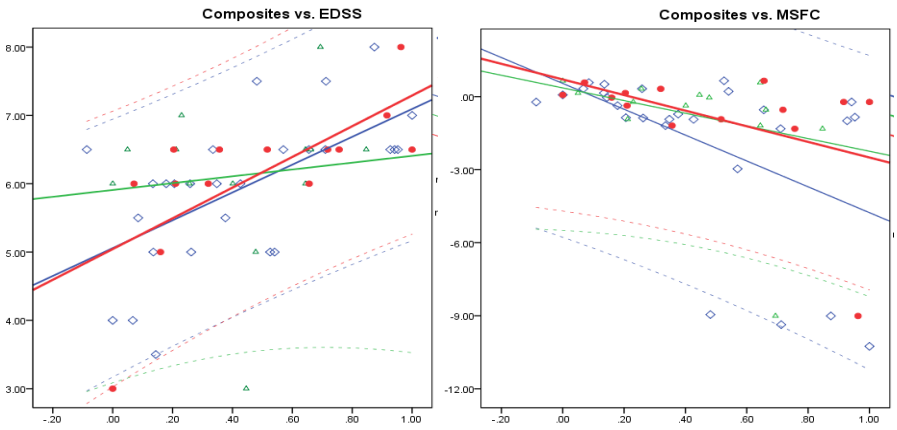


Figure 4 Association of Composite Scores with EDSS & MSFC.

These graphs demonstrate the association between composites and the respective clinical disability rating by EDSS (top) and MSFC (bottom). The green line (and open triangles) represents the imaging MDSS2 data (R^2 .020 r-.279 p>0.5 against EDSS and R^2 .220 r.564 p<.05 against MSFC). The blue line (and open diamonds) represents the S-EPL-Q neurophysiology composite data (R^2 .372 r.748 p<.005 against EDSS and R^2 .264 r-.574 p<.005 against MSFC). The red line (and full circles) represents the BDSS cross-modality composite data (R^2 .483 r.808 p<.005 against EDSS and R^2 .313 r.580 p<.05 against MSFC). In both situations a greater degree of variance was accounted for by the BDSS system.

DISCUSSION

With a view to generating a biomarker of physical disability in PMS which strongly associates with standard clinical outcome measures we attempted to combine structural imaging and functional neurophysiological metrics of disease-related damage into a novel single cross-modality composite. We purposely included both the EDSS and MSFC for comparison given their current use as trial outcome measures. Association between individual conventional MRI metrics with disability was minimal with respect to cranial metrics. Atrophy at all cord levels examined was correlated with physical disability measured by both EDSS and MSFC however this only achieved statistical significance at the level of c5/6 (r=-.544 p<.01, and r=.71 p<.005 respectively). This pattern mirrors the weighting of the EDSS in particular toward myelopathy(314).

Given the independent dynamics of cranial and cord volumetric changes in multiple sclerosis (226) and the very modest association of imaging composites reliant on only cranial parameters (187, 429), as suggested by Bakshi et al.(186) it is appropriate to include cord metrics. Our attempt to use the described MRDSS-2 system was hampered by attrition, predominantly at the post-acquisition stage of metric abstraction and attributed largely to the scan duration and consequent movement likelihood. Nonetheless positive association with physical disability was seen, significantly and more so with the MSFC, unsurprising given greater contribution to that score by cerebral performance as captured by the PASAT.

In contrast, MMEP batteries were attained on all participants, making no contribution to dataset attrition and displayed moderate to strong and globally significant associations with disability measured by both EDSS and MSFC, irrespective of the actual means of abnormality quantification employed. Our results from stepwise linear regression modelling would suggest that again indicators of long tract integrity within the cord are dominant contributors to such association. Afferent and efferent pathways to the lower limbs are also of paramount importance. Using the derived coefficients to proportionately weight MMEP composites, further improved already strong association with clinical disability scales.

We arithmetically combined the individual components of the MRDSS-2 and S-EPL-Q as both rely on normalised interval measurements in an unweighted fashion. The resultant BDSS score displayed generally superior significant correlation with EDSS and MSFC than either the separate imaging or MMEP composites alone. Most notably, it also accounted for more observed variance (R^2) in disability than either modality separately suggesting a genuine gain in information from their combination.

Examination of the absolute standardised regression coefficients produced by attempting to weight the contribution of the imaging and MMEP composites to a cross-modality surrogate of EDSS suggests an approximate 2:1 ratio of influence from functional EP metrics compared to structural MRI parameters. In an attempt to overcome the effects of MRI attrition we applied the established technique of Multiple Imputation to reduce the effect of data absence in our cohort and explore the association of composite scores and disability in a larger group. Again, the BDSS performed significantly strongly in correlation with physical disability ratings and usefully accounted for much variation in these outcomes. In addition to their impressive capacity to reflect physical dysfunction in Multiple Sclerosis, MMEP are safe, widely available and comparatively inexpensive.

Our own local experience suggests they are also well tolerated. Logistically, the relatively small additional costs of MMEP acquisition in the clinical trial setting could be ultimately offset by savings realised by consequent reductions in study scale conferred by a more sensitive cross-modality instrument.

The dynamics of change in various parameters, particularly cord volume is recognised to be non-linear, with the majority and greatest rates of atrophy being seen early in the disease course around the time of initial injury before subsequent structural changes almost plateau (196, 197) in a manner strikingly akin to the diminution of the Retinal Nerve Fibre Layer after Optic Neuritis (397). Cord-dependent evoked potentials appear to correlate with ongoing myelopathic functional decline which produces worsening disability after a point at which such structural plateaus have been reached. More detailed understanding of the time-course and relative contribution of structural changes to disability in PMS would be offered by a larger scale investigation of MMEP and MRI of brain and cord metrics. Indeed, with additional MMEP inclusion (which has no discernible negative effect on either recruitment or attrition) alongside routinely acquired datasets from future phase II and III PMS trials could rapidly provide a wealth of longitudinal data. Such analysis would also offer the opportunity to derive appropriate weightings for individual components of a Biomarker Disability Severity Scale system.

Cross-modality integration would hopefully not only offer tighter correlates of physical disability with a causal association but feature elements with pathological specificity for aspects of disease activity. Rather than solely illustrating presence or absence of irreversible structural loss, through incorporation of EPs such a unified composite would possess a dynamically responsive means of detecting *repair*. Indeed, as recent studies (337, 433) have suggested important remyelination and axonal preservation effects evident on EP studies may otherwise go clinically and radiologically undetected.

CONCLUSION

In this study we have shown a cross-modality composite Biomarker Disability Severity Scale derived from conventional MRI and MMEP elements confers advantage over using each modality in isolation in ability to reflect clinical disability. Both structural and functional metrics attest to the dominant disabling effect of myelopathy in PMS. A larger investigation will enable accurate quantification of individual parameter contributions to disability and appropriate weighting of a widely practicable and likely most informative biomarker.

V

The Growing Potential of Long Tract Neurophysiology in Primary Progressive Multiple Sclerosis – A Prospective Investigation over 3 years

Objective To evaluate the longitudinal relationship of multimodal evoked potential abnormality burdens with progression of clinical disability and assess their candidacy as surrogate biomarkers of disability for translational research in Primary Progressive Multiple Sclerosis (PPMS).

Background Evoked Potentials sensitively reflect the functional integrity of pathways which centrally determine physical disability in Multiple Sclerosis. It is hoped they may offer a surrogate outcome able to meaningfully associate with disability and through deterministic association accurately predict findings in large trials using FDA and EMA accepted clinical outcomes.

Method 28 patients with PPMS were recruited and underwent clinical disability rating by EDSS and MSFC at baseline and 6, 12, 24 and 36 months subsequently. Contemporaneous Multi-modal Evoked Potential recordings were also made at each time point and abnormalities quantified by the established semi-quantitative Multimodal EP Score system (MEPS). The EP battery comprised bilateral visual, brainstem auditory, 4 limb somatosensory and 4 limb TMS-evoked Motor evoked potentials. The relationship between composite MEP score and time to confirmed disability progression was explored.

Results Combined sensory and motor potentials of all 4 limbs (MEP-4) demonstrated consistent cross-sectional relationship with disability over time (Spearman's Rs=.325 to .747 p<.005). Individuals with a higher baseline MEP-4 manifested greater and more accelerated progression by both EDSS and the newer EDSS-Plus criteria (Log Rank p<.05 and Area under the ROC of .71). There was fair agreement between MEP-4 change and pattern of disability change over the first 12 months (Cohen's k=.34) and subsequent year (Cohen's k=.35). The average rate of MEP-4 worsening was approximately steady for the whole cohort and separate high/low MEP-4 cohorts over time, increasing by an average of 1 point per year.

Conclusions Focussed neurophysiological interrogation of the spinal afferent and efferent long tracts offers meaningful quantitative information about the key determinants of disability detected by the accepted clinical trial outcome measures. Higher MEP-4 burden conferred a worse prognosis, with subjects displaying shorter times to progression and ultimately worse levels of clinical disability. The average rate of neurophysiological decline is also congruent with the observed natural history of the PMS phenotype. Evoked Potentials offer a means to prognosticate, enrich trial cohorts for those most likely to manifest clinical outcomes and track changes (including improvement) over desirable intervals.

Plain Language Summary Having identified a meaningful relationship between the severity of physical disability and the cumulative extent of dysfunctional signal transmission in patients with Multiple Sclerosis whose clinical function had worsened progressively since onset, we sought to explore the behaviour of these measures over time.

At regular intervals over a period of three years a group of patients with progressive Multiple Sclerosis underwent dual evaluation of their physical disability and assessment of conduction along the pathways mediating sensation from and control of their limbs. Despite marked individual fluctuations in signal conduction over time, collectively there was a progressive decline in transmission which to a degree mirrored and heralded subsequent physical decline in the group. Additionally, those persons with more abnormal signal conduction at the start demonstrated more rapid worsening of their physical disability also. The findings suggested it might be possible to use such signal conduction measures in early, smaller scale clinical trials to predict response to treatment when tested against clinical outcomes of disability in large scale studies. Also, given their ability to offer prognostic information about who is more likely to display progressive disability earlier they might serve to enrich recruitment into studies of those participants more likely to worsen without intervention and as such might also offer a route to shortening and shrinking the size of studies, making them more achievable and rapidly informative.

Team & Contributions

Dr Luke Canham	Data Collection, Clinical Ratings & Analysis
Dr David Cottrell	Project Design, Funding, Ethics & Recruitment
Dr Nick Kane & Dr Stuart Butler	Project Design, Conduct of TMS-MEPs (Dr Kane)
Mrs Jenny Homewood	Study Administration
Dr Paul White	Statistical Oversight
Dr K Inglis, Dr H Faulkner & Dr J Witherick	Clinical Ratings
Dr Agyepong Oware	Conduct of TMS-MEPs
Mr Peter Walsh & Miss Kelly Blake	Conduct of SSEP, VEP and BSAEP

INTRODUCTION

Treatment for Progressive Multiple Sclerosis represents one of the greatest of unmet needs in modern neurological practice (178). Paradoxically, the very methodology which informs the design and conduct of clinical trials in this context is likely more limiting of available therapeutics than any lack of putative treatments worth trialling (169, 203, 213, 216). Growing patient demand for an effective intervention, coupled with a long-overdue shift in industrial focus (178, 179) and technological developments which promise accelerated drug discovery (434) all underscore the pressing need for a means to conduct more rapid and smaller scale translational research than EDSS-based evaluation can permit.

The older age and attendant comorbidity (151, 169) of patients in the Progressive phase poses a further significant limitation to the conduct of translational research in this group. The proportion of those affected eligible for trial participation is considerably smaller in comparison to their younger Relapsing-Remitting counterparts. Furthermore, specific focus on the behaviourally-distinct (148, 169) but pathologically-equivalent (1, 46) individuals with a Primary Progressive phenotype to avoid the statistically confounding effects of relapse and interrogate the clinically purest manifestation of the mechanisms underlying progression offers even greater restriction (169). An ideal biomarker surrogate of disability (the ultimate and required test of efficacy at phase III) would reduce the impact of such limits at earlier phases and minimize the hazard exposure inherent to any clinical trial by requiring less sustained recruitment to achieve statistical power.

One approach has been to incorporate limb function elements (425) from the validated MSFC (399) with the standard disability rating system of the EDSS (210) to derive more stringent thresholds and sensitive detection of clinical progression (425). Such a method is being increasingly deployed within translational research (ASCEND NCT01416181) but may only offer part of the solution. It remains inherently vulnerable to the effects of subjectively rating EDSS functional system components and the all too familiar variability in performance due to factors affecting effort during task performance.

Evoked Potentials have a long history of use in the context of Multiple Sclerosis as a consequence of their remarkable sensitivity to the dysfunction directly arising from the pathological hallmark of Multiple Sclerosis, namely demyelination (182).

There is much to commend their consideration as a useful biomarker candidate in the setting of PMS research. The dependence on the functional integrity of long tract pathways in both Multimodal Evoked Potentials (MMEP) and disability (314) suggests an ability to preserve the former in phase II *should* deterministically predict attenuation of the latter at phase III given their shared underpinnings. The cross-sectional association of MMEP batteries and their composite elements with disability rated by the accepted outcome measures has been reliably, repeatedly and independently demonstrated by several groups (162, 181, 184, 188, 435). A small number of investigations have demonstrated impressive prognostic capability (189-191) and their use as outcome metrics in various forms has seen some albeit limited exploration in therapeutic trials(337, 387), also with promising results. Their capacity to demonstrate not only deterioration but also dynamic improvements indicative of remyelination and repair is likely to drive the current resurgence in their investigative use. (74, 337, 393, 436). The literature on performance of multimodality evoked potentials as a biomarker of disability in the context of primary progressive multiple sclerosis is small (181, 191). The optimal battery of acquisition and means of quantification also remain undefined. We sought to longitudinally evaluate the relationship between evoked potential abnormalities and progression of clinical disability within a cohort of Primary Progressive MS patients. We aimed i) to test the hypothesis that MMEP would be sensitive to the incremental dysfunction underlying worsening disability, ii) identify the most useful practicable battery for performing translational investigations and iii) estimate the statistical parameters required for doing so.

METHOD

Following ethical clearance (NHS Frenchay Research Ethics Committee ref.06/Q2007/7) 28 patients with an established diagnosis of Primary Progressive Multiple Sclerosis consistent with McDonald criteria (9) were recruited from our tertiary clinical cohort. Each underwent a multi-modality battery of evoked potentials by a trained neurophysiologist prior to clinical disability rating by trained neurologists according to the Expanded Disability Severity Scale (EDSS)(210) and Multiple Sclerosis Functional Composite (MSFC)(415) systems. Clinicians and neurophysiologists were blinded to results outside of their respective modality. The multi-modality potential battery comprised visual and brainstem recordings bilaterally in addition to somatosensory and transcranial magnetic stimulation induced evaluations from all four limbs. Potentials were attained according to IFCN guidelines (309) with the protocol as used in Rice et al. (337) and graded according to the documented semi-quantative Multimodal Evoked Potential Scale (183) (table 12) based on appropriately height adjusted (413) and normalised (309) results. This scale was selected on the basis of its superior association with disability in comparison to other systems in our previously published work (314). Clinical and electrophysiological assessments were made at baseline (T0) and 6, 12, 24 and 36 months subsequently.

Clinical Progression was judged as >6 month confirmed sustained worsening of disability by worsening of one point on the EDSS scale from below 5.5 and half a

point increment above that point in keeping with the system employed in clinical trials (425). The latest modification of EDSS based outcome assessment was also used (425); herein termed 'EDSS-Plus' by which Confirmed Disability Progression was achieved if subjects met the above criteria *or* demonstrated a >20% worsening in the 25 Foot Timed Walk *or* 9-Hole Peg Test performance compared to their baseline MSFC.

Statistical analysis was performed in SPSS v.21, critical level of alpha was set at 0.05 and bootstrapping with 2000 iterations was employed where necessary to overcome assumptions related to normalcy of parameter distributions.

Table 12 The Acquired Multimodality Evoked Potential Battery performed

*ULN (Upper Limit of Normal) taken as 2.5sd above population mean (309).

Multimodal Evoked Potential Battery	
Modality	**Acquisition**
Visual(VEP)	Checkerboard (30 degree of retinal field), 2Hz flicker, 1 degree of arc check size, white brightness of 150cdm^{-2}, contrast 87.5% N75-P100 recorded at Oz (Fz reference)
Brainstem (BSAEP)	Monaural Stimulation via earphones with rarefaction click stimuli of 0.1ms duration and intensity of 75 dB above subjective hearing threshold. The contralateral ear was masked with white noise. Wave components I,III & V
Somatosensory (SSEP)	Square wave electrical stimulation with pulses of 0.2ms duration. Applied at median nerve to yield N13 and N20 for upper limb And posterior tibial nerve to yield P37 for *lower limb *ULN taken as 25.7ms x height in meters as per (183)
Motor(MEP)	9-cm circular Magstim Coil over vertex, 140% threshold stimulation. EMG recordings made over Abductor pollicis brevis and Abductor halluces in the foot. Central Motor Conduction Time =1/2(M+F+1); F as minimum F wave latency. Lower limb CMCT upper limit of normal taken as: =(0.08ms/cm * height)+3.7ms As per Claus et al.,(413)

Multimodal Evoked Potential Score	
MEPS	**Feature**
0	Normal
1	Pathological Assymetry of Latency
2	Latency between ULN* and 1.1xULN or >50% difference in bilateral amplitudes
3	Latency 1.1xULN to 1.3xULN
4	Latency >1.3xULN
5	Absent Component

The Multimodal Evoked Potential Scoring System. *This semi-quantitative rating method as described in (183) was applied to each component of the MMEP battery.*

RESULTS

Demographics. Of the 28 subjects there was an equal gender balance (14 Female, 14 male). The mean age was 50.1 years (range 31.5-66.8) with a estimated disease duration of 9.12 years (range 1-30) since first symptoms. The average EDSS at baseline was 5.3 (range 2.5-6.5). 22 patients participated until completion at 36 months. Discontinuation was attributed to substantial progression (2 cases) and change in personal circumstances unrelated to the trial (3 cases). One subject manifested a liability to seizure during the course of investigation which contraindicated the use of transcranial stimulation. The conduct of all elements was reportedly well tolerated.

Clinical Progression. By standard Confirmed Disabiltiy Progression criteria using the EDSS alone, 60% of participants reached this end point over the 36 month period. The pattern and frequency of decline was in keeping with larger Natural History cohorts (148, 151). A substantially greater proportion (90%) passed the EDSS-plus threshold in the same epoch; with most (60%) having done so by 12 months. Time to disability endpoint was greater and more accelerated when judged by this method (P=.008 Log Rank (Mantel-Cox)). The Survival Function of the individual components of the MSFC within this cohort demonstrate expected patterns of deterioration mainly in Timed 25 foot Walk and 9-Hole Peg Testing with minimal contribution from the PASAT, supporting the exclusion of the latter in the composite EDSS-Plus threshold. This cannot be taken as evidence of no cognitive decline in this group but instead reflects anticipated (437) behaviour of the measure due to practice effects (see figure 5).

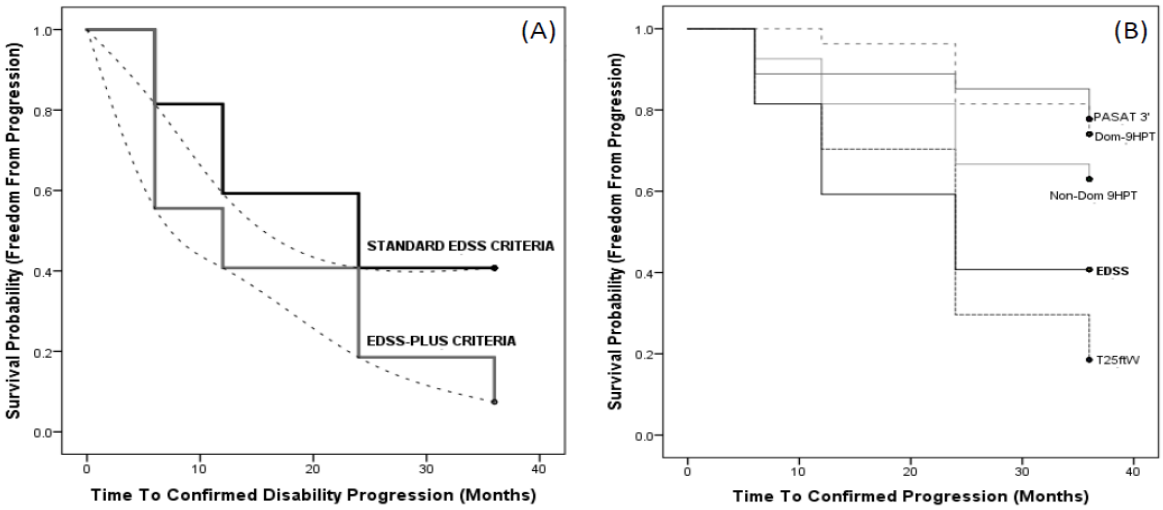


Figure 5 Clinical Progression

(A); Survival curves of time to disability criterion measured by EDSS alone (black) and EDSS plus (grey) (including the T25ft walk and 9HPT); the latter was significantly more sensitive to decline (Log Rank Test p=.008).(B); Survival curves of time to threshold for individual components of MSFC; The majority of decline was evident in the walking and upper limb tasks. The performance on PASAT did not substantially decline in the cohort over time.

Cross Sectional Relationship. Although composites involving all EPs recorded manifest association with physical disability, this appeared mainly driven by the sensorimotor potentials from all four limbs and these as the MEP-4 were the focus of all subsequent analysis. A consistent and significant pattern of association between MEP-4 scores and physical disability rating was observed over the entire course of the investigation. This was apparent when considering the cohort as a whole and solely those who completed the study. A closer association with final EDSS at 36 months than baseline clinical status at T0 was evident (see table 13).

	MEP-4 vs. EDSS (r_s)	
Time Point	*All n*	*N=22*
T0	.467**	.325
T6	.747***	.730***
T12	.594***	.608***
T24	.612***	.577**
T36	.622***	.622***

Table 13 Cross-Sectional Correlation between clinical disability rated by EDSS and MEP-4 score

Values represent Spearman's Rank Correlation coefficients (* P<.05, ** P<.01, *** p<.005)

Cohort Behaviour Over Time. Individual MEP4 scores demonstrated marked fluctuation over the course of the study period (see fig 6). All improvements were spontaneous and by the nature of the quantification system applied evidently greater than the normal standard deviation associated with recording in each modality. It is reasonable to infer reductions in neurophysiological scores therefore, at least partially, represent restored functional integrity to a degree. Behaviour of MEP-4 scores at the group level however manifest slowly progressive decline congruent with the natural history of PPMS. The cohorts mean average change per year was a steady MEP-4 increase of 1.03 (sd 0.52). This rate was fairly consistent even when groups underwent median split into albeit smaller high and low MEP-4 at baseline and tracked longitudinally over 36 months (see fig 7). Simple retrospective modelling based on such estimates suggest the cord demyelination likely began an average of two decades prior to the study period when our subjects were likely in their late 20s and early 30s. This in agreement with the average onset of disease phenotypically classified as relapse-remitting in natural history studies (12, 14).

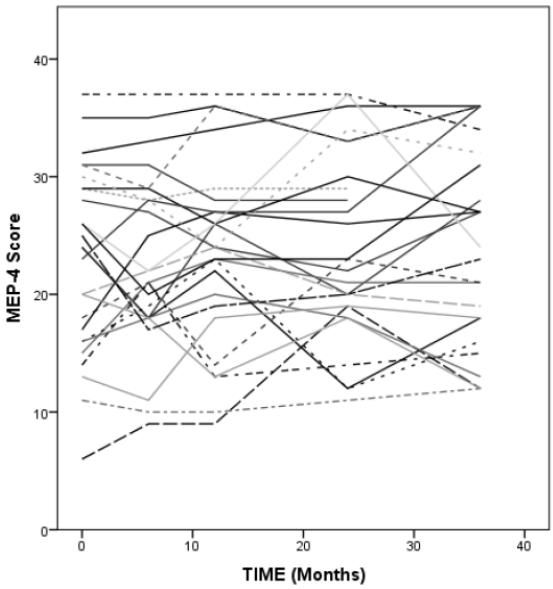


Figure 6 Fluctuation of individual MEP-4 scores over time.

Spontaneous improvements in MEP-4 composite scores were seen within individuals, despite an overall trend to worsening long tract neurophysiological function.

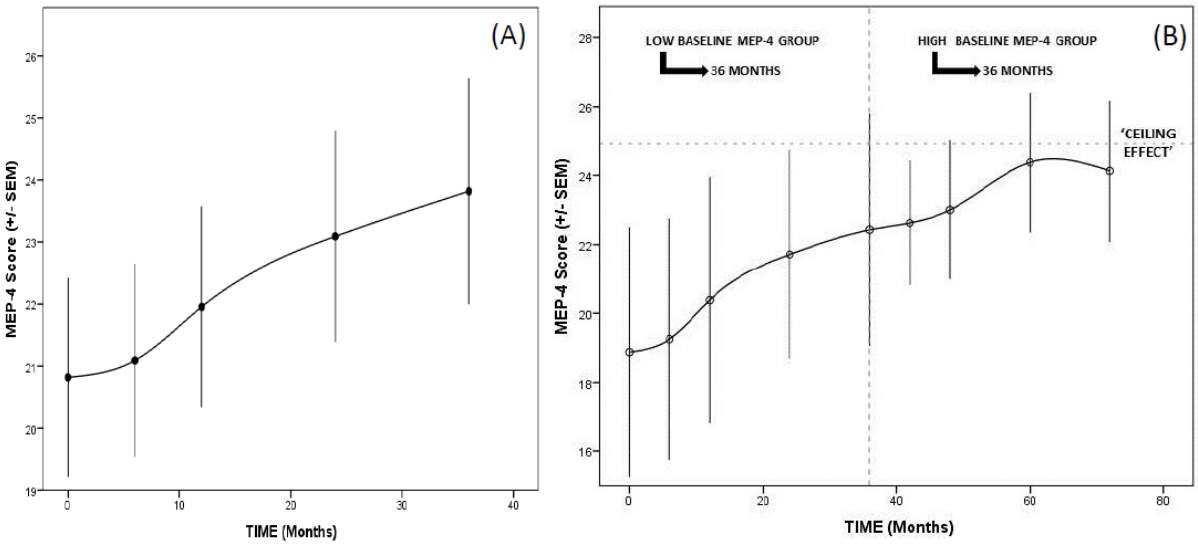


Figure 7 MEP-4 change over time

(A) mean MEP-4 of whole cohort (+/-SEM) at each time point. (B) Performing a median split of the whole cohort according to their MEP-4 score at baseline into 'low' and 'high' groups enabled modelling of the trajectory of change longitudinally over the subsequent 36 months. After a steady trend of deteriorating MEP-4 over time a 'ceiling' effect is suggested.

MEP-4 and Effect on Clinical Progression. The area under the receiving operating curve was .705 for the ability of MEP-4 to discriminate between individuals more likely to demonstrate faster clinical disability progression in the study period. A cut-off MEP-4 of 16 (out of a possible 40) offered 75% sensitivity and 63% specificity for indicating those likely to progress faster. At this cut-off significant differences were seen in survival curves against both EDSS (Log Rank p=.043) and EDSS-Plus (Log Rank P=.01). MEP-4 greater than 16 at baseline was associated with double the likelihood of reaching the standard EDSS progression threshold (80% vs. 40% for MEP-4 <16, HR 2.0) over the 36 month period and shorter time to progression by EDSS Plus criterion (by an average of 18 months) (see fig 8a-c).

HIGHER RELATIVE MEP-4 effect on Progression. Those individuals with an above average MEP-4 score for their given EDSS at baseline (based on a simple linear regression model EDSS=3.57+0.06*MEP-4) demonstrated a trend to faster progression judged by the EDSS-Plus criterion (Breslow Test, p=.07) reaching this threshold an average of 6 months before those with low MEP-4 relative to starting EDSS. This effect was not seen with EDSS as the sole disability measure; the ordinal nature of this measure and small cohort size makes such relative determinations very approximate (see fig. 8d).

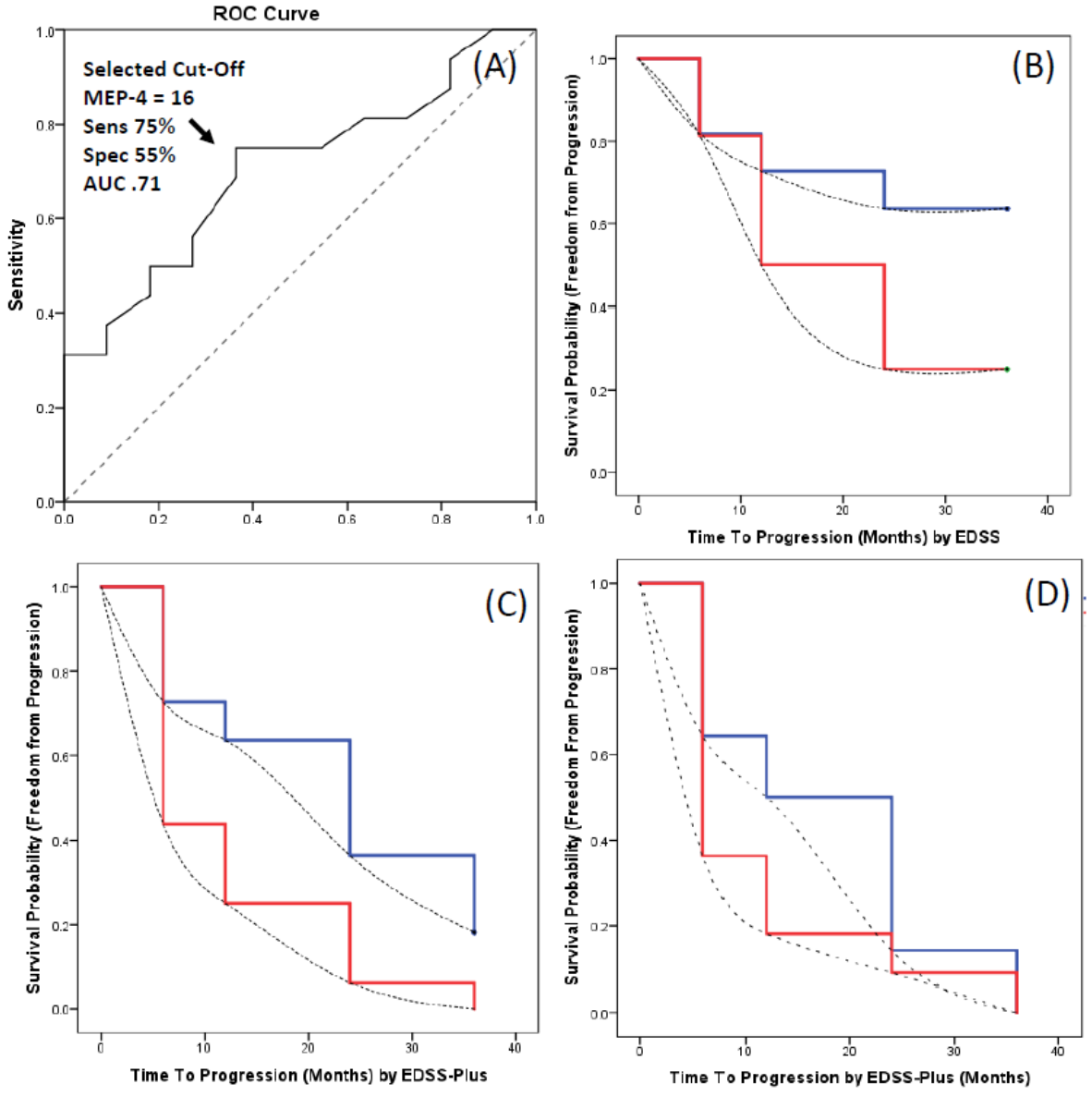


Figure 8 Effect of MEP-4 Burden on Progression

(A) The Receiver Operating Curve for the ability of the MEP-4 to predict faster time to confirmed disability progression suggested at least fair discrimination (AUC=0.7). Applying a cut-off MEP-4 of 16 to separate the cohort into high/low MEP-4 groups (red/blue respectively) revealed significantly shorter time to progression by standard EDSS (B), Log Rank p=.043) and EDSS-Plus criteria (C), Log Rank p=.01). (D) Patients with Higher Relative MEP-4 for EDSS at baseline (RED) progress faster by the EDSS Plus criteria compared to Lower Relative MEP-4 for EDSS patients (BLUE) (p=.07 on the one-tailed Wilcoxon Breslow Gehan Test.

Agreement. The agreement between neurophysiological rating and clinical rating of change (in categorising 'improvement', 'deterioration' or 'remaining stable') over the first 12 months of the investigation was fair (Cohen's Kappa=.34). Notably, the neurophysiological change in the first year displayed an almost identical level of agreement with pattern of clinical change over the second (Cohen's Kappa .35). In this cohort, over longer periods and larger intervals, agreement between clinical and neurophysiological modalities was not observed and confidence intervals were additionally too wide to make conclusive assessment.

Power. Based on the data collected a sample size estimation and power calculation was performed (in G*Power 3.1.9.2 ©University of Kiel, Germany) to gauge the approximate recruitment needed to detect a partial ability of a putative intervention to attenuate worsening of MEP-4 score over a 12 month interval with 80% power. Trial arm sizes would be taken as equal numerically and matched demographically. Alpha is set at .05 for an independent between-groups analysis. The mean annual cohort MEP-4 increment was taken as 1.04 (with SD 0.52) with anticipated sustained recruitment of 93% per year, as experienced here. Given the aforementioned results above, an ability to slow MEP-4 accrual by at least 30% should translate into a minimally important clinical difference on both the EDSS and EDSS-Plus scales albeit in larger scale studies (see table 14).

Effect Size	*Group Sample Size*	*Total Recruitment Required For **12** Month Investigation*
0.3	139	299
0.4	78	168
0.5	51	110

Effect Size	*Group Sample Size*	*Total Recruitment Required For **24** Month Investigation*
0.3	62	164
0.4	35	94
0.5	23	62

Table 14 Estimated recruitment required for a two equal-arm interventional trial using MEP-4

As a primary outcome measure to achieve 80% power of detecting minimally clinically important effect sizes. left parameters for a 1 year and right parameters for 2 year investigations.

DISCUSSION

Our results suggest that MMEP not only significantly associate with physical disability as rated by the EDSS but also have a significant predictive value with respect to prognosis for ensuing clinical disability progression over the time-frame of a standard clinical trial. These findings are concordant with results reported elsewhere(191) and across wider MS phenotypes(162, 181, 184, 188, 190).

The particular EP battery of most use in this cohort of PPMS patients involved particular focus on the sensorimotor long tracts carried within the spinal cord. This is perhaps unsurprising given that myelopathy and consequent limb dysfunction are the central drivers of disability detected by the clinical rating scales employed. Whilst VEP and Brainstem potentials do offer information about the functional integrity of their respective pathways(329), as has been the case

with radiological cranial metrics their modification at phase II may not relate to phase III outcomes(231) because similarly they are minimally related to the disability captured by accepted clinical rating scales(319).

The marked fluctuation of individual potential recordings over time in each modality is striking. The tendency to spontaneous shortening of latencies has been interpreted as evidence for delayed persistent remyelination when observed in longitudinal VEP cohort analysis elsewhere (395, 396). That such positive changes are evident on the MEP-4 scale by its nature suggest the reductions in latency are often several times greater than the normal standard deviation (seen in health) and therefore likely more than simple physiological variability. However, this inference needs to be tested with the variance in disease adequately demonstrated.

The use of the MEP-4 semi-quantitative scoring system also confers some advantage when summating data across cohorts (because of the very broad range of latencies and occasional physiological absence of responses) and in this case demonstrated a slow but steady consistent worsening of the group's cumulative long tract neurophysiology over time in a manner compatible with the clinically familiar natural history of this disease.

Although having a maximum upper MEP-4 value of 40 does in itself place a limit on achievable scores, our cohort suggested that in practice the ceiling seen in trial settings would be lower. Indeed as individuals pass into EDSS 7 and above their sustained participation drops off and consequently very high MMEP burdens are less frequently observed.

Below such a ceiling the dynamics of electrophysiological deterioration in this phenotypically representative cohort were approximately linear and unremitting over time. This is particularly interesting and relevant for clinical trials for several reasons.

Firstly, it is consistent with the model of damage accrual being relatively steady even though the crossing of clinical disability thresholds clearly is not.

Secondly, that a) higher MMEP burden is associated with faster time to confirmed disability progression (by both EDSS and fused EDSS-MSFC rating systems), b) MEP-4 bears cross-sectional association with greater EDSS score and c) electrophysiological change appears to antecede clinical changes by several years, supports the contribution of ongoing insidious inflammatory demyelination to delayed manifestation of disability. This view is supported by old (438) and emerging data (218) from clinical investigations of anti-inflammatory agents studied outwith the typical duration of standard clinical trials demonstrating delayed albeit modest benefit unapparent during the initial 24 month treatment window. Therefore although axonal loss is a clear associate and functional determinant in the context of progressive MS, as pathological studies (1) and response to anti-inflammatories (173) would suggest demyelination is also likely a

driver even in later stages (46). The consistently observed prevalence of inflammation-related contrast enhancement at baseline in the large scale PPMS treatment trials (231, 281) (and the positive treatment response particularly where this is present(280)) also supports this view. MMEP appear usefully sensitive to both processes of initial demyelination and ensuing axonal loss.

Thirdly, structural imaging of the cord would suggest that not only is it affected particularly early in the course of progressive disease (196, 197) but the rate of atrophy is front-loaded before possibly reaching a plateau; akin to the dynamics of RNFL layer atrophy following optic neuritis (397). MMEP appear sensitive to the ongoing functional decline of the cord in a phase after which such a plateau has likely already been reached and patients are in fact typically seen to initially present (151).

With respect to achieving the goal of being able to conduct small but sensitive translational studies with high predictive capacity for gauging phase III outcomes, on the basis of our investigation we posit this may be possible. Employing long tract MMEP sensorimotor batteries to initially enrich for subjects likely to cross a threshold with confirmed disability progression within the immediate 12 month period (by EDSS or EDSS-Plus) could greatly reduce the required recruitment scale. Alternately, those predicted to manifest a longer interval to progression but still likely to do so could be selected for delayed enrolment.

Our results along with those from other groups (162, 181, 183, 184, 188, 189, 191, 315) suggest MMEP are sensitive to long tract demyelination which will likely translate into disability in the near to medium term. Individuals with a higher MMEP burden would therefore make excellent candidates for neuroprotective studies whereas those with lower MMEP burdens, i.e. at an earlier biological stage of disease perhaps make better candidates for anti-inflammatory agents.

In comparison to the EDSS, the finer granularity of objective EP quantification coupled with the steady trend to worsening MMEP burden (in cohorts) over time during the progressive phase would further contribute to a smaller scale of investigation to achieve statistical power. The results of our power calculation compare very favourably to those published for similar cohorts using EDSS based outcomes (213) and composite variations(425). We acknowledge larger datasets would greatly improve the accuracy of such estimations. An additional advantage of MMEP would be the resistance to the under-powering effect conferred by recruiting patients already at EDSS 6.0, who by virtue of the rating scale spend disproportionately long periods at this level (148).

Given the foregoing discussion it is unlikely that an ability to preserve or improve long tract functional integrity would not translate into an outcome of real world benefit on scales accepted by regulatory authorities.

The conduct of MMEP, restricted to the central motor and somatosensory potentials of each limb is readily practicable, logistically widely available and

more affordable than contemporary cord imaging. The recordings are directly quantifiable in a standardised manner and most importantly the technique is extremely well tolerated. No recruited individuals discontinued as a consequence of the chosen methodology.

Consensus on the optimal means of MMEP quantification is outstanding and larger independent datasets are desirable to reaffirm and refine the approach. We have focussed on the MEP system on the basis of our previously published comparison to other methods. However, incorporation of MMEP as an outcome measure, alongside conventional EDSS and MSFC ratings in progressive clinical trials would be broadly useful. Some investigators have already insightfully made such methodological inclusions.

An additionally important focus for future work will be to establish the shorter and medium term variability of EP recordings, attained by ICFN standards in the context of disease and where possible demonstrate cross-correlation with structural indices of myelin integrity, damage and repair along their respective long tracts.

CONCLUSION

MMEP incorporating sensorimotor evaluation of all four limbs display moderately strong cross-sectional correlation with physical disability, track with underlying progressive deterioration and predict likelihood of clinical progression in the near, medium and (as seen elsewhere) longer term. The technique also offers sensitive scaled quantification of disease-related dysfunction over the time period and disability range typically encountered during translational investigations. This heralds a possibility of reducing clinical trial sizes in the PPMS phenotype with an attendant reduction in risk and increase in feasibility with recruitment of the small available pool into broader and more numerous clinical investigations. Further exploration of this promising technique, at this challenging time is surely warranted.

VI

Closing the 'Cognitive Gap' in Multiple Sclerosis

Cognitive dysfunction is highly prevalent in patients with Multiple Sclerosis, being present in all stages and phenotypes however becoming more apparent and disabling with advancing progression (166, 249, 423, 439-447). Large sampling studies (448-450) suggested the existence of a **'cognitive footprint'** of MS, with particular difficulties across the domains of processing speed and episodic memory for verbal and visual elements. Unfortunately most other cognitive faculties are seen to be additionally affected, including executive function and whilst there is considered to be some relative preservation of language and longer term memory (29) they do not escape unscathed.

The consequences of MS related cognitive impairment (CI) are profound and have only in recent years begun to receive the acknowledgement and attention they deserve. Much, if not most, focus has been on the physically disabling nature of the condition (249, 268), as typified by the strong bias toward such problems in the clinical disease severity rating scales such as the EDSS. However given that half of those people who lose employment due to MS do so at or before the 'mild' physical disability rating of EDSS 3 (29) suggests the impact of CI in MS is at least equally devastating as the physical symptom burden.

Even prior to losing employment outright, MS patients commonly experience restrictions on working hours (205, 449) and productivity whilst facing escalating risks of adverse work-related events of various types including accidents(451). Outside of the working environment, CI heralds significant reductions in social activities and frequent need for assistance with activities of daily living (ADLs) (29, 449, 452). All of these are accompanied by a profound fall in Quality of Life brought by CI and are independent of the general effect of concurrent physical disability (418). From a practical perspective CI also carries implications for medication concordance and driving safety (249).

Estimates of the actual prevalence of measurable CI amongst MS patients typically vary between 30-70% (401, 441, 448, 453) and much of the variation is attributable to inter-sample differences, the neuropsychometric properties of the cognitive testing batteries used and the actual classification criteria deployed (441). Generally the prevalence amongst hospital samples is greater than those in the community (50-60% vs. 40% respectively (448, 454)).

It is recognised that MS related cognitive difficulties are initially subtle and confined to specific domains early in the clinical course of the disease (423, 441, 445, 455) but unfortunately become more extensive and severe with disease

progression (249, 439). Notably, in comparison to other functional systems impacted upon by MS, decline in the cognitive domain is particularly unlikely to remain stable and rarely improves with time (29).

Following the general acceptance that routine neurological evaluation is relatively insensitive to the presence and severity of CI (401) and that standard bedside cognitive evaluation with tools such as the Mini-Mental State Examination was almost equally unreflective of the dysfunction attributable to MS drove the development of three major neuropsychometric initiatives in the field of MS cognition.

Firstly the Brief Repeatable Battery (456) comprised five elements targeting processing speed, episodic memory and language and was superseded by the consensus driven collection of seven tests into the 'Minimal Assessment of Cognitive Function in Multiple Sclerosis' (MACFIMS) which additionally interrogated spatial processing and higher executive function (416, 450). Despite being compiled with the hope of engendering wider deployment of a standard set of neuropsychometric tools sensitive to the particular difficulties in MS, the ongoing specific requisite of a neuropsychologist to both administer and interpret the results in addition to the average performance time of 60-90 minutes has not facilitated broad use and was recognised as a serious limitation to its use for routine clinical screening for CI(401). This prompted further consensus driven refinement of the initial 7 components of the MACFIMS down into the 3 felt to be most sensitive and relevant to MS, namely the SDMT, BVMT-R and the CVLT-2 (401, 457). Collectively these tests form the Brief International Cognitive Assessment in Multiple Sclerosis (BICAMS) and excluding any delayed-recall elements used in their original formulation take approximately 15 minutes to perform and can be administered by non-specialist healthcare providers (401).

Following their recent advent, at the time of writing international normative data is currently being acquired and validation is underway(457). Several important questions remain unanswered regarding the use of these tests. Notably from a measurement perspective although the instruments appear more suited toward detecting change over time rather than absolute presence or absence of MS related CI *per se* the optimal test-retest intervals remain undefined (Dr. M. Newson, *personal communication*). Additionally, the use of such a battery in the routine clinical care of MS patients brings an ethical challenge to replace the logistical obstacle of its predecessor. Although the latest NICE guidelines for MS care delivery advocate the general pursuit and assessment of cognitive difficulties (NICE 2014), given the absolute lack of any meaningful licensed therapy, pharmacological or otherwise to improve such problems(38, 458)(see table 16 for a review of agents trialled) such recommendations are somewhat at odds with the generally accepted Wilson Screening criteria (459) still adopted by the World Health Organisation. Whilst it is recognised that cognitive performance may be related to a number of frequently co-existing factors which are equally 'invisible' to routine clinical evaluation, namely depression, sleep disturbance and fatigue,

and intervention aimed directly at these may produce secondary cognitive benefits, they are all screened for in their own right and as such additional cognitive 'screening' may be unnecessary from this perspective. The prospect of very real psychological harm arising from awarding a diagnosis of cognitive impairment or even pursuing it, without a therapeutic avenue available should not be underestimated. The contemporary example of the impact of the Directed Enhanced Service initiative of dementia screening by General Practioners for NHS England has already begun to highlight this (460).

Table 15 Randomised Controlled Trials of Candidate Therapeutics to Improve Cognition In MS. Studies have been heterogeneous both with respect to paradigm and recruitment, in addition to being generally small, short and without clear relation to indices of real-world function and clinically-meaningful change (37). Whilst a recent RCT of Dalfampridine (n=120) has demonstrated significant benefit over placebo at 12 weeks on the SDMT, this work presented at the 2017 AAN(476) has not been published at this time. Also despite a growing literature of studies examining the potential benefits of cognitive rehabilitation the evidence base supporting their general application in practice at this time is considered weak (477).

CLASS	AGENT	Exploration	Outcome Metric	Findings
Stimulant	Modafinil	4 Blinded RCTs(461-463), Peak duration 4 months, Total N=129	SDMT, PASAT, Trail making, Alertness Testing, Cognitive Fatigue on MFIS	only 1 positive trial
	Amantadine	1 Blinded RCT(464), Duration 6 weeks, Total N=16	Multiple Tests	Negative Outcome
	l-amphetamine	1 Blinded RCT with dual analysis and 1 within-subjects placebo-controlled design study (465-467), Peak Duration 4 weeks, Total N=108	SDMT in one study, CVLT2 and Visual Memory Testing in other	Positive only for CVLT2/Visual Memory Testing
	Methylphenidate	1 single dose RCT(468), Total N=14	PASAT pre and post dose	Improved attention
Cholinesterase Inhibitor	Donepezil	2 Blinded RCT(469, 470)s of 24 weeks duration, Total N=96	Selective Reminding Test and Self Report	only one positive
	Rivastigmine	1 Blinded RCT(471), 12 weeks duration, Total N=30	Weschler Memory Scale	Negative
	Memantine	1 Blinded RCT(472), 16 week duration, Total N=58	PASAT and Selective Reminding Test	Negative
Potassium Channel Blocker	Aminopyridine	3 Blinded RCTs(473-475), Peak duration 6 months, Total N=97	Mixed Psychometric Tests	Negative

The Basis of MS related Cognitive Impairment (MSCI)

The advancing frontiers of neuroimaging have revealed numerous insights into the pathological substrate underlying CI in MS. The model of dysfunction has evolved from slowed processing being a consequence of delayed conduction following demyelination giving rise to largely 'subcortical' cognitive phenotype to a far more complex picture, involving disconnection (478-480) and failed integration of information both locally and globally within the cerebrum as a consequence of various pathological activities distributed heterogeneously and strategically throughout the brain substance (249, 268, 481-483).

With the traditional view of Multiple Sclerosis being predominantly an inflammatory condition affecting white matter of the CNS and with this tissue component being most amenable to visualisation by conventional acquisition methods on contemporary neuroimaging (249, 268), initial investigations sought to identify structural correlates of damage therein relating to cognitive dysfunction.

Historically the global macroscopic burden of white matter change evident on T2 MRI sequences revealed only very modest association with objective cognitive performance (196, 484, 485). Although one might initially ascribe such a dissociation to the heterogeneous pathological processes at work within T2 lesions (demyelination, oedema, remyelination, gliosis and axonal loss) (69) addition of T1 hypointensities (reflecting more substantive axonal loss and outright tissue destruction) did little to improve the degree of cognitive-imaging association and account for the degree of CI in MS (268, 427).

With the advent of techniques able to visualise and quantify pathological changes within white matter that appears otherwise 'normal' on conventional sequences (NAWM) such as Magnetisation Transfer Ratio (MTR) – a metric of myelin content derived from estimations of magnetisation transfer beteen free and bound protons (249, 263, 268) – has demonstrated a stronger degree of correlation between macroscopic white matter damage burden and cognitive performance than conventional metrics (485). Interrogation of white matter chemical composition and metabolism by means of MR Spectroscopy and Proton Emission Tomography respectively has further supported at least a moderate degree of association between global white matter disease and cognitive dysfunction (486).

More detailed exploration of the contribution of MS-related white matter pathology at the microscopic level *in vivo* has been afforded by the recent emergence of Diffusion Tensor Imaging and reconstructive tractography (180, 267, 271, 480, 487, 488). By magnetic resonance derivation of water molecule diffusion characteristics within submillimeter cubic volumes, voxels, it is possible to both

visualise tract presence, orientation and most importantly relative structural integrity (489).

Although general reductions in white matter integrity reflected by such metrics are commonly found within MS patients who are both cognitively preserved and impaired when compared to healthy controls (249, 268), several components of the white matter fibre skeleton are found to be more severely damaged in those patients with objective cognitive impairment. Namely these are the corpus callosum, forceps major, cingulum bundle, corticospinal tracts, fornices and both the inferior and superior longitudinal fasiculi (267). Additionally white matter pathways related to the brain stem, cerebellum and particularly the thalamus appear to be more significantly damaged in those patients exhibiting cognitive impairment (267). That lesion location determined by conventional MR sequences only partially correlates with tractographic white matter abnormalities speaks to the importance of lesion-independent NAWM abnormalities with respect to cognitive impairment in MS (268).

Although the presence of both macroscopic and microscopic damage in cerebral white matter, particularly that occurring within strategically vulnerable areas central to cognitive processes can be readily appreciated to explain a significant proportion of the cognitive burden in MS, it has not been seen to provide a complete explanation, or indeed account for much of the variance in cognitive performance observed in patient samples.

Further insights have emerged from consideration of the grey matter burden of disease in Multiple Sclerosis (490). As an entity it has been acknowledged from the very earliest clinic-pathological reports (namely from Dawson in 1916(491)), however with the relative insensitivity to grey matter pathology of routine para-clinical investigations its contribution to progression and cognitive impairment in MS went largely unexplored.

The past decade has seen a resurgence of focus on the paramount importance of grey matter pathology (82, 171, 175, 266, 298, 492-496) both that within the cerebral cortex and the deeper grey structures of the basal nuclei (497), deep cerebellar nuclei (498) and especially the thalamus (267, 426, 499). Disease activity within the grey substance which had previously gone unobserved because of technical factors such as small lesion size, poor contrast against the background of normal tissue and partial volume effects from adjacent cerebrospinal fluid (328, 500) has been rendered visible by use of double-inversion recovery acquisition and the improved spatial resolution possible with incremental magnetic resonance field strength (263, 501). This said, post-mortem correlations with ultra-high field MRI findings suggest that even the most contemporary approaches still fail to visualise a considerable proportion of grey matter pathology for the reasons above (266, 298, 501). Given the putatively central role for grey matter degeneration underlying part of the progressive aspect of MS (82, 490, 491, 494, 502, 503) and the consistent association with

cognitive embarrassment in the disease generally (496, 504-506) this insensitivity is unfortunate.

Accompanying the broad array of techniques quantifying parenchymal properties has been an assortment of volumetric approaches characterising the final outcome of neuronal loss, atrophy. This has been applied globally to the cerebrum((180, 246, 256-259, 268, 361, 426, 507)), to tissue subcompartments of grey and white matter (180, 186, 220, 225, 494, 502, 504, 508-513), to cortical areas and deep nuclei (267, 271, 426, 493, 499), by various means of abstraction including Voxel-based morphometry (514) yet with reasonably consistent results. Cortical atrophy, as evidenced by cortical thinning does demonstrate a relationship with cognitive performance (504) as does whole brain atrophy (268) albeit some years after disease onset likely after exhaustion of the functionally adaptive capacity of the cerebrum (59, 377).

Atrophic changes are seen throughout the entire natural history of MS (220), having been observed from the stage of Clinically Isolated Syndrome (159) and even clinically silent Radiologically Isolated Syndromes (515), forward into the relapsing-remitting phase (246, 259) before becoming most profound in progressive phenotypes (157, 196, 197, 226, 262, 516, 517). The typical rate of whole brain atrophy in MS is 0.5-1% per year (428) (normal health related decline is ~0.3%/year after attaining peak brain mass at 25 years (518) and appears to worsen relentlessly at a similar rate almost independently of the disease phenotype considered (519). Its deployment as a biomarker is not without significant methodological challenges (195) or susceptibility to artefact - particularly patient hydration status (520) but has nonetheless been met with some modest success in the phase II setting as exemplified by the recent and successful MS-STAT trial exploring a putative neuroprotective effect of statins in secondary progressive disease (521). However the ability to favourably change the dynamics of atrophy measures by pharmacological intervention in other phase II treatment trials has not translated into clinically meaningful success in pivotal phase III trials aimed at attenuating disability accrual (INFORMS) and a substantial and sustained cognitively-preserving effect of any agent remains equally undemonstrated(458), given the typically short 24 month trial durations and minimal use of validated cognitive outcome measures in most published studies to date (522).

Also, examination of the combined explanatory power of Brain Atrophy and Lesion Load measurements would suggest an ability to account for only approximately 20% of the variance of performance on objective cognitive tests (523), suggesting other properties likely exist with a greater potential to account for cognitive dysfunction in the setting of Multiple Sclerosis.

Whilst MMEP have much in their favour as candidate biomarkers of physical disability, our own analysis demonstrated no meaningful relationship with cognitive performance (314). This is unsurprising and highlights the need for an

additional approach to capture this important domain which becomes impaired in the majority of patients (29). The growing appreciation of the contribution of MS related cognitive impairment to real-world disability(524), occupational loss(418, 449), health economic burden and most importantly the quality of life of patients (418, 452, 525, 526), has not been met by a parallel surge of translational studies for cognitive interventions, with methodological constraints likely representing a greater barrier than any lack of putative candidates worthy of test (527).

The tightest relationship between cognitive performance and conventional imaging metrics are those of atrophy, in the cortical and deep grey structures particularly (249, 267, 322, 426, 427, 483, 485, 496, 505-507, 523, 528, 529). Newer modalities such as DTI and MRS which disclose pathology in 'Normal Appearing White Matter' and 'Normal Appearing Grey Matter' also bear some relationship to cognitive performance particularly in frontal and limbic regions (267, 426, 480, 530-533). However, such volumetric properties typically represent substantial and to date irreversible tissue loss with the result being an inability to manifest dynamic response to intervention, especially over short term intervals.

Cerebral tissue is the structural medium from which cognitive processing arises, however it is both the quantity and quality of functional coupling within and between specialised regions that provides the actual substrate of human thought (534, 535). Measurement of such coupling should offer superior relationships with objective cognitive performance compared to structural metrics and this has recently been demonstrated through the application of fMRI to key processing regions (536). However the temporal resolution of this modality is fundamentally constrained by the dynamics of neurovascular coupling which generate its output (537), and which are also demonstrably perturbed in Multiple Sclerosis (538).

In contrast, neurophysiological output is time-locked directly to the cortical neuronal activity generating cognitive processes(296). Any inferiority in spatial resolution is more than compensated for by superior temporal (millisecond) resolution – which is of heightened relevance in the investigation of a condition wherein the dominant feature of its 'Cognitive Footprint' is reduced Information Processing Speed (IPS) (479).

It has long been appreciated that Cognitive Evoked Potentials can be elicited in an almost identical manner to those in the primary afferent pathways considered above (313). In this instance the stimulus is typically a modality-independent discrepancy or 'oddball' embedded within a stream of 'regular' presentations. The attention-based decision in cognitive recognition of difference elicits a characteristic time-locked positive waveform ~300msec later (539). Several studies have demonstrated a prolongation of such P300 latencies in the setting of MS in a manner associated with IPS(424, 540-543). Recent methodological consensus(539) and normative data (544) from large cohorts have also emerged. It has been observed to dynamically improve over short intervals in response to the use of Methylprednisolone for MS relapse (540) and also Modafinil for MS

Fatigue(545). The impact of formal immunomodulatory therapies on P300 latency has been explored in small cohorts with varying outcomes (546, 547) from which it is difficult to make conclusive inference.

Understanding of the physiological mechanism underlying the P300 waveform itself remains incomplete (548, 549) which is perhaps a limitation to making deduction about effects and its wider implementation, nonetheless it does – at least at the group level, offer an index of attentional decision making speed (550).

A lower-level sub-awareness response to detection of novelty or change with otherwise identical auditory paradigms is Mismatch Negavity (MMN), seen as a negative deflection typically 200msec post stimulus (539). Although the neuroanatomical basis of this passive response is better delineated and less dependent on any active engagement its exploration in MS to date is limited. Nonetheless its aberration has demonstrated positive cross-sectional relationship to severity of cognitive dysfunction in a modest sized cohort of MS patients and more widely in a broad range of neuropathological settings (253, 551, 552).

Examination of the standard resting state clinical electroencephalogram by means of spectral decomposition using familiar Fourier Techniques and similar has demonstrated a consistent slowing and weakening of power particularly in the human alpha band (8-12Hz) in 40-79% of MS subjects in a manner which meaningfully relates to burden of cognitive dysfunction (553, 554).

However, the most powerful application of EEG to generating biometric indices of cognition may come not from routine specialist evaluation or such quantitative analysis but from using time series data recorded at each scalp electrode to represent nodes in a network and statistical dependencies between such series to weight the estimates of functional coupling between them (534, 535).

Cross Channel Coherence analysis had already demonstrated reductions in large scale connectivity as a corollary of cognitive dysfunction in MS subjects (555) prior to the recent larger scale investigation of 349 patients by Schependorm *et al.* (478) wherein newer less-biased connectivity estimations of Synchronisation Liklihood (556) and Phase Lag Index were applied to routine clinical EEG recordings and used to form the basis of network models analysed by standard Graph Theoretical techniques. Now it has been possible to demonstrate that MS not only confers a quantitative reduction in connectivity (555, 557) but also a degradation in the qualitative arrangement of remaining couplings, which itself is quantifiable by Graph Analysis (478), yielding objective metrics which positively relate to cognitive performance in their own right.

Fulfilling the very real promise that logistically simple but computationally complex EEG based brain network analysis has for the study of MS (akin to that seen in Alzheimer's (558), Fronto-Temporal Dementia (559) and other settings(560, 561)) will require overcoming similar challenges to those seen with MMEP batteries. Choice of optimal reference, coupling measure, resting state

condition and graph metrics – including thresholds, weighting systems and similar, will all need clarification (562, 563) and ultimate consensus agreement. Establishing the relationship to cognitive outcomes and dynamics over time will also be essential.

The model of initial brain network adaptation prior to decompensation is similarly advocated (59) for the cognitive outcomes of MS but output from fMRI based network analysis to date is recognised to have been conflicting. This is likely consequent of general methodological heterogeneity between such studies (59) and also the peculiar dissociation in MS between neuronal activity and the metabolic demand which drives neurovascular coupling, itself the surrogate of fMRI (538). In contrast, the picture emerging from EEG and MEG studies to date is supportive of functional disconnection and network collapse underlying cognitive failure in MS (478, 554, 564-570).

Given that the very architecture of the brain is organised into a hierarchical network (535) with qualities including so-called small world architecture (571) conferring its efficiency, and the very integrity of this system rests upon the presence of selectively emplaced long range fibres with myelination tuned (within individuals) to confer precise communication and integration of information over vast spatial extents with millisecond timescales (307), a cognitive biomarker using this as its conceptual basis is likely to be particularly useful in MS.

Identification of a reliable cognitive surrogate, through EEG analysis would enable application of a widely available and inexpensive technique with unbiased outcome production. It would also enable inclusion of the not insignificant number of patients currently excluded from clinical trials on purely mobility grounds (148, 213). Given the broad range of electrophysiological candidates available for test and a similarly heterogeneous approach for clinically gauging cognitive dysfunction, as a prelude to further neurophysiological exploration we should first attempt to define if possible a unified construct of MS Cognitive Impairment and with it a composite metric with which we can judge the potential meaning of candidate surrogates of cognitive dysfunction thereafter.

VII

Towards A Global Cognitive Outcome Measure in Multiple Sclerosis by Means of Principal Component Analysis: The MS-Q Score

Objective To derive a weighted composite metric of Multiple Sclerosis Cognitive Impairment (MSCI).

Background Principal Component Analysis is an established technique for quantifying key factors responsible for variance across distinct but related domains.

Method 100 Cognitive datasets from MACFIMS and BICAMS psychometric assessments were collated with the accompanying demographics required for scaling against established regression based norms. Principal Component Analysis was performed to yield a composite MSCI score herein termed 'MS-Q'.

Results Multi-domain cognitive impairment was identified in 76-83% of the cohort. A single extractable primary component was found to account for 65% of variance ($p<.001$) in the three BICAMS subtest scores. This factor (the MS-Q) strongly correlated with the number of abnormal BICAMS tests ($Rs=-.912$ $p<.001$) and number of domains impaired on the more extensive MACFIMS battery ($Rs=-.883$ $p<.001$). A cut-off MS-Q score above 1.97 predicted freedom from multi-domain impairment with 89% specificity, (AUC .938 $p<.001$).

Conclusions After adjusting raw scores for demographic factors known to affect cognitive performance it is possible to generate singular composite scores which account for an impressive majority of the variance observed across the familiar psychometric batteries, thereby quantifying the abstract property of MSCI as desired. Adjustment for individuals' premorbid intelligence is especially critical.

Plain Language Summary A key challenge in deriving a biological measure which meaningfully reflects the amount of thinking and memory difficulty commonly experienced by patients with Multiple Sclerosis is that many different techniques have been employed to detect impairments across the broad range of abilities humans make use of in their day to day lives and occupations. To identify and in doing so quantify the amount of cognitive impairment related to Multiple Sclerosis in an individual we used a validated statistical technique which took a person's performance across a range of commonly used tests to extract a single factor which reflected the scale of gap between how they fared versus how they might have performed if they were not affected by disease.

This single factor was seen to significantly relate to the number and extent of failures on the many standard thinking and memory tests used in the field of Multiple Sclerosis research and after appropriate adjustment for age demonstrated a cumulative worsening with likely disease duration. The identification of a meaningful single measure of cognitive disability was an important precursor to the pursuit of other biological features which might relate to it. How such scores relate to a person's function in the real world will be a focus of future work.

Team & Contributions

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Dr David Cottrell & Dr Kirsty Inglis	Project Oversight
Dr Paul White, Dr David Western	Statistical Oversight
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INTRODUCTION

Cognitive Impairment affects the majority of patients with Multiple Sclerosis (29, 166, 212, 423). It is responsible for much of the unemployment (418, 440, 449) associated with the disease, is present from the earliest stages of the condition (441, 444, 445, 525, 565) and contributes heavily to the misery experienced by patients (418, 449, 452, 525) and those who love them. Currently there are no proven interventions which prevent or meaningfully ameliorate cognitive decline due to Multiple Sclerosis. Methodological issues have been cited as central limiting factors hindering effective translational research in this important field (572).

Although several neuropsychometric tests have demonstrated sensitivity to the various domains affected by the *'cognitive footprint'* of Multiple Sclerosis (573) their use as candidate primary outcome measures for clinical trials alone has not been accepted by regulatory authorities. Efforts to combine them into psychometric batteries (401, 450, 456) have offered systems of assessment which are sensitive to the presence and burden of MS-related cognitive impairment (MSCI). Such approaches have given insight into the phenotype (29, 448), natural history (418, 439-441, 455, 529, 574) and consequences of MSCI (418, 449) but not as yet offered a singular composite metric to serve as a primary trial outcome measure. Several published attempts to generate such metrics have used arithmetic averaging of scores normalised against individual's healthy demographic peers (575, 576).

Although offering a scaled interval system of measurement such attempts do not necessarily have the required ecological validity a desired cognitive outcome requires for conducting effective translational studies, as would be demanded by regulatory bodies.

Improving real-world cognitive outcomes will require a metric that is sensitive to the difficulties bestowed by MSCI but weighted in a manner that respects their individual contributions to cognitive disability. Epidemiological studies have demonstrated the relative primacy of reduced information processing speed (479) in this regard over other domains such as visuospatial function and particularly linguistic function. MSCI is the construct representing the *reduction in cognitive function from where an individual would be were it not for the presence of disease*

and is that which we wish to address by intervention. The impact of MSCI on all cognitive domains is *important* but certain effects are clearly more disabling (574, 577) in the real world than others. No single test sufficiently captures the breadth of MSCI. Averaging across multiple tests with and without criterion based classification of impaired vs. preserved inappropriately equates change within separate domains and ultimately lessens our handle on that which we hope and ultimately need to measure.

In recent years two cognitive batteries, the Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS (450)) and subsequently the Brief International Cognitive Assessment in Multiple Sclerosis (BICAMS (401)) have been compiled to aid in the diagnosis and monitoring of MS cognitive deficits. The latter comprises the three most sensitive and deployable component tests of the former larger system of seven tests with delayed recall elements. Normative data are established for the component tests, with regression based norms allowing scaled adjustment for age, gender and years of education (400). Both are included in the AAN 2015 MS Quality Measurement Set and have been used in our local tertiary practice and research. The BICAMS in particular is developing a growing international pattern of use and implementation given its simplicity of administration and cross-cultural versatility (578). Its elements have already seen experience as secondary outcome use in large scale therapeutic trials ('EXPAND' NCT NCT01665144, 'ORATORIO' NCT NCT01194570). Given the use of this consensus driven (401) instrument in trials is likely to grow and National guidelines for care of MS patients (579) recommend the routine assessment and identification of cognitive dysfunction within our patients we sought to explore a means to generate a composite score from such batteries. We selected Principal Component Analysis (PCA) to generate a singular metric of MSCI by identifying the presence of a key factor driving a significant majority of the variance in scaled performance scores across tests from the two batteries.

PCA is a well-established and widely used statistical method for quantitatively revealing the internal structure of multivariate datasets the variance of which is often related to a smaller number of factors. Similar Factor Analysis techniques have been applied broadly and successfully in other contexts including psychosis (580), traumatic brain injury (581) and non-MS cognitive disturbance (582) to permit quantification of various abstract constructs. To our knowledge this has not as yet been performed in the context of MSCI.

METHOD

We pooled anonymised cognitive datasets (n=100) from two recent observational clinical investigations conducted at the Bristol & Avon MS centre (UK REC Ref.15-NI-0042 SWiNd-MS and UK REC ref. 06/2007/7 Multimodal Neurophysiology in Primary Progressive Multiple Sclerosis) and cognitive assessments performed following clinical indication by our resident clinical neuropsychology colleagues. Permission for the sampling was granted by the Research & Innovation Department of North Bristol NHS Trust (with United Kingdom Health Research

Authority Approval IRAS 208519) and all analysis was conducted at the Bristol Brain Centre of Southmead Hospital, Bristol.

Data collated included age, gender, MS phenotype, use of DMT, years of education, score on the Test of Premorbid Function, estimated disease duration and EDSS where available. All subjects were actively under the care of Neurologists from the local Bristol & Avon MS (BrAMS) service and had established diagnoses of Multiple Sclerosis consistent with McDonald Criteria (8).

Subjects also typically had subjective evaluations of their cognitive dysfunction according to the Perceived Cognitive Deficits Questionnaire (PDQ) and had rated their affective burden on the Depression, Anxiety and Stress Scale (DASS-21(409)). Cognitive data was only included from subjects without significantly confounding affective disturbance and where there were no concerns about motivation having limited performance.

The objective tests comprising the MACFIMS include the Paced Auditory Serial Addition Test (PASAT, 2 second and 3 second versions), Symbol Digit Modalities Test (SDMT), California Verbal Learning Test – 2nd Edition (CVLT-2), Brief Visual Memory Test – Revised Edition (BVMT-R), Controlled Oral Word Association Test (COWAT), Judgement of Line Orientation (JOLO) and Delis-Kaplan Executive Function Sorting Test (DKEFS). The CVLT-2 and BVMT-R feature delayed recall elements. Their direct recall components coupled with the SDMT comprise the three tests of the BICAMS.

Raw scores were converted to scaled scores on the basis of cumulative frequency distributions attained in Healthy Controls and published by Parmenter *et al.* (400).

Two separate approaches were then used to derive T scores quantifying the difference in standard deviations between where a subject's performance was and its predicted outcome. These are outlined in figure 9.

The first employed the continuous regression based norm equations, again published by Parmenter *et al*., (400). These generate predicted scaled scores on the basis of gender, age, age squared and years of education.

Additionally, estimated premorbid IQ was used to refine the evaluation of the disparity between anticipated and actual performance on individual tests. Scaled scores >1.5 SD below IQ adjusted means were considered abnormal. Premorbid IQ had been estimated by either using the above demographics coupled with an occupational score as described by Crawford et al., (583) or alternately where available with performance on the Test of Premorbid Functioning (TOPF) as described by (584). Importantly the former also features age adjustment to yield a demographic estimate of IQ.

To our knowledge IQ-adjusted normative data for the MACFIMS tests do not yet exist, however it is appreciated that application of demographic based norms combined with the arbitrary 1.5sd cut-off threshold for defining impairment may

result in a considerable and functionally relevant degree of impairment going undetected. We therefore sought to address this by incorporating predicted scaled scores based on established techniques for estimating an individual's general intelligence relative to the population. We readily acknowledge the familiar disparity between *general* intelligence and performance on domain-specific testing, however this is still likely a smaller confounding factor than otherwise disregarding it entirely.

All statistical analyses were performed in IBM SPSS v.23. In tests of correlation Pearson's Coefficient and Spearman's rank were derived with bootstrapping involving 2000 iterations. Principal Component Analysis was used to identify the sought factor thought to represent MSCI. Kaiser-Meyer-Olkin tests of sampling adequacy and Bartlett's tests of sphericity were performed in addition to derivation of coefficients for each component test used in the analysis. The Direct Oblimin method was selected for dimensional rotation as the separate psychometric scores likely had non-orthogonal relationships. Data from complete MACFIMS and BICAMS batteries were considered separately aside from the overlap conferred by the inclusion of BICAMS tests within the MACFIMS.

All subjects had suitable BICAMS evaluations featuring the SDMT, CVLT2 and BVMTR. 54 of these had sufficiently complete MACFIMS assessments with the additional PASAT 3sec, PASAT 2sec, JOLO, DKEFS sorts and DKEFS description tasks, the COWAT and delayed recall elements of the BVMT-R and CVLT-2 tests. For the purposes of this analysis outputs from individual tests were grouped into five key domains and with impairment considered present if any test within a group was below the 1.5sd cut-off. Information processing speed (IPS) was measured by PASAT and SDMT performance, Frontal-Executive function by the DKEFS and COWAT, Visuospatial by JOLO and BVMTR, Language by the CVLT2 and Memory by the delayed recall elements of the BVMTR and CVLT2.

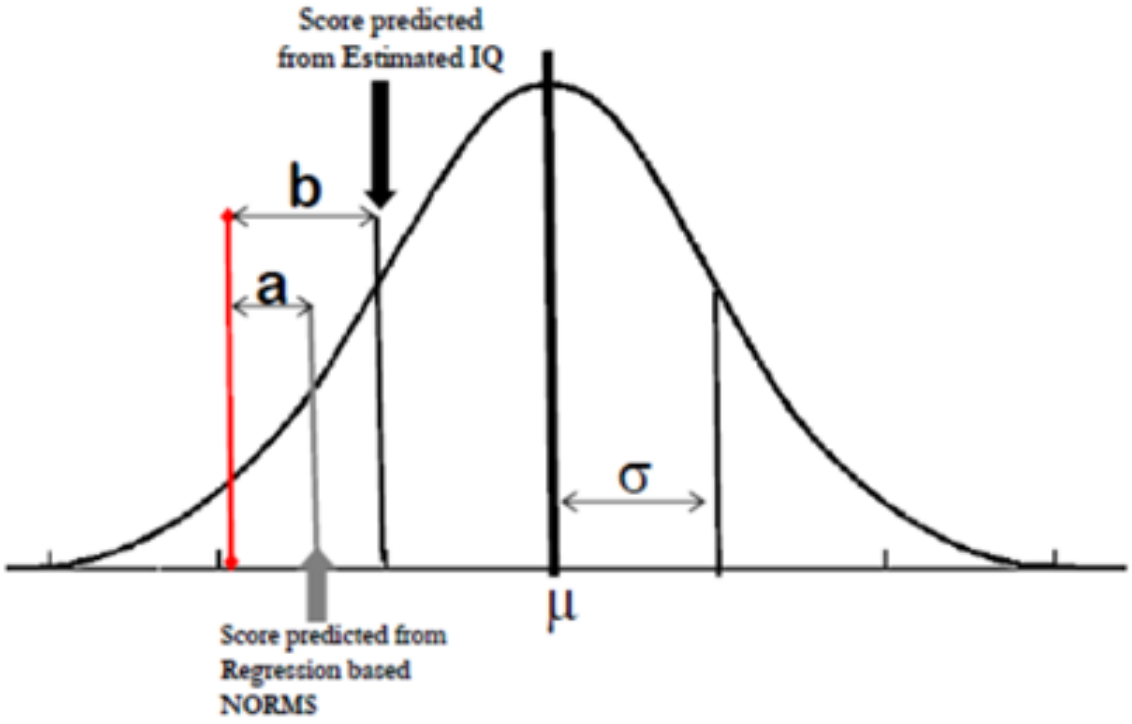


Figure 9 Determination of Cognitive T-Scores

For individual cognitive test outcomes the separation between actual performance (red line) and that predicted is of interest and considered indicative of MS related impairment. The difference (a) represents the number of standard deviations below a predicted scaled score based on the regression formulae published by Parmenter et al. (400) which take the form *Predicted Scaled Score = Constant + (β_1* age) + (β_2*age^2) + (β_3*gender) + (β_4*Years of Education).* The standard deviations, constants and coefficients are different for each separate MACFIMS component, are derived from healthy controls and published therein. The difference (b) represents the number of standard deviations below a predicted scaled score based on an estimation of a subject's premorbid IQ. In this case this either follows the form of *Est IQ=Constant+(β_1*age)+(β_2*gender)+(−β_3*Occupational Score)+(β_4*Years of Education)* as published by Crawford et al., (583) or the formal Test of Premorbid Functioning IQ scoring system (584). Based on the subject's IQ, their place within the population distribution of general intelligence is derived (mean 100, Sd 10) and transposed onto a predicted scaled score based on a mean of 10 and standard deviation as published by Parmenter (400). The cut-off for abnormal was set at >1.5sd below the mean in this case being a T-score <35.

RESULTS

Demographics. Datasets were examined from 100 patients with established diagnoses of Multiple Sclerosis compatible with McDonald Criteria. The mean age was 48 (SD 11) years, with a gender balance of 62 female and 38 male typical of our clinical cohort. The phenotypic spread had a similar real-world distribution with 43 relapsing and 57 progressive patients. The mean EDSS (recorded in the preceding 6 months) was 5 (range 1-8). The mean estimated premorbid IQ was 104 (SD 10.3), derived in 40 subjects by using the TOPF and in 60 by employing the Occupational score coupled with demographic determinants. The proportions of the cohort without and with mono- and multi-domain impairments expectedly varied by system of norm applied. Using the continuous regression based norms alone 76% had multi-domain impairment across the MACFIMS battery. Use of estimated IQ based cut-offs increased this to 83%, with both prevalence estimates being broadly consistent with the wider literature (29, 448).

Principal Component Analysis. PCA of the T Score differences between observed and predicted scaled scores on the BICAMS from 100 datasets was performed for both regression based norm values and also those using estimations of premorbid IQ. This was also performed using the T score differences across the whole MACFIMS battery with both systems in the 54 cases available. In each case formal indicators of factorability (Kaiser-Meyer-Olkin Measure of Sampling Adequacy and Bartlett's Test of Sphericity) were good and the residuals indicate the solution was acceptable. Scree plots of each PCA demonstrated dominant primary components with Eigenvalues >1 and given the nature of testing and adjustments made these were considered to quantitatively represent MSCI. Outcomes of the four analyses are in table 16 below. The component loadings are in table 17 with the system for deriving MS-Q outlined subsequently. The MS-Q was taken as the dominant principal factor from the BICAMS battery, herein termed MS-Q (Reg. Norms) when derived from regression based norms and MS-Q (Est IQ) when from the estimated premorbid IQ set. It is a standardised multi-decimal number ranging between +3 and -3 in this cohort.

System	KMO Sampling Adequacy	Bartlett's Test of Sphericity	Component 1 Eigenvalue (% Variance)	Component 2 Eigenvalue (% Variance)	Component 3 Eigenvalue (% Variance)	Cumulative % Variance by First 3 Factors
BICAMS (Regression Based Norms)	.651	P<.001	1.838 (61.9%)	.662 (22.1%)	.500 (16.7%)	100%
BICAMS (Est IQ)	.669	P<.001	1.943 (64.8%)	.601 (20.0%)	.456 (15.2%)	100%
MACFIMS (Regression Based Norms)	.739	P<.001	4.851 (44.1%)	1.738 (15.8%)	1.026 (9.3%)	69%
MACFIMS (Est IQ)	.827	P<.001	5.632 (51.2%)	1.367 (12.4%)	.936 (8.5%)	72%

Table 16 Results of Principal Components Analysis

Psychometric Test	MS-Q (Est IQ)	MS-Q (Reg. Norms)	MACFIMS Primary Factor (Est IQ)	MACFIMS Primary Factor (Reg. Norms)
SDMT	.791	.823	.788	.584
CVLT-2	.843	.734	.691	.745
BVMT-R	.779	.789	.783	.672
PASAT #3	-	-	.796	.723
PASAT #2	-	-	.796	.745
JOLO	-	-	.517	.486
DKEFS Sorts	-	-	.678	.733
DKEFS Description	-	-	.695	.686
COWAT	-	-	.543	.671
CVLT-2 Delayed Recall	-	-	.857	.736
BVMT-R Delayed Recall	-	-	.644	.434

Table 17 Primary Component Factor Loadings

Figure 0-2 The MS-Q Cognitive Composite Score

i. General Form:

$$MS-Q\ Score = \left(\frac{(SDMT_T - mean) * load}{sd} + \frac{(CVLT2_T - mean) * load}{sd} + \frac{(BVMTR_T - mean) * load}{sd}\right)/2$$

ii. For Use with Regression Based Norms to establish predicted scores:

$$MS-Q\ Score\ (Reg.Norm) = \left(\frac{(SDMT_T - 34.23344) * 0.823}{13.8765} + \frac{(CVLT2_T - 42.54364) * 0.734}{13.23594} + \frac{(BVMTR_T - 37.28002) * 0.789}{15.85125}\right)/2$$

iii. For Use with Estimates of Premorbid IQ to establish predicted scores:

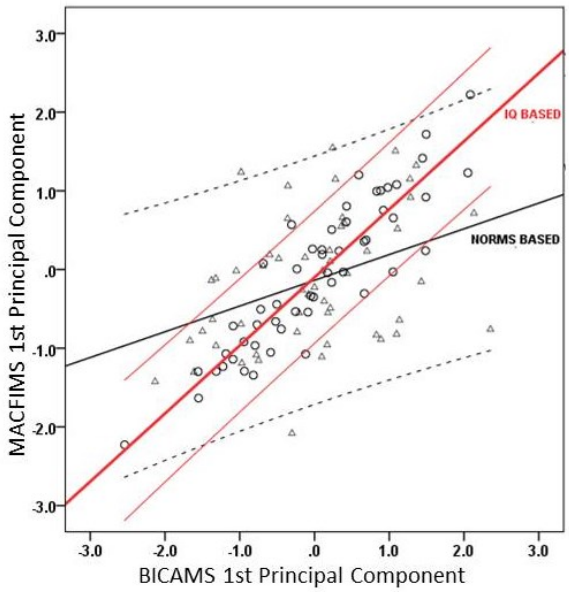
$$MS-Q\ Score\ (Est.IQ) = \left(\frac{(SDMT_T - 29.74833) * 0.791}{14.52328} + \frac{(CVLT2_T - 35.8217) * 0.843}{13.61011} + \frac{(BVMTR_T - 33.46135) * 0.779}{15.22564}\right)/2$$

Where $TEST_T = 50 + \left(10 * \frac{Scaled\ Performance\ Score - Predicted\ Scaled\ Score}{SD\ of\ Test\ Scaled\ Score\ in\ Healthy\ Controls}\right)$

Relationship between Factors. To examine the hypothesis that the principal component extracted from the BICAMS (and termed MS-Q) was sufficiently similar to that extracted from the larger MACFIMS battery, association between the two factors was examined. The Pearson's correlation between the MS-Q and MACFIMS principal component was modest at *r*=.431 (P=.001, n=54) in the case of applying regression based norms to generate predicted scaled scores. The association between MS-Q and the MACFIMS primary factor increased to *r*=.933 (p<.001, n=54) on introduction of IQ based scaled predicted scores. Our interpretation is that with this IQ based adjustment MS-Q is sensitive to (and predominantly driven by) the same construct producing the majority of variance in performance across the more extensive MACFIMS, namely MSCI (See figure 10) .

Figure 10 Correlation between Primary Components of BICAMS and MACFIMS

MS-Q is on the vertical and MACFIMS primary component is on the horizontal axis. The black line represents the linear association between factors derived by regression based norms (with 95% confidence intervals, r=.431 p=.001). The red line represents the far stronger linear association achieved by utilising IQ based predicted scaled scores (with 95% confidence intervals, r=.933***, p<.001).



Spearman's Correlation (r_s)	No. of Abnormal Tests on BICAMS (range 0-3)	No. of Abnormal Tests on MACFIMS (Range 0-11)	No. of Abnormal Cognitive Domains (Range 0-5)
MS-Q (reg. norms based)	-.863*** (p<.001)	-.816*** (p<.001)	-.827*** (p<.001)
MS-Q (Est IQ based)	-.912 ***(p<.001)	-.890*** (p<.001)	-.883*** (p<.001)

Table 18 Correlation between MS-Q and Cognitive Impairment on BICAMS & MACFIMS

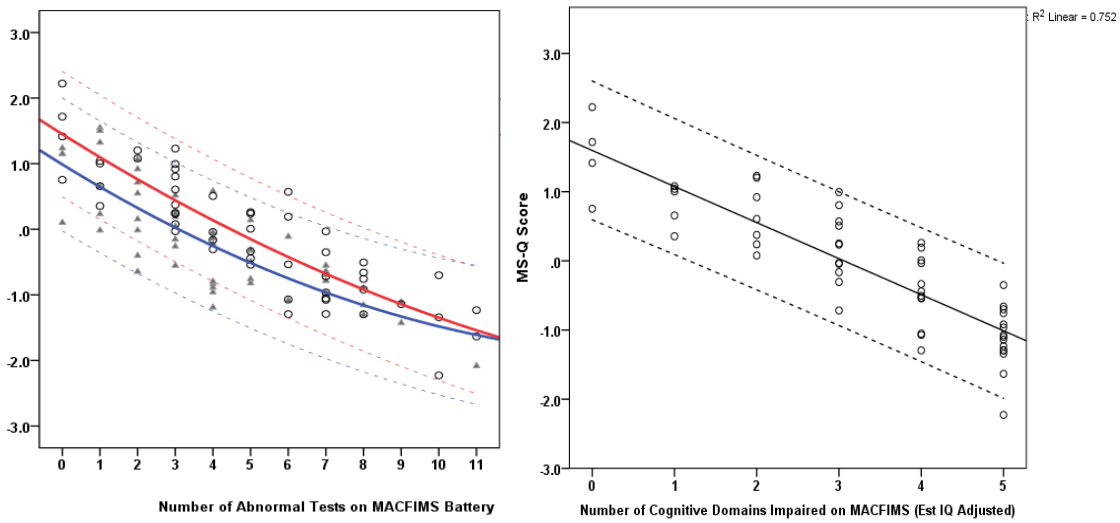


Figure (11) Left, Figure (12) Right.

Figure 11 MS-Q score against number of abnormal tests on MACFIMS

In this graph the values based on regression norms are in grey circles with the blue line representing the non-linear association with the number of abnormal tests on the MACFIMS battery, including 95% confidence interval (dashed lines). The darker circles and red line represent results from the estimated IQ predicted score approach. Although both systems display strong association, the IQ based system appears to account for a greater proportion of the observed variance (R^2 .670 versus .773 respectively).

Figure 12 MS-Q Score against number of cognitive domains affected.

The MS-Q score displays a near-linear association with the number of cognitive sub-domains considered abnormal on the full MACFIMS battery.

Relationship of MS-Q and Cognitive Impairment. The MS-Q displayed significant association with both the number of tests considered abnormal on the BICAMS and MACFIMS and also the number of separate cognitive domains affected, see table 18. This was the case for both the regression based norms and estimated IQ predicted scaled scores (see figures 11 & 12). For the MS-Q based on the latter it was possible to identify a cut-off score of 1.97 above which predicted freedom from multi-domain MSCI with 89% specificity. The area under the receiver operating curve was an impressive .938 (p<.001).

Relationship of MS-Q and other MS factors. The mean MS-Q was significantly worse in those patients with progressive disease compared to relapsing counterparts (-0.17 vs. 0.27, Student's T-test p=.021), however as expected there was a considerable degree of overlap. The association with EDSS in this cohort was weak (r_s=-.20 P=.05). It is well recognised that estimation of MS onset is notoriously unreliable, with the frequent presence of likely longstanding changes identified on neuroimaging at the time of index presentation. For the purposes of this analysis, given the marked longitudinal association of disease progression with age we took the latter as a surrogate co-variate of the former. A composite cognitive outcome system should display a negative association with age, once

natural effects of age had been accounted for. We found this only to be the case for the MS-Q system based on premorbid IQ estimations (r_s=-.49 P<.001), and the effect was even more pronounced (r_s=-.58 P<.001) when only considering those 60 subjects whose IQ estimations featured formal age adjustment in their derivation. See figure 13 (a&b). No significant association with subjective rating by the PDQ-5 (cognitive deficits) was observed by either system of MS-Q derivation in the 89 subjects in whom this parameter was available.

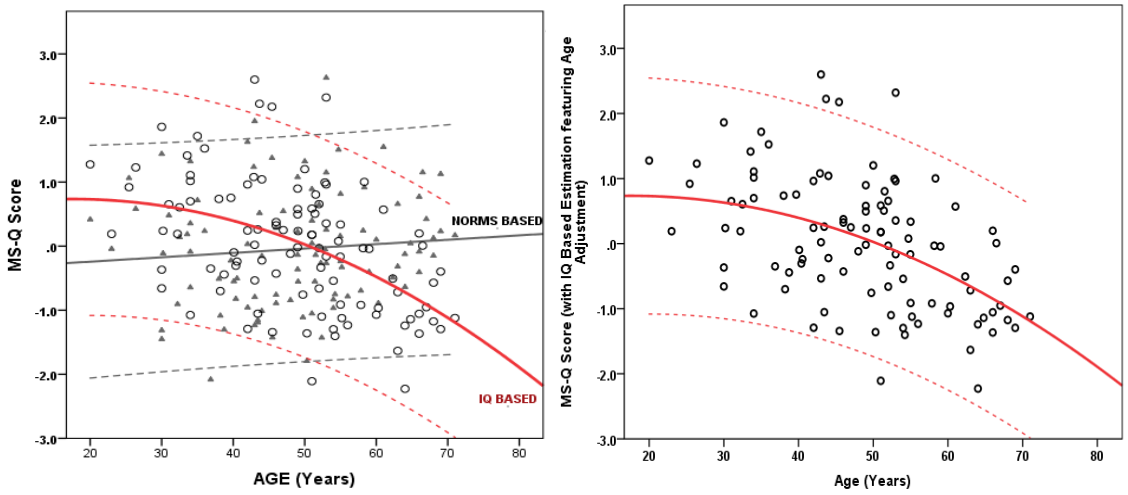


Figure 13a The Relationship of MS-Q and Ageing (left)

The small grey triangles represent values from subjects graded according to regression based norms, the solid black line represents the degree of association (or lack thereof) between age and MS-Q with this system. The black circles are values from MS-Q scores derived by IQ-estimation. The solid red line represents the non-linear decline of MS-Q over time not accounted for by age alone.

Figure 13b The Relationship of MS-Q and Ageing (right)

In this figure only MS-Q scores based on IQ estimated predicted scores which feature age-adjustment are represented (n=60). The degree of association is even stronger (r_s=-.583 p<.001) between the co-variates in this situation. The decline with age is considered to reflect the non-linear increase in cognitive burden over time with advancing disease beyond that attributable to natural ageing.

DISCUSSION

We sought to identify a single factor driving a significant majority of the variance in performance across the BICAMS battery which would directly reflect the extent of MSCI after other key determinants of cognitive performance had been taken into consideration. In this instance, from a clinically and demographically typical patient cohort, the standard technique of Principal Component Analysis was able to extract such a factor which we have termed the MS-Q. After further refinement and necessary validation it may serve as the desired composite cognitive outcome for the purposes of translational research and para-clinical biomarker evaluation. The MS-Q is an objective, scaled metric with fine granularity. It bears strong correlation with the number of abnormal tests on both the BICAMS and wider MACFIMS batteries. The linear and non-

linear association with the number of affected cognitive domains is equally strong. These relationships exist with both regression based demographic norms and the use of IQ-based predicted scores. Moreover, although one acknowledges the two batteries share some structural properties in their data, the relationship between the separate main principal components from the BICAMS scores and the broader MACFIMS is much stronger when estimated IQ is taken into account. This suggests that only after such adjustment are both Principal Components being driven predominantly by the same shared construct, here considered to be MSCI.

Collectively these findings suggest it is most unlikely that a substantial improvement in MS-Q would not translate into a real-world benefit.

Although the MS-Q is seen to be significantly worse in patients in the Progressive phase of MS, compared to their 'earlier' Relapsing counterparts the relative dissociation between this cognitive metric and the standard disability outcome of EDSS is in keeping with the wider literature. The subset of predominantly myelopathic yet cognitively intact patients (some of which featured here) who are typically excluded from conventional trials consequent of having an EDSS>6.5 is also painfully familiar. The lack of useful association with subjective rating of perceived cognitive deficits seen here is also recognised and if anything questions the utility of such self-reported outcome scales rather than the composite built on sensitive and validated objective tests.

The move away from discrete to continuous normative data by means of regression based modelling has been an important step in reducing instability in the estimates of predicted performance (400). Further adjustment for the important effect of the similarly continuous distribution of intelligence appears not only useful but necessary if we are to attain a cognitive composite sensitive to effects over time that might otherwise be 'drowned out' by the temporal co-variate of natural ageing. The optimal method of premorbid IQ estimation and adjustment remains to be clarified. This said, the relatively simple systems based on occupational attainment and/or familiarity with irregular words coupled with demographic factors as used here are rapidly performed in the clinic or bedside in a matter of minutes, with minimal training and participation requirements. Although imperfect, they may nonetheless prove quite sufficient. Furthermore, from a longitudinal perspective, aside from the age component they will not change after being initially established.

At present national MS practice guidelines advocate the proactive detection and assessment of cognitive difficulties, which will affect many but by no means all patients. The MACFIMS, by its nature is considered a 'Minimal Assessment' and its conduct requires considerable testing time (~2 hours) from trained neuropsychological staff, logistically in short supply. The MS-Q appears to have impressive specificity for detecting which individuals are likely to be free of significant multi-domain impairment on the wider MACFIMS (even when using arguably more stringent cut-off criteria). The contributing BICAMS tests can be performed by non-psychologist staff within the confines of a short 15-20 minute consultation. International norms are increasingly

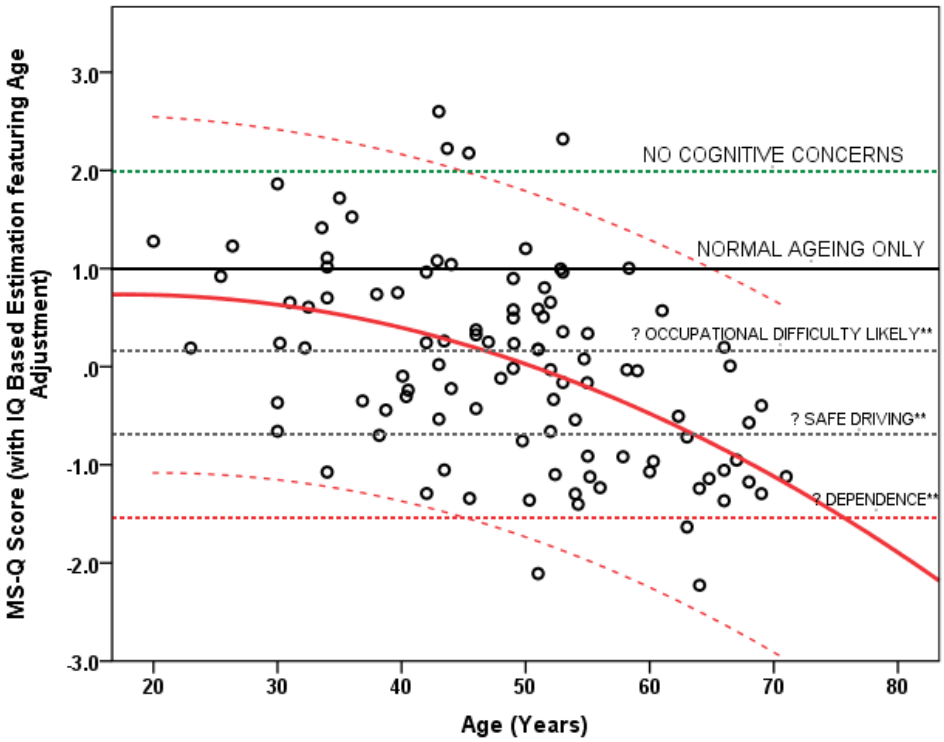
available and the actual formula based derivation of the MS-Q lends itself well to application-based tools on portable digital media such as tablets and worksheets.

The MS-Q metric is a standardised parameter, itself based on the extracted principal component analysis and in our cohort varies between +3 and -3 with a gradation of several decimals therein. It would be a simple matter to introduce a constant and coefficient to produce positive whole number scores with preserved granularity of measurement. Issues related to test-retest reliability, short-term variability and practice effects on the individual BICAMS components are if anything likely to be reduced by the combined steps of alternating stimulus forms, applying scaled scores and subsequently deriving a higher-order composite score. This likelihood warrants further evaluation. It is already acknowledged tablet-based application delivery may enable automated ratings to overcome challenges of inter-rater variability with the subjective aspect of the BVMT-R (585). Importantly the automatic output of a singular index of MSCI could enable clinicians to both capitalise on the many psychometric and practical advantages of the BICAMS instrument (585) to more clearly inform real-world decision making, related to counselling on occupational factors, driving safety and care dependence. Defining '*probability of difficulty*' thresholds for each of these key areas and for the presence of MSCI is at the least theoretically possible (see figure 14) and would likely have high utility and greater accuracy than the standard neurological clinical judgments (586).

Larger datasets would define such thresholds and also enable refinement of the factor coefficients used to derive MS-Q scores. Characterising the formal longitudinal change over time would also inform the statistical parameters needed for translational research, elucidate the relationship of MS-Q to the pivotal real-world outcome of employment status and further ratify the model of gradual and non-linear decay in cognition over time, from the earliest stages of the disease.

Figure 14 A Model of Using the MS-Q to define 'Probability of Difficulty' Thresholds amongst MS patients using the BICAMS panel

*** such values are currently undefined and therefore hypothetical.*



The non-equal weightings of the various scaled scores to the MS-Q reflect the informative contribution each test makes but in a manner perhaps more congruent with functional outcomes. Deficits in information processing speed should not be equated for arithmetic convenience with those of visuospatial function for instance and the MS-Q approach respects this; all scores provide influence in a proportionate and non-equal fashion. With a growing legion of putative para-clinical biomarkers of cognition emerging from neuroimaging, biochemistry and neurophysiology, identifying those which meaningfully relate to the construct of MSCI as opposed to a confusing subset of the MACFIMS or BICAMS batteries (possibly arising just by chance from multiple comparisons) will also be increasingly important.

CONCLUSION

A single dominant factor underlying much of the variance in performance across the BICAMS and MACFIMS battery has been identified by Principal Components Analysis. This composite cognitive outcome score has been termed the MS-Q and it is strongly associated with the global burden of impairment across multiple domains. The MS-Q scale offers high granularity of measurement, is rapid to acquire and simple to derive. Its promise as a composite cognitive outcome in MS surely deserves further exploration.

VIII

Are There Smaller Worlds in Neurodegeneration ? : A Pilot Exploration of Electroencephalographic Connectivity & Cognitive Evoked Potentials in Multiple Sclerosis

Aims To develop a neurophysiological cognitive biomarker system to facilitate translational research in Multiple Sclerosis. **Background** MS related Cognitive Impairment (MSCI) is a common feature of the condition. A lack of adequate metrics has been prohibitive of the necessary translational investigation. Our group has explored the utility of neurophysiological evoked potential techniques as surrogates of disability in progressive MS patients with some success. Identification of a neurophysiological cognitive biomarker which may complement the existing physical MMEP battery is desired. **Method** 30 patients with Multiple Sclerosis were recruited (20F,10M, Age 45.4y+/-11, Phenotype 13 RRMS,17 PMS) to undergo resting-state EEG (10:20 system, 512Hz Sampling, 0.1-70Hz, nasal reference) before and after neuropsychometric evaluation with the Minimal Assessment of Cognitive Function in Multiple Sclerosis battery to yield indices of MS Cognitive Impairment gauged by test failure and the derived MSQ Score. Cognitive Evoked potential batteries were also performed using an auditory stimulation paradigm to yield N200 Mismatch Negativity and P300 responses (P3a and P3b). Subjective ratings of important covariates of cognition, namely affective status and fatigue were attained using the DASS and NFI-MS respectively. EEG analysis focussed upon between channel intra and inter-hemispheric connectivity and the use of such measures to evaluate higher-level network qualities by graph theoretical analysis. Cerebral dynamics were also explored by examination of oscillatory phase changes (phase slip) within the alpha band and the relation to cognitive Information Processing Speed evaluated. **Results** Cognitive Evoked Potential scores, both individually (MMN, P3a, P3b) and collectively as a composite CEP-3 score demonstrated significant association with the extent of MSCI as judged by test failures on the MACFIMS battery. The relationship to MSQ was not significant. Average Phase Slip rates over the frontal and temporal regions bore significant positive correlation with Information Processing Speed as measured by performance on the most discriminating PASAT '2 test. Whilst metrics of quantitative and qualitative network based assessments of large scale connectivity did in some conditions demonstrate significant associations with extent of MSCI, these findings were not consistent. Dynamic changes in metrics between the pre- and post-test conditions did not associate with subjective tendency to MS fatigue ratings. A shift in spectral power toward a greater theta:alpha ratio was associated with more severe MSCI in some instances. **Conclusions** Whilst a range of neurophysiological techniques may individually offer routes to partially index MSCI, a number of significant methodological challenges remain to be solved to overcome issues relating to state variability and connectivity abstraction. It is unlikely a simple loss of connectivity alone underlies MSCI and a more sophisticated conceptual framework is required.

Plain Language Summary In seeking a measurable biological property which could reflect dysfunction and performance across the broad range of thinking and memory faculties affected in patients with Multiple Sclerosis we sought to explore a range of techniques which examine the naturally occurring patterns of electrical activity on the human scalp which directly arise from brain function inside the skull. Small responses reliably provoked by the detection of a difference in a series of simple sounds did display some relation to cognitive performance in a group of patients with different types of Multiple Sclerosis. However, a clear relation of thinking difficulties to the amount and balance of naturally occurring oscillations in the spontaneously occurring electrical activity of the brain was not so clear. Similarly, more sophisticated approaches examining how the activity at different points on the scalp is connected to that at the others in the form of a network did not offer measures which related to cognitive performance. Although the rate of change of electrical activity bore some direct relationship to how patients fared on tests of information processing speed, collectively the findings suggested a reconsideration of the basis of cognitive impairment in Multiple Sclerosis was required in the next instance.

Team & Contributions

Dr Luke Canham	**Project Design, Ethics, Recruitment, Data Collection & Analysis**
Dr David Western	**Auditory Stimulator Construction & Signal Processing**
Dr David Cottrell	**Project Oversight & Recruitment**
Dr Kirsty Inglis	**Recruitment**
Mr. Peter Walsh	**Neurophysiological Training and Procurement**
Dr Nick Kane	**Neurophysiological Methodological Advice**
Ms. Alzbieta Klicnikova & Mr. Matthew Harland	**Psychometric Testing**
Dr Laura Hanley	**Psychometric Training**

Introduction

Multiple Sclerosis related Cognitive Impairment (MSCI) is a common, debilitating and many-faceted aspect of the condition (212, 448, 449, 587, 588). The general correlation of MSCI with physical disability appears not as strong(29) as might initially be anticipated given that impairment across the two domains appears almost independently driven by encephalopathy(443, 483, 514, 523, 536, 589) and myelopathy(196, 219, 226, 228, 229, 362, 386, 432, 517, 590-597) respectively. This perspective is supported by both our own local investigation and a wider body of corroborative neuroimaging findings.

Notwithstanding possible benefits from psychological support, rehabilitation(477, 598) and limiting confounding contributions from other symptoms and medication; the currently advocated approach to limiting the consequences of MSCI is to effectively minimize (as far possible)the accrual of injurious cerebral inflammation by application of disease modifying therapies in the relapsing phase wherein they are all licensed(36-38, 599). However, the effectiveness of this prophylactic approach to limit MSCI is by no means supported by as much empirical data(37) as that which exists for the benefits of such licensed agents in limiting the key clinical outcomes of general relapse suppression(100, 522, 600) and attenuation of confirmed disability progression over short and longer (218)intervals. No sufficiently large disease modifying therapy trials have used cognitive preservation as a *primary* outcome in either relapsing or progressive phenotypes (based on comprehensive review of *all* Multiple Sclerosis investigations listed within the National Clinical Trials registry up to 31st January 2018 and those historically reviewed by others(91) prior to 1997).

While they have not infrequently been incorporated as secondary outcomes in more recent translational studies including the EXPAND(601) and ARPEGGIO trials (NCT 02284568, TEVA Pharmaceuticals) either as the PASAT component of the MSFC or a variable combination of elements from the BICAMS battery it is not clear if one can be wholly confident that sufficient adjustment for demographic modifiers of intelligence, adequate test-retest intervals and methods for adjudicating clinically meaningful change have been applied given the relationship between deterioration on SDMT performance and employment loss(577) has only been suggested in the past few years. Similarly, the practice effect seen with re-testing on the PASAT which confers 'insufficient change' over time intervals of years (also evident in our work) has been a reason for its disregard in the newest modification to EDSS criteria(602) (EDSS-Plus) which nonetheless is otherwise able to utilise the other components of Timed 25' Walk and 9-Hole Peg Test from the MSFC in confirming the outcome of disability progression.

The focus on apparently 'hard' but nonetheless important disability milestones by application of EDSS criteria heavily weighted toward myelopathic damage has through their centrality to trial inclusion requirements effectively excluded many patients at no-less risk of MS related encephalopathy and the information they might bring with them. It is noteworthy that the phase III success of Ocreluzimab in Progressive Multiple Sclerosis(96-99) and the benefits of other agents (Rituximab) in such patients with more inflammatory para-clinical profiles(280) (including evident intracranial disease activity) is a strong testament to the fact that a possibly preventable driver of the encephalopathy underlying MSCI persists into the phase where physical progression is being contemporaneously driven by additional less-inflammatory neurodegenerative mechanisms(1, 85, 176, 308, 530); with such a variable pathological overlap acknowledged to exist through the entire natural history(1, 46, 72, 176, 515, 603) of the condition which clearly further complicates matters.

An argument could be made that if putative disease modifying or reparative therapies could be successfully identified with established and widely understood trial paradigms using physical disability as primary outcome measures then such positive results should prove sufficiently generalizable; i.e. 'if it is good for walking it *should* be good for thinking' and one could almost forego the need to focus on the comparatively less straightforward issue of attempting to measure human thought.

There are however several decisive counter-arguments. Firstly, from a practical perspective therapies are typically only licensed for use in patient groups clinically akin to those in which agents were tested and for the indications that were under test. For example, patients manifestly progressing as a consequence of *progressively* worsening myelopathic sequelae will potentially meet therapeutic 'stopping criteria' even whilst the possible opportunity to preserve cerebral integrity exists(604). The ability to continue therapeutics with the intention of cerebral protection in the face of myelopathy which may not benefit from such could only ever be considered justifiable when good data (using valid measures) supported such a course of action. This is therefore needed.

Secondly, although tempting for conceptual convenience it would potentially be an erroneous oversimplification to consider the only difference between the encephalopathy and myelopathy of MS as one of topography.

The myelinated spinal long tracts are particularly clinically eloquent(2) both as a consequence of their importance to ambulation and also their significant vulnerability to demyelinative attack across the vast extent of their considerable length(182). The metabolic demands of energy maintenance and protein synthesis, and dependence on mechanisms of axonal transport and trophic support from ensheathing myelin in such pathways must be comparatively vast, particularly for the metre-long giant pyramidal cells of Betz which constitute the cortico-spinal tract, the particular lack of calcium buffering within which also renders them especially vulnerability to mitochondrial dysfunction(605). Pertubation of all these factors have been identified as potential contributors to the neurodegenerative change, dysfunction and axonal loss underlying progression (85, 605, 606). Is it really conceivable that all such neuronal types and particularly those both far more numerous and smaller in the cortex are all *equally* vulnerable to such effects?

Outwith the literature on Multiple Sclerosis, differential neuronal vulnerability has been indentified in a range of neuropathological settings, ranging from the neurodegenerative proteinopathies (607), motor neuron diseases (608) and in response to epileptic (609), ischaemic (610) and other forms of metabolic derangement or toxic insult (611).

The relevance of this matter becomes apparent when one considers that the current dominantly accepted trial paradigm(178, 179) is effectively designed to test the efficacy of candidate therapies in their ability to protect arguably a comparatively small minority of especially vulnerable neurons in the hope of maintaining the clinical functions they serve.

At first glance this is not an unreasonable objective; a positive effect in such a paradigm might indeed suggest an ability to protect less vulnerable parenchymal constituents for example.

However, one has to acknowledge that with differential vulnerability comes a directly related difference in capacity for salvage and protection. Therefore it is wholly conceivable that candidate therapeutics, supported by a rigorous scientific rationale may yield encouraging findings in pre-clinical studies (albeit using nervous systems of similar morphology but vastly

different scale) and translational biomarker studies and yet ultimately fail when trialled against the very high watermark of being able to effectively protect the very most vulnerable neuronal structures. In the forum of clinical research such failure is understandably, without good evidence to the contrary taken as *complete*.

The adequate powering of large scale clinical trials is an inbuilt feature to minimize albeit incompletely the risk of such failures arising purely due to chance alone (612, 613). Therefore placing such possibilities aside, the presence of either known or unknown factors should exist to account for any observed discrepancy between pre and post phase III findings. Using a biomarker at phase II which does not relate to the outcome measured clinically at phase III (c.f. The INFORMS study of Fingolimod in PMS(614, 615)) is one example and the disproportionate use of SOD1 mutant mice as disease models which lead to many failed trials in human Amyotrophic Lateral Sclerosis (wherein SOD1 accounts for a minority of cases) provides many others(616).

There is however the real possibility that failure may not be so very complete. If the underlying model is empirically supported and the method effective; apparently negative findings may simply arise from issues relating to measurement (179, 604) rather than the whole approach being 'wrong' *per se*.

It is conceivable that the most vulnerable cell groups are beyond the point of demonstrable benefit from candidate therapeutics; their fate effectively sealed by the aforementioned host of mechanisms before participation in any respective clinical trials. A metric based primarily on their integrity will therefore demonstrate failure *almost as a foregone conclusion*. However, the other remaining and less vulnerable neuronal elements may still be *saveable*(604) and possibly amenable to restoration; but such successful benefits would be completely overlooked and ultimately discounted unless adequately clinically detected.

This notion of such differential salvage potential is empirically supported to some degree by the findings of the ASCEND (of Nataluzimab's efficacy in progressive MS) trial wherein lower limb function deteriorated relentlessly in a manner wholly unaffected by treatment whereas some significant preservation of upper limb function (mediated by markedly shorter sections of the long tracts) was observed in PMS(604). However, as with all studies it was powered with respect to its primary (ambulation) and not secondary (upper limb function) outcomes and caution against over-interpretation is mandated.

Thus, objective clinical and biomarker indices of encephalopathy and the resulting MSCI may indeed have much to additionally offer with respect to the translational inquiry and subsequent management of these problems and possibly the condition as a whole.

A consilience of findings from neuropsychometric evaluation of persons with MS (PwMS) has supported the view of a neurocognitive footprint(29) of a condition typified by difficulties particularly relating to working memory, attentional control and information processing speed (416, 448, 455, 456, 587, 617).

The criticality of these faculties which are both integral aspects of many broader cognitive functions(618) and key aspects of executive function in their own right, cannot be underappreciated. Whilst a 'footprint' may possibly exist no cognitive domain ultimately passes unscathed in MSCI(29, 400). This is likely both as a result of direct injury to the areas which mediate certain faculties (i.e. the mesial temporal structures(531, 619-621) which support

anterograde episodic memory) and importantly the loss of careful executive control(536, 622-624) over cognitive processing resources across the cerebrum.

Deriving effective clinical indices and biomarker surrogates of MSCI that may have subsequent application in translational endeavour can immediately be recognised as a major challenge that will consistently demand respect of its inherent multi-dimensional nature.

Several different approaches are possibly valid. One could not unreasonably focus on a specific and perhaps paramount aspect of the cognitive footprint, namely information processing speed(479). Candidate therapies could be trialled against outcomes measured by individual validated tests of this, namely the PASAT or SDMT and such an approach has very recently suggested some symptomatic benefit may be offered by Fampridine(625) in a comparatively short study. The generalisability of such findings to other domains and relation to real world benefit remains unclear. Alternately one could use a battery of cognitive tests to serve as outcome measure; therein one would face interpretative difficulties if differential effects were seen across the component tests. What would be the inference if a drug were seen to significantly improve performance on only one test and not others, performance on which might even worsen? Also there is the problem of *meaning*; is it reasonable to assume performance changes on all tests are equally important to patient's function or relate to the same degree of pathological change intracranially?

Furthermore, in using multi-component batteries to identify causal associations between clinical variables and *families* of biomarkers (159, 236, 240, 626-628) from a range of modalities without some form of dimensional reduction as an attempt to best capture the underlying construct of MSCI there is considerable risk of type I error from multiple comparison analysis.

A resolution to the challenge of how best to clinically index MSCI remains outstanding but this should not necessarily preclude exploration of surrogate biomarkers which may deterministically relate to it.

Although structural metrics of intracranial atrophic loss of particularly cortical (220, 246, 503, 505, 506, 511, 515) and especially subcortical grey matter structures(490, 509, 629-632) including the thalamus(499, 630-640) and striatum(497, 632, 633, 641) most strongly and consistently associate with MSCI on neuroimaging, such tissue loss is by modern standards irreversible and incapable of displaying the dynamic change over short time spans that might be associated with symptomatic improvement. Exploration of functional neuroimaging techniques has also not offered a sufficient convergence of findings that would support the use of one methodology over another as a cognitive biomarker(624).

With the field therefore remaining still open to new possibilities and in response to the limitation of our deployed multi-modality evoked potential battery in failing to index cognitive function whilst successfully capturing the physical, we have elected to pursue further electrophysiological techniques in an attempt to close this 'cerebral gap'.

A very considerable range of possibilities exist; all stemming from the underlying principle that with artefact excluded, all of the dynamic changes of electrical potential across the cranium are a direct result of cerebral function and hence its integrity(642, 643).

The encouraging local experience with multi-modality evoked potential batteries, with respect to their logistical acquisition and relation to clinical disability in the PPMS cohort suggested that exploration of a cognitive evoked potential method to sit alongside such a battery may hopefully prove similarly effective.

Although conventional visual interpretation of general electroencephalographic recordings from PwMS by trained neurophysiologists is seen to offer comparatively little in the way of additional information or findings (with the exception of 'non-specific' slowing of the brain's natural oscillatory rhythms)(644) advances in computation have yielded a great many interesting and potentially useful techniques for abstract analysis(562, 645-649). This recent renaissance in candidate EEG-derived biomarkers has seen some impressive initial success in the neurodegenerative conditions of Alzheimer's(650-654) and Prion disease(655).

Herein, we therefore elected to explore the possible utility of cognitive evoked potentials and a number of EEG-derived metrics in their respective capacity to index MSCI.

COGNITIVE EVOKED POTENTIALS

The same principle of extracting a response time-locked to a triggering stimulus(656) which underlies physical evoked potentials may be employed to elicit scalp-recorded electroencephalographic potentials in response to a variety of stimuli which trigger cognitive processes(657).

The cognitive event-related potentials selected for exploration herein are seemingly provoked by the cerebral detection of *difference*, largely independent of the modality (auditory, visual or somatosensory) in which the different stimuli are presented(539, 549, 658). The *oddball paradigm* is typically employed, wherein a series of majority '*standard*' stimuli are punctuated by a much less frequent '*target*' stimulus which the participating subject is asked to attend to (with such attention focussed by a need to push a response button or similar when a target is presented)(657). Back-averaging over a sequence of repeated trials yields a characteristic surface positive deflection in the order of 10-20 microvolts (544) which typically arises after a latency of approximately 300 milliseconds specifically after presentation of the target stimulus; hence its designation as the P300 response (657). The inclusion of a third '*deviant*' stimulus (which is similarly non-standard in being uncommon and also non-target requiring no response) into an oddball paradigm demonstrates an apparent dissociation of responses around the similar 300 millisecond latency into a P3a waveform topographically distributed frontocentrally in association with deviant responses and a slightly later P3b response evidently greatest over the posterior parietal regions and evoked particularly by those target stimuli requiring a directed response (549). Efforts to source localise the underlying neural generators of these responses have employed approaches of standardised Low-Resolution Brain Electromagnetic Tomography (sLORETA)(659) which seeks to offer realistic solutions to the classic pitfalls inherently associated with the inverse problem of attempting to localise underlying dipoles from a surface potential distribution (642). There is a significant caveat attached to efforts which employ point localisation techniques in pursuit of what are likely *distributed* neural generators(660). However, application of sLORETA to P3a responses suggests predominantly anterior activation within the frontal and cingulate regions in addition to some right parietal activation (548, 661). The spatio-temporal pattern of the P3a response has supported the assertion it is a correlate of undirected attentional switching, such as an alerting response (548, 549). In contrast, the more posterior P3b associated with 'sought' target stimuli appears more strongly associated with activation of the parietal structures and posterior cingulate gyrus; however activation of the dorsolateral and ventrolateral prefrontal cortices and the anterior cingulate is also notable (548, 662, 663). The general association of these areas with voluntary attentional processes and the executive allocation of cognitive resources is increasingly well recognised (535, 618), with the P3b consequently being considered to possibly offer an index of such a complex distributed process(664).

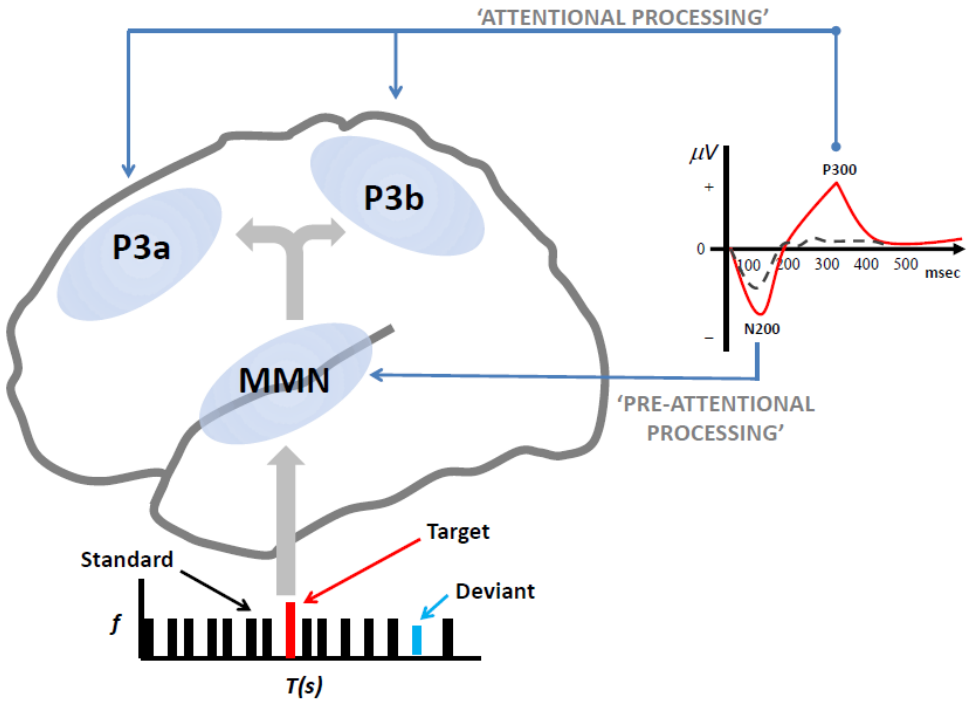
The conceptual parallels between physical evoked and cognitive event related potentials begin to breakdown at the point where responses become dependent on multiple higher-order responses involving disparate cortical regions as seen in the case of attentional processing (with P3a and P3b(661, 664)) in contrast to the comparatively simple (and hence far shorter latency) responses associated with the journey of somatosensory stimulation to the primary sensory cortex(329). However, response dependence on stimulus characteristics may be even more marked in the case of cognitive potentials, with response amplitude being greatly enhanced by the degree of challenge associated with task at hand, in addition the relative likelihood of target presentation and the amount of information conveyed within a stimulus (539, 657).

A possible route to circumvent some of the variability inherent to the multi-region interaction that underlies attentional processing may be to focus on the initial cortical but *pre*-attentional detection of stimuli difference which occurs prior to the events occurring around 300 milliseconds(539, 551).

The presentation of infrequent target stimuli interspersed within a background of frequent standard stimuli can similarly evoke a specific time-locked surface negative potential peaking at approximately 200 milliseconds post-stimulation, designated as the N200 (253, 551) and seen to indicate the detection of 'mismatch' between the probabilistically anticipated standard and occurrence of a qualitatively different target (657) even in the absence of attention (551, 665)(see figure 15). There is nonetheless some evidence (666, 667) that possible influence of directed attention on observed MMN responses does exist and therefore interaction between the processes underlying the MMN and P300 cannot be perceived as independent of one another even if the exact relationship remains unclassified (665); this will therefore have implications for paradigms attempting to use *singular* procedures to elicit both (as undertaken herein).

Figure 15 Cognitive Evoked Potentials

Mismatch Negativity, P3a and P3b arising from infrequent target and deviant stimuli intermixed with frequent standard stimuli of different tonal frequencies in an Oddball Paradigm



Akin to P300s the MMN response can be elicited by various perceptual modalities including those of audition (253, 657), vision (668) and somatosensation (669). However, most experience is with the original auditory stimulation paradigms (668). The application of focussed beamforming acquisition techniques to magnetoencephalographic datasets of individuals exposed to an auditory oddball paradigm has demonstrated the MMN responses arise not simply from activity within the superior temporal cortices mediating the primary bottom-up processing but are contemporaneously accompanied by activity from nearby inferior frontal cortex presumed to be mediating an early top-down processing response related to the occurrence of mismatch (665). Indeed, these findings are congruent with an earlier MRI-EEG co-registration exploration of the auditory MMN response by Schonwiesner *et al.,* (670) which lead to the conception that mismatch detection is distributed over at least three successive hierarchical steps; of detection in the primary auditory cortex followed further processing along the aforementioned superior temporal gyrus and adjacent planum temporale prior to a 'final' decision on whether or not adequate novelty is present to warrant attentional allocation by the ventro-lateral prefrontal cortex(670). Although presented as a spatial distributed hierarchy, the overlapping temporal sequencing of their respective activations (even on MEG) (665) does attest to a likely high degree of simultaneity in their activities.

Although increasingly sophisticated stimulus paradigms are being explored to evaluate sensory processing hierarchies within the visual system particularly (i.e. through presentation of standard and target pictures with different categorical representations for example (671) for the purpose of identifying a widely deployable and practically quick test which could index the *pre* and *pos*t attentional processing stream which follows cognitive events and which already has some empirical support in the field of MS (253, 424, 529, 540-542, 545, 547, 658, 672, 673)we elected to explore the relationship association of MMN and P300 with MSCI when elicited from a singular auditory oddball paradigm.

The sheer variety of means by which general electroencephalography might be employed to explore cerebral function is at once both remarkable and daunting. It has clear established clinical utility in the domain of epilepsy (644, 674, 675), a growing role in anaesthetic monitoring (676) and has a place in international diagnostic criteria for the neuro-degenerative diagnosis of sporadic Creutzfeld-Jacob Disease (644). Despite a variety of typically non-specific electroencephalographic changes being evident to specialist visual inspection in a wide variety of neuropathologies of various types (2, 644, 675) for a number of reasons it has not as yet found a concrete place in the translational research to aid such conditions, including Multiple Sclerosis.

Despite clearly established consensus based practice standards for the technical acquisition of EEG (406, 677-679) a similarly broadly accepted post-processing analysis technique or framework to guide it remains outstanding (645). One approach adopted with some success in the setting of Alzheimer's Disease has been the automated collation of an entire series of classifier properties extracted from EEG time-series of such patients which appears resultantly '*tuned*' toward the extent of cholinergic deficit characteristic of that particular disease(680, 681); such specific pathophysiological abnormality is not clearly evident in MS and such a relatively unconstrained approach remains similarly untested that setting also.

Attempting a focussed approach in the first instance seemed most appropriate; seeking to examine a key pathophysiological consequence of the disease which may contribute to the cerebral dysfunction underlying MSCI.

With the majority of the initially most evident demyelinating pathology arising within the heavily myelinated white matter of the cerebral hemispheres(1, 39, 176, 247, 268, 496) which in turn comprises the many cortico-cortical fibres mediating trans-hemispheric communication(136, 535, 642, 682, 683), examination of the coupling between disparate regions is an intuitive primary step.

Connectivity is however a broad entity; which may be judged on the basis of direct physical coupling of two or more entities (*structural connectivity*), the undirected statistical dependence between the patterns of activity between such entities (*functional connectivity*) or presence of directed causal influence of one elements activity on another (*effective connectivity*)(535, 684).

The cerebral structural connectivity is undoubtedly disturbed in Multiple Sclerosis(487, 536, 630, 685-687). Serial examination (at month/several month intervals) of the white matter tracts by high resolution diffusion tensor imaging is able to reveal abnormal increases in the transverse diffusivity of water in white matter tracts indicative of the purported (688) 'pre-lesional' pathologic cascade within the ensheathing myelin *months* before the appearance of frank demyelinating lesions which enhance with gadolinium signalling the typical accompanying blood brain barrier encroachment (55). As also acknowledged, structural connectivity pertubations as gauged by such DTI techniques revealing increased mean diffusivity and loss of fractional anisotropy compatible with demyelination and loss of axonal integrity respectively do demonstrate an association with MSCI particularly when present in the frontal subcortical regions (267, 689-691).

However, the spatial resolution of EEG is also far more limited than the exquisite millimetre precision afforded by modern topographic imaging(624, 642). This is not solely as a consequence of the anatomical deformation of field lines and electrical filtering afflicting the dipoles arising from the secondary ionic currents around the active pyramidal cells producing them (642, 675, 692); it is an inherent consequence of such dipoles emerging from *distributed s*heets of oscillating neurons whose ensemble synchrony (642, 660, 692) cumulates to produce the barely detectable microvoltages on the scalp. Coupled with the matter of distributed sources is the compounding issue of instantaneous parallel detection of signals at dispersed recording points as a consequence of volume conduction (693, 694).

Nonetheless, a small number of studies (653, 695, 696) comparing functional connectivity estimates derived from EEG (or MEG) and structural connectivity ascertained by high-resolution DTI sequences *do* support an association between them *in vivo*; in the same manner that interdependence between functional connectivity from fMRI and structural DTI, where the former is to a partial extent constrained or shaped by the latter has also been previously observed in healthy controls (697-699).

A study of 36 subjects (20 healthy controls and 16 with amnestic MCI) (653) demonstrated significant correlation between interhemispheric connectivity measured by electroencephalographic coherence in the alpha band particularly and the integrity (as judged by diffusivity) of the anterior and posterior white matter callosal structures linking the respective regional cortices of each hemisphere. A similar pattern of findings was identified with a more focussed coupling assessment applied to specific cortical regions of particular interest in relation to amnestic difficulty co-examined structurally by DTI and functionally by MEG (in this instance using a simpler correlation of amplitudes approach rather than phase-based coupling) in a cohort of 51 individuals (26 amnestic MCI and 31 healthy controls) (695). Once again, the association was mainly apparent in the alpha frequency band (695). Further exploration of EEG-DTI relationships in a cohort of 40 patients featuring a broader spectrum of

MCI-AD severity (n=31) alongside healthy controls (n=9) further suggested a degree of matching between structural DTI of white matter structures and EEG based functional coupling metrics (651). In this particular instance the similar pattern of findings arrived at by the application of different coupling measures is perhaps encouraging. A smaller study using retrospectively attained tractographic and high-density montage (70 electrode) data from a demographically narrow cohort of 7 young adolescent females enrolled into an epilepsy surgery programme was employed by Chu *et al*.(696) for the similar purpose of examining the relationship between electrophysiological and structural white matter connectivity; in this instance by taking advantage of the comparatively more spatially accurate electrical source imaging techniques possible with such high-density recordings. Their work demonstrated a very particular dependence on the integrity of structural connectivity in enabling the presence of functional connectivity (as judged by a linear coupling method) at higher frequencies within the EEG(318). Most notably, the structural-functional interdependence became increasingly more marked and significant in a frequency dependent manner above the middle of the alpha band(318). Two sets of observations must be kept in mind when considering such a finding; firstly their subject number was small and featured a particularly refined cohort at ages well before final cerebral maturation (with respect to myelination and neuronal arborisation (307, 682, 700-702) and adult patterns of long range functional connectivity (as judged by EEG) are established (702). Secondly, the temporal frequencies of EEG oscillations are accompanied by an observable related spatial wavelength with the two being inversely related (642, 700); therein slower oscillations are not only proportionately larger in amplitude but also have a broader *spatial* extent over the cortex (642, 700). Therefore if spatial frequency (which may be centimetres) is of a similar order of magnitude to the separation of recording electrodes it will directly contribute to a perceived statistical dependence being observed between such pairs and achieving stable phase relationships at lower frequencies is proportionately generally much easier to achieve also (642, 703).

All considered, it is not therefore unreasonable to infer that an important causal relationship does exist between the two levels of description offered by functional and structural connectivity (696) which could be exploited in the field of MSCI.

Furthermore, a range of functional EEG connectivity methodologies have been explored in the setting of neurodegenerative diseases (559) and MS in particular (182, 252, 478, 555, 704, 705), demonstrating some degree of association with cognitive impairment. Whilst this small encouraging convergence of EEG findings in MS patients is in general agreement with patterns seen on the similarly functional but sharper spatial resolution modality of MEG (564-566, 568, 570) and stands in contrast to the absence of consensus on connectivity evident from systematic review of the MS MRI literature to date (59, 706), there are still major methodological challenges.

Firstly, the EEG time-series recorded at each channel, after exclusion of transient phenomena and artefact of non-cortical origin is demonstrable by Fourier decomposition to represent a summation of different frequency components formally divided into broadly accepted frequency bandwidths of delta (0.5-3.5Hz), theta(4-7Hz), alpha(8-12.5Hz), beta (13-20Hz) and gamma(>20Hz)(675, 700, 707). These may be further subdivided into additional subsets of lower and high alpha (8-10.5Hz and 10.5-12.5Hz respectively) for example. There is not a completely clear one-to-one mapping of frequency to function(642, 660) or indeed of frequency to its genesis(307, 708) and there are also characteristic topographical variations (642, 675, 700, 707) in the relative distributions over which this family of oscillations is observed. Also the changing relative dominance of the respective rhythms is also a defining electrographic feature

accompanying the dynamic clinical state changes associated with the sleep-wake cycle and other pharmaceutical and pathological alterations of consciousness(644, 675).

Therefore in examining EEG connectivity there is the clear possibility of differential effects amongst the various frequency bands, an effect already observed by others (182, 555). In turn a fundamental shift in the power-frequency spectrum characteristics of the underlying cortex due to pathology may similarly contribute to an apparent disturbance in the measured degree of coupling. Indeed, the success of various quantitative EEG (qEEG) studies examining the power ratio between the slower and faster frequency bands (as delta-theta:alpha-beta for example) in various neurodegenerative(655), ischaemic(709) and traumatic brain injury(710) settings is based on exploitation of this very real and *aetiologically non-specific* spectral shift phenomena. Any exploration of EEG connectivity should therefore necessarily examine effects across a range of frequency bands with accompanying evaluation of any shift in the power spectrum.

In addition to the appropriate derivation of measurement properties that would subsequently follow on from identifying a potentially useful connectivity based approach, and the equally significant preceding challenges associated with selection of epochs, artefact rejection methods and technical acquisition parameters(645, 646), two more fundamental scientific questions must first be addressed – firstly, *how* can connectivity between EEG series best be ascertained? And secondly, *what* is the most useful method of abstraction collectively applied to such outputs? Utility being considered as the capacity to provide a surrogate measure of MSCI and offer meaningful insight into its pathophysiological substrate.

A range of possible solutions to each question may exist. Different approaches may indeed be particularly more effective in some conditions than others and also differentially best suited to examination of the various frequency domains present (711). As such, a 'one-size-fits-all' set of answers may not exist.

The assessment of dynamic coupling between EEG time-series has been explored by a range of techniques, which can be collectively divided into older linear and newer non-linear functional connectivity estimators and an additional class of more sophisticated metrics which attempt to gauge directed influence and thereby aim to capture effective connectivity(556, 648-650, 693, 712-714).

Attempts to use older linear connectivity estimators such as examination of direct statistical correlation between EEG signals (which may appear to serve adequately in fMRI studies) are beset by a major vulnerability to the aforementioned effects of volume conduction (642). The more elaborate evaluation of coherence, wherein the temporal stability of power and oscillatory phase relationships between frequency bands at separate (or even the same) sources are examined is arguably less vulnerable to volume conductive effects (642, 693). It has a longer history of use and has previously been applied locally in the context of traumatic brain injury prognostication (715) and to explore interhemispheric communication in the setting of Multiple Sclerosis (555, 557). However, whilst mathematically more tractable it rests on an unsubstantiated assumption that interactions between remote cortical regions are linear in nature; when given the recognised complexities involved this is very unlikely to be the case (649, 716).The epoch duration required to generate the overall estimate of stability of phase/power relationships(642) may also substantially *exceed* any natural underlying transient stationarity in the signal structure arising over the scalp(717-722). Indeed far from being pathological, a constant change in the phase/power of oscillations punctuated with short periods where they achieve stationarity (oscillate with the same phase and variation of

magnitude) is observed naturally (717-719, 722-724) occurring at timescales below the temporal resolution at which reliable estimates of coherence are typically attained (642).

Appreciation of these demonstrable phase transition patterns consisting of alternating periods of more prolonged phase-locked stationarity with interspersed with short instances of re-tuning by phase shift is in itself potentially extremely useful(717-723). Derived by application of a Hilbert Transform to yield an analytic signal of the instantaneous frequency of the original band-passed EEG signals in the frequency range of interest (717, 718, 723), in the context of developmental neurocognitive disorders they have already demonstrated some capacity to index cognitive performance particularly with respect to information processing speed (725). They effectively offer a *signature of the temporal dynamics* within cerebral function and given their putative influence on estimations of connectivity and relationship to cognitive speed outwith the context of MS it is similarly appropriate to also explore them herein.

In an attempt to overcome pitfalls associated with such linear approaches and to engender sensitivity to non-linear effects the instantaneous frequency output of Hilbert transformation may similarly be explored between two signals to quantify the statistical dependence of the phase relationships between them, independent of amplitude fluctuations (713). The ability to examine particularly short intervals bestows less vulnerability to the aforementioned consequences of non-stationarity and metrics related to how likely signals are to be locked in phase (Phase Locking Value) or alternately be relatively fixed out of phase (Phase Lag Index) can be derived(713). A challenge with these approaches is the possible misattribution (and subsequent rejection) of apparent zero-phase lag between regions as being solely due to volume conductive effects – even if this mechanism may indeed make a contribution(712). A significant proportion of work supports the model of disparate cortical regions forming functionally coupled assemblies by the very act of synchronisation (307, 726-741) therefore it would be erroneous to fully disregard zero-phase lag couplings as merely artefactual in nature. Notably, there are also good reasons to suspect that non-linear methods applied to EEG data may not be wholly superior to linear approaches (as suggested by sensitivity to noise and performance on modelling studies)(703).

The Synchronisation Likelihood approach first described by Stam (556) is a further non-linear technique which effectively examines the similarity between signal structures when they are rendered into a higher-dimensional phase space. The approach is sensitive to likelihood of co-occurrence of trends, motifs and oscillatory phases between signals(742) and the selection of the so-termed embedding dimension for the phase space is heuristically driven. It is particularly noteworthy that with allowance for a scaling factor the Synchronisation Likelihood is essentially akin to the more fundamental property of Mutual Information (556, 557) which is a direct quantification of the entropy shared by two independent variables or in this context time-series (743). Wholly independent, separated variables have zero Mutual Information(744).

All such functional connectivity methods by their nature yield measures of the undirected coupling between time-series data(535). Whilst the degree of reciprocal wiring within the cerebrum is readily acknowledged to be very high (136, 700, 745) there is clear desire to identify the direction of influence, or travel of *information* between cortical areas.

The use of TMS pulses to non-invasively stimulate the cortex can be used to directly observe the integrity of such effective connectivity between disparate cortical regions (746-749). This has demonstrated the attenuation of such in a range of clinical states associated with impaired conscious processing(749), however the experimental paradigm appears to require high-density EEG recording arrays and may in a manner akin to the challenge with Motor Evoked

Potentials(363) be vulnerable to errors in relation spatial navigation. Whilst such a concern has not as yet been verified through its application to date with different stimulation sites yielding similar results the patients under test have all been classified into gross clinical states of awareness (749); application to neurodegenerative contexts where differences may be *far more subtle* may indeed necessitate the use of advanced and particularly costly TMS-MRI co-registered navigation systems.

It is believed that some inference of directed influence can be derived by exploration using the general Nobel-Prize winning principle of Granger Causality (750, 751) originally applied to complex economic time series; wherein events which reliably antecede others and which therefore have predictive value for them *may* indeed make a contribution to causing them. In the context of wishing to derive multiple estimations of effective connectivity between many regions simultaneously and non-invasively(750), accepting the limits of such a principle is not unreasonable.

The Granger Causality Index (GCI) between two time-series is derived by taking the logarithm of the ratio between prediction error associated with forecasting the output of one series based solely on its past values and the prediction error observed when additional information from a second time series is also used in such a forecast(703).

Whilst this holds for a *purely* two channel model, Granger readily appreciated the relationship is only reliable in the absence of other influences; i.e. no other unaccounted for channels with causal effects on the system under examination (703).

A partial solution is therefore to necessarily construct a multivariate autoregressive (MVAR) model featuring all the channels under consideration with the transfer matrix arising from such yielding information about the individual relationships between all considered channels(703). The approach is at once both seemingly robust against noise and simultaneously vulnerable to any extraneous introduction of artefactual correlation from use of reference electrode channels within the dataset under test; thus common average and bipolar derivations are possibly to be avoided (703). Partial Directed Coherence can be derived directly from the phase relationship information within the transfer matrix and is but one method of estimating effective connectivity between channels. Another, more elaborate application of the MVAR-derived transfer matrix information is the Directed Transfer Function which identifies causal influences between channels at specific frequencies. It has been applied to estimate cortical connectivity and with sufficient sampling resolution is able to demonstrate not only coupling but cascade flows of propagation (750) between regions and has some resistance to the effects of volume conduction(703).

As with the Granger Causality model they sprang from, the mathematical elegance of both PDC and DTF as attempts to quantify effective connectivity is accompanied by vulnerability to the influence of hidden drivers which may exert causal influence on the assessed time series(703) . Therefore application to scalp EEG montages which are relatively insensitive to the inferior and deep medial cortices and the subcortical structures encased within(642, 644, 675) does introduce a significant caveat for their deployment. This said, DTF based coupling metrics have demonstrated some empirical promise in relating clinical cerebral state(716, 752, 753) and cognitive function in neurodegeneration (754)to estimations of cerebral connectivity and thus remain worthy of further exploration.

The second question of '*how*' best to utilise any attained functional or effective connectivity derivations remains.

In keeping with the pursuit of a neurophysiological signature of the disconnection of disparate cortical regions by demyelination a first approach would be to examine the long range coupling afforded at least in part by the intra-hemispheric association fibres which constitute the superior and inferior longitudinal fasiculi running longitudinally in an antero-posterior orientation between the fronto-temporal and posterior cortical regions including the parietal lobe (682). The common and near characteristic injury to the corpus callosum(267, 510, 685, 755) seen in MS suggests that the horizontal inter-hemispheric coupling(756-759) afforded by this and adjoining white matter forceps(481) structures should also be evaluated. This low level of abstraction based on gross large scale anatomy has offered insight in MS (182, 252, 555) and other neuropathologies previously (715) and may possibly be at the very limit of the several centimetre spatial resolution offered by EEG (642, 760) without further source localisation refinements to improve it.

However, it may also be possible to compliment functional exploration of the integrity of larger white matter structures with a higher-level abstraction to evaluate the entities such tracts are in place to sub-serve *in vivo*; namely the Intrinsic Cortical Networks (ICN)(534, 535, 684, 761, 762) of the cerebrum.

A large and growing body of work from various structural and functional neuroimaging modalities attests to the existence of functionally specialised networks amongst the vast interconnected mosaic of cerebro-cortical regions (534, 535, 684, 761, 762). The spatially distributed large-scale ICNs are defined in part by the cerebral functions they serve as a substrate for and the membership of their components, which is reliably delineated both by the identification of a statistical-dependence in electrical-metabolic activity between them, and/or direct structural anatomic coupling(534, 535, 684, 761-763). The networks identified to date demonstrate a broadly hierarchical relationship and defined at higher levels of processing by involvement in task-postive engagement of directed tasks or task-negative 'resting state' matters, such as introspective thought and the like(534, 535, 684, 762).

The appreciation of large-scale network structures within the brain brings with it an entire field of concepts and methodologies for analysis from the domain of Graph Theory which may serve to both quantify and qualify key attributes of such systems and which may in turn offer objective evaluation of their functional integrity in a manner which may relate to functional outputs, such as cognition in the case of the brain(534, 650, 651, 764, 765).

The fundamental components of networks (or graphs) are nodes and the edges or vertices which serve to couple them(534).

Applied to EEG the recording electrode positions in sensor-space are typically taken to represent nodes and then the coupling between any two nodes is inferred from the connectivity method applied to their respective time series(478). A threshold is typically applied to exclude weaker couplings and the length of the edge between them may either be taken a standard unitary value or a product of the strength or *weight* of the coupling(535).

Such processing applied to the adjacency matrix of the couplings between each node and every other will yield a graph of the network embedded amongst the nodes assessed(535).

From here it is then possible to identify the *average path length* (being the shortest distance between two nodes) which offers an insight into both its spatial topological extent and the functional 'closeness' of elements, particularly relevant with respect to communication networks(535).

The presence and extent of spatial segregation within networks can also be quantified by examination of *clustering* (if one node is connected to two others, what is the likelihood they are also directly coupled themselves forming a triangular motif) and *modularity*; which captures the extent to which the network is divided into subgroups or communities by deriving a ratio for the balance of within group and between group connections.

The number of edges or connections falling onto a node is typically taken as a marker of its *degree* and from here one can examine the degree distribution of nodes within a given network and additionally examine the extent to which nodes of different degree couple with each other (*assortativity)* or more specifically the extent to which nodes of high-degree selectively connect with the same, to offer a *rich club coefficient* (535).

Cytoarchitectonic, fMRI and DTI studies collectively support the existence of an uneven, skewed degree distribution in the connectivity of the brain(534, 535, 684, 761, 762). Furthermore, they indicate the presence of such a *rich club* of cortical regions, particularly in the anatomical mid-line(535). The high internal coupling between these areas, which simultaneously serve as hub regions coupled to many others outwith the club is considered to afford *integrated* communication across the entire cerebral mosaic(534, 535, 684, 762). The high neuronal density and metabolic demand of both constructing and sustaining such functionally important areas does unfortunately appear to come with heightened vulnerability to the toxic sequelae of hypoxia, hypoglycaemia and the toxic accrual of by-products from neuronal metabolic activity including amyloid(535, 766, 767).

The strongly conserved(136) nature of this arrangement suggests that the functional benefit it bestows should greatly exceed such inherent costs(766) and it is now exactly two decades since the arrival of the Watts-Strogatz (768) model of '*Small Worldness*' which offers some account for how that might be the case.

Beginning with a simple network wherein nodes are regularly connected only to their local neighbours the average path length between any two nodes is comparatively long, the number of edges falling on each node is the same (i.e. the degree distribution is flat) and the clustering (relative proportion of connected triangular motifs) will be high(571, 768).

If one then models how the behaviour of these three properties change in relation to each other as random re-wiring of connections takes place with increasing probability, until a point of maximally random wiring across the entire network is evident, a characteristic pattern is seen to arise(571, 768).

Firstly, average path length progressively reduces with the introduction of long-range connections between disparate network regions; concurrently the degree distribution will become less homogeneous and thirdly with interruption of the tripartite couplings the degree of clustering will progressively fall away (571, 768). In networks (and the systems in which they are embedded) where *segregated* functional subspecialisation is as important to the overall operation as the need to facilitate long range integrative cooperation between the regions where such processing takes place it becomes apparent

that an optimal balance between these two opposing but complimentary requirements should be found (534, 535, 762, 766)(see figure 16).

Achieving short communication path lengths in the brain through the deployment of wholly random connections would lead to an absence of distinct functions (744) even if the prohibitive metabolic, spatial and resource constraints could be overcome (136). This singular optima(571), wherein average short path lengths are balanced with minimal compromise of clustering is achieved by the placement of a comparatively small number of long range connections and networks with such a balance are considered to have a small world architecture.

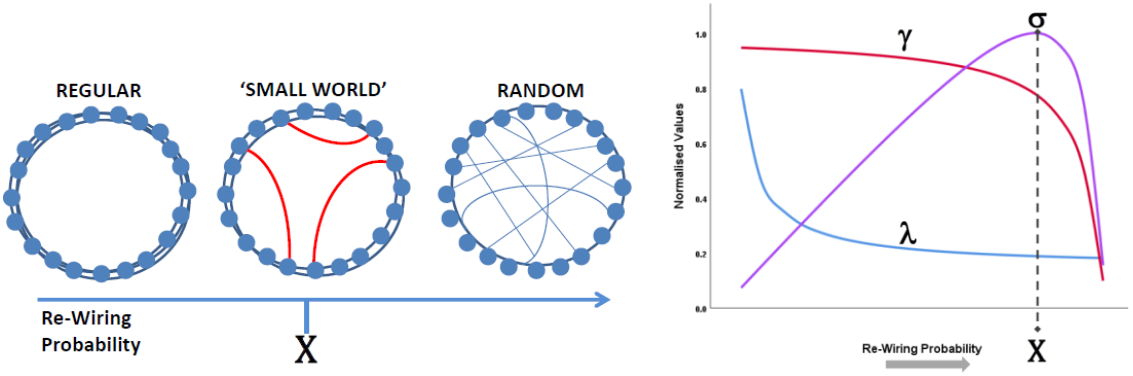


Figure 16 The Watts-Strogatz Model of Small-Worldness

Herein γ represents normalised clustering, λ normalised path length and the ratio between them σ is the index of small worldness. Adapted from(571)

They appear remarkably prevalent outwith the domain of neuroscience (535, 762, 769, 770). However, the practical quantification of this property is non-trivial (771) despite in essence being taken as the ratio of clustering to path length (571). Coefficients of clustering and path length are derived from the respective values from networks under investigation which are normalised against wholly random Erdos-Renyi graphs applied to the same node structure(571). Notably, the definition and construction of such random constructs is also unhelpfully currently lacking clear consensus (771). One can appreciate that a modest variance in the values of clustering or path lengths from such random graphs, can through their role as denominators in normalisation produce substantial differences in the corrected parameters and the final value assigned to small worldness (771).

$$effectively \quad \sigma = \frac{\gamma}{\lambda} \quad where \quad \sigma = \frac{C/C_{random}}{L/L_{random}}$$

Clustering may be taken as the ratio between the number of triangle motifs and the number of paths of length 2 (571) or other methods may be employed (768). If small worldness and the balance between clustering and path length it represents is a *fundamental natural organising principle* underlying cerebral architecture and function (136, 700, 762) then it is reasonable to question whether or not it is perturbed in relation to MSCI as it has been seen to be in other disorders (Stam (558, 559, 772-774).

Structural connectivity analysis based on grey matter thickness (775) and more recent FMRI based functional connectivity analyses (776) do suggest some perturbation toward a more *'disorganised'* state may be observed in MS however wider review and larger meta-analysis of fMRI connectivity has not yielded the anticipated clarity of view(59, 624, 706) and there are those who both question whether the brain is indeed inherently small world (777) and even *if so* whether we have the methodological capacity to capture it *in vivo* by neuroimaging (771). The unresolved connectomic challenges and pitfalls laid out clearly for MRI based work (with respect to how best to define edge weighting and similar) which still apply (778) hold almost equally for EEG (562, 645).

On the basis on the considerations above the following questions were asked:

1. *Can a single auditory oddball paradigm offer cognitive evoked potential indicators of pre-attentional and directed-attention processing which associate with MSCI in a meaningful fashion which may serve as an adjunct to the physical multimodal evoked potential battery?*

2. *Is there an association between MSCI and EEG-derived indices of inter- and intra-hemispheric functional connectivity?*

3. *Can such connectivity indices serve to allow meaningful abstraction of network properties which may reflect qualitative alterations of such in association with MSCI? More specifically, is small worldness lost in association with worsening cognitive function in MS?*

4. *Is there a significant deterioration in the dynamics of phase shift behaviour in association with MSCI?*

Study Design. A prospective single visit cross-sectional observational study (outlined in figure below) within a typical cohort of mixed phenotype MS patients was planned. In the absence of established measurement properties to guide power calculation sample size was based on numbers which had proven informative in settings of cognitive evoked potentials and EEG analysis elsewhere in the context of MS (figure 17).

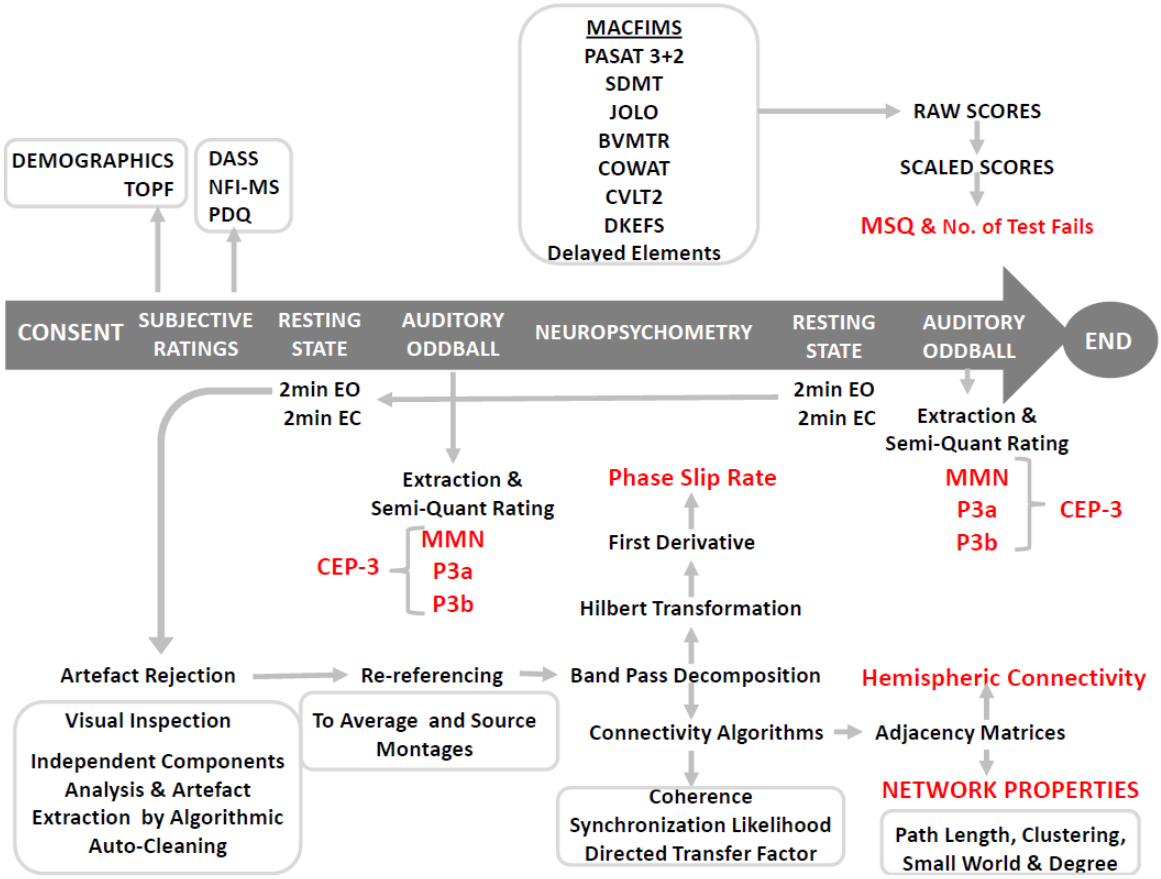


Figure 17 Study Outline

METHOD

Ethics approval was awarded by the NHS Northern Ireland Research Ethics Committee ref.15-NI-0042. 31 subjects of assorted MS phenotypes were recruited from the local Bristol and Avon Multiple Sclerosis Service, a tertiary specialist facility based at the Bristol Brain Centre. Participants were directly invited to participate by the healthcare provider team and issued with formal patient information materials detailing the purpose, nature and intended benefits of investigation. No significant risks were anticipated.

For inclusion participants were required to have a previously confirmed diagnosis of Multiple Sclerosis in agreement with the Modified McDonald criteria (2010) (8) and be registered under the neurological care of a North Bristol NHS Trust employed Consultant Neurologist.

Additionally participants were required to be between 20-66 years of age and have capacity to provide informed consent. Speaking fluent English and an absence of clinically apparent deafness were also necessitated by the nature of the neuropsychometric and cognitive evoked potential studies.

Absolute exclusion criteria were a concurrent history of active or past other neurological disease aside from MS; including significant head injury, epilepsy or other neurodegenerative disorder.

To avoid electrical contamination artefacts participants were not allowed to have indwelling electrical pacing devices such as cardiac pacemakers, deep brain stimulators or similar. Subjects were free to terminate participation on request at any point had they wished. After acquisition of signed informed consent subjects provided a series of demographic variables (age, occupation, time of first symptom, MS phenotype, years of education, ambulatory capacity and medications).

The Test of Premorbid Functioning (TOPF)(405) was then administered and a series of subjective rating scale questionnaires administered; these were the Neurological Fatigue Index – Multiple Sclerosis (NFI-MS)(411), the Depression, Anxiety and Stress Scale (DASS)(408) and the Perceived Cognitive Impairment Questionnaire (PDQ) and included its 5-Item short form (PDQ-5)(410, 779). The TOPF score was utilised in conjunction with gender and years of education to yield estimates of Full Scale pre-morbid Intelligence Quotient (FSIQ) by the accompanying large scale regression based formulation:

Where YOE= Years of Education, Gender = 1 for female and 2 for male, and TOPF is the raw score on testing(405);

$$Est.FSIQ = \sum 29.991 + (2.09426 * TOPF) + (-0.0404559 * TOPF^2) + (0.000340705 * TOPF^3) + (1.4617126 * YOE) + (4.925 * Gender)$$

The NFI-MS raw scores were scaled according to the Rasch-based conversion scheme derived by the test originators(411); this yielded interval outputs for cognitive and total fatigue severity. The DASS and PDQ were scored to generate values for their respective constructs and subdomains (which include subjective gauges of memory, attention and executive difficulties in the case of the latter).

Electroencephalographic recording was undertaken using the standard international 10:20 montage(406) (figure 18) with a nasal reference for acquisition. Ag-AgCl electrodes were utilised in conjunction with a Lifelines R40 device. Recording impedence of <5KOhms was ensured with filter settings of 0.1Hz for low pass and 70Hz for high pass; the sampling frequency was 512Hz which met the Nyquist criterion(642).

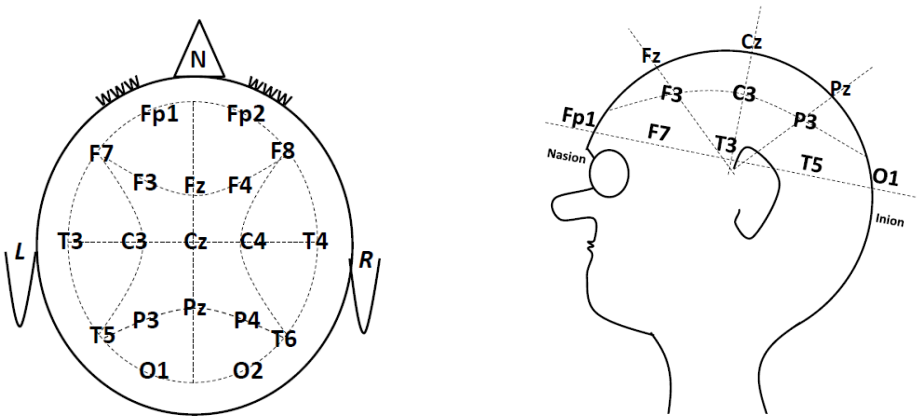


Figure 18 International 10-20 Electrode Placement System

Adapted from (406).Wherein the distances between the nasion-inion circumferentially in the sagittal and axial planes and the distance between the auricular points in the coronal plane are divided into 20% intervals to facilitate the size-adjusted positioning of the electrode positions in the figure above after establishing a 10% margin away from the aforementioned measurement landmarks.

Subjects initially underwent a triple-stimulus **Auditory Oddball Task**. Three distinct tones were played binaurally through headphones above sensory threshold between 50-70 dB. The standard tones of 1000Hz, occurring with a probability of 0.8 were interspersed with Target tones of 1500Hz and Deviant tones of 500Hz each occurring with probabilities of 0.1. The inter-stimulus interval was 1 second. Subjects were required to press a button on audition of the target stimulus only; this was directly relayed to the acquisition device as was the programmed Arduino© which provided precise temporal markers for the onset of the stimuli. 50 target stimulations were provided yielding a variable testing period of 6-8 minutes.

Resting state EEG data was then acquired; 2 minutes of sampling with eyes open (allowing for blinks) and then 2 minutes with eyes closed.

Neuropsychometric testing was then undertaken with the MACFIMS(400) battery. The order of conduct was for the PASAT '3, PASAT '2, SDMT, JOLO, BVMT-R, COWAT, CVLT-2, DKEFS (sorting task, then descriptions) finishing with delayed elements of the BVMTR and CVLT-2. This variably took between 70-90 minutes proceeding in accordance with pace appropriate to each participant.

Further resting state EEG data was then acquired, again with 2 minutes of sampling with eyes open, followed by 2 minutes of eyes closed recording. A repeat of the Auditory Oddball paradigm with the same parameters, but similarly randomised sequencing was finally undertaken prior to completion of the study visit.

Neuropsychometric scoring. After testing raw scores were awarded in line with publisher instructions for each test prior to subsequent scaling and award of demographically adjusted T-Scores for each test in line with the MS-specific data tables of Parmenter *et al.*(400). A test performance was taken to indicate impairment if it fell <1.5 standard

deviations below the mean in line with the criteria of Amato *et al.,*(441, 780). An alternate cut-off based on estimated premorbid IQ was also derived as outlined in the chapter detailing the derivation of the MSQ score. Such composite scores to index the degree of MSCI were also used herein (using the previously derived factor loadings) to provide a singular scaled metric alongside the total number of test failures in each of the MACFIMS and BICAMS batteries as rated by each criterion ('population' or 'self').

EEG post-processing. Direct visual inspection of the EEG was undertaken at the time of acquisition and subsequently to identify and minimise interference of artefact. The raw data files from each participant were each segregated into six files respectively containing recording from the four resting state conditions (pre and post with eyes open and closed) and the cognitive-evoked potential recordings from related to the oddball paradigm undertaken before and after the neuropsychometric battery. Each of the six EEG files from each subject were in turn additionally passed through the Harvard Automated Processing Pipeline for Electroencephalography (HAPPE) which employs an array of validated and peer-reviewed techniques for algorithm based artefact extraction, pre-abstraction data opitimisation and quality control(781). Sequentially, each dataset underwent 1-48Hz bandpass filtering (to exclude environmental and mains electrical artefact), prior to identification of 'bad channels' (by classifying those greater than three standard deviations in log power away from the montage average in any frequency band) and non-cortical artefacts by means of Independent Components Analysis which were then amenable to identification by the Machine Learning-trained MARA classifier (which was 'educated' on expert ratings of over 1000 different artefactual components)(782). Subsequent application of wavelet-enhanced Independent Component Analysis (wICA)(783) enabled fine extraction of low-frequency phenomena considered artefactual with restitution of the remaining higher frequency EEG signals deemed physiological. Further transient artefacts were extracted by segmenting each record into 2 second epochs and rejecting those where *any* electrodes amplitude exceeded 100μV; this was considered a conservative but necessarily rigorous approach at this pilot stage.

Additionally to maintain consistency of EEG montage between samples the bad channel data was substituted for an interpolation derived from the remaining channel data. In the overall dataset the average number of rejected channels was only 0.33 in each record and the maximum of 4 (of a total 19 channels) rejections arose in only 2 file samples. It was considered that the minimal loss of information accompanying this rejection-interpolation procedure was acceptably small; however future work will be able to examine the validity of this assertion. Processed EEG was then re-referenced into average and current source density montages and contiguous sections of EEG were reconstituted from the previously separated epochs. For analysis of the resting state data only sections of 30 seconds of artefact-free data were retained with a view to prioritising the longest contiguous sections to avert as best possible any boundary effects related to epoch selection, segregation and re-integration. Any resting state samples not meeting this 30 second criterion were excluded from subsequent abstract analysis.

Cognitive evoked potentials were extracted by back-averaging applied to temporally-coded triggering information derived from direct coupling of the Arduino tone stimulator and the R40 device. EEG data containing responses to the standard, target and deviant tones were able to be discriminated by the presence of stimulus-specific event markers from the connected Arduino device. If the aforementioned automated processing stream yielded less than 20 artefact-free responses for any type of stimulus, estimates of its parameters would be considered unreliable and were thus excluded; this again was considered conservative. Samples underwent further smoothing by 12Hz low-pass filtering to reduce ambiguity around the identification of the zenith or nadir of each cognitive evoked response. As per standard evoked potential processing, all acceptable sufficiently artefact-free samples underwent back-averaging to derive typical response waveforms.

Amplitude and latency characteristics were extracted in an automated fashion by algorithmic criterion-based identification however each datum additionally underwent direct visual inspection of the identified wave characteristics on annotated wave-plots to ensure validity of derived waveform parameters (figure 19).

Data on button-press accuracy with respect to target presentation was similarly afforded by its coupling into the R40 at the time of acquisition. As with physical, long tract evoked potential studies and particularly within the context of MS(182) there are occasions when a response (either for MMN, P3a or P3b) is absent. In this cohort this effect was present in a significant proportion of the dataset; with 35%, 27% and 18% of trials failing to yield unambiguous MMN, P3b and P3a responses respectively. Consequently, application of purely quantitative analysis would prove insensitive to the information represented by such absent responses. Therefore, as effectively applied to the MMEP in our earlier work, a semi-quantitative scaling system was applied akin to the MEP-4 used previously for the long-tract evoked potentials. The absence of reliable normative data in the wider literature precluded a direct transposition of the rating system used by Jung *et al.* (183) on short-latency EP responses. Hence, a novel 5 point scaling system was devised based on the pre- and post-test datasets collected herein. For each cognitive potential (both with respect to its latency and amplitude) the 25^{th}, 50^{th} and 75^{th} centiles of available responses were used to define thresholds for scores 1-4; with 5 points being awarded for an absent response (tables 19-21). Each cognitive potential was graded with respect to amplitude and latency; the sum scores for MMN, P3a and P3b were then summed into a combined CEP-3 score (in the manner akin to the previous scoring(183) of the MMEP).

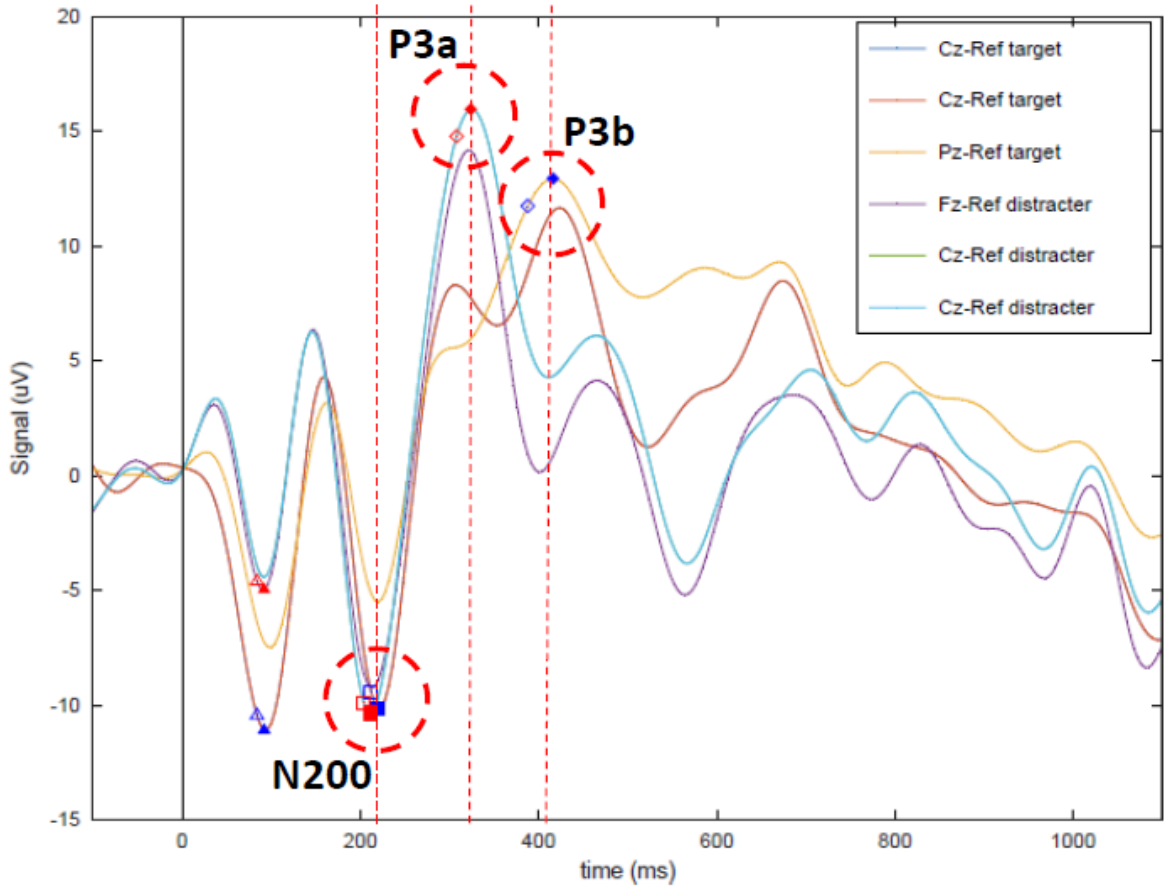


Figure 19 Typical Cognitive Evoked Potential Waveforms

The N200 (Mismatch Negativity), P3a (in response to non-target distractor) and P3b (in response to Target Stimuli) from The employed triple-stimulus Auditory Oddball Paradigm. Waveform plot Court. Dr. D. Western, Annotation from the author. Example directly from study participant.

Score	Latency	Amplitude
1	<25th Centile	>75th Centile
2	<50th Centile	>50th Centile
3	<75th Centile	>25th Centile
4	>75th Centile	<25th Centile
5	Absent Response	Absent

Table 19 Semi-Quantitative Cognitive Evoked Potential Scoring Scheme

		N200 Latency (msec)	N200 Amplitude (μVolt)	P3b Latency (msec)	P3b Amplitude (μVolt)	P3a Latency (msec)	P3a Amplitude (μVolt)
N	**Present**	39	39	44	44	49	49
	Absent	21	21	16	16	11	11
Mean		208.62	-9.71	371.27	10.97	346.29	8.36
Std. Error of Mean		2.26	0.946	10.239	0.658	8.018	0.566
Median		208.00	-8.82	366.00	10.14	340.00	7.56
Std. Deviation		14.084	5.909	67.917	4.364	56.089	3.962
Variance		198.348	34.93	4612.67	19.05	3146.00	15.70
Range		68.00	20.11	244.00	19.46	244.00	17.79
Minimum		180.00	-20.93	256.00	0.93	256.00	0.84
Maximum		248.00	-0.83	500.00	20.39	500.00	18.63
Percentiles	**25**	200.00	-12.45	316.00	9.15	306.00	5.58
	50	208.00	-8.82	366.00	10.14	340.00	7.56
	75	212.00	-4.41	411.00	13.93	382.00	10.86

Table 20 Result Characteristics for collected Pre and Post Test Cognitive Evoked Potentials in this cohort

Score	MMN Lat. (msec)	MMN Amp. (μV)	P3a Lat. (msec)	P3a Amp. (μV)	P3b Lat. (msec)	P3b Amp. (μV)
1	<200.01	< -12.45	<306.01	>10.86	<316.01	>13.93
2	>200.00	> -12.46	>306.00	<10.86	>316.00	<13.94
3	>208.00	> -8.82	>340.00	<7.56	>366.00	>10.14
4	>212.00	> -4.41	>382.00	<5.58	>411.00	<9.15
5	Absent	Absent	Absent	Absent	Absent	Absent

Table 21 Resultant Scoring Thresholds applied to this cohort

$$CEP-3 = MMN_{EP\ Score} + P3a_{EP\ Score} + P3b_{EP\ Score}$$

Formula for generating Summed Cognitive Evoked Potential Score (CEP-3) used herein.

Phase Dynamics. Hilbert transformation was applied to the raw acquired EEG after it had been alpha-band passed (8-12.5Hz) to yield analytic signals. The first derivative was then taken to yield rate of frequency change over time to identify periods of phase lock and phase shift (figure 20). A threshold of any time the instantaneous frequency deviated from the filter band by more than twice the filter band width was specified as a cut-off to identify significant phase slip events and the number of events per second was taken as the phase slip rate. This was applied to data from all channels. Values for each region were taken as the average of several channels; frontal (Fp1, F3, F4 and Fz), Central (Cz, C3, C4, Fz, Pz), Occipito-Parietal (O1, O2, P3, P4, Pz), left temporal (T3, T5, F7), right temporal (T4, T6, F8) and temporal (T3, T4, T5, T6, F7, F8). Analysis was applied to the collective resting state data in the pre- and post-test conditions.

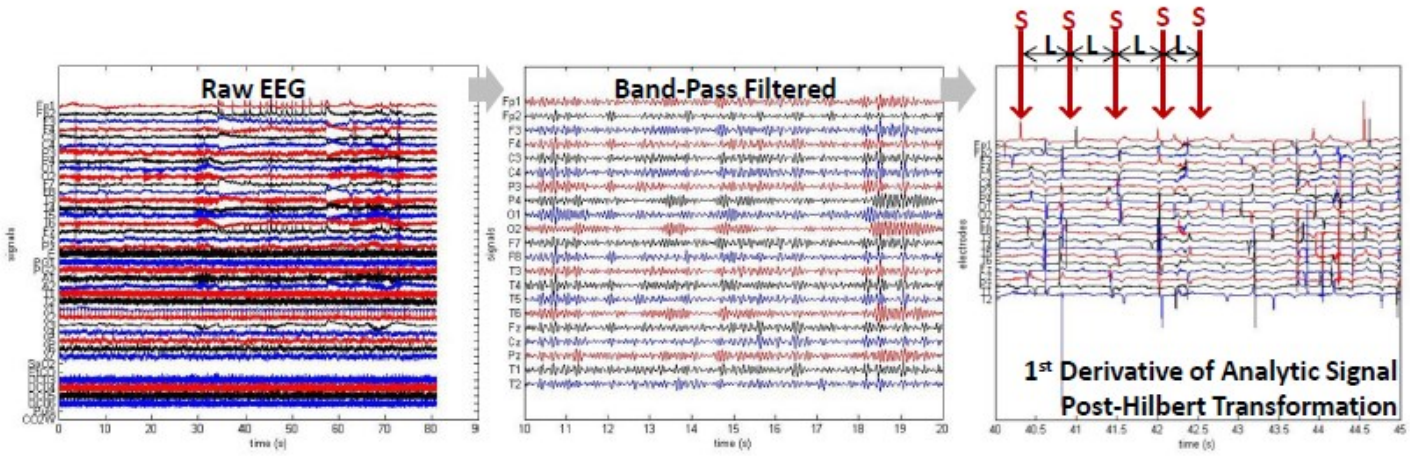


Figure 20 Identification of Phase Slip events within EEG

Connectivity. The resting state data were re-referenced by a Laplacian algorithm to a Current Source Density montage and then, for each condition of eyes open (EO) and eyes closed (EC) for both the pre- and post-test conditions, the connectivity between the time-series from each electrode and every other was estimated by application of three alternative metrics: coherence (Coh), the Directed Transfer Function (DTF), and Synchronisation Likelihood (SL) (tables 22). These distinct approaches were selected on the premise that they would complement each other with regard to linearity and causality.

As discussed above, Coh and SL evaluate linear and non-linear connectivity respectively without discerning causality (directional influence). Conversely, DTF quantifies linear causal interactions (for example whether the behaviour within Channel X offers predictive information about that within Channel Y). Coh and DTF were calculated based on a Multivariate Autoregressive (MVAR) model of the kind described above which here was fitted to each multi-channel recording, using Faes and Nollo's eMVAR Toolbox for Matlab(784).

Of note Coh and DTF produce different values for each frequency in the EEG time-series spectrum. Interactions within separate alpha (8-12.5Hz) and high alpha (10-12.5Hz) bandwidths underwent aggregation by summing the magnitude of connectivity across all frequency bins within the respective band. As SL does not discriminate between coupling at different frequencies the bands were necessarily assessed separately by band-pass filtering the signals prior to SL being calculated utilising the Neurophysiological Biomarker Toolbox(785). Thus each technique yielded an adjacency matrix of inter-channel connectivities, for each frequency band, in each condition (figures 21 & 22).

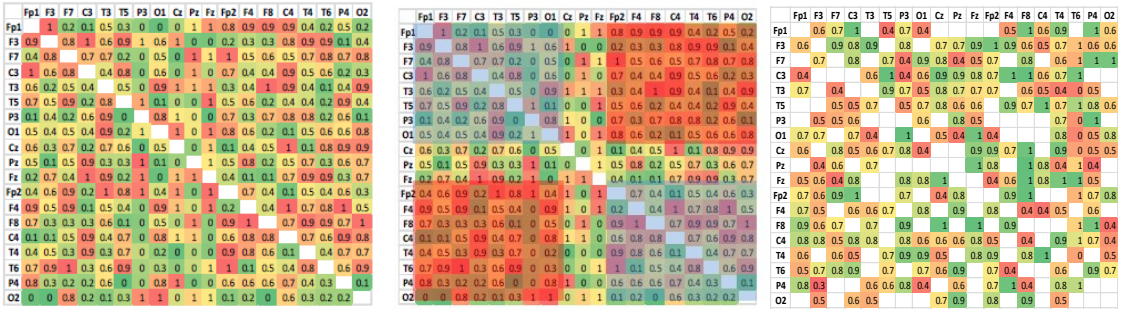


Figure 21 Adjacency Matrices

(a) *values from connectivity estimation by various methods were used to construct adjacency matrices for each condition prior to application of thresholding to remove weak connections prior to graph theoretical analysis (left). (b) direct functional hemispheric connectivities were separately derived by assessing intrahemispheric (blue highlighted) and interhemispheric (red highlighted) couplings respectively (middle).(c) After application of a threshold, remaining couplings in the matrix then serve as the basis for graph theoretical network analysis (right).*

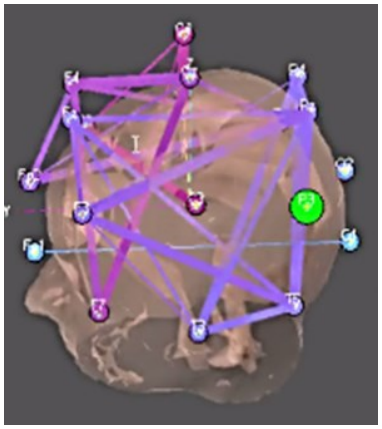


Figure 22 Illustrated Example of Graph Derivation derived from EEG Recording Montage

Image Court. Dr. D. Western; rendered from participant data.

Table 22 Applied Connectivity Metrics

Metric	Formula
Coherence(694) (Coh)	$\gamma_{uv}^2(f) = \frac{\lvert G_{uv}(f)\rvert^2}{G_{uu}(f)G_{vv}(f)}$
Synchronisation Likelihood(711, 742) (SL)	$SL_n = \frac{1}{N'} \sum_{\substack{v=1 \\ w_1<\lvert n-v\rvert<w_2}}^{N} \theta(\varepsilon_{y,n} - \lvert y_n - y_v\rvert)\theta(\varepsilon_{x,n} - \lvert X_n - X_v\rvert)$ $where\ N' = 2(w_2 - w_1 - 1)P_{ref}.\lvert.\rvert\ is\ the\ Euclidean\ distance$ And $\theta\ the\ Heaviside\ step\ function\ \theta(x) = 0\ if\ x \leq 0\ and\ \theta(x) = 1\ otherwise.$ $w_1\ and\ w_2\ are\ windowing\ parameters.$
Direct Transfer Function(703) (DTF)	$DTF_{j\to i}^2(f) = \frac{\lvert H_{ij}(f)\rvert^2}{\sum_{m=1}^{k}\lvert H_{im}(f)\rvert^2}$

Table 23 Applied Graph Theoretical Network Abstractions

Metric	Formula
Degree(535) (*D*)	*= No. of links (L) on a node I; However in the case of weighted connectivity applied here this taken as the* $\sum_i \sum_j L_{ij}$ *; except where* $i = j$
Degree Variance	*=variance (D)*
Global Efficiency (786) (GE)	$GE = \frac{1}{n} \sum_{i \epsilon N} E_i = \frac{1}{n}$ *Where* $E_i = \sum_{i \epsilon N} \frac{\sum_{j \epsilon N} d_{ij}^{-1}}{n-1}$ *Is the efficiency of node i ; where dij is the combined length of the shortest path from i to j, taking the length of each link as 1/Wij*
Average Path Length (786) (Lw) (where W=weight)	*Where* $L = \frac{1}{W}$; $L = \infty \; if \; W = 0$ $Lw = \frac{1}{1/(N(N-1)) * \sum_{i=1}^{N} \sum_{j \neq i}^{N} (1/L_{ij})}$
Average Clustering (where Cw=mean(C_i) (786)	$C_i = \frac{\sum_{k \neq i} \sum_{\substack{l \neq i \\ l \neq k}} W_{ik} W_{il} W_{kl}}{\sum_{k \neq i} \sum_{\substack{l \neq i \\ i \neq k}} W_{ik} W_{il}}$
Lambda (Lw normalised against random graphs)(571)	$\lambda = \frac{L_w}{L_r}$
Gamma (Cw normalised against random graphs)(571)	$\gamma = \frac{C_w}{C_r}$
Small Worldness (Sigma)(571)	$\sigma = \frac{\gamma}{\lambda}$

Evaluation of Long Range Hemispheric Connectivity. Two approaches were taken to examine general and specific inter-regional coupling. Firstly, a gauge of intra-hemispheric connectivity was derived from the adjacency matrices by summing coupling strengths for connection pairs within the right and left hemishpheres collectively. Conversely, inter-hemispheric connectivity was derived by summing only left-right channel interactions.

Specific inter-regional long range couplings were also examined between electrode pairs served putatively served by the superior longitudinal fasiculus (F3-P3 and F4-P4) which connects the Dorsolateral Prefrontal Cortex and Parietal regions(180); and the symmetrical pairings of each side which are putatively dependent on the corpus callosum(275, 276, 311).

Networks. A series of cardinal graph theoretical metrics were algorithmically derived for each network; these were degree distribution, clustering, average path length and small world index; itself a ratio of the clustering and path length values normalised against average counterparts from a set of 50 Erdos-Renyi random graphs (generated by utilizing

the distribution of connectivities attained for each sampling but randomly allocated to alternate node pairs to maintain overall 'connectivity' values) to yield respective coefficients(289). Additionally measures of Global Efficiency (GE), average Degree (Deg), and degree variance (Deg Var) were also attained. The former offering not just an index related to average path length between two nodes but a quantitative comparison of *all* minimal internodal distances compared to an ideal minima for each particular network were all nodes directly coupled; with closeness to such a minima being regarded as more efficient. These parameters were derived for the alpha and high-alpha bandwidths in each condition (eyes open/closed and pre/post-test). The formulation applied for each metric is as outlined in table 23.

Statistics. Analysis of demographic, cognitive evoked potential and phase dynamic behaviour in relation to clinical-metric cognitive outcomes was performed in SPSS v.23 (IBM). To avoid potentially erroneous assumption of normality, bootstrapping with 1000 iterations was utilised in each analysis.

RESULTS

i. Demographics. 31 subjects were successfully recruited into the study. 1 subject elected to withdraw participation due to excessive apparent somnolence limiting his capacity to engage with the initial electroencephalographic recordings; there were however no features of drowsiness evident on his EEG. The clinical impression was of a functional behavioural disturbance.

The remaining 30 participants yielded complete datasets of subjective rating, neuropsychometric and electrophysiological recording. The balance of gender (20 Female:10 Male), age (Avr. 45.4y, Range 25.4-66.5 years) and phenotype (13 RRMS; 17 PMS) was considered comparable to that seen in the larger BrAMS patient cohort. Furthermore, the range of premorbid IQ estimates (mean 102.2, S.D. 12.2) is not dissimilar from that of the general population (mean 100, S.D. 15) (table 24 & 25, figure 23).

	Age (Years)	Full Scale Estimated IQ	Years of Education	Estimated Disease Duration (months)	Functional Subsystem Ambulation
Mean	45.4	102.2	14.6	108	3
S.D.	11.1	12.2	3.5	2	3.4
Min.	25.4	80.9	11.0	110	0
Max.	66.5	125.3	26.0	77	10
Range	41.1	44.4	15.0	48	10

Table 24 Demographic Characteristics of Tested Cohort

	Female	Male	RRMS	PMS
N	20	10	13	17
%	66.7%	33.3%	43%	57%

Table 25 Gender and Phenotype Composition

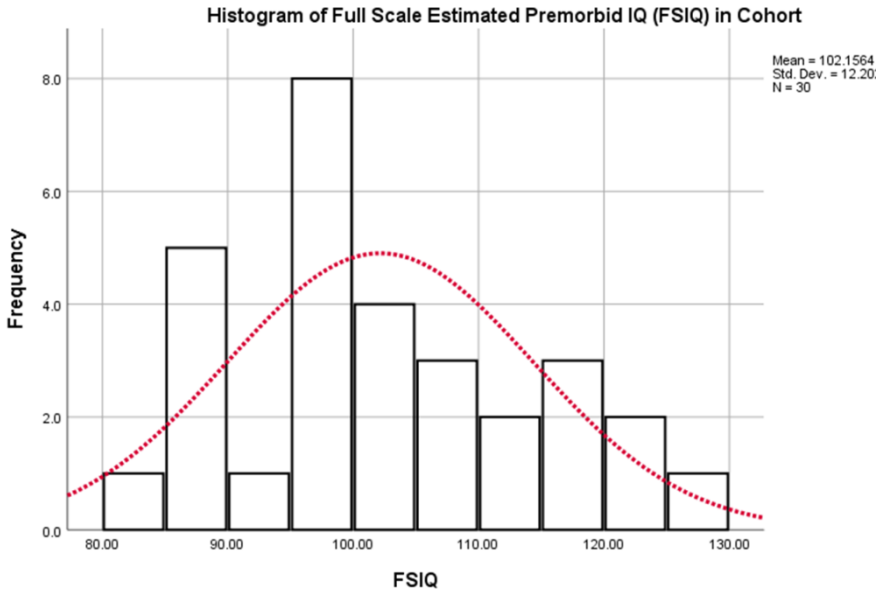


Figure 23 Distribution of Estimated Premorbid Intelligence in Cohort under Investigation

The subjective burden of cognitive difficulties, rated by the PDQ suggested a broad spectrum of issues across the domains of retrograde memory, anterograde memory, attentional capacity and executive function with a corresponding spectrum in perceived severities across the cohort also (table 26).

Similarly, fatigue was both clearly prevalent as expected in this group with an accompanying range of severity; with a comparable pattern of affective disturbances also. On review of the latter contemporaneously at the time of acquisition no cases were judged sufficiently severe to warrant exclusion from the group dataset (tables 27 & 28).

	Attentional Capacity	Retrograde Memory	Anterograde Memory	Executive Difficulty	PDQ Total	PDQ-5 Total
Mean	10.5	9.7	8.0	8.6	36.8	9.4
S.D.	3.8	4.6	3.9	4.5	15.2	4.5
Min.	4.0	2.0	2.0	1.0	9.0	1.0
Max.	17.0	17.0	17.0	18.0	69.0	18.0
Range	13.0	15.0	15.0	17.0	60.0	17.0

Table 26 Burden of Perceived Cogntive Deficits in Cohort by Subjective Rating (PDQ)

	Physical Fatigue	Cognitive Fatigue	Relief by Rest	Abnormal Somnolence	Total
Mean	16.7	8.2	11.6	9.9	19.7
S.D.	3.9	2.7	3.4	2.3	5.9
Min.	8.1	1.4	1.7	4.6	1.0
Max.	24.0	12.0	18.0	15.0	30.0
Range	15.9	10.6	16.3	10.4	29.0

Table 27 Burden of Fatigue in Cohort by Subjective Rating (NFI-MS)

	Depression	Anxiety	Stress
Mean	5.4	5.0	6.9
S.D.	5.0	3.4	5.1
Min.	0.0	0.0	0.0
Max.	18.0	14.0	19.0
Range	18.0	14.0	19.0

Table 28 Burden of Depression, Anxiety and Stress in Cohort by Subjective Rating (DASS)

ii. Burden of MSCI. The distribution of MSQ scores in the cohort appeared normally distributed (mean 0.52, s.d. 0.935, figure 24) in a manner akin to estimated Full Scale IQ (FSIQ), with a highly significant negative correlation between the two (r-.48 p .007) being evident despite the former being inherently adjusted for the latter; perhaps congruent with a protective effect of intelligence against MSCI as described by others. Similarly, even though the MSQ also features adjustment for age, linear regression modelling with independent variables of age and FSIQ are able to account for 34% of the variance in MSQ, (*r* .58 p<0.001) when explored in the larger n=100 MSQ calibration cohort explored in the previous work.

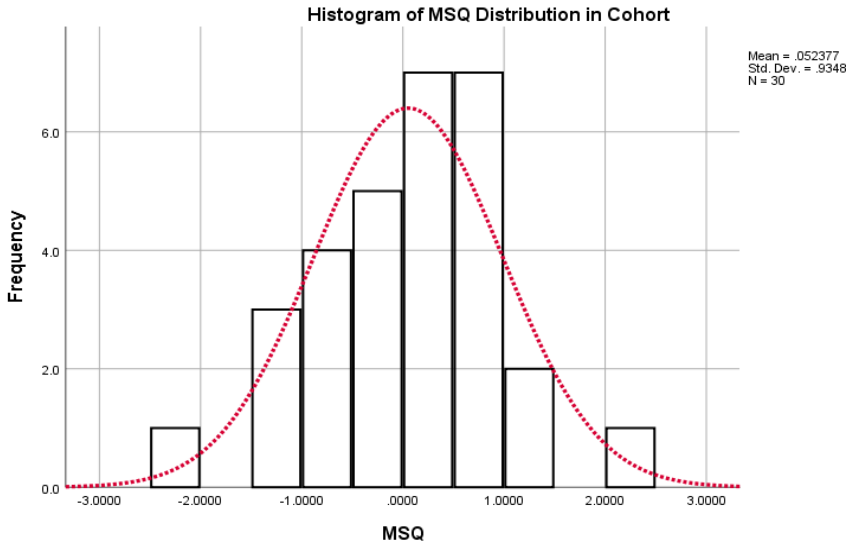


Figure 24 Distribution of MSQ Scores in the Cohort under Investigation

The extent of MSCI in this cohort, as judged by consideration of number of tests in each battery which exceed 'fail' thresholds is greatly dependent on which criteria are utilised. Although the number of fails on the MACFIMS and BICAMS as judged by 'population' or 'self' criteria are significantly correlated (*r* .647 p<0.001 and *r* .593 p .001 for the MACFIMS and BICAMS respectively) there are nonetheless significant differences in the mean number of fails judged by each standard (p<0.001 for both MACFIMS and BICAMS)(table 29, figure 25). The use of 'self' criterion produces a far greater apparent fail rate than the >1.5 s.d. below the mean population based criteria, evident across all tests (table 30, figure 26).

The distribution of failures across the testing battery both reconfirmed its sensitivity to the neuropsychometric pattern of MSCI and confirmed the presence of the typical '*cognitive footprint*' (29, 401) within the cohort under test. Information Processing Speed, Verbal-Visual Memory and executive dysfunction revealed proportionately greatest impairments.

Battery	Criteria	Mean Number of Tests 'Failed'	*Min.*	*Max.*	*Range*	*N*	Std. Deviation	Std. Error Mean	Pearson's *r* between Failures	*p*
MACFIMS	**Population**	5.2	0	11	11	30	3.02	0.55		
MACFIMS	**Self**	9.1	2	11	9	30	1.85	0.34	0.647	<0.000
BICAMS	**Population**	1.7	0	3	3	30	1.17	0.21		
BICAMS	**Self**	2.6	0	3	3	30	0.66	0.12	0.593	0.001

Table 29 Extent of Multiple Sclerosis Cognitive Impairment in Cohort by Neuropsychometric Battery and Criteria

Population cut offs of >1.5sd below mean applied to test scores adjusted for demographic norms. Self – cut off below test score predicted based on estimate of Full Scale IQ.

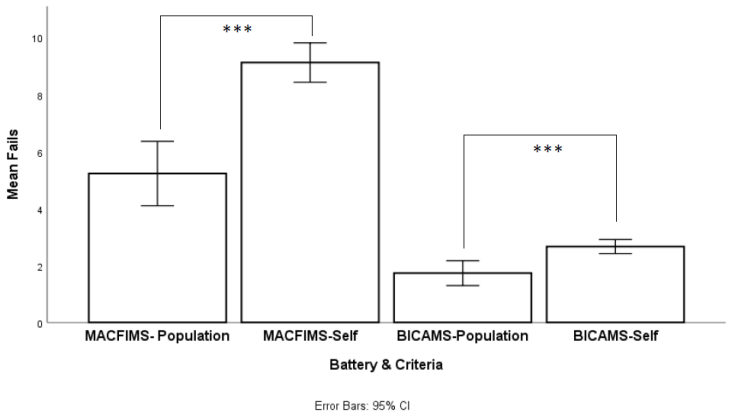


Figure 25 Number of Test Failures in Relation to Neuropsychometric Battery and Criteria

Test	Population Criteria	%	Self Criteria	%
PASAT 3'	8	27	27	90
PASAT 2'	17	57	26	87
SDMT	21	70	26	87
BVMTR	18	60	28	93
BVMTR-DR	18	60	27	90
JOLO	3	10	13	43
CVLT2	13	43	26	87
CVLT2-DR	14	47	25	83
COWAT	10	33	23	77
DKEFS Sorting Task	13	43	24	80
DKEFS	22	73	29	97

Description	

Table 30 Number of Failed tests across Entire Cohort on MACFIMS Battery by Criteria (Population vs. Self).

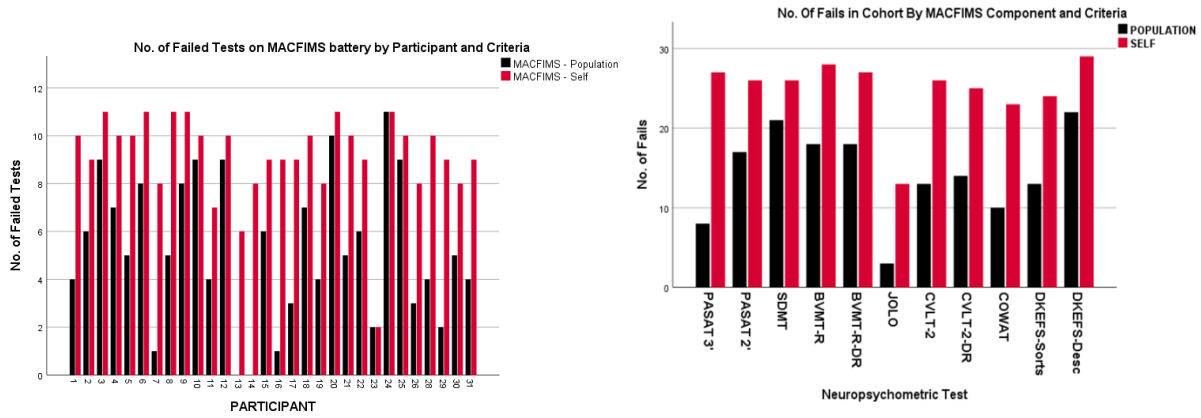


Figure 26 Burden of MSCI in Test Cohort

(a) Number of failed tests on MACFIMS battery in each participant by criteria (left). (b) Number of fails on each component test across cohort (by criteria) demonstrating the typical 'cognitive footprint' of MSCI in this cohort.

iv. **Relationship between MSCI and Cognitive Evoked Potentials.** The performance on tests of working memory and information processing speed (PASAT 3','2 and SDMT) was inversely associated with indices of subject accuracy of button-press in response to target stimuli (False Positive % *r* -.474 p.008 to -594 p.001) in a manner not seen in relation to MSQ scores (table 31).

		Hit Rate	False +ve	Mean Reaction Time	Hit Rate	False +ve	Mean Reaction Time
PASAT 3 sec	*r*	0.193	**-.496****	-0.005	0.246	-0.263	-0.104
	p	0.308	0.005	0.980	0.198	0.168	0.591
	n	30	30	30	29	29	29
PASAT2 sec	*r*	0.285	**-.474****	-0.075	0.307	-0.356	-0.184
	p	0.127	0.008	0.693	0.106	0.058	0.338
	n	30	30	30	29	29	29
SDMT	r	0.350	**-.594****	-0.138	**.532****	**-.505****	-0.353
	p	0.058	0.001	0.467	0.003	0.005	0.060
	n	30	30	30	29	29	29
MSQ	r	0.099	-0.267	-0.093	0.262	-0.213	-0.279
	p	0.601	0.154	0.626	0.170	0.268	0.143
	n	30	30	30	29	29	29

****. Correlation is significant at the 0.01 level (2-tailed).**

***. Correlation is significant at the 0.05 level (2-tailed).**

Table 31 Target Response Accuracy on Auditory Oddball Paradigm

In the conditions of Pre and Post MACFIMS Neuropsychometric battery.

MMN scores were consistently associated with the IPS and Working Memory test scores and indeed the number of failed tests on the MACFIMS battery by both population and self -criteria, on the pre-test condition particularly and to a lesser degree on the post-test sampling.

The extent of association with the P3b (Classic P300) response scores was significant in both the pre and post-test conditions in relation to the number of fails on the MACFIMS and BICAMS by population criteria (missing significance despite a similar trend against self-criteria). The significant association with the CVLT2 element of BICAMS in the pre and post conditions of the P3b response is also interesting.The pattern of association between P3a and MSCI appeared generally less marked despite significant correlation between the overall burden of abnormalities on MACFIMS by either criteria, in the pre-test condition. The combination of all cognitive EP scores into a unified (equally weighted) CEP-3 score yielded the greatest and most consistent patterns of significant correlation between the extent of MSCI on validated batteries in the pre and post-test conditions; (*r* .627 p <.001 and r.535 p .002 for the MACFIMS and BICAMS respectively with population criteria) (table 32, figure 27).

Notably, despite modest trends of association there were no significant relationships between the cognitive evoked potentials and the MSQ scores in either recording condition. The mean cognitive evoked potential scores were not seen to be significantly different between the pre and post-test sampling except with respect to the P3b responses (p .007 Wilcoxon Paired Sign Rank Test). There was no significant difference in the mean collective CEP-3 scores between recording conditions, suggesting a degree of stability (figure 28). There was no meaningful association between the change in any of the examined metrics and subjectively rated fatigue (either total or cognitive).

Potential	Condition	MSQ (IQ Adj.)	MACFIMS (Self)	BICAMS (Self)	MACFIMS (Population)	BICAMS (Population)	PASAT 3' (Raw)	PASAT 2' (Raw)	SDMT (Raw)	CVLT2 (Raw)	BVMTR (Raw)
P3a	Pre	-.159	**.466****	.204	**.390***	.251	-.133	-.289	-.220	-.192	-.086
		.403	**.009**	.279	**.033**	.181	.483	.122	.243	.310	.650
	Post	.105	.266	-.031	.105	.050	-.063	-.160	.088	.013	.172
		.580	.155	.869	.582	.793	.742	.397	.645	.947	.362
P3b	Pre	-0.75	.277	.193	**.379***	**.427***	-.293	-.305	-.116	**-.432***	-.159
		.694	.138	.306	**.039**	**.019**	.116	.101	.540	**.017**	.400
	post	-.200	.307	.232	**.366***	**.429***	-.259	-.283	-.195	**-.388***	-0.50
		.289	.099	.218	**.047**	**.018**	.166	.129	.303	**.034**	.792
N200	Pre	-.148	**.472****	.184	**.499****	**.381***	**-.446***	**-.584****	**-.490****	-.178	-.125
		.436	**.008**	.239	**.005**	**.038**	**.014**	**.001**	**.006**	.346	.510
	Post	-.258	.200	-.071	.299	.253	-.327	-.265	**.586****	**-.437***	-.204
		.168	.288	.711	.108	.178	.078	.156	**.001**	**.016**	.280
CEP-3	Pre	-.201	**.533****	.316	**.627****	**.535****	**-.450***	**-.587****	**-.443***	**-.409***	-.226
		.287	**.002**	.089	**.000**	**.002**	**.013**	**.001**	**.014**	**.025**	.230
	Post	-.246	.340	.127	**.435***	**.386***	-.331	-.325	**-.464****	**-.459***	-.148
		.190	.066	.505	**.016**	**.035**	.074	.080	**.010**	**.011**	.434

Table 32 Correlation Matrix of Cognitive Evoked Potential Scores against Indices of MSCI

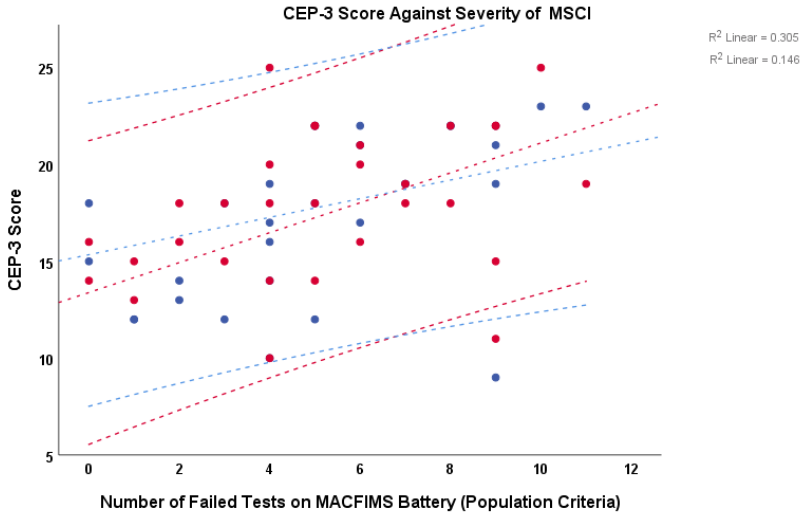


Figure 27 Composite Cognitive Evoked Potential Score (CEP-3) against burden of MSCI

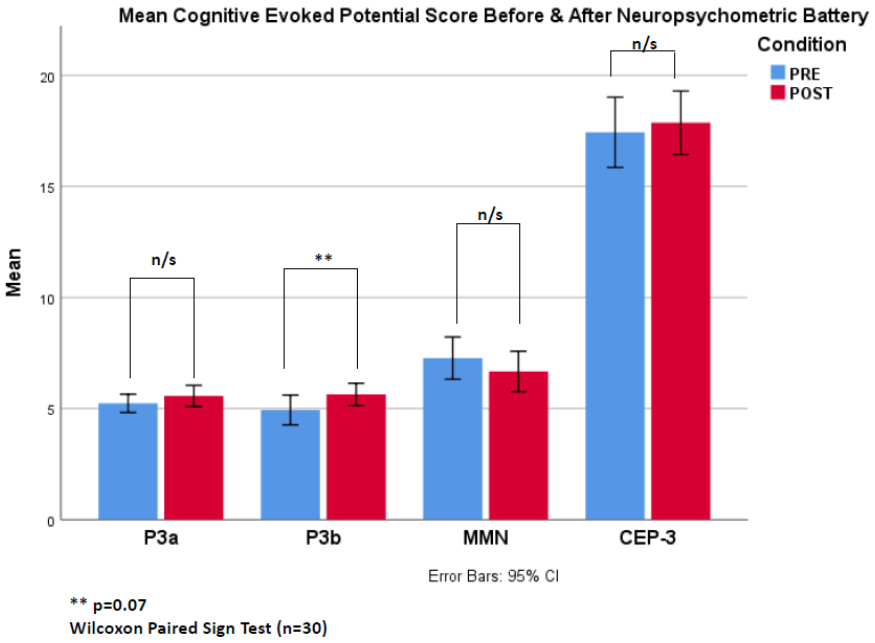


Figure 28 Variability of Cognitive Evoked Potential Scores between Pre and Post Test conditions

iv. Relationship of Information Processing Speed to Phase Slip Rate.

Phase slip rate in the alpha band was examined in the resting state EEG time-series to yield median averages for the frontal, central, occipito-parietal and temporal (left, right and average) regions and compared to performance on the primary tests of Information Processing Speed.

REGION	Condition	SDMT (Raw)	PASAT 3' (Raw)	PASAT 2' (Raw)	REGION	Condition	SDMT (Raw)	PASAT 3' (Raw)	PASAT 2' (Raw)
FRONTAL	Pre	.199	.268	**.498****	TEMPORAL (LEFT)	Pre	.242	.316	**.504****
		.301	.160	**.006**			.205	.095	**.005**
	Post	.360	**.402***	**.521****		Post	.227	**.377***	**.498****
		.055	**.031**	**.004**			.237	**.044**	**.006**
CENTRAL	Pre	-.330	.057	.230	TEMPORAL (RIGHT)	Pre	.293	.288	**.491****
		.866	.769	.230			.123	.130	**.007**
	Post	.069	.169	.307		Post	.253	.306	**.372***
		.724	.380	.105			.186	.107	**.047**
OCCIPITO-PARIETAL	Pre	.151	.183	.358	TEMPORAL (Average)	Pre	.269	.307	**.494****
		.433	.341	.056			.158	.106	**.006**
	Post	.175	.282	**.391***		Post	.276	.326	**.456***
		.363	.139	**.036**			.147	.084	**.013**

(Pearson's *r*, *<.05, **<0.01)

Table 33 Relationship of Average Phase-Slip Rate by Region with Indices of MSCI.

There was a significant and consistent positive association between phase slip rate particularly in the fronto-temporal regions and performance on the most discriminating of the three tasks, the PASAT '2. This was evident in both the pre and post-test conditions. This pattern was evident to a lesser degree on the PASAT '3 and only as weak insignificant trends in association with the SDMT (table 33). Comparison of regional average phase slip rates demonstrated the only significant changes between pre- and post- test occurred in the frontal (p .022) and central (p.048) regions as judged by paired-samples T-Tests (figure 29). A general pattern of faster average phase slip rate across all regions in the post-test condition was observed. Again, there was no significant association between the change in phase slip rate (in any region) between conditions and the severity of reported fatigue or its cognitive subcomponent.

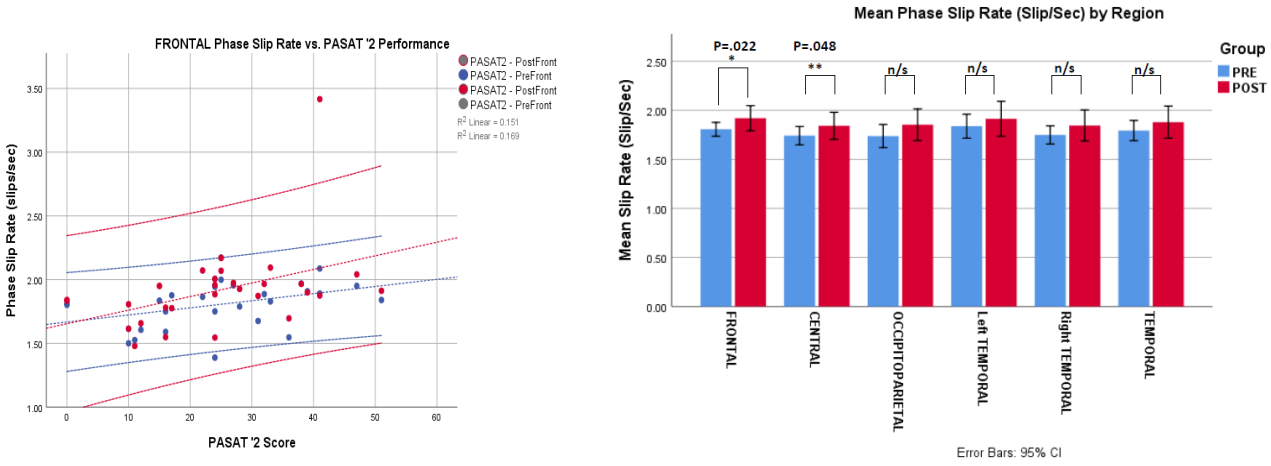


Figure 29 Relationship of Regional Phase Slip to Information Processing Speed

(a) (Frontal vs. PASAT ‘2) (left). (b) Variability of Average Regional Phase Slip between Pre and Post Test Conditions (right); significance tested by paired sample T-Test.

v. Spectral Analysis. The relative spectal power was examined between the theta:alpha-beta and high:low alpha frequency band widths, in the eyes open/closed pre and post-test conditions. Considerable intra-condition variance and inter-condition variability was observed albeit without significant differences (on paired samples T-Test) between the mean group values for matched pre/post test samples. There was no significant difference between any condition or band-power ratio on comparison between groups of high or low MSQ by the independent samples T-Test. Association with the number of failures on the MACFIMS battery was seen only in the *post*-test Eyes Closed resting state for the theta:alpha and theta:alpha-beta power ratios (*r* .527 p.004 and *r* .584 p.001 respectively)(table 34); no other states bore a consistently significant relationship with the MSQ or fatigue metrics.

EYES	OPEN	CLOSED

Table 34 Descriptive Statistics for Quantitative Spectal Band Power Ratios by Sampling Condition in MS Cohort

CONDITION		PRE			POST			PRE			POST		
		θ:α	Hi:Lo α	θ:α+β	θ:α	Hi:Lo α	θ:α+β	θ:α	Hi:Lo α	θ:α+β	θ:α	Hi:Lo α	θ:α+β
N		28	28	28	27	27	27	27	27	27	28	28	28
Range		32.59	2.55	46.03	8.62	13.50	48.42	120.69	113.82	128.15	222.65	22.94	222.83
Minimum		0.03	0.02	0.09	0.19	0.03	0.35	0.05	0.01	0.08	0.04	0.01	0.07
Maximum		32.62	2.58	46.12	8.81	13.53	48.77	120.75	113.83	128.22	222.70	22.95	222.90
Mean		4.54	0.61	6.49	2.15	1.27	5.58	6.60	5.37	10.39	11.55	2.44	13.40
	S.E	1.56	0.12	1.96	0.44	0.51	1.81	4.42	4.18	4.68	7.93	0.83	7.91
Std. Deviation		8.25	0.64	10.36	2.31	2.64	9.39	22.97	21.71	24.32	41.98	4.37	41.88
Variance		68.12	0.41	107.30	5.32	6.98	88.24	527.42	471.34	591.37	1761.98	19.14	1753.75
Skewness		2.97	1.53	2.89	1.96	4.25	4.05	5.09	5.17	4.70	5.07	4.11	4.98
	S.E	0.44	0.44	0.44	0.45	0.45	0.45	0.45	0.45	0.45	0.44	0.44	0.44
Kurtosis		8.42	2.36	8.72	3.26	19.35	18.39	26.21	26.81	23.36	26.26	19.08	25.62
	S.E	0.86	0.86	0.86	0.87	0.87	0.87	0.87	0.87	0.87	0.86	0.86	0.86

vi. Association between General Connectivity and MSCI.

No consistent association with cognition and coherence derived quantitative connectivity metrics in either the alpha or high alpha bands was observed, with the exception of antero-posterior coupling in both bands in the post-test eyes open state.

Synchronisation Likelihood demonstrated no consistent correlation between any cognitive metric or coupling pattern, except for a singular unrepeated association of intra-hemispheric coupling on the left side in the post test eyes closed condition.

Analysis by DTF did demonstrate a more consistent trend in both the full and high alpha bands for antero-posterior connectivity for both the eyes open and eyes closed state in the post-test condition.

By this method coupling between horizontally symmetric pairs in the pre and post conditions for the eyes closed states also associated with the number of fails on the MACFIMS battery also (tables 35-37). No meaningful or consistent associations with general fatigue or cognitive fatigue were observed when similarly examined, even in relation to those few coupling metrics which demonstrated significant change between pre and post-test conditions on DTF (figure 30). High

		f	ALPHA					Alpha				
			Int.Hem. RIGHT	Int. Hem. IHL	INTER HEM	SYM PAIRS	A-P	Int.Hem. RIGHT	Int. Hem. IHL	INTER HEM	SYM PAIRS	A-P
	EO	PRE	.055	.147	.172	.192	.286	-.089	-.016	.010	-.005	.218
		p	.782	.456	.380	.327	.141	.654	.936	.960	.978	.265
	EO	POST	.367*	.334	.308	.340	.481*	.288	.281	.248	.241	.409*
MSQ		p	.055	.082	.111	.077	.010	.137	.148	.202	.216	.031
	EC	PRE	.059	-.271	-.099	-.044	.125	-.001	-.193	-.130	-.102	-.142
		p	.767	.162	.616	.825	.525	.998	.325	.509	.606	.470
	EC	POST	-.114	.107	-.018	-.002	-.081	.078	.108	.055	.025	.076
		p	.562	.589	.930	.991	.682	.694	.585	.780	.899	.702
	EO	PRE	-.081	-.217	-.278	-.327	-.215	-.056	-.136	-.238	-.292	-.190
		p	.681	.268	.152	.089	.272	.776	.490	.222	.132	.332
MACFIMS	EO	POST	-.062	-.162	-.091	-.089	-.274	.058	.005	.037	.072	-.119
Fails		p	.756	.409	.646	.651	.158	.769	.981	.851	.717	.547
Population	EC	PRE	-.042	.205	.088	.084	.106	.044	.148	.033	.052	.102
Criteria		p	.833	.296	.656	.672	.591	.825	.452	0.869	.794	.606
	EC	POST	.167	.045	.135	.033	.236	-.024	-.039	-.027	-.045	.254
		p	.395	.820	.494	.869	.227	.904	.845	.892	.819	.192
	EO	PRE	.125	-.128	-.131	-.182	-.095	.096	-.120	-.037	-.117	-.119
		p	.525	.517	.507	.354	.632	.625	.542	.851	.552	.545
MACFIMS	EO	POST	-.044	-.155	-.055	-.126	-.297	.016	-.108	-.017	-.042	-.224
Fails		p	.824	.432	.782	.524	.124	.937	.585	.931	.832	.251
Self	EC	PRE	.037	.264	.169	.140	.057	.132	.285	.232	.210	.139
Criteria		p	.852	.174	.389	.476	.771	.504	.141	.234	.283	.482
	EC	POST	.182	-.116	.026	-.089	.018	-.028	-.188	-.118	-.114	-.013
		p	.354	.556	.895	.653	.929	.886	.338	.551	.563	.947

Table 35 Relationship of MSCI to Quantitative Metrics of Connectivity Derived from COHERENCE (Coh)

		f	ALPHA					High Alpha				
			Int.Hem. RIGHT	Int. Hem. IHL	INTER HEM	SYM PAIRS	A-P	Int.Hem. RIGHT	Int. Hem. IHL	INTER HEM	SYM PAIRS	A-P
	EO	PRE	.180	.140	.267	.361	.197	.082	.087	.130	.172	.042
		p	.361	.477	.170	.059	.315	.678	.660	.511	.380	.830
	EO	POST	-.151	-.034	-.034	.172	.054	-.066	-.166	-.136	.118	-.010
MSQ		p	.443	.862	.864	.380	.784	.740	.397	.491	.549	.958
	EC	PRE	-.173	-.078	-.143	-.151	-.047	-.229	-.264	-.231	-.219	-.064
		p	.379	.692	.468	.445	.812	.240	.175	.237	.262	.748
	EC	POST	-.083	-.084	.002	.088	-.048	-.141	-.296	-.116	.062	-.381
		p	.676	.672	.993	.657	.808	.475	.127	.556	.755	.045
	EO	PRE	-.064	-.041	-.054	-.359	-.179	-.112	.125	-.015	-.195	.026
		p	.747	.837	.785	.060	.362	.570	.526	.941	.321	.894
MACFIMS	EO	POST	.203	.196	.177	.022	.142	.225	.404*	.321	.121	.067
Fails		p	.299	.318	.367	.912	.472	.249	.033	.096	.541	.737
Populatio n	EC	PRE	.065	-.008	.039	.026	-.177	.059	.183	-.004	-.041	.131
Criteria		p	.743	.967	.844	.896	.368	.765	.352	.983	.836	.505
	EC	POST	.340	.388	.265	.129	.045	.354	.532	.311	.215	.325
		p	.077	.042	.173	.514	.821	.065	.004	.108	.272	.092
	EO	PRE	-.158	-.056	-.093	-.310	-.158	-.103	.096	-.008	-.055	.020
		p	.421	.776	.639	.109	.422	.600	.625	.968	.781	.920
MACFIMS	EO	POST	.297	.228	.274	.158	.143	.303	.334	.374	.167	.181
Fails		p	.125	.244	.158	.423	.467	.117	.082	.050	.395	.358
Self	EC	PRE	.092	.031	.042	.063	-.183	.151	.164	.132	.096	.075
Criteria		p	.642	.877	.831	.750	.352	.442	.405	.503	.625	.705
	EC	POST	.231	.217	.190	.018	.102	.234	.227	.064	-.048	.326
		p	.236	.268	.334	.929	.606	.230	.245	.745	.809	.091

Table 36 Relationship of MSCI to Quantitative Metrics of Connectivity Derived from SYNCHRONISATION LIKELIHOOD

		f	ALPHA					High Alpha				
			Int.Hem. RIGHT	Int. Hem. IHL	INTER HEM	SYM PAIRS	A-P	Int.Hem. RIGHT	Int. Hem. IHL	INTER HEM	SYM PAIRS	A-P
	EO	PRE	.079	.404*	.400*	.414*	.135	.142	.300	.411*	.439*	.063
		p	.690	.033	.035	.029	.495	.472	.121	.030	.019	.748
	EO	POST	-.090	.092	-.035	-.080	.418*	-.186	.083	-.054	-.131	.547***
MSQ		*p*	.648	.642	.860	.686	.027	.343	.676	.784	.507	.003
	EC	PRE	.291	.067	.229	.177	-.067	.361	.084	.299	.133	-.045
		p	.134	.736	.242	.367	.734	.059	.672	.122	.500	.818
	EC	POST	.141	.199	.279	.345	.446*	-.011	.118	.106	.285	.410*
		p	.475	.309	.150	.072	.017	.956	.551	.593	.142	.030
	EO	PRE	.045	-.200	-.062	-.075	.017	-.003	-.139	-.043	-.015	-.069
		p	.819	.306	.753	.703	.930	.988	.481	.826	.938	.727
MACFIMS	EO	POST	.188	-.092	.055	.147	-.200	.186	-.088	.092	.218	-.257
Fails		*p*	.337	.642	.781	.455	.306	.342	.656	.642	.265	.186
Population	EC	PRE	-.281	-.351	-.438*	-.461*	-.045	-.330	-.324	-.460*	-.416*	-.059
Criteria		*p*	.147	.067	.020	.014	.819	0.087	.092	.014	.028	.765
	EC	POST	.104	-.169	.049	-.124	-.510**	.184	-.150	.149	-.416*	-.455*
		p	.598	.391	.806	.530	.006	.349	.447	.448	.028	.015
	EO	PRE	-.026	-.073	-.023	.032	.131	-.072	-.044	.005	.061	.066
		p	.894	.711	.906	.871	.507	.717	.825	.980	.758	.737
MACFIMS	EO	POST	.061	-.150	-.059	.011	-.065	-.005	-.175	.006	.095	-.225
Fails		*p*	.756	.446	.766	.956	.742	.981	.372	.975	.631	.250
Self	EC	PRE	-.284	-.203	-.337	-.345	.072	-.327	-.133	-.334	-.289	.134
Criteria		*p*	.142	.300	.079	.072	.716	.089	.498	.083	.136	.496
	EC	POST	.041	-.038	-.069	-.198	-.294	.110	-.072	.017	-.220	-.327
		p	.836	.847	.726	.313	.128	.579	.714	.931	.262	.089

Table 37 Relationship of MSCI to Quantitative Metrics of Connectivity Derived from DIRECT TRANSFER FUNCTION (DTF)

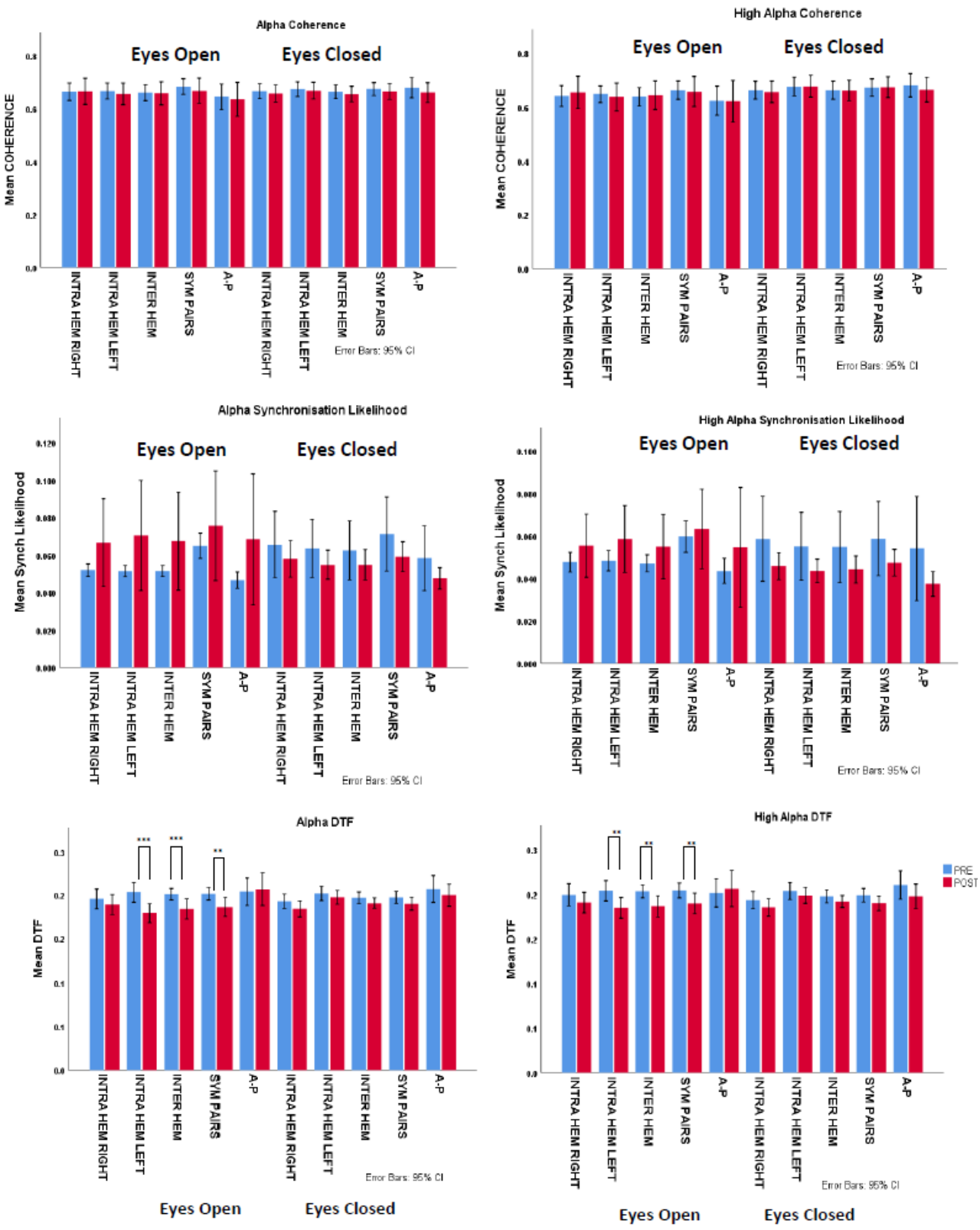


Figure 30 Variability of Quantitative Connectivity Metrics between Pre and Post Testing Conditions

In both Eyes Open and Eyes Closed by bandwidth of interest (Alpha & High Alpha) and coupling derivation (Coh, SL and DTF).

vii. Insights from Network Abstractions.

Performance of classical graph metrics derived by coherence in the alpha and high alpha bands did not demonstrate any consistent pattern of association with MSCI indicators.

Significant pre-test associations between lambda and MSCI although in keeping with the notion of disconnection were not reproducible.

Synchronisation Likelihood based derivations offered a suggestion that reduced Global Efficiency within the high alpha band in the post-test setting is associated with MSCI as may path length have been; however once again this was not a consistently reproducible pattern.

Application of DTF did not clarify matters. Again, no strong consistent patterns were observed.

An increased lambda was seen in association with MSQ in the high alpha pre-test eyes closed setting and similarly approached significance for the eyes open setting also. The observation of several significant associations both in positive association with MSQ and conversely the number of fails on the MACFIMS battery suggests the findings are inconsistent (tables 38-40). Again significant pre/post-test variability where seen (mainly in the DTF derivations) did not associate with fatigue ratings; but is evidence of a dynamic change in functional network coupling between states.

			ALPHA								High ALPHA							
			APL	CLUST	Deg	Deg V	G.E.	λ	γ	σ	APL	CLUST	Deg	Deg V	G.E.	λ	γ	σ
MSQ	EO	PRE	.166	.200	.187	.301	-.104	-.336	.237	.321	-.074	.095	.000	.226	.116	-.533***	.291	.353
		p	.397	.308	.342	.120	.598	.080	.225	.096	.707	.632	.000	.247	.557	.003	.134	.065
	EO	POST	.323	.336	.311	-.013	-.316	.006	-.152	-.171	.268	.267	.271	.116	-.24	-.08	-.07	-.12
		p	.093	.081	.107	.949	.102	.974	.441	.383	.168	.169	.162	.557	.217	.670	.711	.540
	EC	PRE	-.084	-.085	-.086	.257	.158	-.070	.278	.269	-.04	-.112	-.105	.34	.056	.039	.313	.28
		p	.670	.666	.662	.186	.421	.723	.152	.167	.851	.570	.597	.077	.778	.844	.105	.149
	EC	POST	-.029	-.006	-.018	.152	.039	.054	.109	.109	.041	.056	.054	.157	-.021	-.302	.059	.056
		p	.883	.976	.930	.441	.844	.784	.581	.581	.836	.776	.786	.426	.916	.118	.767	.778
MACFIMS Fails Population Criteria	EO	PRE	-.227	-.232	-.243	.029	.225	.436*	.119	.037	-.33	-.245	-.247	.096	.301	-.07	.157	.139
		p	.245	.234	.214	.885	.249	.020	.546	.853	.086	.209	.204	.628	.119	.741	.424	.482
	EO	POST	-.092	-.081	-.083	.024	.103	.080	.130	.157	.010	.013	.017	-.023	-.011	-.122	.024	.080
		p	.643	.684	.675	.903	.603	.685	.511	.425	.959	.946	.931	.908	.956	.537	.902	.685
	EC	PRE	.128	.164	.156	-.167	-.181	-.105	-.221	-.195	.064	.168	.131	-.16	-.05	.164	-.16	-.14
		p	.515	.405	.428	.395	.357	.595	.259	.320	.747	.394	.507	.423	.792	.405	.410	.479
	EC	POST	.199	.162	.188	-.224	-.188	.031	-.202	-.209	.005	-.007	-.004	-.280	-.002	.160	-.259	-.249
		p	.311	.409	.339	.253	.338	.875	.304	.286	.980	.972	.983	.149	.992	.416	.183	.202
MACFIMS Fails Self Criteria	EO	PRE	-.063	-.084	-.073	.026	.035	.318	.044	-.024	-0.1	-.05	-.038	.119	.103	-.03	.129	.091
		p	.752	.670	.711	.895	.859	.100	.825	.903	.599	.801	.848	.547	.601	.884	.513	.645
	EO	POST	-.073	-.059	-.066	-.047	.098	-.045	.049	.068	-.05	-.06	-.045	-.14	.059	-.29	-.05	.059
		p	.710	.765	.740	.813	.618	.820	.804	.732	.786	.762	.820	.491	.766	.133	.795	.766
	EC	PRE	.159	.220	.178	-.089	-.209	.050	-.176	-.166	.193	.309	.268	-.100	-.185	.126	-.149	-.151
		p	.419	.260	.366	.652	.285	.802	.370	.399	.324	.110	.168	.614	.347	.523	.450	.443
	EC	POST	.057	.026	.050	-.096	-.047	.012	-.050	-.057	-.085	-.098	-.088	-.319	.076	.005	-.208	-.192
		p	.771	.894	.802	.625	.811	.951	.799	.775	.667	.619	.656	.098	.702	.982	.289	.328

Table 38 Relationship of MSCI to Qualitative Metrics of Connectivity Derived from Coherence (Coh).

			ALPHA								High ALPHA							
			APL	CLUST	Deg	Deg V	G.E.	λ	γ	σ	APL	CLUST	Deg	Deg V	G.E.	λ	γ	σ
MSQ	EO	PRE	.209	.278	.255	.300	-.205	.053	.284	.207	.090	.164	.138	.205	-.065	.085	.205	.161
		p	.286	.152	.190	.121	.296	.791	.144	.291	.648	.404	.484	.296	.744	.668	.296	.412
	EO	POST	-.044	-.008	-.047	.123	.049	.027	.147	.158	-.203	-.122	-.107	-.049	.166	-.155	-.073	.014
		p	.825	.969	.814	.532	.806	.892	.455	.421	.301	.536	.587	.806	.399	.430	.713	.945
	EC	PRE	-.133	-.167	-.159	-.065	.121	-.545**	-.039	.135	-.359	-.357	-.349	-.402*	.347	-.105	-.424*	-.402*
		p	.500	.394	.418	.742	.540	.003	.844	.495	.061	.062	.069	.034	.070	.597	.025	.034
	EC	POST	-.036	-.137	-.123	-.188	-.016	.126	-.248	-.182	-.213	-.227	-.215	-.160	.160	.060	-.177	-.165
		p	.855	.486	.534	.339	.936	.523	.203	.355	.275	.246	.273	.415	.415	.761	.368	.402
MACFIMS Fails Population Criteria	EO	PRE	.027	-.135	-.056	-.274	-.035	-.092	-.312	-.280	.078	-.052	.019	-.145	-.132	-.137	-.211	-.173
		p	.890	.492	.778	.159	.861	.642	.106	.150	.692	.794	.923	.461	.503	.488	.280	.380
	EO	POST	.085	.111	.146	.028	-.092	.011	-.031	.030	.407	.262	.291	.163	-.414*	.290	.084	.012
		p	.668	.575	.458	.887	.640	.956	.874	.880	.031	.178	.133	.408	.028	.134	.671	.951
	EC	PRE	.069	.016	.057	-.044	-.069	.219	-.070	-.071	.182	.197	.194	.247	-.174	-.183	.204	.234
		p	.727	.935	.775	.823	.726	.263	.723	.720	.354	.314	.322	.205	.376	.350	.298	.231
	EC	POST	.235	.288	.334	.277	-.201	-.223	.204	.236	.390*	.386	.401	.291	-.364	-.161	.189	.266
		p	.228	.137	.082	.154	.306	.255	.297	.227	0.040	.043	.034	.132	.057	.414	.335	.171
MACFIMS Fails Self Criteria	EO	PRE	-.060	-.222	-.149	-.367	.051	-.026	-.367	-.345	-.033	-.073	-.023	-.119	.041	-.148	-.166	-.119
		p	.762	.255	.448	.055	.796	.896	.055	.072	.866	.712	.906	.548	.837	.452	.398	.546
	EO	POST	.266	.122	.208	-.025	-.269	.077	-.203	-.161	.457*	.283	.300	.165	-.450*	.427*	.053	-.030
		p	.171	.536	.289	.899	.167	.698	.299	.412	.014	.145	.121	.402	.016	.023	.788	.880
	EC	PRE	.040	.050	.050	-.085	-.012	.271	-.163	-.209	.195	.255	.237	.366	-.192	.065	.390	.339
		p	.842	.802	.799	.666	.953	.163	.406	.285	.320	.189	.225	.055	.327	.742	.040	.077
	EC	POST	.120	.225	.218	.243	-.068	-.189	.209	.214	.164	.208	.169	.229	-.143	.050	.197	.180
		p	.542	.249	.266	.213	.732	.335	.285	.275	.403	.289	.390	.241	.469	.802	.314	.359

Table 39 Relationship of MSCI to Qualitative Metrics of Connectivity Derived from Synchronisation Likelihood (SL)

			ALPHA								High ALPHA							
			APL	CLUST	Deg	Deg V	G.E.	λ	γ	σ	APL	CLUST	Deg	Deg V	G.E.	λ	γ	σ
MSQ	EO	PRE	.284	-.027	.270	-.223	-.357	-.355	-.244	.329	.209	-.155	.200	-.265	-.291	-.368	-.238	.358
		p	.143	.890	.165	.255	.062	.064	.212	.087	.287	.431	.307	.172	.134	.054	.224	.061
	EO	POST	-.027	-.049	-.099	.105	.009	.043	.074	.281	.009	-.002	-.088	.089	-.002	-.027	.076	.402*
		p	.892	.803	.616	.597	.965	.829	.709	.147	.965	.993	.658	.654	.993	.890	.700	.034
	EC	PRE	.239	-.028	.250	-.213	-.282	-.277	-.252	.346	.117	-.148	.293	-.304	-.236	-.383*	-.305	.433*
		p	.221	.886	.199	.277	.146	.154	.196	.071	.555	.451	.130	.116	.227	.044	.115	.021
	EC	POST	.259	.098	.323	-.172	-.181	-.122	-.148	-.187	.142	.158	.166	.096	-.048	.060	.038	-.044
		p	.182	.620	.093	.382	.356	.536	.451	.342	.470	.423	.399	.626	.808	.763	.847	.825
MACFIMS Fails Population Criteria	EO	PRE	-.077	.016	-.035	-.091	.061	.023	-.090	-.165	-.005	-.067	.072	-.108	.013	-.049	-.126	-.084
		p	.698	.937	.861	.646	.757	.907	.647	.401	.981	.733	.715	.586	.947	.804	.523	.669
	EO	POST	-.011	.144	.034	.069	.035	.053	.046	-.118	.030	.160	.043	.061	-.001	.082	.070	-.068
		p	.957	.465	.863	.726	.858	.791	.814	.549	.881	.415	.827	.759	.994	.680	.725	.729
	EC	PRE	-.292	-.069	-.290	.275	.274	.316	.288	-.143	-.261	-.074	-.332	.266	.305	.356	.318	-.266
		p	.132	.727	.135	.157	.158	.101	.137	.467	.179	.707	.084	.171	.115	.063	.099	.172
	EC	POST	.020	.023	-.005	.092	-.064	-.098	.016	.498	.116	-.044	.079	-.054	-.180	-.137	-.035	.186
		p	.919	.906	.980	.641	.748	.621	.935	.007	.557	.825	.690	.783	.361	.486	.859	.344
MACFIMS Fails Self Criteria	EO	PRE	-.014	.076	.039	-.070	.026	.010	-.071	-.053	.057	.024	.140	-.061	-.040	-.088	-.110	.030
		p	.944	0.701	.845	.722	.896	.959	.720	.790	.772	.905	.477	.757	.841	.657	.579	.880
	EO	POST	-.004	.071	.029	.030	.061	.057	.026	-.155	-.047	.082	.015	.028	.079	.112	.039	-.222
		p	.983	0.720	.882	.878	.756	.772	.897	.431	.814	.680	.941	.886	.691	.570	.845	.256
	EC	PRE	-.177	-.004	-.223	.190	.185	.206	.227	-.077	-.076	.049	-.243	.227	.145	.274	.261	-.138
		p	.367	.983	.254	.333	.347	.292	.246	.698	.700	.806	.212	.246	.462	.159	.179	.484
	EC	POST	.015	-.186	-.005	-.110	-.149	-.266	-.174	.509**	.095	-.225	.053	-.234	-.224	-.318	-.190	.398*
		p	.938	.344	.981	.577	.448	.172	.377	.006	.631	.250	.790	.232	.251	.099	.332	.036

Table 40 Relationship of MSCI to Qualitative Metrics of Connectivity Derived from Direct Transfer Function(DTF)

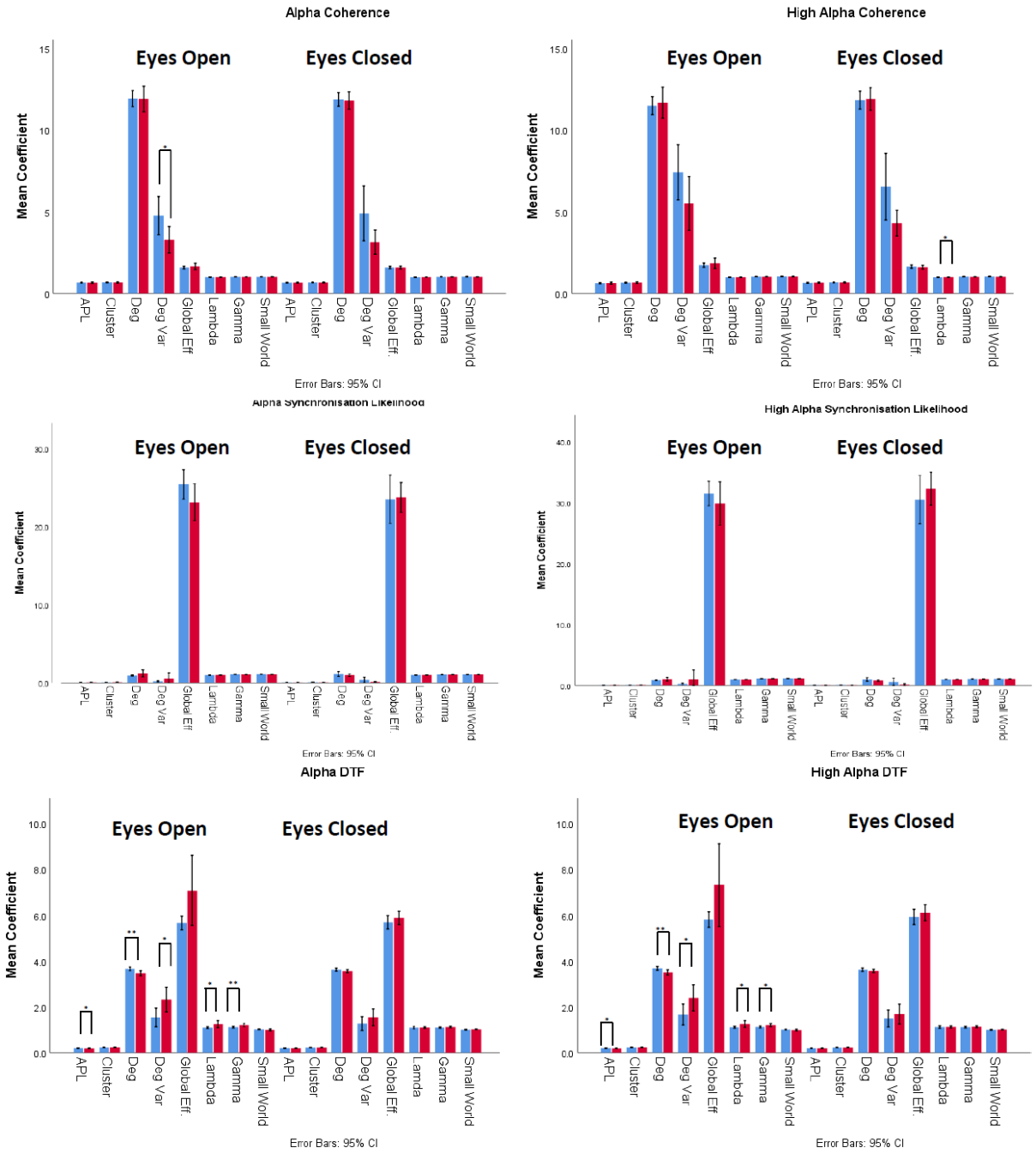


Figure 31 Variability of Qualitative Connectivity Metrics between Pre and Post Testing Conditions

In both Eyes Open and Eyes Closed by bandwidth of interest (Alpha & High Alpha) and coupling derivation (Coh, SL and DTF)

DISCUSSION

The recruited cohort sample appeared typical of that seen in routine clinical practice (when compared to the wider BrAMS clinical cohort (n=1800) when contemporaneously examined as part of service planning for 2016) with respect to demographic balance and phenotypic spread. There was a possible underrepresentation of individuals with more severe physical disability. The inclusion criteria demanded the capacity to provide informed consent and engagement with the psychometric tasks; this effectively precluded ascertainment of neurophysiological data from patients with frank MS-related dementia and in future work it would be useful to establish the electrophysiological characteristics of this as the opposing end of the spectrum to that possessed by healthy control series.

The prevalence and severity of MSCI in this group was felt to be sufficiently mixed. The comparison between conventionally applied and individually tailored criteria for judging the presence of cognitive impairment on tests reveals a significant disparity. Whilst readily acknowledging the possible methodological weakness inherent in attempting to derive effective estimates of an individual's premorbid general intelligence and then using this singular index to serve as a standard against which their performance across a range of differing domains is then judged there is conversely, as seen here a likely failure to detect that which may be very relevant to the *individual* when comparing them against otherwise demographically matched peers, especially when they may appear to do 'favourably' in such comparison. A large scale study by Jennum *et al.,* (787) demonstrated that employment in patients with MS begins to steadily fall in the 8 years *prior to diagnosis* relative to controls and continues to steadily drop thereafter. This is clearly in an interval before physical disability becomes a limiting factor – 'subclinical' cognitive decline must be a prime candidate as a driver of this and the identified prevalence of MSCI amongst patients with 'incidentally' identified radiologically isolated syndromes (788, 789) lends support to this argument. One acknowledges fatigue has an independently recognised bearing on employment also (790, 791) but this influence is more established in post-diagnosis studies.

The very nature of occupation and hierarchical status attained therein (with accompanying responsibilities and fiscal reward) particularly within the typically service-led economies characteristically found in countries where MS is particularly dominant(3, 22, 124), whilst heavily determined by motivation is nonetheless highly dependent on the cognitive ability of the individual concerned.

The very Darwinian nature of promotion and competition within work environments ensures (with an acknowledged interplay of many other economic and personal factors) that individuals will typically enter roles highly matched to their cognitive ability. The *occupational tolerance* of an individual losing cognitive performance maybe far less than that based on a demographically matched population based criterion of the kind used in many psychometric studies. Simply put, they may pass clinical testing thresholds but still experience limiting difficulties in the real world; indeed, in addition to the hard outcome

of occupational loss, MS patients experience a greater general incidence of negative work events also whilst occupation is maintained(451). Difficulties with learning new knowledge and skills may further prohibit entry into new roles once old ones are necessarily abandoned and the 'disability trap' of unemployment is recognised in the MS literature (792). The particular focus on employment is not simply or primarily one of cold health economics; employment status and household income are very strong determinants of quality of life amongst MS patients (793, 794).

Resolving these issues and achieving the most practically useful 'optimal' clinical cognitive outcome measure will require further exploration of approaches such as the MSQ or similar to ultimately arrive at some form of methodological consensus and prescription of thresholds with established confidence intervals of measurement. The current heterogeniety of psychometric batteries(407, 416, 448) and cut-off criteria can only contribute to the sometimes conflicting variability of findings amongst investigations of therapy and biomarkers in relation to neuropsychometric performance(59, 624, 795).

Our pursuit of electrophysiological biomarkers of MSCI was motivated by prior experience of evoked potential techniques and awareness of a growing literature suggesting promise (253, 424, 545, 551). The more classical approaches of cognitive evoked potentials were included in the hope they may sit as natural partners alongside the physical MMEP battery given some guidance for their standard acquisition (539) exists in addition to empirical findings suggesting an association with cognitive dysfunction in this disease setting (253, 424, 540, 543, 545-547, 552, 796) and other neurodegenerative diseases (549, 797).

At the outset certain recognised limitations(657) were acknowledged to exist which had to date restricted general implementation of cognitive potentials into the sort of routine clinical and investigative practice observed with visual and somatosensory evoked potentials. Firstly, in contrast to the MMEPs used in our other works which are all effectively passive requiring little active participation (aside from minor active participatory facilitation for MEP acquisiton) on the part of the patient to yield outcomes (313, 329), cognitive EPs do demand the active attention of subjects (539, 549, 550, 658) particularly with respect to the elicitation of the more widely explored P300 response. The lack of attentional requirement is seen as a possible major advantage of the 'pre-attentional' MMN response (551, 657) hence its exploration in a broad range of conditions where even conscious allocation of attention may not be possible(551).

In an attempt to discern, if technically possible, separate P3a and P3b components a three-component auditory oddball paradigm was employed (using standard, target and deviant tonal stimuli). The observed performance accuracy was imperfect; even with a brief introductory familiarisation period and the inclusion of those without subjective auditory impairment. One could make the argument that this in itself is informative and that performance on a beep-discrimination task in itself could perhaps constitute a useful clinical test of auditory attentional capacity; effectively obviating the requirement for analysing the accompanying evoked potential response. Indeed the false positive hit

rate did significantly relate to worsening performance on the information processing and working memory tasks. Notably the *challenge* associated with using such 'active' paradigms is perceived to be a factor limiting the application of cognitive evoked potentials in patient populations generally (657) as once accuracy breaks down completely response characteristics cannot be interpreted reliably.

The utilisation of the cognitive ERP and EEG recording techniques were extremely well tolerated by all participants in our study and encouragingly, herein each of the three cognitive potentials bore a significant relationship with extent of MSCI, however in a manner that appeared moderated by both testing condition (pre and post battery) and cognitive rating criterion. The pattern of association was generally strongest for MMN and least for P3a. Nonetheless combining them into the composite CEP-3 score (as we had previously done for MMEP) provided both stronger and more consistent patterns of association with indicators of MSCI.

These heartening findings must be balanced against a number of caveats. Firstly, whilst the use of a semi-quantitative scoring system based on a spectrum of results as applied here is in principle a reasonable method for handling informative absent responses, the thresholds used here have by necessity been derived from the same cohort. It is quite conceivable that the quartiles for the respective parameters might be different in alternate datasets with a resultant opportunity for modifying the strength of association.

The impact of age is acknowledged to be significant with respect to the P3b response(544), with attenuating amplitude and progressive latency naturally evident after the middle of the third decade. Regression age-adjusted normative thresholds for the P3b were not used herein and such have not been published for the MMN or P3a responses either.

At the time of conduct outside of our study normative data on MMN were not established and had been studied in less than 100 PwMS globally (253, 552). Some normative data for how the mean and the upper limit of the classic P300 (3b) vary with age between 20-80 years had been published along with general guidelines for their acquisition (657) however it was dually recognised the very nature of the tonal stimuli, particularly with respect to its frequency and presentation probability significantly modify evoked responses as does the duration of the test itself and the degree of novelty of the stimulus, which naturally decreases over time in a manner suggestive of habituation(539, 549). A contemporaneously identified meta-analysis(544) examining the P300 (3b) technique reinforced both the significant influence of age on the latency and amplitude characteristics of these potentials. Latency reduces during the early years of life, reaching a nadir at around 20 before again slowing increasing with advancing age; with an exactly converse pattern being displayed for amplitude(544). Such a pattern appears to match well developmental patterns of myelination (136, 701) and cortical network sculpting (702, 798, 799) which support increasingly *integrative* processing prior to their subsequent decline with senescence(701). However, the spread of data across the 75 separate studies included within the meta-analysis of P300s (544) does raise a very real concern that such a marker may not possess the measurement properties

required to perform small signal studies of sufficient statistical power. Particularly as the observed overlap between cognitively impaired and healthy controls may indeed be significant (657). Although notably, the variance of cognitive potential scores (including the CEP-3) did not significantly vary between the pre- and post-test conditions (separated by ~90-120 minutes) with the exception of the P3b response. Criterion based scoring may therefore help overcome some of the natural variability associated with electrophysiological measurement.

Nonetheless, the existence of reliable demographic norms would allow a more rigorous semi-quantitative scoring system to be applied to all three potentials and it is perhaps the absence of this approach which accounts for the lack of significant association between our cognitive evoked potential ratings and the MSQ scores; which by their nature feature adjustment for age to a greater degree than the population-based cut-offs used in the thresholds for judging MACFIMS failures and which is completely absent from the raw cognitive test score performances. This factor may account for the association of these two features with cognitive potential scores in the seemingly paradoxical absence of an association with the MSQ.

Effectively, measured by the method used here (without the benefit of more precise normative adjustment) cognitive potentials may index raw intellectual function to a greater degree than the *deficit between raw and predicted* performance attributable to MSCI.

An additional critique is the absence of accompanying objective audiometric analysis to assess capacity for frequency perception in the tested frequency ranges. Clinical audio threshold testing was employed however and all subjects could reportedly hear the provided tones. A recent attempt (800) at normative data collection for the auditory MMN response in 40 young healthy adult subjects suggested no significant differences between dominant and non-dominant hemisphere generally but an effect of gender did appear to exert a significant influence on MMN latency in the non-dominant hemisphere, having tested each ear separately; female non-dominant MMN appeared faster. Our study issued stimuli to both ears synchronously, however we have focussed on the left hemispheric response in an attempt at consistency. Given the influence of frequency, stimulation intensity and attention (which although not required for MMN does nonetheless have a modifying effect) on MMN responses(539, 551) the direct application of such norms to the exact sort of attention-directed triple-stimulation paradigm used here is open to question and ideally *paradigm-specific norms* should be acquired. This said, stimulus novelty(539, 549) exerts a modifying effect particularly on the P300 responses which may produce a confounding effect at repeat interval testing. A 1000Hz tone may simply not elicit the same target response every 3-6 months over the course of a longitudinal study; misinterpreting this '*loss of novelty*' as an index of '*worsening*' would be erroneous. However, the effect of stimulus novelty on P300 amplitude is accompanied by a simultaneous and possibly related effect of '*task difficulty*', with more cognitively demanding tasks eliciting higher amplitude responses(539, 549). Interestingly, far from seeing a diminution of response amplitude due to loss of novelty (for P3a or P3b) over the course the ~90-120 minutes test re-test interval our cohort

demonstrated significantly increased amplitude scores for the P3a and P3b waveforms after the cognitively tiring batteries (Wilcoxon Signed Rank Test p .06 and p .013 respectively), congruent with it posing more of a challenge after cognitive exertion. Again, there was no association between the magnitude of this objective change and subjective ratings of total or specifically cognitive fatigue on the NFI-MS.

These important influences of novelty and task-complexity on variability, whilst potentially limiting are nonetheless very tractable to evaluate and possibly surmountable. For instance, in construction of norms one could employ different stimulation paradigms across a sufficiently large number of demographically varied people; this would be akin to the *'multiple forms'* approach utilised for the neuropsychometric tests. It would also be useful to identify over what interval the effect of novelty is lost.

A caveat to this is the assertion by some investigators(801) that an increased amplitude of cognitive potentials in earlier disease (as seen in MS subjects versus healthy controls for a behavioural error-related negativity which arises fronto-centrally in response to error detection may represent some form of unspecified adaptive compensatory mechanism. Such that, rather than a simple linear alteration of cognitive EP properties across the MSCI severity spectrum, we may observe initial increases of amplitudes prior to diminution with advancing disease. Some support for this pattern was seen in an additional study of auditory and visual oddball within RRMS and healthy control subjects; notably frontal P300 amplitudes were greater than in controls and positively correlated with performance in patients but not controls (673) and this was interpreted as sign of additional *adaptive cortical recruitment* to maintain faculties before such capacity is exhausted and impairment arises. On fMRI a pattern of *increased* cortical activation by MS patients in comparison to healty controls in response to cognitively demanding tasks (particularly over the frontal and parietal regions) has been recognised for some time (623, 802) in a manner that is very much less evident with more severe MSCI. Compensatory effects on latency are less clear and thus at the expense of losing information conveyed by EP amplitude focusing particularly upon temporal properties alone may therefore ultimately and usefully simplify matters.

Although the waveforms of MMN, P3a and P3b have been derived from recording channels specified in acquisition guidelines, in a manner which respects the presumed topography of their cortical generation it would be appropriate in future work to confirm the regional basis of their generation is both congruent with that advocated in the healthy control literature and how this relates to structural indices of MS pathology. One might question if the P3a and P3b (evoked by deviant and target stimuli respectively) have indeed arisen from frontal and more postero-central sources in the paradigm and disease setting explored here. Application of source topography and co-registration with neuroimaging would help substantiate this.

Exploration of the phase slip rate as an objective electrophysiological metric of cerebral dynamics did demonstrate a significant degree of association with clinical tests of information processing speed; in a fashion that was most evident with the most

challenging and hence discriminating PASAT ‘2 task and particularly over the fronto-temporal regions. This is the first exploration of this property in the setting of MS. Its derivation here was focussed on the alpha band and within individual channels whose rates were then subsequently averaged.

EEG is however able to offer some assessment of spatial extent and going forward it would be appropriate to examine the number of adjacent channels manifesting synchronous phase slip events to offer insight as to the spatial scale of such interactions across the cranium in addition to their rate. There is some emerging evidence that the number of regions recruited into such shifts (putatively related to the effective connectivity between neighbouring cortico-thalamic units) has a positive association with intellectual function in its own right(803).

The exact physiological substrate of these abrupt shifts in oscillatory frequency, which may not be wholly confined to the alpha band, is not completely clear(717, 718, 723) however the mechanism of phase encoding as means to maintain segregation between processing of distinct stimuli or information streams has been demonstrated by LFP recordings *in vivo* within non-human mammals(708). Whether the slower average phase slip rates seen in relation to worsening MSCI are a cause or simply an accompaniment of slowed information processing speed remains unclear. The sampling of phase slip rates during active tasks as opposed to resting state conditions as analysed here may help define the relationship to a stronger degree. The presence of movement artefact during the tasks themselves was considered prohibitive to doing so here; nonetheless paradigms using hand based responses rather than verbal report and minimal head movement would facilitate such an exploration. Aside from the BVMT-R task none of the psychometric tests used herein required any manual dexterity to minimise the influence any concurrent ataxia (a common feature in MS(644)) may have had on resultant scores; allowance for such was made in rating BVMT-R outputs to limit any confounding effect.

Note is made of an outlier in one condition of the phase slip averaging, over the frontal region. The degree of association with PASAT ‘2 performance was the same on Pearson’s and Spearman’s Rank correlation. Outwith this outlier the average range of phase slips over the frontal regions was 1.4-2.2 slips per second. The regional average phase slip rate was not significantly different in the pre and post-test conditions between most regions, with the exception of the frontal and central areas. Notably once again, the extent of variability in phase slip rates was not associated with any subjective rating of total or cognitive fatigue. The effect of threshold for defining a phase slip and the optimal bandwidth for its derivation will need to be examined further also.

The findings also suggest that at least the connectivity aspect of the inquiry was under-powered to a degree which likely varies by each metric considered. To fully characterise the associations between electrophysiological connectivity would likely take a cohort several-fold larger. Without question the ascertainment of normative healthy control data would be equally crucial.

A strength of the paradigm however, was the inclusion of a second set of resting state 90-120 minutes after the first set, following cognitive exertion.

Interestingly no meaningful associations of subjectively rated cognitive fatigue were identified; even where dynamically changing electrophysiological properties were seen these did not significantly associate with fatigue, a property that is ubiquitous amongst all MS cohorts(30, 804-806). With respect to fatigue our failure here may relate to acknowledged methodological weakness or an absence of connectivity relating to this symptom; equally and has been argued elsewhere a major problem may arise in both the validity of the construct and the rating instruments which purport to 'measure' it(11). We employed the NFI-MS given its supporting literature and the Rasch analysis involved in its development(411). However, any future explorations should ideally focus on a more objective aspect of this symptom, namely *stamina* and employ a more targeted paradigm to explore this nebulous but very disabling phenomena which also significantly contributes to loss of employment (790, 791).

The pre/post assessment of variability and correlation did however suggest that single one-off recordings could potentially have made data perhaps appear far more significant than the picture from repeated sampling would imply.

A real-world neurophysiological biomarker will theoretically be applied to people at various circadian phases and differing levels of prior cognitive exertion. The *system* level assessment we seek must somehow adjust for this if possible in a sophisticated manner that will need to go far beyond mere averaging; some form of *state* level correction is needed.

A further contributor to the failure connectivity analysis as seen here is indeed the application of averaging in derivation of these metrics (particularly in the linear analysis of coherence) which have an inherent assumption of quasi-stationarity (642, 720) of the respective EEG channel time-series. The albeit modest success seen with results from our average phase slip rate analysis is a testament both to the non-stationarity of these oscillations *in vivo* and their apparent disturbance as a possible contributor to MSCI.

The *dynamics* of the system in question, beyond those of the recorded oscillations are effectively wholly overlooked by these approaches in a manner which is likely methodologically fatal to the desire to adequately index cognition and its impairment.

The inherent pitfalls of multiple comparisons analysis(612) are duly appreciated. Even in the absence of correction techniques (such as the particularly conservative Bonferroni adjustment (612, 613) or more forgiving hierarchical false detection rate minimisation approaches(807)) it is immediately apparent that even acknowledging the inherent variability of EEG time-series and caveats attached to the various connectivity measures, in this modest size cohort of typical MS patients a strong signal of association between MSCI and *connectivity* (measured by linear, non-linear and multivariate autoregressive approaches) did not present itself. This is keeping with an earlier coherence based analysis of (557) in a similar group. Notably significant associations were found between increased functional connectivity and MSCI within the beta band by Tewarie *et al* (564)*.,* using the alternate metric of Phase Lag Index and alternately using synchronisation likelihood applied to MEG by Schoonheim *et al.,* (808). This high dependency on choice of connectivity is well recognised (478, 557, 809).

Indeed, aside from a possible, modest and not wholly consistent association between antero-posterior coupling (putatively those regions served by the superior longitudinal fasiculus) and MSCI the convergence of findings here would be to suggest that MSCI is perhaps *not* primarily driven by long range disconnection *per se,* at least in the commonly accepted sense (478, 536, 810).

This said, the earlier positive alpha coherence findings reported by Leocani *et al.*(555)*,* were from a progressive cohort and such measures may indeed have more discriminative sensitivity to damage where it is more severe, hence its demonstrated efficacy in cases of traumatic brain injury for example (715). This said, interhemispheric alpha band coherence has been observed to fall in a cohort of RRMS patients (811), however MEG was deployed in this setting and this is in many respects a more sensitive method particularly when utilising higher density arrays(694, 812).

Our exploration was initially focussed on the alpha and high alpha bands because of the putative role of synchrony in this band supporting long range coupling between interacting regions (307, 700, 707, 813, 814), and the particular weakening and slowing of this oscillatory band being seen in the context of MS by other investigators (Van Der Meer (554, 555, 815). Also electrophysiological studies, of quantitative and qualitative connectivity have suggested specific abnormalities in this frequency band (568).

All of these analyses are readily applicable to higher (beta) and lower (theta) band widths, however the cohort size is not sufficient to validly support such a broad range of uncorrected association analyses. And furthermore, this was not part of the original question under test and there is already adequate information (from the phase and CEP findings) to support pursuits in a much larger group with healthy matched controls where all such analyses can be performed to yield more reliable outcomes. Additionally, in our network modelling we have effectively given equal consideration to the output from and interactions between all available channels; this is in keeping with the approaches of others ((478, 648)) and yet somewhat counter to the general notion that certain regions (particularly those in the midline *rich club*) have greater relative importance to cerebral network integrity and function(684, 766, 816); whether focussed regional network interactions may have greater yield is a worthy consideration given the changes in network morphology (namely altered centrality and of the default mode network regions(817) and generally increased modularity(818)) that have been described in MS in addition to qualitative changes in connectivity(478, 624).

Two further important considerations that have become apparent with exploration of current and emerging literature are firstly the appreciation that not all oscillations at a given frequency are necessarily of the same genesis(307, 700, 708, 814) and therefore their diminution may arise by multiple different pathophysiological mechanisms and due to varied matching between task and frequency have equally varied consequences. Secondly, whilst focus on individual bandwidths is an intuitive first step, *in vivo* there is much to suggest cross-frequency coupling between oscillations of different frequencies at the same and across locations is a more accurate description of the interactions which underlie cognitive processing (708, 727, 732, 813, 819, 820).

Deriving indices of such '*multiplexing*'(813) of signals from EEG time-series arising from individual channels is tractable, indeed cross-frequency bi-coherence between high and lower frequencies is seen to vary as a function of level of consciousness and is increasingly explored in simple montages in the field of anaesthetics(821). However expanding this principle to larger arrays of multichannel data, is far more challenging and introduces an additional set of methodological permutations. It would certainly demand a larger dataset but nonetheless remains an area of consideration for our group.

Our application of classical graph theoretical metrics did not yield consistent patterns of association with indices of MSCI. This is likely multifactorial and comes with the acknowledgement that even outwith this enquiry, at least across many studies in the field of functional neuroimaging a consistent pattern of association between MSCI and graph metrics has not emerged either(59, 624, 706) in the same manner as has been seen in more archetypal neurodegenerative conditions(535). A singular but larger enquiry of graph modelling in EEG applied to MS had been conducted shortly prior to our explorations, which made use of different cognitive outcome metrics and an alternate array of coupling metrics in addition to synchronisation likelihood as used here(478). We did not fully recapitulate their findings (and indeed did not explore theta/delta coupling wherein much of their associations were found), which generally suggested a perturbation of clustering in relation to MSCI, putatively as a consequence of disconnection.

A rejection of the small world conceptual framework in the MS field would however be extremely premature. The use of electrode positions (in 'sensor space') to serve as nodes of a brain network model is a simplification of convenience, however even application of higher-density arrays in this manner would not necessarily overcome the possible flaws this crude approximation to the cerebral networks brings. Mapping of electrophysiological data onto structural imaging may enable construction of real-time functional networks with a more realistic node arrangement based on *brain-space* rather than a complete abstraction largely related to low-resolution sampling points on the scalp.

The very definition of what constitutes an 'edge' linking nodes and the ascription of their length or weighting also remains methodologically open; we have applied established techniques for threshold identification prior to its application to the adjacency matrix but various thresholds could be applied each with knock-on consequences for resultant connectivity and graph property measurements(652). A possible reason for the modestly superior results seen with DTF here is that by its nature its offers more potential links (given it is a directional measure) between nodes than the simpler linear and non-linear techniques which are non-directional functional coupling methods. The number of links emerging from adjacency matrix will clearly have a bearing on the opportunity for generating small worldness(571) and other properties and one cannot be wholly certain that normalisation against randomly constructed graphs of similar numbers of elements fully correct for this effect.

An important exploration in any posited future work would be to evaluate varying thresholds on network outcomes and to identify if possible whether a more optimal method for judging edge length or weight exists. We have notionally taken stronger couplings to infer shorter edge lengths, but the exact mathematical function linking the two properties is certainly open to recalibration. One must also remain realistically conscious that EEG based network measures derived from scalp recordings applied without spatial localisation may simply not demonstrate either small worldness or other useful metrics despite various efforts at methodological tuning.

Further still, the analyses applied here and predominantly elsewhere have been built on an assumption of relative spatial stationarity and *three-dimensionality* of the networks they are seeking extract graph metrics for; this is increasingly recognised as being a probable false approximation of *in vivo* behaviour(822, 823). Examination of spatially extensive networks which have directional coupling to others at different times via shared nodes is an advocated form of *hyper-graph* analysis (824). This approach is still in its infancy but would greatly benefit from the temporal resolution of EEG. The application of such a higher-dimensional hypergraph approach to large fMRI data series has already shed insight recently into significant patterns of even more deeply embedded higher-level organisational change across the cerebrum which accompany neurodevelopment and which are not apparent by simpler three dimensional abstraction (824). Application of such techniques to neurodegeneration remains unexplored, but sensitivity to higher-dimensional network changes with developmental construction does strongly hint toward utility when this effectively '*runs in reverse'* with degenerative deconstruction. On reflection we should perhaps not expect reductive low dimensional attempts to measure the high dimensional property of cognition to yield adequate biometric indices of it.

A further general methodological spectre that will haunt all neurophysiological pursuits seeking to define outcome measures will be the absolute requirement to establish not simply how such candidate metrics change with age over years but more pertinently how they naturally vary, both in health and disease (as the two may not be equivalent) over intervals of hours, days, weeks and months. Natural circadian, ultradian and infradian rhythms of cerebral physiological function are well recognised (700) and will exert corresponding influence over cognitive performance and in all likelihood the evoked potential correlates of such processes. The same blessing-curse relationship will unavoidably exist for any metric based on the reasonably tight causative association between cognitive processes and their accompanying physiological substrate, including those utilising EEG generally. A possible solution may arise in being able to make adjustment of observed physiological responses not only for the 'external' factors known to contribute to its variance, such as age, but also for the 'internal' state of the system under test; by extracting other electrophysiological indices more directly related to state rather than response generation *per se*. Whether this is viable remains to be seen.

An age-old question which has accompanied neurophysiological efforts in functional brain cartography is the selection of which electrical reference system to use to generate the very time-series for subsequent abstraction (646). Artefactual enhancement

of the correlational structure between electrodes by volume conduction (760) and shared reference effects (703) will inadvertently skew emerging data leading to a misperception of functional integrity. If connectivity measures are to serve as the foundation for higher level abstractions into networks and similar they must be as methodologically robust as is achievable to limit the *propagation and magnification of error* up the processing hierarchy. The very strength of EEG lies in its reported high temporal (535, 642, 700) resolution yet in the context of analysing signals, which are by their nature indivisibly *spatial* (and) *temporal* structures the dichotomy is not so sharply defined and the inappropriate choice of referencing system can have adverse effects on both domains concurrently (760). The application of Laplacian derived estimates of Source Current Density have a number of advantages in this regard (642) and have performed reasonably herein; however in contrast to simpler bipolar, common average and linked-ears reference systems there is a far greater variety of different algorithms available each with associated methodological pros and cons (825).

Achieving consensus on any metric will require equal accord on the systems of acquisition and abstraction to derive it (646).

The plethora of acquisition and virtually unlimited processing permutations brings with it statistical challenges that have been appreciated from the earliest days of topographic analysis of electrical brain activity(646). The risk of type I error from small scale studies subjected to multiple comparisons analysis is considerable(612); herein application of conservative thresholds imbued by Bonferroni corrections would have proven prohibitive. Having considered this problem in depth, any efforts going forward should incorporate not only larger datasets but application of established hierarchical systems of analysis(807) to minimise our false detection rate. This is not a perfect solution and desire to minimize type I error must be balanced against the equal desire to minimize errors of the second type. The examination of larger data series would as in most cases support both requirements, however *in absentia* of a definitively established metric of known statistical properties of average value and natural variance it is difficult to perform the necessary formal power calculations *a priori.* The cohort size selected here was based on the samples which had yielded useful findings in the earlier work of others (555, 557) and that applied to our preceding MMEP work; the absence of a defined power estimate is a recognised limitation of this exploratory endeavour and even estimates derived in a *post hoc* fashion will vary by outcome variable. The general '*reproducibility crisis*' amongst EEG findings (645, 826) particularly those using increasingly sophisticated derivations of abstract properties applied to standard EEG time-series remains a contributing factor behind their general lack of clinical application in neurocognitive disorders (644) despite a long held view that such approaches given their inherent reflection of brain function 'should' ultimately prove useful (646, 707, 743). Whilst factors such as the common reliance on small group sizes and the 'filtering effect' of publication bias toward only positive replications has in part contributed to the current *status quo* (644) so equally has a relative paucity of data on the natural short and longer term reliability of candidate EEG metrics applied to the same patients(645, 809).

Only very recently have efforts been undertaken to explore such variances in the case of connectivity based derivations. A study (809) of 60 subjects featuring a mix of healthy controls, individuals with subjective cognitive complaints and patients with temporal lobe epilepsy examined a series of 14 different connectivity metrics applied to two standard 27 channel montage EEG recordings a fortnight apart. The measurements were collated into a multivariate regression model and their respective inter-recording correlations examined (admittedly without higher abstraction into graph theoretical properties). Remarkably strong (rho >0.9) correlations were seen for spectral, coherence and direct transfer functions(809) of the kinds applied herein. However, it is particularly noteworthy that the presence of *clinical dysfunction attenuated the reliability* within different frequency bands and measures depending on aetiology, with less inter-test consistency seen in the delta band couplings of patients with mild cognitive impairment for instance(809). A smaller (n=10) preceding comparison of re-test reliability utilising MEG(827) was less encouraging and systemic review of the reproducibility of graph theoretic properties based on connectivity measures reported across 23 studies suggested, as one might perhaps anticipate are less reliable when applied to functional connectivity measures than when applied to structural derivations from DTI and similar (826). However, most studies included in this analysis(826) were fMRI based and not electrophysiological in nature or clearly focussed on specific components and instead may have represented more global analyses.

The more recent work of Pinter *et al.* (828) in examining the reliability of connectivity derivations applied both generally across the cerebrum and more specifically across the nine main intrinsic resting state networks to fMRI data from both stable PwMS (n=20) and healthy controls (n=14) over a test re-test interval of three months is illuminating. Whilst the general assessment of connectivity did *not* appear to show an acceptable degree of agreement on repeat testing, correlation was generally very strong when examining the specific regions of interest in the ICNs suggesting potential for high reproducibility(828). This is both encouraging for those pursuing reliable functional indices and a lesson that *targeted assessment on key areas* may yield best results.

The notably lower reliability of low frequency EEG connectivity indices seen in the context of mild cognitive impairment by Holler (809) stands against a consistent background of findings from others (827, 829-831) suggesting an inherently superior degree of reliability of connectivity indices when examined in the slower frequency bands than those above alpha, ie. beta and gamma. This may in part relate to the sheer technical difficulty in accurately recording gamma activity on the scalp given the substantial temporo-spatial filtering effects imposed by the very conductive properties of the skull (642), band-pass filtering of EEG signals at acquisition and overlap with the frequencies of electromyogenic artefacts particularly outwith the central verteceal regions (675). Given the putative central role of gamma frequency oscillations in cortical processing (52, 307, 700, 708, 728, 732, 733, 735, 737, 740, 814, 832-835), this is a very unfortunate and well recognised limitation of scalp electroencephalography (642).

More broadly, even outside the gamma band the contribution of signal-to-noise ratio exerts a demonstrable influence on reliability of connectivity estimates (831) as do the

number and durations of epochs examined (830, 831, 836). Thus, it is reasonable to simply summarise that the influence of *any* factor which has a bearing on the initial ascertainment of a connectivity estimate (such as electrode separation in the case of EEG (642)) will be *magnified* when the procedure is repeated for comparison.

Adherence to the tightest consistency of measurement techniques is a central principle in the daily practice of general clinical neurophysiology (313) precisely to avoid such magnification of error (results disparate from the *'truth'*) in the delivery of direct patient care. Such high standards will similarly need to be followed in the pursuit of candidate electrophysiological metrics particularly if results are to be compared between studies with any validity.

On reflection it was perhaps overly optimistic to anticipate possible strong identification of a small-world signature in networks of comparatively small size (19 nodes) when consensus has not been achieved on fMRI approaches which have up to 10^5 nodes available to them for such analysis (771). However, bearing in mind the established *decay* of small worldness which arises with increasing edge density (571) examination of these properties at this scale was perhaps not wholly unreasonable. Nonetheless, one must also appreciate the influence of smaller network sizes in generally placing an inherent constraint on path lengths such that clustering exerts a disproportionate influence over small worldness, a recognised effect (837) which may certainly therefore have been at work in our initial exploration here.

Further, the application of graph theory to EEG based networks in the context of Alzheimer's by Stam *et* al.(652) demonstrated the very strong modifying effect of threshold setting on the significance of differences in a between-groups comparison against subjects without multi-domain cognitive impairments. Herein a singular method for threshold determination was applied and the utility of other thresholds remains unexplored. Although the values of Synchronisation Likelihood attained appear 'low' in contrast to other metrics, nonetheless they are comparable to those in other studies (647).

Moreover, relationships between the properties underpinning small-worldness, namely clustering and path length were seen to have some albeit modest but inconsistent association with MSCI in such low node density paradigms both here and in other EEG based work (478) and MEG studies(568) wherein MSCI appeared associated with a general move toward both increased clustering and average path length (but in a manner that varied with frequency band). Findings from this latter study(568), which identified that only such a pattern in the alpha band as being of particular significance (and which employed Synchronisation Likelihood) were interpreted as a move toward a more '*regular*' , less *complex* and less disorganised network by the investigators. The possible influence of a shift in the power spectrum effectively inflating the lower alpha component at the expense of deflating the higher alpha component was acknowledged by the authors (568).

To adjust for such an effect one might consider 'tuning' the alpha coupling to the respective alpha peak observed within each individual. However, with disparate roles

and topographies so far attributed to the recognised *family* of alpha frequency rhythms (700, 707, 814), it is not clear that would be wholly appropriate even if on *prima facie* it may accommodate changes in the EEG power spectrum accompanying the encephalopathy which produces MSCI.

The use of individual alpha peak frequencies and a move away from the fixed classical bandwidths has recently gained (838, 839) but the identification of such peaks, if and where they exist is again open to methodological variability. They have however, along with a transition frequency tuned to the individual's delta very recently been successfully applied in conjunction with LORETA to Multiple Sclerosis by Babilioni *et al.* (815). Their EEG analysis of 36 RRMS and 23 PMS patients in comparison against a further 41 healthy control subjects recapitulated not only the quantitative abnormalities in the power spectrum but also significant topographic differences in the *topography* of such changes even between earlier relapsing and later progressive patients, with particular emergence of greater slow activity over the centro-parietal and limbic territories of the latter(815). Such a spectral deterioration over 'the rich club' territories purportedly central to bestowing small world architecture and integrative function is in itself an intriguing result.

All such findings however must be reconciled against the seemingly mixed pattern of quantitative EEG results described by Keune et al. (840) wherein SDMT performance (taken as a sole surrogate of information processing speed) was *positively* associated with a frontal *increase* in low and high alpha activity whilst simultaneously being related to an increase in theta:beta ratio (an increased slow, reduced fast activity balance) which was in turn related to performance on an objective test of attention in PwMS. Whether such an apparent partial discrepancy is related to heterogeneity in methods of topographic analysis, acquisition paradigm, or simply greater synchrony of 'idling' pyramidal cells in more poorly engaged cortex of those with MSCI, or some other unknown factor remains to be seen.

The direction of any observed association between small worldness and MSCI also clearly needs interpretation with caution. Whilst a single maxima for it exists falling values may arise from differential change in the measured balance between clustering and path length, with the optimal system for judging the latter far less clearly established than the derivation of the former (571, 768).

Further caution is warranted against over-ascription of the paramount importance that small-worldness and its drivers may contribute to information processing in the cerebrum (771) despite the popularity this topological 'universality class' has attained on the basis of its compatibility with intuitive notions of network function and apparent widespread prevalence across contexts (535, 770). Indeed, the pattern of *branched loop* systems with *re-entrant* interactions between and within cortical and subcortical structures is a far more striking coupling motif (700, 744, 841-845) which are recognised targets of MS pathology(529, 564, 567, 632, 846) and which may generally play a far more pivotal role in cerebral *integrative* function than abstract network properties possibly evident across the cortical surface. Furthermore, such arrangements of re-

entrant loops and branched networks are fundamentally united in their activities and the necessary attempts to 'untangle' such 'knots' (744)will again be far from straightforward.

Our initial expedition into the applications of graph theoretical approaches to EEG datasets has focused primarily on an examination of spatial structure, albeit using temporally-variant signals, to generate what may be termed '*stationary connectivity'* (847). Whilst acknowledging the importance of seeking an indicator of larger scale global dynamics in the form of phase alterations, which do show some significant relationship to information processing speed in a manner that warrants further examination in its own right, a more rigorous assessment of cerebral dynamics in Multiple Sclerosis is mandated. Assessment of the Global Field Potential across the scalp by EEG has demonstrated the appearance of an ever changing distribution which is regularly punctuated by periods of transient stationarity, termed microstates of ~100 milliseconds in duration(848-850). Careful regressive analysis to overcome the differing temporal resolutions of co-registered EEG-FMRI dataseries has demonstrated a fair matching between the activity of resting state networks and specific microstate topographies within the scalp Global Field Potential. (848-850) As yet microstate dynamics in the setting of MSCI remain unexplored despite the promise already demonstrated in the setting of gross encephalopathy, neuropsychiatric disease and dementia (851). A first initial exploration recently conducted by Gschwind *et al.*,(852) using high density EEG in 102 subjects (53 with RRMS and 49 healthy controls) has demonstrated some association between microstate features with some general disease characteristics relating to global disability severity but no clear association between the occurrence or frequency of certain microstates with cognition as measured solely by SDMT alone. It is a reasonable proposition that the utility of such dynamic EEG features will lie in examining their rates of change(848, 853, 854) rather than component features *per se.* Notably, microstate adjusted connectivity assessments have also recently been demonstrated to offer a superior degree of association with cognitive function in the context of Alzheimer's(851), in a situation where without such 'microstate correction' relationship to connectivity was not evident at a group level. Our group attempted to apply established microstate characterisation algorithms to our MS dataset for this very purpose however extraction into characteristic microstate topographies was not felt sufficiently reliable in our cohort. Given the foregoing discussion of increased (spatially distributed) cortical activation arising in the context of MSCI as seen on fMRI (623, 802) the difficulty with global field power characterisation we encountered here may not be a chance finding but an electrophysiological correlate of such distributed cortical activation. Assessment of concurrent fMRI and EEG microstates in MS subjects would be required to test this supposition.

Difficulties with spatial microstate characterisation will not however prohibit evaluation of their *dynamic* patterns of change over time. However exploring the promise of microstates will bring the increasingly familiar multiple-approaches problem.

A further dynamic property of networks worthy of exploration is resilience; or capacity to maintain integrity in the face of possibly adverse change. A recently described

framework (855) suggests this inherent property of all functional networks can be reliably indexed by examining their behavioural trajectories *en route* to collapse. The simplification of various sophisticated heterogeneous network models down into a two dimensional state-space of effective connectivity and effective node activity on which such a trajectory is mapped and then measured to yield a singular index of network 'health' is an appealing prospect with a strong theoretical underpinning(855). Whether it is tractable with EEG dataseries or of use in MS is an intriguing question certainly amenable to exploration.

Future work should also seek to explore the relationship between identified neurophysiological properties of relevance to MSCI with those suggested to have similar relationships on neuroimaging. Not only would such attempts at cross-modality validation add further conceptual support to the proposed use of such measures but it may also offer insights into the relative importance of the various components which collectively constitute the physical substrate of MSCI (including atrophy of the cortical and deep grey matter structures particularly in addition to the cortico-cortical white matter fasiculi). The availability of a contemporaneous structural neuroimaging dataset may also facilitate attempts at cross-registration with EEG datasets for the purposes of source localisation by the aforementioned sLORETA (659) technique or similar. The same connectivity algorithms used to characterise coupling between time-series recorded at disparate topographic locations on the cranial surface can similarly be applied to oscillating targets localised by imaging bounded-reconstruction to the cortical structure itself (856, 857). The insights already afforded by such work do support the concept that large scale distributed intrinsic cortical networks fundamentally utilise synchrony between regions as a substrate for their dynamic formation (728). However, whilst such techniques may in part overcome the acknowledged variability inherent in the limits of spatial localisation afforded by the 10-20 system (classic *ex vivo* work demonstrates at the very least a centimeter of variance of electrode positions in relation to the main Sylvian and Rolandic fissures of the cerebrum (406)), every level of abstraction in post-processing appears to serve as an opportunity for error, attrition and noise. A recent comparison of the significantly discrepant findings between different methodologies applied to identical datasets is also not wholly encouraging (858).

Herein some attempt was also made to limit strong confounding influence of centrally acting drugs by the exclusion of individuals with a liability to seizure who may require anticonvulsant therapy (and similarly possess inherently different electrophysiological network signatures). However MS patients commonly utilize agents for neuropathic pain, anticholinergics for neurogenic sphincter dysfunction and centrally acting agents for spasticity(28) all of which may exacerbate effects of MSCI or potentially alter EEG findings. This is an acknowledged limitation and almost unavoidable if one wishes to examine typical patients; also some argument could be made that alterations of electrophysiological patterns should be accompanied by influences on cognitive performance by such drugs and *vice versa* which may attest to a strength rather than a weakness of the technique in question. No patients on prescribed stimulants were examined however subjects were not screened for recent caffeine or other alerting agents which may be acquired over the counter. Whilst a growing literature on the

cognitively modifying effects of such substances is appreciated(859), particularly with respect to positive effects on attention and uncertain attenuation of dynamic aspects of cognitive control and flexibility (860, 861) the same rationale applied to prescribed medications outlined above may similarly hold here in this instance. Going forward however formal handling of such potential influences is indicated.

All said therefore, a deeper consideration of the nature of MSCI is required with a move toward constructing a more refined working model or framework which whilst respectful of a contribution from disconnection is not solely based upon it but is perhaps focussed on *higher-dimensional system level features* which are conceptually closer to the actual physical substrate of cognitive processing.

CONCLUSION

MSCI is a critically important feature of the condition. Patients, clinicians and investigators alike could be well served by the availability of effective clinical and biomarker surrogate measures of it. Derivation of such metric techniques will be far from straight forward and would benefit from establishing both a rigorous conceptual model as to the essential nature of MSCI and directed efforts to divine an approach of abstraction based on such a framework.

The initial exploratory pilot endeavours described herein, in combination with a growing body of work elsewhere suggest that the central question in relation to the utility of electrophysiology in neurodegenerative disease is perhaps not one of '*if*' it can be useful but very much '*how*' best to make it so.

LJWC

IX

Towards A Measure of Brain Functional Measurement Based On Capacity for Qualia

'It is always the unknown that leads us'

Jean Martin Chauvet

Aims To develop a desired biomarker surrogate of brain functional integrity and cognitive capacity to aid acceleration of translational research in Multiple Sclerosis. **Background** Complex systems are partly defined by their capacity to generate emergent properties not demonstrable by reduction to exist within constituent parts. Consciousness (the generation of qualia) is the most emergent phenomenon produced by the brain. A non-invasive electroencephalographic index based on the framework of Integrated Information Theory and calibrated against limiting cases on the clinical spectrum of consciousness may yield the desired metric of brain functional integrity for use in disorders such as Multiple Sclerosis which are particularly characterised by heterogeneities in distribution of pathology and resultant cognitive sequelae arising from systematic functional disintegration. **Hypotheses** 1) The maximal capacity for measurable integrated information (Φ) is reduced in a scaled manner in neurological states associated with impaired consciousness. 2) There is a positive association between the structural spatiotemporal complexity of electroencephalographic activity and the capacity for integrating information (Φ). 3) These metrics are reduced (compared to healthy controls) in patients with Multiple Sclerosis and MS-Related Cognitive Impairment (MSCI) and yet remain greater than in subjects without clinically demonstrable awareness (conscious experience). **Method** After generation of a system-level framework to capture essential features of MSCI, standard clinical EEG datasets (10:20 montage) recorded from 20 patients in states of Slow Wave Sleep, REM, Wakefulness and various aetiologies of Coma (n=20) underwent processing to yield surrogate metrics of Φ ($\Phi*$ across an atomic partition) and Complexity (Local Channel Higuchi Fractal Dimension and Global Dynamics captured by Phase Slip Avalanche Size Ratios) along with datasets from Healthy Controls (n=108) and patients with Multiple Sclerosis and various degrees of MSCI (n=30) before and after cognitive exertion. **Results** A highly significant pattern of association between level of consciousness and the Fractal Dimension and $\Phi*$ was seen, with the behaviour of the latter being akin to a form of system level mutual information between past and present states. MS subjects were most clearly distinguished by a pattern of suppressed large scale dynamics at a point of criticality which bore relation to their information processing speed clinically. They also displayed increased system level mutual information between past and present states in the alpha and theta bands which inversely correlated with cognitive performance; both findings were independently consistent with reduced temporal complexity of global cerebral dynamics in association with MSCI. **Conclusion** A Framework based on Integration Information Theory coupled with a physical conception of Complexity and Entropy may offer the necessary metrics to capture the cerebral dysfunction underlying the disintegration which characterises MS related Cognitive Impairment. However, $\Phi*$ is an inadequate surrogate of Φ as conceptualised and further exploration is warranted.

Plain Language Summary With the most evident injury of Multiple Sclerosis arising amongst the pathways in the brain which interconnect the regions on its surface and deeper structures within, the concept that thinking and memory difficulties in this disease arise from a disconnection of components naturally developed. However, the recognition that the disseminated damage also proportionately arises in such components in addition to their connections and that the collective architecture naturally serves to produce integrated processing with cognition arising in an emergent fashion from such interactions stimulated the perspective that Multiple Sclerosis related thinking impairments result from a disintegration of normal function. Recognising the brain as a complex system from which thinking emerges lead to pursuit of a system of analysing the electrical patterns of brain activity on the scalp based on the extent of their complexity and dynamical change over time and how these things vary with differing levels of consciousness, taken as the most emergent property to arise from the brain. Some initial promise for this framework is demonstrated from work in a number of subjects in various states and conditions. Plans for future endeavours are outlined.

Team & Contributions

Dr. Luke Canham	**Conceptual Framework Development, Project Design, Ethics, Recruitment, Data Collection & Statistical Analysis**
Dr. David Western	**Signal Analysis**
Mr. Adam Pearson	**Clinical EEG Dataset Acquisition of Non-MS Subjects**
Dr. David Cottrell & Dr. Kirtsy Inglis	**Recruitment of MS Subjects**
Mr. Peter Walsh & Dr. Nick Kane	**Neurophysiological Methodological Advice**
Ms. Alzbieta Klicnikova & Mr. Matthew Harland	**Psychometric Testing of MS Subjects**

Special thanks are extended to the Research & Innovation department of North Bristol NHS Trust for their sponsorship of this work, in particular Mr. Jamie Methuin thereof for his permission. **Declarations –** All datasets used herein were irreversibly anonymised at point of collection.

INTRODUCTION

Global Climate Change, Economic Crisis and Neurodegenerative Disease arguably represent three of the greatest challenges to the current generation and all share the singular theme of complex systems undergoing collapse (59, 862, 863).

Our stated goal as a motivated collection of clinicians, scientists and stakeholders in a better future for persons with MS (PwMS) is to overcome delays in translational research conferred by current approaches to measurement. International efforts in this field have focussed on a broad array of possible indices of various disease features across a catalogue of modalities (159, 236, 240, 291, 514, 624, 626, 628, 864, 865). Our group has focussed on neurophysiological techniques and identified a degree of potential therein.

There is meaningful coupling between Evoked Potential responses conveyed by the long tracts and physical disability (181, 183, 188, 190, 191, 254, 315, 435). The relationship to cognitive dysfunction appears somewhat less reliable – even when cognitive evoked potential techniques are employed. Our labours and considerations initially lead to exploration of quantitative changes within the electroencephalographic (EEG) architecture amongst affected patients; this offered modest yield and it is not clear how this might be taken further or what, in isolation, such changes *'mean'*.

Following the advocated premise that cognitive dysfunction in MS emanates from a '*disconnection* syndrome' (478, 480, 536) driven by the disproportionate attack upon myelin (the dominant purpose of which is to facilitate timely communication by neurotransmission (307, 700)) we explored longer range connectivity in brain function.

This was achieved by examining the statistical dependence in the behaviour between electroencephalographic signals recorded across the head and analysing their relationship to MS related cognitive impairment.

Alongside the even more modest promise this offered, in parallel it posed a new and serious problem – what is the *best* method of deriving connectivity measures from the gamut of the many evolved techniques available?(562, 703). Our experience highlighted a significant variation in outcome dependent on method which is therefore a significant limitation when drawing inference and making conclusions.

Achieving similar results with different methods would typically engender greater confidence as to what was really being measured and its meaning (866) as it has here with MMEP, however such a 'convergence' has not been observed with connectivity metrics so far, either locally or elsewhere (59, 624, 706).

This issue has extra importance when one is making use of connectivity measures not only as possible outcomes in their own right but particularly so if they are serving as the foundation on which higher-level abstract network metrics are based. The consideration of which system to utilize must also focus not only on mathematical efficiency but applicability at spatio-temporal scales relevant to natural human cognition(642, 700, 814, 841, 867) and vulnerability to the many types of artefact(644, 675) which can plague EEG recording and interpretation.

Furthermore, as with our earlier studies on physical disability, findings from multiple domains, modalities or dimensions have proven collectively more informative than mono-parametric indicators; as reflected in their capacity to explain a greater fraction of observed clinical variance and offer insight into the key drivers of disability.

We should perhaps *not* expect that a single mono-parametric variable would be able to sufficiently index the scope of human cognition to serve as a translational biomarker; particularly in the context of our primary disease of interest. For it is reasonable to reflect that the very challenge of MS lies in the dissemination of its pathology at different times and places throughout the central nervous system(24, 26). This stands in stark contrast to many other archetypal neurodegenerative diseases which despite some heterogeneity of manifestations phenotypically do nonetheless have *almost invariable targets* which are affected early, progressively and consistently i.e. the primary motor cortex in Amyotrophic Lateral Sclerosis (868, 869), the substantia nigra in Parkinson's Disease(870) and the precuneus and medial temporal lobes in Alzheimer's disease(26, 871).

Whilst an MS specialist may immediately cite the cervical cord and optic nerve as similarly cardinal targets for pathology in MS(23, 25), charting the tempo and course of decline arising in these regions has not as yet proven clinically *useful* in the setting of Progressive MS even though one acknowledges the emerging benefit of structural and functional optic nerve assessment to translational endeavours in earlier disease(74, 872).

It is also important to consider that although one can construct lesion *probability* maps for cohorts of patients(267, 514, 619, 630, 873-875) these highlight *trends* in spatial dissemination which can nonetheless be vastly heterogeneous between patients. Also the consequences of lesions are not as readily predictable from location(876) as one might imagine unless embedded within strongly eloquent territories, which most are not (1, 877). Similarly, recognised effects from retrograde and trans-synaptic degeneration(220, 630, 878-882) on distant and neighbouring tissues connected to lesions are not clearly quantified either. Further still, in addition to recent appreciation of a large burden of disease affecting otherwise 'normal appearing' white and grey matter(82, 883) there appears to be a profound variety of dysfunction *at all levels* and

one therefore reaches the conclusion that pursuit of a single, analytically simple and low-level property is likely not optimal.

Rather than focussing on a single property in the hope it may function as a surrogate of a vastly more sophisticated and seemingly immeasurable organ it might serve to acknowledge the defining feature of our target system and indeed the very immune process attacking it – com*plex*ity(535). By this it is not meant that we shall resign ourselves to it being too difficult and conversely we shall be under no illusion that this is just a com*plic*ated problem – one that with enough steps taken to 'divide and conquer' will reasonably yield(156). No, in our pursuit we should next respect the brain as a Complex Adaptive System (CAS) in the proper sense (156, 744, 884)and similarly regard the immune cascade as another CAS which collides with it. By doing so we can immediately make use of the established scientific complexity framework that exists in part(156, 885-890) and come to have insight into why this disease is so variable and unpredictable, why treatment comes with such risks for typically incomplete benefit and most importantly open a potentially useful way forwards.

Multiple Sclerosis Cognitive Impairment:

A Syndrome of Disintegration, not simply Disconnection.

The central proposition herein is based on three main tenets; firstly, that the brain's capacity for consciousness is a product of its facility for *differentiating*, *integrating* and ultimately *specifying* complex informational constructs(744, 841, 891-896).

Second, it may be practically achievable to derive neurophysiological indices which reflect these key properties and which are in turn deterministically associated with level of consciousness.

Thirdly, if MSCI arises from pathological functional disruption of these key properties it is arguable that level of consciousness may similarly be reduced and the aforementioned neurophysiological indices may serve effectively as a biomarker surrogate of MSCI also.

However, is it reasonable to argue that MSCI is associated with reduced consciousness? An initial response, based on a classic, binary clinical perspective (of consciousness vs. unconsciousness) coupled with a sympathetic view that although patients with MSCI have cognitive difficulties '*they are no less conscious*' would be '*no*'.

Nonetheless, the notion of consciousness being a non-binary phenomenon which exists on a spectrum of varying content has gained increasing acceptance (749, 867, 897-906) and is of direct clinical utility(907). Indeed, a vast assortment of neurological syndromes are characterised by the subjective loss of phenomenal conscious experience (*qualia*) of various types consequent of localised brain injury, secondary to stroke or other acute pathology(26). Prosopagnosia (loss of facial perception due to fusiform gyrus dysfunction(841, 908)), achromotopsia (loss of colour perception from cortical area 4 damage)(909) and complete cortical blindness (from occipital lobe injury) are such examples(26). Moreover, losing the cortical representation of any part of the visual field

produces corresponding anopic regions of proportionate size(26) and an absence of appropriate qualia therefrom.

On a more global scale, there is much to suggest the multi-domain impairments of cognitive faculty and personality change accompanying the more classic dementias are also accompanied by subtle progressive disturbances of conscious experience(910) in addition to the more florid hallucinosis that may typify the synucleinopathies(26). Although subjective self-report of experience has recognised limitations(898, 911) and generating external representations of internal concepts may be greatly hindered by accompanying failings of praxis(912), the progressive qualitative decline seen in the self-portraiture and similar creative expressions by dementia sufferers (913) (and similarly those with Multiple Sclerosis (23)) does at least suggest a falling in the qualitative richness of their conscious experience. Moreover, the familiar bedside clinical tests using 'fragmented forms' to detect aperceptive agnosia and presenting multiple stimuli to bring out the simultagnosia commonly observed in dementia(912) are essentially testing a failure of conscious *integration* of the provided stimuli into the appropriate unified whole that should be experienced.

In the context of MS, the more readily discernible (by MRI) demyelination afflicting the commisures, association pathways and projection fibres of the cerebrum(25) has supported the model of MSCI being driven primarily by 'disconnection' (478, 480, 536, 706) in a manner wholly akin to the classical '*disconnexion*' syndromes introduced by Wernicke in 1874 and established into a larger clinical framework by Geschwind in 1965(914, 915). The initial focus on the genesis of various neurological syndromes arising from the break in communication between cortical areas rather than their own loss *per se*(915)(table 41) laid the foundation for appreciating the role of association areas and the interconnecting pathways between them in higher cognitive functions. This in turn engendered a move away from classical localisation models in support of the topologically distributed nature of many faculties(535, 916).

Table 41 Classical Disconnection Syndromes Associated With Subcortical Pathology

Adapted from (915) and (756, 810, 914, 917, 918)

Syndrome	Nature	Locus of Lesion	Descriptor
Conduction Aphasia	Impaired Repetition	Arcuate Fasciculus	Wernicke
Visual Agnosia	Impaired Feature Recognition without visual loss *per se.*	Inferior Longitudinal Fasiculus	Lissaeur
Alexia without Agraphia	Loss of reading ability with preserved linguistic representational ability	Splenium of Corpus Callosum	Dejerine
Non-Dominant Apraxia	Impairment of sequential cognitive- motor tasks on non-dominant side	Anterior Callosum	Liepmann
Tactile Anomia	Inability to nominally identify sensory stimuli presented to non-dominant side	Posterior Callosum	Geschwind

Over the period of many decades a growing recognition of the very particular expansion of the association structures during our evolution to *Homo sapiens* (136, 307, 700, 898, 919), has been paralleled by the similarly important appreciation of the cerebrum's achievement of integrated functional subspecialisation by arrangement into constituent interacting networks (534, 535, 684, 761, 762, 765, 766, 920).

Indeed, the original cytoarchitectural cortical mapping of Korbinian Brodmann (illustrated in (682)) has recently been augmented by a new cerebral cartography with delineation of regions based not only on their direct structural characteristics (relative thickness and myelin density) but also their structural connectivity with myelinated tracts rendered by DTI(921).

The function of the 180 distinct cortical regions identified in *each* hemisphere(921) is therefore appreciably a product both of the local circuitry within such modules and moreover the arrangement of white matter connections intrinsically coupled to them.

The role of myelinated fibres is generally considered one of facilitating rapid communication over distance(307, 683, 700); the benefits of saltatory conduction (922) are appreciated as are the resource costs (136, 766) and exquisite tuning of myelin thickness (307) to maintain cortico-cortical conduction delays with growing cerebral size. Indeed the ratio of grey to white matter across the vertebrate lineage (during which time the solution of myelination has arisen *but once* in contrast to several separate instances of gyration to optimise spatial utilisation(136)) appears tightly governed by a natural power-law (923) which further speaks to its *fundamental* importance.

The role of myelin in memory acquisition is also becoming recognised(924) alongside the range of trophic and metabolic support offered to enclosed neurons by the sheathing oligodendrocyte cells principally bearing it(71).

In contrast to the impression clinical MRI and gross macroscopic histological examination may offer the viewer, the white matter architecture could not be further from a homogenous unstructured mass(682, 686, 925). The long projection fibres (such as those of the pyramidal corticospinal tract) traversing vertically to and from the cortex, which are particularly clinically eloquent in the case of sensorimotor interruption (2) are accompanied by a great many more white matter pathways which are arguably less eloquent (and often go un-interrogated on routine clinical examination) but are nonetheless important.

For example, the family of commissures (comprising the corpus callosum, habenular, anterior and posterior commissures) facilitate horizontal interhemispheric communication (682, 757, 758, 926). All of these structures are recognised targets of MS pathology (180, 510, 755, 927, 928) however the subcomponents of the corpus callosum, namely the forceps anterior coupling each frontal lobe, the forceps posterior linking the occipito-parietal regions and the tapetum of fibres linking the temporal lobes, which collectively comprise a mere 250 million axons (929) appear particularly affected (481, 690, 930, 931).

Longitudinal intra-hemispheric cortico-cortical coupling is afforded by a further family of long association fibres coupled into fasiculi running antero-posteriorly; namely the superior and inferior longitudinal fasiculi, the arcuate fasiculus, cingulum bundle, perpendicular fasiculus and the uncinate fasciculus running between the frontal and temporal polar regions(682, 917, 932) (see figure 32).

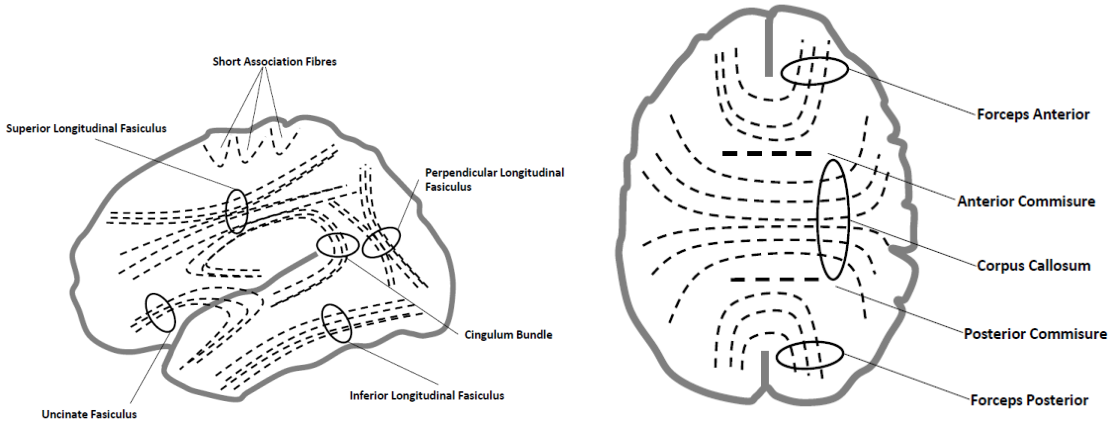


Figure 32 The Main Long Range Cortico-Cortical Pathways

Adapted from (682, 917)

All are recognised (192, 267, 271, 873, 883) as almost indiscriminate targets of MS as are the numerous short association U-fibres sitting immediately beneath the cortex (933)providing cortico-cortical coupling (934).

A number of higher-faculty impairments in the form of disconnection syndromes are also seen to arise from localised injury to these longitudinal pathways, such as visual object agnosia and prosopagnosia following damage to the inferior longitudinal fasiculus and ideomotor apraxia may similarly result from arcuate fasiculus injury also(810, 915, 917, 935).

The spectrum and severity of the various apraxias, agnosias and linguistic deficits arising from callosal injury has become a large field of inquiry in its own right following the Nobel prize-winning work of Sperry and Gazzaniga amongst others(756) into the neuropsychological deficits in callosotomy patients therapeutically lesioned in an effort to palliate refractory epilepsy.

The insights from such patients are not without some significant relevance for patients with MS given the almost characteristically high prevalence of injury to the callosum in this condition(26); likely consequent of both its proximity to ventricular structures and its representation of being a sizeable portion of cerebral myelin content(25).

Corpus callosotomy patients experience a variety of neuropsychological effects particularly with respect to diminished ability to report that which is processed in the hemisphere contralateral to cortical language representation(756). Such deficits develop alongside a range of apraxic phenomena arising from altered control of non-dominant limbs also and higher visual perceptual deficits may additionally occur(756).

The heterogeneous completeness of the commissurotomy in such cases, which are also relatively few in number offers some explanation for the degree of observed clinical variability(756). Also even in nature's own 'experimental' setting of the rare individuals born without a callosum due to congenital dysgenesis capacity for interhemispheric information transfer was recently demonstrated on functional and diffusion tensor imaging (936) as being facilitated by aberrant changes within the preserved connections of the anterior and posterior commissures of those patients.

Such findings highlight both the strong biological drive to establish interhemispheric communication and the potential for adaptive coupling to arise in the absence of callosal fibres.

Whilst the disconnection framework offers insight into the origin of various forms of apraxia, agnosia, aphasia and alexia, and how these emerge from focal disruption in the aforementioned white matter cortico-cortical fasiculi both highlighting their importance and in some cases offering clinical localising value(912) such classical syndromes are apparently *not* a common feature in routine MS clinical practice(26) despite the prevalence of injury to the relevant fibres.

Two complimentary reasons for such an observation are firstly that this may reflect reporting and ascertainment bias and secondly the lesions are frequently incomplete (39). Not all plaques lead to complete pathway transection of fibres passing through them and therefore the real-world presentation of such partial '*disconnection by degrees*' is likely to be far milder than the dramatic phenomena such as Alien Hand Syndrome resulting from more complete antero-middle callosal injury(937); indeed subtle pathology may produce corollary subtle signs such as apraxic difficulty with bimanual tasks needing tight interhemispheric communication between motor cortices(935).

It is noteworthy that despite the great centrality of such bimanual function to occupational roles (typing, driving and craftsmanship) and the progressive atrophic loss of callosal volume on imaging over time with MS(180, 510) such apraxias are not greatly explored by standard clinical review or clinical outcome scales in translational studies. Although notably driving safety is observed to be significantly adversely affected by MS even in concurrent road-users(938-940), this appears to be more significantly associated with cognitive-attentional deficits rather than physical disability *per se* (as gauged by requirement for vehicular modification), however bimanual apraxia has not been clearly evaluated in this context.

Despite offering some initial insight into the specific higher function difficulties which may arise from a collection of named cortico-cortical pathways which are seen to be injured in patients with MSCI, particularly with respect to the anterior forceps (267, 481, 930) and the callosal structures(510, 755, 927), the accounting for MSCI is nonetheless very much incomplete.

There is a degree of mismatch between the neurobehavioural features seen in such cortico-cortical disconnections (912) and those seen in association with MSCI (456)

which can only be redressed at least in part by consideration of the remaining intracerebral white matter tracts and the main subcortical structures they connect to the cerebral cortex, which by extension constitute nodes of cortical networks(535) and are similarly vulnerable to disconnection(810).

Interspersed within the longitudinal association and horizontal commissure fibre scaffold exists a vast array of vertical myelinated projection systems running precisely mapped efferents to the component nuclei of the thalamus (cortico-thalamic fibres) and striatum (via the subcallosal fasiculi of Muratoff)(810, 917, 932).

These fibres are well recognised targets of MS pathology and these deep grey structures have been consistently found to undergo significant atrophic loss in MS in a manner that correlates with cognitive impairment to a degree greater than most other structural radiological findings(267, 426, 483, 497, 499, 502, 567, 630, 632-634, 636-640, 691, 941-945).

This centrality of association is matched by the marked overlap of MSCI with the neurocognitive features seen in other disease states causing fronto-striatal injury (such as Parkinson's disease) namely apathy, bradyphrenia, impaired behavioural flexibility, executive dysfunction and constructional apraxia, along with diminished working memory and capacity for attentional shifting(912, 918, 946, 947).

However, in as much as the cognitive domains we consider central to MSCI are in themselves abstract constructs each dependent on an association of diverse underlying cognitive mechanisms(618), the output and importance of the subcortical components cannot be reductively considered in isolation but only within the context of the *re-entrant systems*(744, 842, 844, 845, 948) (i.e. the source of any original output is, after a series of processing steps also often its destination) of which they are a part.

The '*loop*' motif of efferents running from cortex to basal nuclei, to thalamus and thence back to the cortex is very well established(744, 842-845, 948-950).

The topographical segregation of these loop systems (figure 33) into parallel and functionally distinct processing streams each matched by behavioural-cognitive syndromes in the presence of disease(810) also offers many insights into the substrate of MSCI, as insult *anywhere* within a given loop (to either its components or connections) can have similar final functional consequences(810).

For example, a cognitive-associative loop features projections from prefrontal, posterior parietal and supero-temporal cortices conveying polymodal information to the head of the caudate nucleus which is then processed and through the output projection via the thalamus returns influence to those original cortical source structures(842, 951). Interruption any point therein causes embarrassment of executive and spatial cognition (810, 876), in the same manner that pathology with the limbic loop running between the medial prefrontal cortex and ventral striatum causes features of apathy and abulia(952, 953).

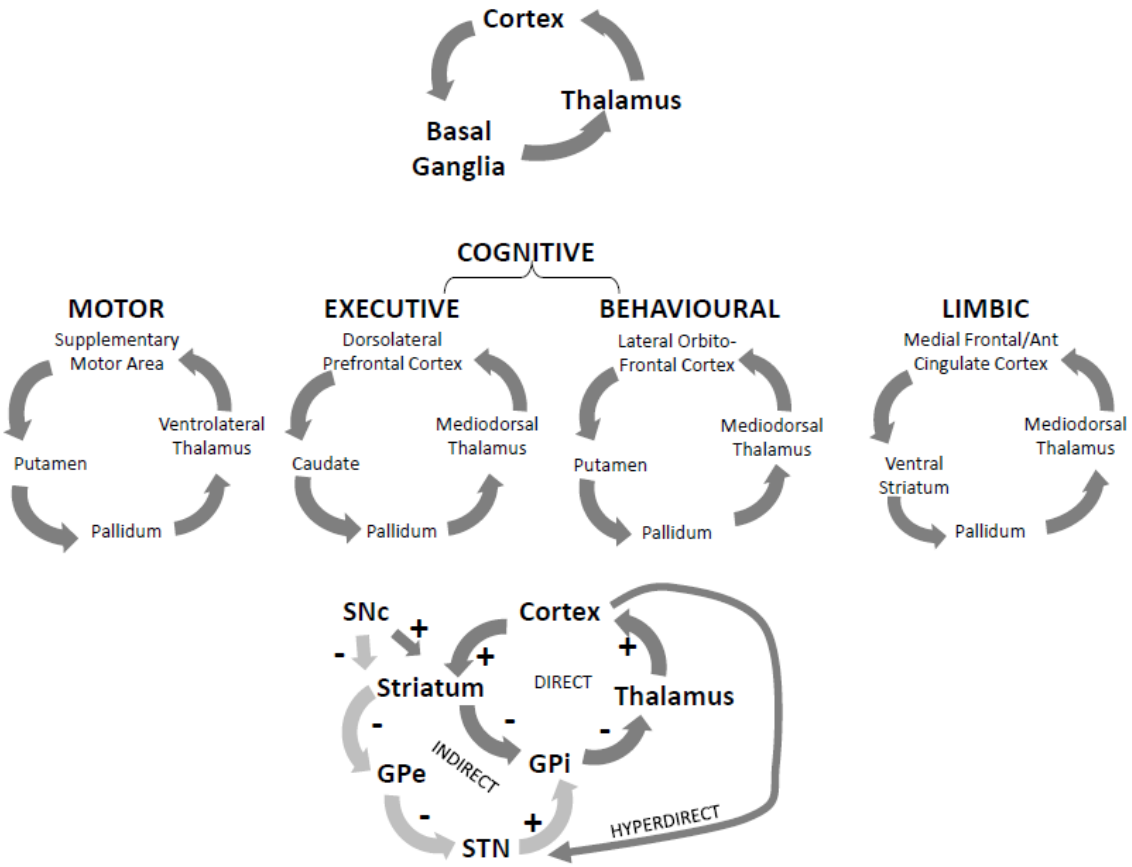


Figure 33 The Cortico-Striato-Thalamic Loop Systems

A series of stereotyped parallel re-entrant loop systems exist mediating motor, cognitive and limbic processing; within each a further series of direct, indirect and hyperdirect pathways contribute to active selection and deselection of relevant cortical activity making use of nigral and ventral tegmental dopamine to enhance (the +/- signs) the discriminatory gain between 'signal' and 'noise'; Adapted from (810, 951, 954, 955).

The central role of the thalamus to MSCI has been underscored by many volumetric and functional imaging studies consistently demonstrating a relationship to the commonly encountered cognitive deficits(426, 499, 564, 567, 630, 632-635, 637-640, 691, 846, 942, 944, 956-958); with the aetiology of thalamic dysfunction considered to be a combination of direct inflammatory attack, secondary decline following Wallerian degeneration of afferents and possible locally cytotoxic effects from local iron accumulation which in turn has been seen to associate with cognitive processing speed independent of thalamic atrophy *in vivo* (956).

Whilst the fortunate placement of the thalamus renders it relatively equidistant from all other cortical areas conferring minimal wiring cost and conduction delays(700), anatomically it directly encapsulates much of the third ventricle(682) making it extremely vulnerable to direct attack (25).

In addition to the classical Dejerine-Roussy Syndrome of contralateral pain and hemisensory loss with possible movement disturbance resulting from infarction of the inferolateral thalamus (810), three other patterns of infarction with distinct neuro-

behavioural consequences further highlight the relevance of this structure to cognitive processes.

Tuberothalamic artery occlusion with resultant anteromedial thalamic injury selectively impairs anterograde memory function, attenuates attentional capacity and damages working memory function; personality changes including a tendency to euphoria, emotional imbalance and impaired spontaneity are also seen(810). Again, all of these features are seen within the cogno-affective phenotype of MS(448, 456, 959).

Paramedian infarction may similarly produce a 'diencephalic' amnesia alongside general confusion, a tendency to anger and disinhibited behaviour; and finally posterior choroidal artery infarction is seen to provoke memory changes, subtle transcortical aphasia and visuo-perceptive changes(26, 810, 912).

As appreciated, the majority of these clinical features are encountered within MS subjects to lesser or greater degree(29, 448, 456).

These broad consequences of thalamic insult are a direct result of the centrality of this structure and its component nuclei to many other interconnected systems; indeed *almost all information* (barring olfaction) reaching the cerebral cortex journeys via the thalamus (700).

Whilst specific sensory nuclei relay information in an equally specific fashion onward to the cortex via the thalamo-cortical projection fibres(682), others participate on the final output stages of the aforementioned cortico-striatal-thalamic loops; with the parafasciular nuclei of the intralaminar nuclear group being relevant to the limbic (ventral striatal) and cognitive (caudate) streams respectively(842, 843, 845, 949, 960, 961).

This striatal limbic loop is accompanied by a number of anterior thalamic nuclear structures which have reciprocal connections with limbic structures of the cingulate, hippocampal formation, entorhinal cortex, restrosplenial cortex, orbitofrontal cortex and the medial prefrontal cortex in addition to the mammillary bodies and amygdala – all of which have well recognised importance to cognitive-emotional processing(810).

Three further features of the thalamus deserve special consideration; firstly the presence of certain 'associative' nuclei(700) believed to *integrate* cross-modality information and share this via reciprocal connections with areas such as the posterior-parietal and prefrontal cortices which are recognised seats of higher-cognitive processing(948, 962).

Then second is a unique architectural feature of the thalamus; namely the presence of a *shell-like* reticular nucleus encasing the other thalamic nuclear groups(700). The GABAergic inhibitory neurons of this structure receive collaterals from thalamo-cortical projections ascending through it *en route* to the cortex and direct feedback from the innermost layer VI(700). Neurons of the reticular shell are coupled by conventional chemical synapses, interneurons and also dendro-dendritic and electrical synapses; they

are therefore *uniquely* positioned to project potentially highly synchronised influence down onto thalamic projection neurons within their resident nuclear subdivisions(700).

These latter cells display a remarkable property of not only producing tonic spike activity when conventionally depolarised by direct incoming stimulation, but also in response to *inhibitory tone* which elicits hyperpolarisation activated Ca^{2+} conductances (I_H) which in turn provokes a low threshold Ca^{2+} (I_T) conductance and similar fast spiking activity prior to the subsequent cessation of the such conductances(700). This mechanism and similar(963), embedded within the unique cortico-thalamic, thalamo-cortical-reticular neuron feedback arrangement (964) predisposes it toward *cyclical* repetition and is considered (at least part) to underlie the natural tendency of thalamocortical units toward *oscillation*(700, 963, 964). The capacity of disparate thalamocortical units to be recruited into synchronous oscillation with each other is furthermore a reflection of and has a dependence on the integrity of fast coupling across and within reticular-thalamocortical units(803, 963, 965).

Of note, the association of microstructural disturbance within the normal appearing grey matter of the thalamus (evidenced by DTI) with physical and cognitive disability in MS (966) is accompanied by a perturbation of its intrinsic oscillatory behaviour evident even at the low frequency (<0.1Hz) resolution of fMRI fluctuations (967). Furthermore, thalamic atrophy is directly correlated with a lesser ability to support faster thalamo-cortical oscillation in the alpha band in MS (564), itself a recognised electrophysiological feature of the condition (554) and a shift which moderately associates with disease related cognitive impairment(554).

The third key feature of thalamocortical projections is a dichotomy between two main types of efferent. 'First order' efferents from thalamic nuclei serving primary sensory modalities which in addition to sending collaterals to the surrounding reticular nucleus project directly to the lower layers of the cortex(700). Such projections are reciprocated with direct feedback from cortical layer VI also, which indeed are much greater in number(700). However, the 'relative' simplicity of these *re-entrant* cortico-thalamic primary couplings stands in sharp contrast to a parallel system of 'higher order' thalamocortical projections – which are conversely driven by cortical fibres from layer V projecting collaterals (*en route* to primary targets within the brainstem) and which in return project efferents to several cortical layers over a *distributed* region(700, 968).

This collection of higher order thalamocortical projections arising within thalamic nuclei attributed to associative function(968) and which have *particularly* increased in number on the primate evolutionary journey towards *H.sapiens* (700) introduces a capacity for *convergence* and *divergence* within the larger cortico-thalamic complex such that regarding the loops between specific cortical regions and related thalamic nuclei as wholly segregated systems with no capacity for integration is both reductive and unfeasible(843, 845, 968).

Similarly, the same consideration applies to the aforementioned cortico-striatal-thalamic loops. Whilst the conserved topographic mapping of cortical to striatal targets initially suggests segregated parallel loops, examination of the connectivity patterns of the

principle medium spiny neurons within the striatum in a manner akin to that of the higher-order thalamocortical projection cells of association thalamic nuclei demonstrates a magnificent degree of convergence at points of interface between seemingly 'separate' loop systems(842, 843, 845, 948, 949, 951, 969).

The importance of such interactions is highlighted by consideration of the general functional purpose of the cortico-striatal complex itself; namely '*selection*' of certain frontal cortical assemblies of activity (relating to motor plans or cognitive choices) by the enhancement of '*signal*' (that which is useful, appropriate or desired) and diminution of '*noise*' (that which is not) (951, 970).

The highly conserved neuro-architectural arrangement of the widely sourced excitatory cortical input descending onto the striatum(136) and thereafter eliciting varying degrees of activity within parallel '*direct*', '*indirect*' and more recently appreciated '*hyperdirect*' pathways effectively serves to enhance certain cortical activities whilst actively deselecting others and additionally makes use of the differential effects of dopamine within the direct (encouraging by excitation) and indirect (discouraging by inhibition) pathways to enhance the '*gain*' in sensitivity (or signal:noise ratio in engineering terms) therein(951, 971).

The acts of selection, deselection and minimisation of noise are *non-linear* processes based on the combined strength and nature of all competing and contributing inputs; appropriately weighting the drivers within the basal ganglia (to achieve optimal selection) demands such input be effectively integrated within the striatum(951).

The lack of balanced integration or poor gain of 'signal:noise' within the striatal complex is as potentially compromising to its function (972)and therein that of the frontal lobe's output as damage to the frontal cortex or severance of its white matter projections(810).

This move away from comparatively simple *linear* arrangements of 'A' stimulating 'B' via a myelinated pathway leading to linearly predictable and 'simple' to measure outcomes to arrangements where the inherently probabilistic output is a product of which optimally weighted convergent subsystem has its activity selectively enhanced whilst others are deselected by inhibition to 'silence' (843, 949, 951, 973, 974)introduces a sharp operational *non-linearity*.

The *beauty* of such an arrangement is matched by both its effectiveness in providing a rapid adaptively-advantageous solution to the 'selection problem'(951) (hence its high conservation in the vertebrate lineage(136)) and the challenge to investigators in seeking to effectively measure its output.

Rather than acting solely as the *output* structure of the basal ganglia relaying feedback to the cortex, a similar series of subcortical loops running counter-current to the cortico-striatal loops appears to exist (975); therein extended limbic structures within the brainstem such as the periaqueductal grey, pedunculopontine and parabrachial nuclei use the thalamus as an *input* route to the basal ganglia.

The centrality of the thalamus is therefore even further extended apparently serving as a hub(968) for cortico-thalamic loops, cortico-striatal loops and a subcortical-striatal system(843, 961); with the grey matter component of each offering facultative *integration* between systems on account of the convergence of connectivity and divergence of axon collaterals and dendritic arbors therein(843, 951).

The mediodorsal nuclei of the thalamus also serve as integral components of the mamillo-thalamic tract running between the fornix of the hippocampal formation and entorhinal cortex to the mammillary bodies(634, 636, 691); the diencephalic pattern of amnestic difficulty seen following injury to such structures is well described in the setting of Wernicke-Korsakoff Syndrome and thalamic infarction(912) as described above and may also be seen in MSCI.

Injury to and atrophy of the parahippocampal formation is well recognised in the setting of MS and correlates with such amnestic difficulties also (619, 636, 690, 691). The myelinated alveus conveying myelinated fibres through the subfields of Ammon's Horn and the dentate gyrus to which it is coupled for the associative integration underpinning anterograde memory function(682, 700) is akin to the thalamus in being regrettably periventricular; and the entorhinal cortex which has widespread reciprocal efferent and afferent coupling with much of the cerebrum via large myelinated fibres is especially vulnerable to disconnection by demyelinative attack and axonal loss(531, 619-621, 690, 957, 976).

A further, final structure utilizing the thalamic nuclei and subsequent thalamocortical projections is the cerebellum.

This infratentorial structure forming the roof of the fourth ventricle is well appreciated as a frequent target of MS pathology(25, 498); with the commonly observed motor features of ataxia, tremor and dysmetria(23) resulting from direct insult to the anterior lobe of the cerebellar hemispheres, the vermis interposed in the midline or the connections therefrom with various brainstem structures(26, 332, 977).

Such features are a reflection of an impairment in the primary role of the cerebellar system – to serve as a *comparator* and where difference between intended and actual movement occurs rapidly introduce a necessary correction(810).

A summation of findings from patients with focal cerebellar injury (either from infarct, tumour, trauma or similar) and genetic disorders with predominant cerebellar dysfunction (such as the Spinocerebellar Ataxias) in recent years has led to the appreciation of a Cerebellar Cognitive-Affective Syndrome (CCAS) originally described by Schmahman and Sherman(810, 978). This features a constellation of executive difficulties (impaired working memory, task-set shifting and perseveration), impairments of visuospatial integration, affective dysregulation with imbalanced control of behaviour and personality change with flattening of affect and disinhibition(810) in a manner considered non-coincidentally familiar to that seen in Schizophrenia(979). Linguistic changes typified by impaired fluency and attenuated prosody have also been

observed(810, 980). Yet again, the neurocognitive and psychological overlap with MSCI(29, 448, 456) is striking.

Whilst the motor and cognitive-affective aspects of cerebellar functioning appear differentially localised to the anterior and more spatially-extensive postero-lateral cerebellar hemispheres respectively(981, 982), they nonetheless appear to utilise a near identical pattern of connectional arrangement yielding what has been described as the '*universal cerebellar transform*'(983). This recognition, along with the nature of clinical findings has led to the interpretation of CCAS being effectively a manifestation of '*dysmetric thought*'(983).

The general (and again from an evolutionary perspective highly conserved(136, 984)) arrangement is of input to the cerebellum entering from pontine nuclear sources via the middle cerebellar peduncle yielding efferents in the form of mossy fibres that either provide a short-loop influence on the deep cerebellar nuclei or more commonly directly synapse with the granule cells in the innermost granular layer of the cerebellar cortex(700, 985). These in turn synapse with overlying Purkinje Cells whose dendritic arbors extend into the outermost molecular layer and which are the principle source of outgoing projection axons synapsing on the deep cerebellar nuclei which thereafter via the superior cerebellar peduncle project to ventral thalamic nuclei and thence directly onward to the cerebral cortex(682) (see figure 34).

The activity of these Purkinje cells is simultaneously modulated by additional efferent supply from climbing fibres originating in the inferior olivary nucleus of the brainstem, which itself receives efferents from the cerebral cortex and spinal cord(682, 700).

Whilst an acknowledged range of cerebellar processing models exist (986) a common theme is that information regarding intended movement is relayed as a form of '*efference copy'* (987)via the pontine nuclei and thence via mossy fibres to granule cells and subsequently Purkinje cells (700); whilst a controlling flow of information conveying actual performance (as a form of 'error signal') travels via the spinocerebellar pathways of the cord and through the climbing fibre system from the Inferior Olivary nucleus which in turn modulates output via the deep cerebellar nuclei and in turn thalamic output to the motor cortices(986).

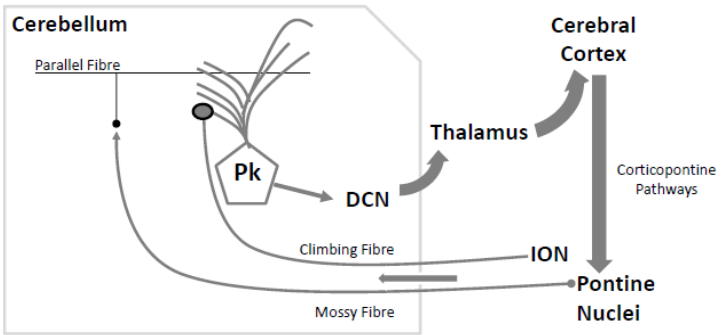


Figure 34 **Schematic of Cortico-Ponto-Cerebello-Thalamic Loops** Adapted from (700)

In the cognitive-affective pathways the association areas of the prefrontal, posterior parietal, polymodal superior temporal and other paralimbic cortices including the cingulate and anterior insula collectively project down into nuclei of the pons via cortico-pontine pathways (988) prior to their input being conveyed by mossy fibres for cerebellar-cortical processing; the output of which is sent to the deep cerebellar nuclei and thence to those thalamic nuclei coupled with the respective association areas(810, 982, 987).

Whilst generally the mapping of cortical zones and functions onto the cerebellum is topographically maintained along its longitudinal extent and divided into a modular arrangement of related composite zones(984), and equally the '*flow*' is generally *feed-forward* rather than *re-entrant*(841, 987) in the manner of the cortico-thalamic and cortico-striatal systems(841, 989), it does nonetheless architecturally and therefore likely functionally display a staggering degree of divergence and convergence on inputs within the cerebellar cortex and subsequently its deep cerebellar nuclear structures(810, 990).

For example, with acknowledged variance of estimates between species(990), several million mossy fibres appear to divergently distribute input to several billion granular cells, the output of which subsequently converges onto 'only' 150 million Purkinje cells; with a further convergence in excess of 10:1 of Purkinje output onto those cells originating within the Deep Cerebellar Nuclei(991).

Such vergence is clearly favourable to *integrative* modulation of output activity; however the necessary rapidity, efficiency and precision of spatiotemporal coding is preserved by maintaining some functional modular segregation into the observed zones and constituent microzones (each containing around 1000 Purkinje cells which share the same somatopic receptive field) (984, 986, 992) within the cerebellar cortex itself and not engendering overly re-verberent(700) activity of the kind seen within the cortico-thalamic and cortico-striatal loop systems.

Indeed, the rapidity of processing within the cerebellar system is fundamental to its function both with respect to immediate performance of motor and cognitive tasks and also with respect to various types of *learning* (987)dependent on activity-dependent plasticity therein (993).

It is with further respect to the issue of temporal precision that one can recognise that a consideration of connectivity alone *cannot* suffice in understanding the consequences of MS on the normal cerebellar physiology.

The performance of rapid motor skills demands even more rapid integration of incoming spatiotemporal information to yield equally rapid and 'correct' output(986).

The dynamic activity patterns within the coupled granule cell, Purkinje cell and climbing fibre inputs are all on a millisecond time-scale(708) and in addition the feedforward

vergent flow described above is possibly shaped by competition from neighbours by lateral inhibition.

Therefore the consequence of even mild delay (from demyelination) within afferent components clearly has the potential to produce erroneous spatio-temporal coding or ultimately be disregarded as weaker 'noise' falling onto hyperpolarised elements suppressed by centre-surround effects.

Such *non-linear* physiological perturbations are likely as relevant as overt structural loss in CCAS and MSCI and also perhaps nowhere more so than in the cerebral cortex itself.

A Necessary Consideration of the Cerebral Cortex

Consideration of the subcortical structures and the re-entrant loop systems they contribute to offers some insight into the *non-linear* nature of the cognitive consequences which arise following the pathological insults of MS; particularly with respect to the thalamus.

Discussion here has not focussed on the many other important subcortical structures such as the amygdaloid complex and other brainstem nuclei which participate in the Reticular Activating System and neuromodulatory projection systems(682, 898). These components similarly both participate in large scale loop systems and fail to escape unscathed by MS(332, 957, 977), potentially further accounting for emotional processing difficulties seen in MSCI and at least some of the sleep architectural disturbance seen commonly in association with the disease itself(994-1004) (with contributions from other factors such as neurogenic bladder disturbance, spasm, pain and intrusive involuntary leg movements also acknowledged). Sleep disturbance undoubtedly brings with it adverse effects on key physiological mechanisms associated with sleep that support healthy cerebral function during the waking state and contribute to accurate consolidation of memory(1005) during sleep itself.

Indeed, the recently identified mechanism of cerebral parenchymal cleansing via the opening of glymphatic channels (1006, 1007) most dominantly during the delta oscillations of slow wave sleep(1008) finally demonstrated an essential restorative mechanism previously unappreciated given the absence of conventional lymphatic drainage in the cerebrum. Impairments of such a mechanism (by sleep disturbance) leading to accumulation of toxic agents, including the amyloid by-products of neuronal activity are increasingly being considered as potential contributory factors in neurodegenerative diseases such as Alzheimer's (1006, 1007, 1009, 1010). The exact role and impact of potentially disordered glymphatic clearance in MS remains unexplored at this time; however its disturbance *in vivo* is seen to directly induce undesirable accumulation of substances that should ideally be removed from the cerebrum(1007, 1009).

Similarly various other physiological components of light sleep (NREM 2)(1011), slow wave sleep (NREM 3)(1012) and REM sleep(1013) are each considered to have a number of important roles in various aspects of memory function and cerebral synaptic homeostasis. It is also noteworthy that the particular pattern of neurocognitive difficulties seen in association with various forms of qualitative sleep deprivation is generally one of '*hypo-frontalism*' with impaired executive function(1014) in a manner not dissimilar from that seen in association with MSCI and similarly associated with specifically reduced functional connectivity in the prefrontal regions on fMRI and EEG (1015).

The interplay between affective state, external psychosocial factors influencing mood, the neuro-modulatory monoaminergic pathways serving as therapeutic targets for treating disturbances thereof and cognitive function is both increasingly appreciated(1016) and far from wholly clarified(1017). Depression, anxiety and stress are all very commonly experienced by patients with Multiple Sclerosis,(456, 526, 1018) both as a consequence of having an irreversible, incurable, life-altering and in some cases life-limiting(24) diagnosis associated with loss of many (and sometimes *all*) of the things that contribute to quality of life, and also as a recognised consequence of having inflammatory activity within the thecal space(1019). The contribution of pain where present and near chronic uncertainty given the marked variability of clinical trajectories(147) are further confounding factors.

This interface between the individual patient's internal and external world is a ubiquitous challenge for all patients; and the direct adverse impact of affective disturbance upon cognitive function is very well recognised both within and outside the confines of MS (167, 507, 1020) and in all fairness whilst seemingly 'complicating' matters further it nonetheless offers a very real treatment opportunity(28) where it can be identified and managed.

Ultimately however, greatest focus should perhaps ultimately fall on the integrity of the very structure served so efficiently by all the aforementioned structures; the cerebral cortex itself.

The critical involvement of cortical grey matter in MS has been increasingly appreciated, with it appearing as targets for intracortical demyelination and neuronal injury and manifesting secondary effects of post-transection retrograde axonal degeneration from white matter lesions(1, 46, 54, 176, 261, 490, 491, 494, 506, 513, 514, 1021).

General relationships between gross grey matter volume and cognitive dysfunction are well established (32, 248, 427, 504, 505, 529, 633, 1021-1023) in addition to relationships with physical disability (509, 1024) indices also.

Histopathologically three distinct patterns of cortical lesion are recognised to occur in MS(1025); those affecting the immediate subcortical white matter of the projection, commissural and particularly short association U-fibres and neighbouring deep cortical layers are classified as Type I.

A second type of lesion is characterised by direct intracortical pathology and finally, and indeed *perhaps* most importantly there are superficial cortical (type III) lesions which are related to sub-pial inflammation(1025).

The particular advent of this latter type of lesion, with the accompanying appearance of ectopic lymphoid tissue inside the thecal space(76, 78) is believed to be both a histopathological signature of MS-related inflammation and also, through such direct cortical injury a major driver of progression in latter more established stages of disease(82, 265). The development of such follicles is seemingly a completion of the 'outside-in' immune migration to the CNS, with such ectopic lymphoid tissue serving as a 'forward operating base' invoking further immune attack via both cellular and humoral antibody mediated mechanisms(78).

The particularly devastating impact of such cortical injury, which is often far more extenstive than can be appreciated on conventional imaging owing to limits of resolution and contrast against local tissue (even targeted double inversion recovery sequences only reveal ~30% of cortical MS burden in contrast to the gold standard of histopathology) (299, 1026)and the substrate of its contribution to MSCI can perhaps only be appreciated by respectfully acknowledging the inherent beauty of the cortex and its function.

90% of the two square metres of 2-4mm thick cortical sheet found in the average adult human is neocortical in type, being comprised of 6 conserved layers histologically defined by relative dominance of various constituent neuronal types therein(136). Running superficially to deep, this sequence of molecular, external granular, external pyramidal, internal granular, internal pyramidal and polymorphic layers(682) further varies in thickness and soma composition in a manner believed to confer the regional functional specialisation seen clinically and in doing so generates a mosaic(921) of diverse capacities across its comparatively vast expanse(744).

One third of the entire cortex is prefrontal by topography(682). Clinico-pathological correlation in the archetypal Fronto-Temporal Dementias and other neuropathologies(26), in addition to innumerable functional imaging studies have consistently demonstrated the central importance of this region to almost the entire array of human cognitive processing(952, 1027-1034), given the implicit appreciation that even tasks typically referred to other regions (i.e. visuospatial processing of the parietal regions and memory functions within the temporal lobes) are critically dependent on *executively directed* attention, context modulation and salience attribution by frontal structures(618).

The crudely tetrahedral arrangement of the three adjoining cortical surfaces united at the apex of the frontal pole is complemented by a functional segregation across the respective orbital, medial and convex lateral surfaces which in turn utilise distinct re-entrant fronto-striatal-thalamic loops as described(843).

The medial prefrontal cortex, significantly composed of the anterior cingulate region and both infralimbic and pre-limbic cortices, is directly coupled to other limbic structures of

the amygdala, hypothalamus, hippocampus, anterior insula and ventral striatum(810, 951).

There is considerable empirical support for an integral role of the mPFC in the consolidation (1035) and recall of memory associated with emotional valence (1036), reward-guided learning (1028) and decision making (1037). The relevance of the anterior cingulate in particular to motivation and processing of emotionally salient information is increasingly appreciated(1038), as is the dysfunction of this area in the clinical setting of depression and the amotivational state of abulia(952, 953). These features are not uncommonly seen in the setting of Multiple Sclerosis (456, 1039).

The adjoining and inferior regions which comprise the orbito-frontal cortex(OFC) are similarly an intrinsic component of the wider limbic system; serving as a further association area and possessing intimate coupling with the other structures therein(1033, 1040).

Whilst classic lesion-behavioural correlations do attest to the provenance of these regions in mediating appropriate social conduct and suppression of inappropriate actions; the function of this area is at once both far broader and less clear than many occasionally conflicting studies would suggest (1040, 1041). A putative solution for the apparently varied roles of the OFC in attributing credit or value, mediating flexibility of choice, response inhibition and predicting error to guide behaviour selection (1040) is that these functions may follow from the presence of a '*cognitive map'* (1031, 1033) related to a particular task at hand, constructed by association from the torrential input from many sources feeding into the OFC where it is formed.

Some empirical support for such an integrated representation has emerged from recent fMRI work in humans (1031) and direct in vivo neuronal recordings from the lateral OFC of rodents has demonstrated the integration of prior and current sensory information is evident within the electrophysiological activity of such populations which also encode subsequent behavioural choices (1041).

The ability to *integrate* appropriately weighted past information on likelihoods of successful outcomes and their emotional and economic values, with current intentions and subsequently make adaptive choices is central to achieving effective goal-directed behaviour (1040). Indeed, the very faculty of being able to manifest a near unlimited repertoire of modifiable, often novel behaviours which can be tailored to suit a breadth of challenges is almost the defining trait of our species (136, 744, 898, 1042, 1043).

It is noteworthy that as a consequence of their anatomical localisation and cortical orientation activity within the cranium the mPFC and OFC is poorly observed by conventional scalp electroencephalography (642, 644, 675) which stands in marked contrast to the lateral PFC residing over the frontal convexity.

The lateral PFC is strongly identified as being central to higher-order cognitive processing and is functionally subdivided into a ventral component (VLPFC) and a dorsal element (DLPFC)(932); crucially these regions are coupled to those similarly higher-order

but sensory associative regions in the posterior parietal regions via the aforementioned longitudinal fasiculi (917).

These two areas in particular are posited to have central roles in two of the cognitive domains most impacted by MSCI, namely attention and working memory(29, 448).

As a construct, the notion of working memory has continued to evolve over the past half century since its inception but is fundamentally more than a faculty of short term recall; indeed its essence is one of active *information manipulation* and processing over a sufficient period of retention typically pursuant of executing goal directed behaviours(859).

The leading Baddeley and Hitch 'Multicomponent Model' of Working Memory has similarly evolved (1044-1047) and currently features four key psychological constructs which collectively contribute to different aspects of working memory faculties. These include a '*phonological loop*' which serves verbal working memory, a '*visuo-spatial sketchpad*' mediating visuospatial working memory and an '*episodic buffer*' facilitating a temporary storage of *integrated* sensory information(859, 1047). Each is putatively mediated by reciprocal interaction between a cortical region of relevant associative processing and the prefrontal regions, wherein also resides a 'central executive' arbitrating attentional control(535, 618, 1048)(see figure 35).

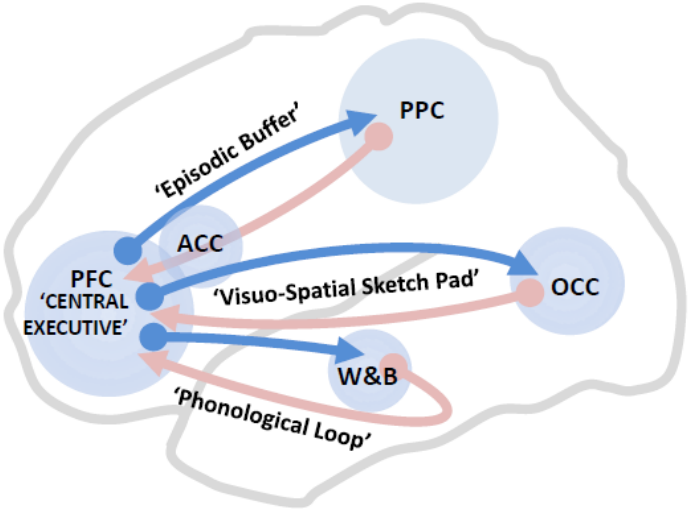


Figure 35 The Baddeley & Hitch Model of Working Memory

Adapted with modifications from (859). A series of Recurrent Loop Interactions Underlie the constructs of a (verbal) phonological loop, Visuospatial Sketchpad and integrated episodic sensory buffer; the collective action of which is governed by central executive influence (attention) mediated by the prefrontal cortices.

An elegant feature of the Multicomponent Model is a degree of matching (1027)between psychological construct subcomponents and network architectures identified *in* vivo by

functional imaging; which highlight coupling between the DLPFC, posterior parietal association cortex and the anterior cingulate cortex as constituting at least part of a '*working memory network*' (859, 1049-1051), which appears at least partly alternately hemispherically lateralised in respect of verbal (dominant) and visuospatial memory (non-dominant)(859). Fronto-partieto-occipital coupling is posited to underlie the aforementioned 'episodic buffer'(1044, 1052). The DLPFC component is particularly relevant to tasks demanding executive control over cognitive resources (1053, 1054) and decision making and is appreciably central to all such loops (1051, 1055) which collectively constitute a distributed a '*workspace*' of *integrated* processing.

Interestingly, although decomposing working memory functions into specific regions is considered somewhat artificial and impractical (1027) there is some evidence of functional segregation of different processes across constituent elements, particularly including the very subcortical structures discussed above. Working memory is however a broad term featuring many processes, including focussed attention, inhibition control, information maintenance and manipulation, salience evaluation and episodic perceptual buffering to name but a few (859) and appreciably differing research paradigms examining working memory place a heterogeneous demand on these capacities which may complicate generalisations from findings(859).

Nonetheless, there is evidence that the medial thalamus appears integral to maintainence of information during working memory tasks (962, 1056), that the cerebellum contributes to temporal aspects of working memory processing (1057) and that activity within the basal ganglia is critical to accurate attentional focus on target, suppression of irrelevant distractors and the ultimate gating of what enters into the loops of working memory activity and is subsequently amenable to retrieval by the DLPFC and PPC (1053).

In addition to the *manipulatory* aspect of information processing which distinguishes it from short term memory, working memory is also defined by its limited transient duration and finite capacity (859). Therefore, the recruitment and allocation of cortical resources from both an informatic and accompanying metabolic perspective must be necessarily optimally controlled which is at least part of what '*attention*' represents; an active executively driven engagement of resources(535, 766, 1038).

It is noteworthy that a loss of such modulatory control over the working memory and other cortical network resources is believed to accompany ageing and has led to the Compensation-Related Utilisation of Neural Circuits Hypothesis or 'CRUNCH' model (1058, 1059) wherein a loss of efficient neural *network control* produces an over-activation with excessive cortical engagement.

Whilst the increased cortical activity demonstrated on the fMRI of MS patients relative to controls (623, 802, 1060) is posited to potentially represent such a compensatory adaptive response (59) which diminishes with advancing disease (1061), in turn it may equally reflect a loss of effective executively-mediated metabolic and information resource control.

This shifting pattern of an initially pathological increase in cortical activation and functional coupling on fMRI prior to a subsequent general diminution with advancing disease has been proposed to account for the contrary findings demonstrating conversely higher (686, 1062, 1063) and lower (536, 1064, 1065) coupling in association with MSCI by some given the studies showing the differential effects can be dichotomised into those with subjects of less than 3 years and more than 5 years disease activity respectively (624). Indeed, a study of structural and functional connectivity changes on imaging of a 138 RRMS patients in the first year of disease demonstrated an increase of intra-network coupling (modularity) and clustering in the absence of clinical deteriorations which may arise subsequently (1066).

Findings from the setting of Traumatic Brain Injury where working memory is frequently impaired (1067, 1068) at least partly support the assertion of adaptation. Heightening of activation and apparent functional connectivity relative to controls is seen in such patients (1069) however estimated directed *flow of information* appears much less efficient (1070). Indeed, the general model of greater cerebral efficiency being characterised by more focussed central recruitment and consequently lower activation is well advocated and empirically supported (1054, 1071).

With over half of the cerebral cortex given over to integrative multi-modal associative processing (921) loss of effective control modulation has clear repercussions for global cerebral metabolic demand over time which are likely compounded by the demonstrably aberrant neurovascular coupling seen in the context of MS (with hypercapnic perfusion imaging) and attributed to chronic habituation to the normal vasodilatory action of nitric oxide as a consequence of persistent inflammation (1072). Chronically excess metabolic demand in the face of pathologically attenuated supply is posited to contribute to the energetic failure underlying neurodegeneration(85). Conversely, cerebral functioning in *health* appears characterised by the timely but segregated activation of the hierarchical family of intrinsic cortical networks evident on fMRI(535, 766).

Whilst the evident modularity of the cortex is reflected in the greater balance of connectivity within areas coupled into functional networks than that connectivity evident *between* areas outwith such network groups (534, 1073), nonetheless these latter *inter-net*work links are critical to supporting integrated behaviour between different subsystems (1074). Information processing within the brain is considered highly dependent on the rapid selection and engagement of the assorted intrinsic networks at its disposal (708). Notably, even in the absence of active task engagement the activity of the resting-state task-negative network (TNN) group fluctuates in an anti-correlated manner with the task-positive collection (535). Furthermore, the *'sharpness'* of such anti-correlation becomes more pronounced with cerebral maturation (1074) and there is much to suggest these patterns of dynamic connectivity are under the executive control of similar fronto-parietal networks to those mediating working memory faculties.

An example of this is the '*Triple Network Model*' (1038)which has arisen from empirical observation of the interplay between the medially residing resting state Default Mode Network which mediates introspective processing (in the medial prefrontal cortex,

posterior cingulate/precuneal and medial temporal regions) and the Cognitive Control Executive Network which resides more laterally and facilitates the aforementioned gamut of executive and working memory processes across the DLPFC and Parietal cortices. The latter network also comprises the Anterior Cingulate Cortex (ACC), however it is the Salience Network in which it also resides (and which additionally comprises fronto-insular, amygdaloid and substantia nigral/ventral tegmental structures involved in the processing of emotionally relevant stimuli) which is demonstrated to exert a dynamically modulating influence over both the DMN and CEN(1038)(see figure 36).

The nature of this modulation is in this instance to reduce the anti-correlation between elements of the DMN and CEN putatively enabling integration between centres given over to high-level introspective processing and those governing cognitive resources in response to detected stimuli carrying significant emotional valence as judged by the Salience Network (1074).

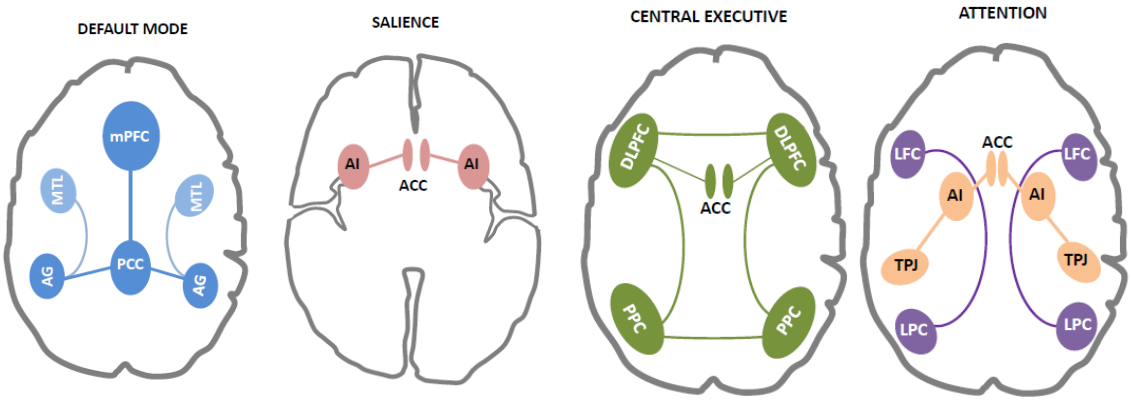


Figure 36 Intrinsic Cortical Networks Underlying Cognitive Processing

Within the Default Mode (task-negative) network the Medial Prefrontal Cortex (mPFC), Posterior Cingulate/Precuneal (PCC) and Angular Gyrus regions serve as dominant integrative hubs in addition to subsystems within the medial temporal lobe (MTL). The Central Executive (Cognitive Control) network features hubs within the dorsolateral prefrontal cortex (DLPFC) and Posterior Parietal Cortext (PPC) in addition to intimate coupling with the anterior cingulate regions (ACC)(534, 535, 766, 1048, 1075). The Salience Network comprises hubs within the anterior insula (AI) and ACC regions; in the Menon's Triple Network Model (1048)the Salience Network is posited to modulate the balance between the task-negative and task-positive DMN and CEN respectively; with imbalance of control being associated with neuropsychopathological sequelae. Hub regions contributing to distinct dorsal (purple) and ventral (peach) attention networks(684) are also illustrated; the former comprising hubs within the lateral parietal cortex (LPC) and lateral frontal cortex (LFC) and the latter comprising similar structures to the Salience network with additional contribution from the integrative centres at the temporo-parietal junction (TPJ).

One can envisage that the closely associated mPFC of the DMN and ACC of the CEN and Salience Network can ultimately serve as modulatory *nexus* between all three subsystems (1038, 1074). One can then also perceive that cognitive-affective sequelae may follow not 'simply' from injury within relevant networks, but *disturbed patterns of engagement-disengagement within and between them*.

This is not purely supposition.

Patients with MS not only have demonstrable difficulties in maintaining task-set (the informatic structure related to a particular procedure) but also in dynamically switching between the sets of distinct but concurrent tasks, in a manner most evident at greater required speed(1076, 1077). Such set maintenance and shifting is highly dependent on the integrity of the PFC (1076) and the apparent pathological increase of coupling within the DMN seen amongst cognitively impaired MS patients has been suggested to greatly hinder the *dynamic switching* toward activity in other networks (1062) whether this is a consequence of pathological over-activity within the Salience Network (a feature strongly associated with disorders featuring anxiety and depression as part of their cogno-afffective phenotype (1078)) or some other mechanism remains unclear.

The identification of significantly prolonged EEG microstate durations putatively in association with some networks at the expense of time spent in others in MS patients(852) is further supportive of this impaired network switching or pathological '*locking*' model as is the recent report that capacity to dynamically modulate connectivity within the DMN (as gauged by fMRI) is significantly positively associated with clinical performance on tests of information processing speed in the setting of MS (847). This latter finding is in turn congruent with the demonstration of higher levels of dynamic change and connectivity coupling between the DMN and the cognitive control fronto-parietal networks being associated with greater cognitive performance and flexibility in healthy subjects (1079).

Thus, it appears MSCI is associated with disturbances of the intrinsic cortical networks, with pathological perturbation of the efficiency of their activation, internal coupling and dynamic modulation of their interaction with other networks. These factors arise alongside more apparent large scale loss of structural integrity in addition to metabolic challenges and general environmental 'hostility' consequent of ongoing inflammation(1019).

However, with respect to accounting for the observed variance in MSCI severity, examination of single property structural models based on grey and white matter integrity or more sophisticated collections of predictors featuring functional connectivity derivations typically yield R^2 typically in the range of 0.2-0.5 (624, 847).

Even though imaging-histopathological cross-examination would suggest the metric of GM volume appears to index the densities of neuronal and axonal elements well, in addition to their size (1080) and GM volumetrics offer perhaps the strongest accounts of variance in cognitive performance (624), considering morphological change therein alone offers an incomplete explanation.

As such, with the modulated interactions between distributed cognitive networks and within cortical modules more fully considered it becomes clear the processes embedded within them are of a far higher-dimensionality than that captured by conventional 4 dimensional electroencephalographic or fMRI time-series (859, 1081) and more revealing

analyses may arise from exploring such higher-dimensional temporo-spatial functional architecture and behaviours (824).

DISINTEGRATION

The necessarily topographically distributed nature of the intrinsic cortical networks which mediate amongst the most sophisticated cognitive processes(534, 535, 766) provides some insight as to why these faculties are so commonly impaired in MS (as indeed they are in many other forms of brain injury) as the dependence on long range coupling clearly bestows a vulnerability. However, the mismatch between the elegance of such reliably constructed node-edge graph representations and their apparent utility to index MSCI(624) may however arise from a failure to capture the pathophysiological disturbances arising within cortical nodes at the cellular level.

As described the cerebral neocortex has a 6 layer structure of varying cellular composition throughout its vast extent which is recognised to be functionally arranged into vertically oriented columns(642, 682, 867). Elucidating a complete model of cortical connectivity and operation in health remains very much an active and extremely large scale human scientific endeavour (761, 1082, 1083). Nonetheless, numerous anatomical, direct electrophysiological and indirect modelling studies have established a number of stereotypical patterns of connectivity within and between disparate cortical columns(700, 708, 750, 1084-1086), the consideration of which may provide further useful insight into the pathophysiology of MSCI.

Attention is broad term effectively describing the controlled top-down influence over information processing within the components of working memory and that incoming via bottom-up pathways. Akin to working memory, it similarly possesses a definite limit to its capacity in any one instant as deficits thereof are a dominant feature of MSCI(29, 448); indeed failure of attention with consequent embarrassment of registration is a well-appreciated contributor to apparent amnestic difficulty (912). The contribution of general neuromodulation by cholinergic and dopaminergic mechanisms to attention is well recognised (976, 1087), however their pharmacological modulation has not engendered consistent findings when therapeutically tested in the setting of MSCI(31, 35-38, 52, 464) suggesting that although possibly relevant, the attentional deficits therein may arise from a different form of perturbation rather than the gross neurotransmitter depletion amenable to intervention in diseases such as Alzheimer's and Parkinson's(26).

It is within the micro-circuitry of the cortical laminae that the flow of incoming bottom-up information encounters top-down modulation by attentional mechanisms (1082). Incoming bottom-up projections relay afferent signalling to the internal granular layer 4, in contrast descending influence from higher cortical areas projects to the external

granular-pyramidal layers of 2/3 and the internal pyramidal layer 5 respectively (832, 1087). Layers 2/3 and 4 are reciprocally coupled, in a manner whereby projections from 4 excite layer 2/3 but in turn the latter appears to inhibit the former (1082). Output from pyramidal cells in layers 2/3, 5 and 6 is believed to then relay information to higher cortical centres via the aforementioned white matter projections (1082). Excitatory pyramidal projections from layer 2/3 additionally mediate competitive lateral inhibition via influence on inhibitory interneurons of layer 2/3 in neighbouring columns (1088).

Detailed modelling of such arrangements suggests positive attentional influence is able to selectively enhance firing from the output projections to higher-centres with the suppression of activity in non-attended columns (1082). Interestingly however, top-down influence via layer 2/3 onto the layer 4 causes a suppressive effect when attention is applied; this is suggested to indirectly 'release' the layer 4 in neighbouring columns in imminent readiness for a *shift* in attention onto the alternative columns (1082). This fine dynamic balance of effects prevents locking into excessive '*winner takes all*' behaviour and endows the system with capacity for rapid attentional switching (1082).

The model above is an acknowledged reduction with recognised limitations in reflection of *in vivo* connectivity schemes (1089). Nonetheless, a growing appreciation of ubiquitous '*dynamical circuit motifs*' (DCM) found through the cerebrum and its cortex has emerged (708). The DCM framework offers insight into both the tripartite relationships between the physical structure, electrophysiological signatures and computational functions of such microcircuits and importantly how they contribute to large scale ICN processing (708).

The motif of High Threshold thalamic neurons bursting inhibitory influence onto the thalamo-cortical relay neurons projecting onto layer 5 at the alpha frequency is empirically demonstrable to be a strong driver of this rhythm's emergence on the scalp EEG (1090).

Intriguingly however the observation of increased coherence between thalamo-cortical spiking and layer 5 local field potentials which accompany directed attention (1091) must be reconciled against the 'alpha blocking' response of clinical EEG (wherein alpha is attenuated with eye-opening) (707) and the more refined observation of increased alpha power in the processing of unattended visual information compared to that which is directed by attention (1092, 1093).

Wolmsdorf *et al*.(708), advocate two distinct alpha generating motifs account for these observations.

Firstly in the absence of attended input pyramidal cells from layer 6 which project vertically through their resident columns induce a general columnar inhibition via coupling through inhibitory interneurons (1094). Notably, these cells also intrinsically oscillate at the alpha frequency (1095) and consequently entrain the column to do so also.

The second alpha-generator is that arising from the thalamo-cortically relayed influence into the middle and superficial cortex on the conveyance of attended input. The higher frequency gamma band activity arising in the superficial layers in response to activation by such incoming input where it is attended is potentially generated by numerous permutations of pyramidal cell-interneuron interactions (708).

Gamma band oscillations are actively enhanced by attention (1096) and demonstrated to have central roles in longer-range '*binding*' of inter-areal activity by synchrony (856).

Critically the gamma band activity in the superficial cortex appears highly gated by or at least coupled to the alpha frequency oscillation arising in deeper cortical layers (1097).

This alpha:gamma cross-frequency coupling or '*multiplexing*' of activity is both empirically evident on tissue local field potential recordings and of direct clinical relevance given the recognised effect of alpha phase-stimulus presentation relationships in human perceptual and working memory performance (813, 1098).

Such physiological relevance of Cross-Frequency Coupling is also increasingly established for the gating of fast frequency hippocampal ripple phenomena by slow-waves which contributes to memory consolidation during sleep(1099).

Collectively, when faced with these observations coupled with the growing diversity of recognised alpha generators *one increasingly comes to doubt the utility of exploring single scalp EEG frequency bands in isolation.*

This notwithstanding, via enhancement of thalamo-cortical input and 'release' from L6 columnar suppression one can appreciate the modulating top-down effect of attention on columnar activity (708).

The *computational tolerance* of delays arising from slowed conduction in the setting of Multiple Sclerosis is likely even less than one might imagine accompanying the temporal gating windows of *alpha* frequency oscillations.

The Pyramidal-Interneuron Gamma (PING) motif is also well described in the superficial cortex, representing feedback coupling between pyramidal cells receiving ascending input and adjacent inhibitory interneurons which they excite and are reciprocally inhibited by (708, 832).

The timescale of this oscillation is in the gamma band and is a function both of $GABA_A$ channel dynamics and the direct input onto the pyramidal cell itself (708, 832).

This coupled oscillator serves to temporally gate responsiveness of the receiving pyramidal cells and by applying a temporal tightening it is also seen to *enhance* the firing precision and likelihood in response to appropriately-timed incoming activation (1100).

Thus gamma oscillation offers a computational enhancement of communication albeit at the expense of reduced response to inputs falling outwith short conducive temporal windows.

As such inter-regional synchrony arising at lower frequency bands, whilst empirically suggested to be important (307, 642, 700, 813, 814, 1101) to cognitive functioning may alone be *insufficient* with intact high-frequency coupling being a further necessary requisite.

The study of *combined* motifs *in vitro* has also illustrated the capacity for sustained '*memory*' to be encoded by resonance of the superficial cortical PING motifs when coupled with intrinsically bursting units within the deeper cortical layers firing in the beta band (1102). Such arrangements have also been observed to enable segregated parallel processing of dual information streams also (1102) leading some to suggest the natural 15Hz beta activity seen in association with working memory maintenance and long-range sensorimotor integration prior to executive action *in vivo* may have a similar genesis (708). The spatial extent of such poly-motif arrangements across the cortical laminae in addition to the cross-frequency coupling with similar laminar dependence only serves to highlight the inherent vulnerability to disturbance by MS pathology at *any* point within the cortical sheet. The observed association of MSCI with cortical lesion load independent of volumetrics is therefore unsurprising (505).

The advent of ectopic follicular lymphoid structures in the meninges is a histopathological hallmark of Multiple Sclerosis and seen in particular association with more established and progressive disease(76). The subpial cortical lesions seen in relation to these centres and difficult to capture on conventional imaging, may indeed be strong drivers of progressive cognitive and physical disability in the latter phenotypes (78, 81, 82, 265, 1103); in addition to the recognised ongoing inflammatory and neurodegenerative processes within the cerebrum and cord(229, 599).

Whilst our discussion of PING and the receipt of descending feedback projections from higher centres arriving in the superficial cortex offers some account as to why subpial lesions are directly troublesome, a greater insight comes from considering the very neuronal groups which by virtue of their vertical alignment and source-sink dipole separation contribute dominantly to the scalp EEG (642, 643); namely the pyramidal neurons of layer 5.

Although the soma of L5 pyramidal neurons may reside within the inner pyramidal layer, they possesses a dendritic tuft which extends into the outermost 'molecular' layer of the cortex, so termed for its relative paucity of cell bodies and high relative composition of interacting axonal and dendritic components. Notably, 90% of the connections onto dendrites within the molecular layer are long-range in nature (1104, 1105).

In addition to receiving top-down influence onto the distal dendrites in the superficial layer 1, the layer 5 pyramidal neurons also receive direct 'bottom-up' feed-forward influence adjacent to their soma; the particular presence of a calcium-spike initiation zone in this region in addition to a sodium-channel based initiation zone in the distal dendritic arbor affords a direct physiological means of association, or *integration* between top-down and bottom-up activity which is then encoded and relayed by the output firing of the pyramidal neuron itself(1106). The empirically supported (1107) mechanism relies on propagation of depolarisation between the distal and apical tuft

initiation regions which both possess voltage-gated channels; activity at either region is able to propagate or back-activate the other, contributing to a modulation of local thresholds. The direct physiological consequence of this, and in particular the switch to a high-frequency bursting output pattern in the presence of the more sustained calcium spikes when coupled with top-down (*attentional*) enhancement arriving via layer 1 is a means for direct modulation and selection/deselection of incoming bottom-up activity(1107). Whether this is bottom-up sensory information arriving from thalamic relay neurons or from cortical regions lower in the hierarchy, the mechanism serves the same purpose (in a manner not wholly dissimilar to the *modus operandi* of a transistor) and putatively contributes in part to integrative '*binding*' of information between disparate sources. Although the schema outlined here highlights the *associative confluence* of ascending and descending cortico-cortical flows, there is a substantial presence of thalamo-cortical projections onto the distal apical dendritic tufts in layer 1 also (1108) suggesting thalamo-cortical processing is both similarly integrative across the *whole cortical extent*, not solely the inner laminae and thus hugely vulnerable to superficial injury.

Critically, whilst such cortical neurons are embedded within columns and regions coupled structurally and functionally into specialised networks(867); we must appreciate that *ultimately these acts of integration occur not at the network level but at the microscopic cellular scale* (1107).

Whilst reducing description of these arrangements to simple '*coincidence detectors*' fails to respect much of their diverse behaviour (i.e. gain control and gating) it does bring home that at this level the synaptic and cellular properties (ion channel conductance dynamics for example) place *fundamental* constraints on the optimal timescales of operation (708) with consequent limits of tolerance with respect to erroneous function in the context of diseases such as Multiple Sclerosis.

Given the established importance of top-down feedback and executive control activity to cognition (1109-1112), conscious perception (1113-1115) and motor control (1116) one can immediately appreciate the devastating effect of subpial MS lesions – even when they remain very superficial and the remaining cortex appears intact; for without distal dendritic modulation in layer 1 the neuronal firing of pyramidal neurons in layer 5 is rendered markedly less effective(1107).

As such, deeper cortical layers may remain structurally present, radiologically visible and metabolically active yet *computationally ineffective* as a consequence of the particularly *subtle knife* that subpial disease represents. This particular loss of integrative function at the cellular level by disturbance of inter-laminar communication, perturbation of motifs like PING and the Back-Propagation Activated Coupling within Pyramidal Layer 5 neurons *and* the *non-linear* consequences of reduced capacity for synchrony afforded by demyelinative conduction delays *in addition* to a probable host of other motif-disturbances *and* other unknown factors may offer a reasonable explanation for the observed inconsistency (706) between volumetric or high-level (tract and network) connectivity analysis and clinical findings.

Whilst it is arguable the progressive accrual of disability which characterises later disease represents a general exhaustion of the brain's capacity to compensate in the face of continued damage(59), the switching of injury-gradient(78) from predominantly periventricular earlier in disease where deeper white and grey structures are injured to a greater relative contribution to peri-meningeal disease and the particularly marked association of subpial injury with progressive phase(76, 82, 1103) and its features is perhaps a signal that *loss of cortical integrative capacity is particularly consequential*.

This is likely both with respect to the normal functions of the cortex, but also relevant to it being the dominant locus of adaptive recruitment and plasticity following past injurious relapse, given that remyelination where it occurs is regrettably often an incomplete repair(65, 68, 72, 395).

Such considerations aside, it appears *simply not enough to be (or at least appear to be) connected*.

A reasonable summary of these initial considerations of the physical substrate of MSCI is that the neurocognitive phenotype at least in part features aspects of hypo-frontalism related to cortico-striatal disturbance, cerebellar cognitive affective syndrome similarly from damage therein and probable higher cognitive deficits related to disconnection phenomena within the white matter callosal and association tracts(see figure 37).

However, the modest association between MSCI and white matter damage on MRI and the limits of connectivity analysis by fMRI (624), and electrophysiology (478) stand in contrast to the stronger association with the burden of grey matter pathology suggesting it is the particular loss of integrative function served *within* and collectively *across* such structures that holds particular relevance for MSCI and why by its very nature it is therefore not likely reducible to or adequately indexed by biomarker surrogates focussing on a single component, or set of elements or indeed in isolation the very macroscopic connections between them. The term '*diaschisis*' (Gr. '*Shocked Throughout*') originally introduced by the classical neuropathologist Constantin von Monakow to describe distributed consequences of localised lesions seems a more apt description than disconnection in this setting and has certainly been applied and recognised in the context of stroke and traumatic brain injury particularly with respect to brainstem pathology (1117-1119). It is thus not unreasonable to assert that MSCI arises from such *diaschisis* and the ensuing consequence of *disintegration* (see figure 38).

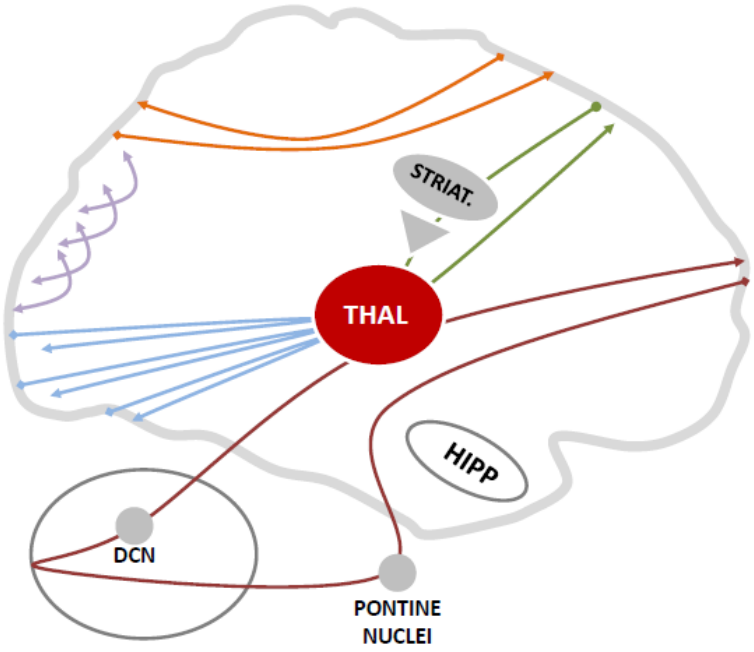


Figure 37 A Collection of Re-Entrant Loop Systems – Substrates of Cognition; Targets of MS

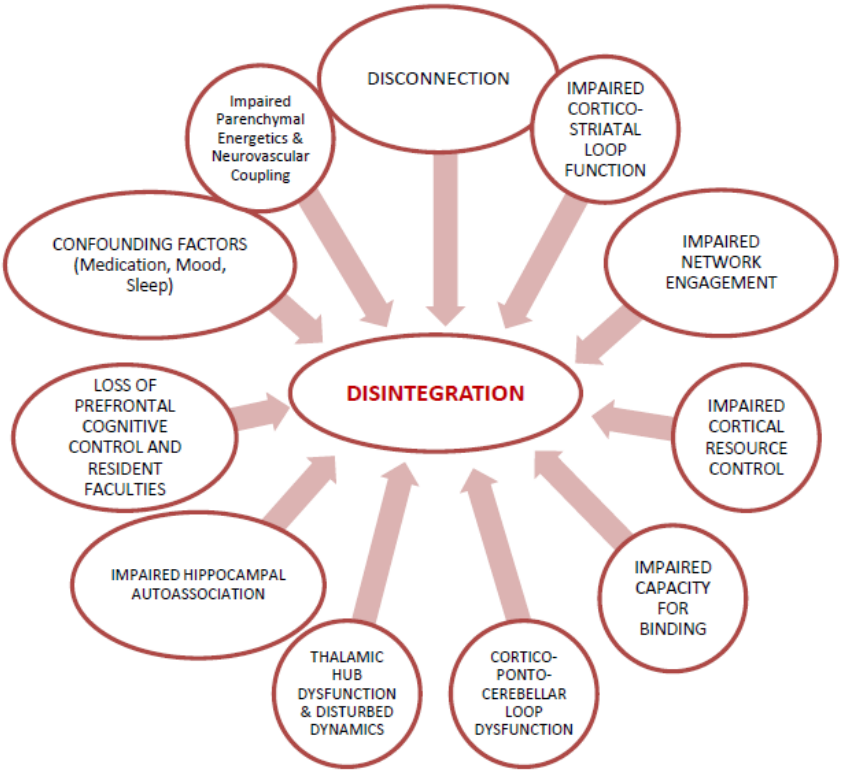


Figure 38 Disintegration

- The final common result of the many factors making non-linear and synergistic contributions to MSCI.

Therefore in seeking such a functional biomarker surrogate of MSCI we are faced with a choice of three possible options; firstly to pursue the strongest associating single observable property (e.g. a particular type of connectivity or similar), the second alternative approach might be to identify a collection of causally related properties and derive a unified and proportionately weighted index of them together which explains the most variance of MSCI or thirdly, we could seek to measure the very property which arises from the cooperative collective action of all the afflicted subsystems when, as *in vivo*, they function not independently in the manner of our abstractions but inseparably, and in unison.

The first option has not proven sufficient here or elsewhere as yet. The second approach, such as collecting a whole set of statistically associated classifiers from EEG which appear to correlate particularly well to a particular feature e.g. the cholinergic deficit in Alzheimer's, has proven moderately fruitful in that disease setting (680). Similar application to the field of MS with machine-learning based classifier extraction has not as yet afforded sufficient accuracy (624). However, although 'feasible' the output of such does not necessarily come with a strong preceding theoretical framework to underpin it which is important when attempting to *interpret* the results of derived metrics in the setting of disease and putative response to treatment.

Therefore we wish to pursue the third option; which demands the initial construction of a theoretical model to serve as a basis of deriving focussed metrics of *systemic health* and its capacity for functional integration.

The first step towards this must be an attempt to rigorously define a workable method of quantifying and in turn understanding what makes something '*complex*'.

COMPLEXITY

The notion of complexity is broadly familiar; intuitively we recognise arrangements which are non-simple(156). Rigorous analysis however necessitates the formulation of a broadly acceptable definition and an accompanying system of metrics to objectively capture such a quality to enable proper comparison between entities considered complex.

Complete consensus on what complexity is and thus how best quantify it remains outstanding (890)despite the centrality of various archetypal complex systems across the broadest spectrum of human scientific endeavour.

Nonetheless, a series of features do exist which are considered quintessentially typical of complex systems; whether these are by their nature '*necessary and sufficient'* for a particular system to be considered complex philosophically however is not fully agreed upon(156, 890).

A principle tenet of complexity is that it qualitatively relates to a form of *structure* which exists on a spectrum between an arrangement of perfect order at one extreme and

an arrangement of complete randomness at the other(887, 888, 890). Neither of these extremes would fit with a conventional notion of complexity.

Other features considered relevant are the often great numerosity of the components which typically collectively engage to form a complex system(890) and more importantly the *non-linear* behaviour that arises from such interactions(885). Concisely, with linear processes definable(1120) as situations where the sum of responses from a collection of individual events is identical whether they occur *simultaneously* or *separately*; In the case of complex systems this superposition principle of linearity does not apply and therefore modelling typically becomes dependent on non-linear differential equations rather than simple linear functions(156, 890).

It is particularly this non-linearity of interactions which deterministically gives rise to the genuinely *chaotic* dynamics so frequently observed in complex systems under operation(155); the evolution of states over time is exquisitely sensitive to initial starting conditions and even seemingly innocuous and small differences in state can lead to distinct onward trajectories of state change which rapidly separate(154). Such non-linearity therefore underlies the severely unpredictable nature of future complex system behaviour even when current state variables are known with a fair degree of accuracy; examples are reflected in the limitations of weather and economic forecasts(154-156).

Further frequently observed traits of complex systems are the appearance of *organisation* which is often both hierarchical and self-generated(156, 890). The appearance of elements closely interacting to form a sub-system of a larger '*whole*' system is frequently observed and provides the opportunity for a wider range of behaviour to result in comparison to a system devoid of any nested hierarchy where all elements interact collectively *en masse* without any capacity for specialisation prior to subsequent production of globally integrated output(535).

The self-organised behaviour which also characteristically arises in an apparently spontaneous manner from complex systems(156), and which may take the form of oscillation or alternation between a range of states is considered a consequence of interactions between system elements that constitute some form of feedback(700).

It can be argued that systems do not necessarily need a large number of elements to produce non-linear interactions with chaotic outputs, the classic double-pendulum arrangement is a fine example of this(154, 155). Similarly, simple couplings of two elements with reciprocal inhibitory and excitatory effects upon one another can produce self-organised behaviour which is oscillatory and frequently complex in nature(156). No consensus exists on the number or nature of interacting elements required to satisfy the notion of complexity(890); nonetheless the output from a single pendulum arrangement is *simpler* than that from a double pendulum; it lacks what is agreed upon to represent chaotic dynamics and instead produces simple harmonic motion which decays to a stop. We can nonetheless reasonably accept that complexity is likely *a non-binary property existing on a spectrum of degree* which may be aided by having more interacting elements but this ultimately is not the sole or central determinant, with the non-linear

nature and nested hierarchical specialisation of their interactions making a dominant contribution(890).

In seeking to yield a metric of complexity some have pursued approaches which endeavour to capture the difficulty associated with understanding or replication of complex arrangements(890). For example, Kolmogorov Complexity is conceptually defined as the shortest binary program which can reconstruct an object; this is a measure of 'algorithmic' complexity and this approach is not readily generalizable or computationally tractable to many systems of interest(890). Successful formulations of this approach which have been derived such as the Lempel-Ziv technique(1121, 1122), which is a ratio between the length of a sequence in question and the program needed to specify it, have had some success applied to neurophysiological data(749) however they are not consistent with the principle tenet of complexity as outlined above in that for random structures they may yield maximal values.

Compatible with our tenet there should be a unimodal distribution of complexity with a zenith between minima at each extreme on the spectrum between order and disorder.

Herein we are seeking a statistical measure of complexity that captures a '*coarse-grain*' or *macrostate level property* of a system determined by the *microstate behavioural interactions* of the constituent elements or parts evident when examined at a finer granularity.

This pursuit is not dissimilar and yet almost the exact reverse of the thermodynamic challenges encountered in the mid nineteenth century(897, 1123). Although empirical systems of useful temperature measurement had evolved considerably to that point, it was ultimately the derivation of the Boltzmann distribution which directly revealed the relationship between the statistical probability of molecules existing at various levels of kinetic energy β (what was occurring at the microstate level) and the macroscopic property of observed temperature(1123);

$$\beta = \frac{1}{kT}$$ (where k=1.38x10^{-23} Joules per Kelvin)

However it is through consideration of the *Second Law of Thermodynamics* that insight toward a useful statistical measure of complexity may be achieved which is wholly compatible with the tenet above.

After establishing the conservation of energy principle underlying the First Law of Thermodynamics, empirical observation had led to the distinctly separate and yet logically equivalent statements of Clausius ('*heat does not pass from a body at low temperature to one at higher temperature'*) and Kelvin ('*no cyclic process is possible in which heat is taken from a hot source and converted completely to into work'*) which laid the foundation for the Second Law in 1850(1123).

Recognising that whilst the First Law governed which processes *can* occur (by non-violation of the aforementioned *quantitative* conservation principle) Clausius demonstrated the tendency of any process to occur spontaneously in nature is governed

by the *qualitative* arrangements of the energy involved. He applied the term '*Entropy*' (Gr. '*In Turning'*) to this characteristic and defined it *macro*scopically as the ratio between the energy transferred as heat and the temperature such an exchange occurred (with resultant units being Joules per Kelvin)(figure 39) (1124). The recognition that spontaneous processes arose only when the overall energy distribution of a system in question changed from an arrangement of ordered higher quality (or lower entropy) to less-ordered low quality (or higher entropy) followed and with it the appreciation that the overall entropy of the Universe must always increase(1123).

$$\Delta S = \frac{\Delta Q}{T}$$

$$\Delta U = T\Delta S - W$$

Figure 39 Formulation for Quantifying Entropy At The Macrostate With Respect to System Level Properties

From (1123); Where S is the total entropy of a system, Q the flow of heat and T the temperature at which it occurs; U is the internal energy of a system and W the energy lost through work.

Consideration of entropy provided a rigorous explanation of the asymmetry of physical processes which occur in one sequence but not reverse and in 1872 Ludwig Boltzmann described the initial statistical (H-Theorem) formulation for absolute entropy which considers the properties of components at the *micro*state scale, i.e. at the level of particles (1123), represented by;

$$S = k\,Log\,W$$

Herein the entropy (the *quality*) of the energy distribution (S) is a product of the logarithm of the *'Wahrscheinlichkeit'* (W) which is the probability distribution of microstate properties, multiplied by the constant *k* for consistency of units(1123). This expression was derived for the model of an ideal gas wherein individual microstates were all considered equiprobable within a given sample(1125). A subsequent and more widely applicable generalisation for systems wherein microstates were not all equally likely followed from Gibbs in the 1870s (1125)(albeit Boltzmann had used near identical relations in work from 1866(1126)) and takes the form:

$$S = -k\sum p_i \ln p_i$$

These relationships not only tied the behaviour at individual particulate *micro*states with the overall system-level *macro*state thermodynamic properties of temperature and entropy but provided the optimal solution for quantifying the abstract and related entity of *information* over half a century later(1127).

In the most general sense information is that which reduces uncertainty and may take the form of messages, observations or deductions for example(1127).

Quantitatively some findings will be more informative than others by reducing uncertainty to a greater degree and offering more suprisal at their occurrence. Similarly a discovery (by message or observation etc.) that confirms an outcome is one option out

of possibly many will be more informative than a finding which identifies one option out of a possible few. By this rationale the occurrence of the unexpected (less likely) is more informative than that which is expected(1127).

In seeking a metric of information to capture these features with a further view to determining the fundamental limits on lossless message compression for reliable transmission over communication channels of finite capacity Claude Shannon constructed his seminal '*Mathematical Theory of Communication*' in 1948(1127).

Therein it was reasoned that a function which quantifies the information which arises from selecting between a set of possible events specified by probabilities of $p_1, p_2, p_3, \ldots, p_n$ should gauge the uncertainty present beforehand and axiomatically possess three additional properties:

Firstly, the function should be a continuous variable across the number (n) of event probabilities. Secondly if all the probabilities are equal (i.e. each is equivalent to 1/n) then as n increases the total uncertainty should monotonically increase in direct proportion to it. Finally, if event probabilities are split into probabilities for sub-events then the overall value for uncertainty (or *Information*) is ultimately equal to the weighted sum of all individual sub-events considered(1127).

Shannon realised that in the same manner that the qualitative nature of distribution of energy within a thermodynamic system can be quantified by the expressions above to yield the quantity of entropy they can equally be applied (with the constant removed) to any probability distribution of possible outcomes to provide a metric of the information provided (and thus amount of uncertainty removed) when one of those outcomes is selected from the range available(1127).

Notably, the Gibbs formulation is the *only* function which meets the three axiomatic requirements, with the *H* taken to represent Shannon Entropy (chosen respectfully after Boltzmann's original H-Theorem, with logs taken to the base of 2 to yield the unit of Bits)(1127).

$$H(X) = -k \sum_{i=1}^{n} p_i \log p_i$$

The choice of logarithms ultimately contributes to providing *higher individual entropy values for lower probability events*, whereupon the negativity which arises (e.g. Log 0.1 = -1) is ultimately countered by the antecedent minus sign in the expression and thus remains in line with the notion of the occurrence of less likely events being more informative (by conferring greater '*surprise*').

Conversely for events which occur as '*certainties*' i.e. with a probability of exactly 1, the value of H is 0 which is fully congruent with the concept of *no additional information* being generated by such an occurrence. Although mathematically the logarithm of a zero-probability would be 'undefined' it is demonstrable by limits that such values can be taken as 0 for the final calculation as:

$$\lim_{p_i \to 0} p_i \log(p_i) = 0$$

Practically the expression also has an advantageous symmetry with respect to its summation; the collective sum of individual entropies is *independent of their considered ordering* and the additive operation with logarithms renders their application far more tractable.

Importantly, it may also provide an essential conceptual bridge to formulating the desired metric of complexity.

There are several reasons why the Shannon Entropy applied to the probability distribution of some form of spatio-temporal arrangement or the Boltzmann/Gibbs relation applied to a molecular system would *not* be sufficient to capture what is meant herein to be 'complexity'. Firstly, there is the very significant issue that it is not appropriate to conflate entropy with disorder *per se*; despite the popular conception fueled by a prevalence of teaching literature to the contrary they are not synonymous (1124). '*Orderliness*' is a very subjective abstraction and it is *not* invariably tied to the qualitative dispersion of energy amongst microstate components underlying the thermodynamic entropy of Clausius et al.,(1124) or to the cumulative probabilistic distribution of uncertainty taken as the basis for information by Shannon.

Secondly, even in settings where subjective notions of 'order' and 'disorder' are generally matched by entropy of their constituent microstates (namely in consideration of extremes such as the order of a crystal lattice at one end of a spectrum and the disorder of a gaseous sample at the other) at no point would entropy or information alone provide an adequate index for complexity as they increased monotonically towards a maximum for arrangements featuring the greatest possible dispersion of energy or equal uncertainty of their probabilities (for entropy and information respectively)(885, 887, 888).

Nonetheless, the importance of Entropy/Information to complexity becomes apparent when one takes the approach adopted by Lopez-Ruiz *et al.* (887, 888); wherein complexity is reasonably considered as yet a further *higher order* qualitative property of an entropy or information distribution; in essence it is the presence of an appreciable ***structure***.

The degree of higher order organisation within an entropic arrangement can be quantified by establishing how far such a distribution is from being maximally dispersed or uncertain (i.e. at *equilibrium* or where all possible microstates are equiprobable). This is termed *disequilibrium* and complexity can subsequently be quantified as a direct product of an arrangement's entropy and this statistical *distance(885, 886).*

Following Shannon, Alfred Renyi(1128) generalised the entropy equations into the standard formulation of:

$$H\alpha(X) = \frac{1}{1-\alpha}\, log\left(\sum_{i=1}^{n} p_i^{\alpha}\right) \qquad H_{1=} - \sum_{i=1}^{n} p_i \log p_i \qquad H_{0=} \log n$$

Wherein α is the order of application; whilst for α=1 (the limit of α→1) the Shannon Entropy of the probability distribution of a set of *X* outcomes emerges (dispersed over *n* possible states), for α=0 the output is simply the logarithm of the cardinality of X, which is *n* ; which provides the '*maximum entropy'* of the arrangement(885, 886). Disequilibrium then is the result of the distance between these two entropies H_1 and H_0, which can be expressed as:

$$D = \left(\sum_{i=1}^{N} (p_{i-1/N})^2 \right)$$

In accordance with Lopez-Ruiz *et al.* (887, 888) if we take Complexity (*C*) to be the product of Shannon Entropy (H_1) and disequilibrium (*D*) then:

$$C = H_1 \, . \, D$$

Expressed as:

$$C = -\left(k \sum_{i=1}^{N} p_i \log P_i \right) \left(\sum_{i=1}^{N} (p_{i-1/N})^2 \right)$$

However, a definitive reason for their use of quadratic distances $(p_i\text{-}1/N)^2$ is not provided and using the modulus instead $|p_i\text{-}1/N|$ appears equally reasonable (when considering *N* states which have only orthogonal relations) to yield:

$$C = -\left(k \sum_{i=1}^{N} p_i \log P_i \right) \left(\sum_{i=1}^{N} |p_{i-1/N}| \right)$$

Complexity can then be normalised against *N* by using(887, 888):

$$\overline{C} = \overline{H} \, . \, D \qquad where \qquad \overline{H} = \sum_{i=1}^{N} \frac{p_i \log P_i}{Log \, N}$$

Importantly, in deriving H_1 our *N* is the number of states that a particular probability distribution covers, whereas in our derivation of H_0 this becomes *all* possible states (which will each have a probability p_i of *1/N*) and is therefore typically greater unless at the point of equilibrium where $H_1=H_0$.

Modelling the performance of these expressions yields a desirable metric for complexity which is minimal at either extreme of entropy and has a unimodal distribution with a singular zenith between them and is therefore so far cogent with our initial notion of this property(887, 888) (figure 40).

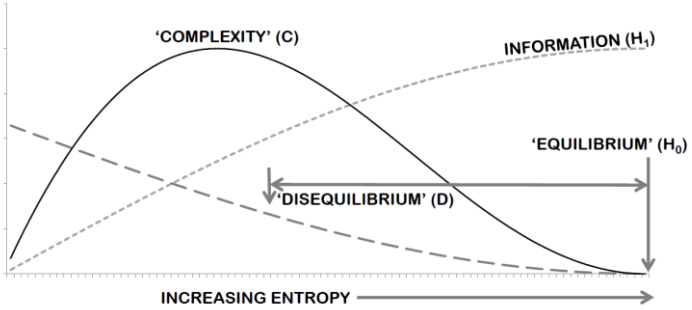


Figure 40 The Complexity-Entropy Relation Adapted from(888)

From this relationship it becomes evident that for any given value of H_1 (or equally S) there are a broad range of potential values for complexity, consistent with the principle that some distributions of information or entropy can possess *greater higher-order degrees of structure* than others.

Correspondingly, the route to increasing complexity is to imbue the available information-entropy distribution with *more structure within* it.

One must appreciate that by their very formulations the absolute values for H_1 and H_0 are highly dependent on the selected number of available microstates (N) under consideration. At a given level of quantisation or discretisation (the '*granularity*' of measurement) there will be N_a available states within the probability distributions under examination which will correspondingly produce entropies H_{1a} and H_{0a}, and further a certain level of Disequilibrium and ultimately a particular value for Complexity.

However, as we examine the distributions in question at a higher granularity, the N will increase to N_b, with accordingly higher values for H_{1b} and H_{0b}, but the *relative distance of disequilibrium* will not necessarily change nor the value of complexity (once normalised for N).

Thus, simply examining a probability distribution of entropy-information at higher resolution (*discretisation*) will *not* in itself increase the relative value for complexity.

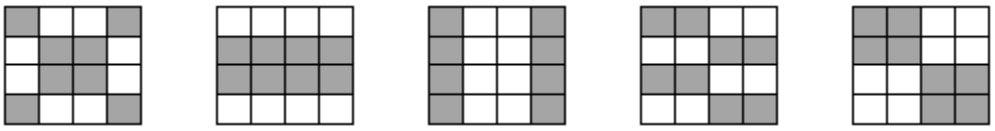
Conversely, if as one examines a distribution in question at higher levels of discretisation (increasing values of N for the same original arrangement) the entropy does *not* increase as a linear function of N because sub-distributions of less than maximal entropy become apparent within it, then whilst the relative value for Equilibrium (H_0) increases the relative value for H_1 *decreases*; this results in an apparent relative increase in disequilibrium, with the result being an *increase in complexity*.

Again, complexity is therefore increased by featuring informative (less entropic or uncertain) structure to arrangements *across a range of scales* (within the same ultimate boundaries as the original) that therefore become evident when examined at greater resolution (higher N).

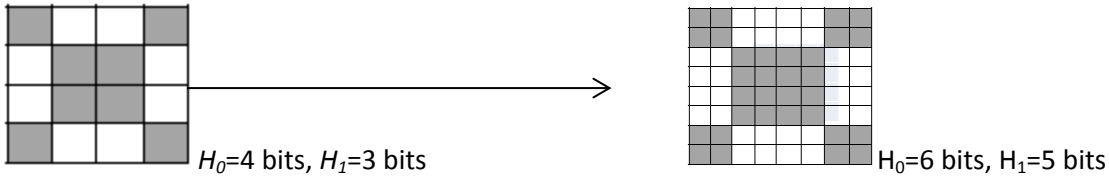
A worked example may clarify things further.

If we consider a simple 4 x 4 square, wherein half of the possibly available 16 component squares are darkened and take this to represent a probability distribution (the sum of which is 1) then the probability of selecting *any particular* dark square of those dark squares available is 1/8.

Therefore, the H_0 will be Log_2 16 = 4 bits whilst H_1 = - (8 x (0.125 $\log_2$ 0.125)) = 3 bits, and this holds irrespective of the exact spatial arrangement of the distribution within its boundaries. Such that all the following can be considered to have *equal* entropy:



If however we divide the original arrangement into an 8 x 8 grid, the *N* rises to 32 for deriving H_1 and 64 for H_0, with resultant values for H_1 = 5 bits and H_0 = 6 bits.



Effectively, at higher levels of discretisation one is considering the entropy distribution within finer regions which would be graphically represented as such (figure 41);

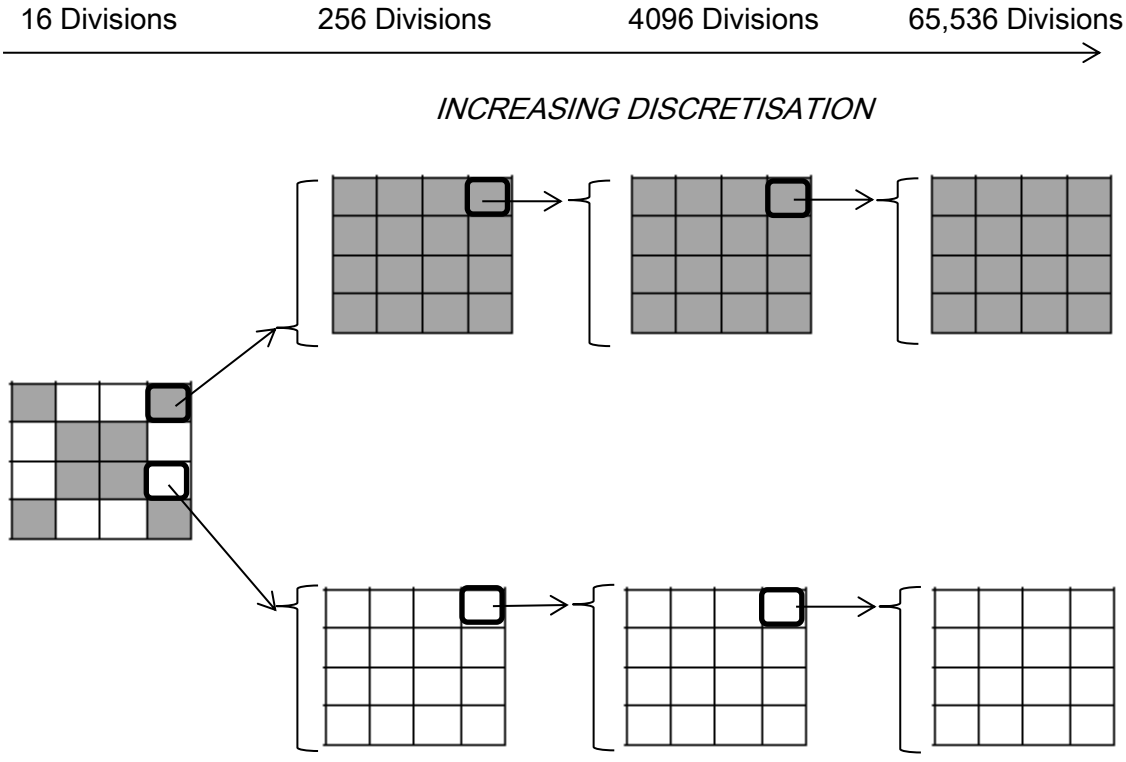


Figure 41 Illustration of the effects of increasing discretisation

It is possible to derive the H_0 and H_1 for such distributions spread across the original 4x4 square arrangement, for the full number of permutations of total squares darkened (i.e. 1/16 to 16/16 of the *original* 4x4 grid) and calculate how their respective entropies increase with such increasing discretisation, as is shown below (table 42, figure 42).

Table 42 Effect of increasing resolution (discretisation) on the entropy of a distribution

Where values in each column (16-1) are the H_1 for that distribution, characterised by how many squares n/16 are darkened at the most superficial level of the 4x4 grid; as one would expect for 16/16 the $H_1 = H_0$

Level	*Divisions*	H_0	*16*	*15*	*14*	*13*	*12*	*11*	*10*	*9*	*8*	*7*	*6*	*5*	*4*	*3*	*2*	*1*
1	16	4	4	3.91	3.81	3.7	3.6	3.5	3.3	3.2	3	2.8	2.6	2.32	2	1.58	1	0
2	256	8	8	7.91	7.81	7.7	7.6	7.5	7.3	7.2	7	6.8	6.6	6.32	6	5.58	5	4
3	4,096	12	12	11.9	11.8	12	12	11	11	11	11	11	11	10.3	10	9.58	9	8
4	65,536	16	16	15.9	15.8	16	16	15	15	15	15	15	15	14.3	14	13.6	13	12
5	1,048,576	20	20	19.9	19.8	20	20	19	19	19	19	19	19	18.3	18	17.6	17	16
6	16,777,216	24	24	23.9	23.8	24	24	23	23	23	23	23	23	22.3	22	21.6	21	20
7	268,435,456	28	28	27.9	27.8	28	28	27	27	27	27	27	27	26.3	26	25.6	25	24
8	4,294,967,296	32	32	31.9	31.8	32	32	31	31	31	31	31	31	30.3	30	29.6	29	28
9	68,719,476,736	36	36	35.9	35.8	36	36	35	35	35	35	35	35	34.3	34	33.6	33	32
10	1,099,511,627,776	40	40	39.9	39.8	40	40	39	39	39	39	39	39	38.3	38	37.6	37	36

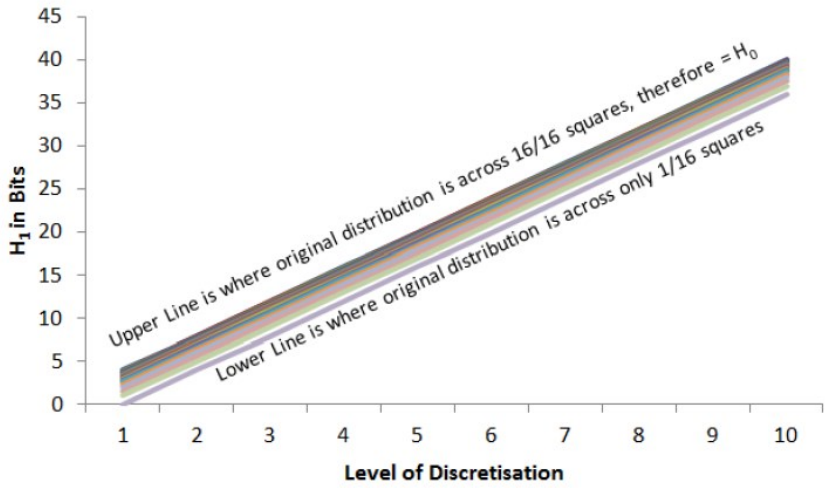
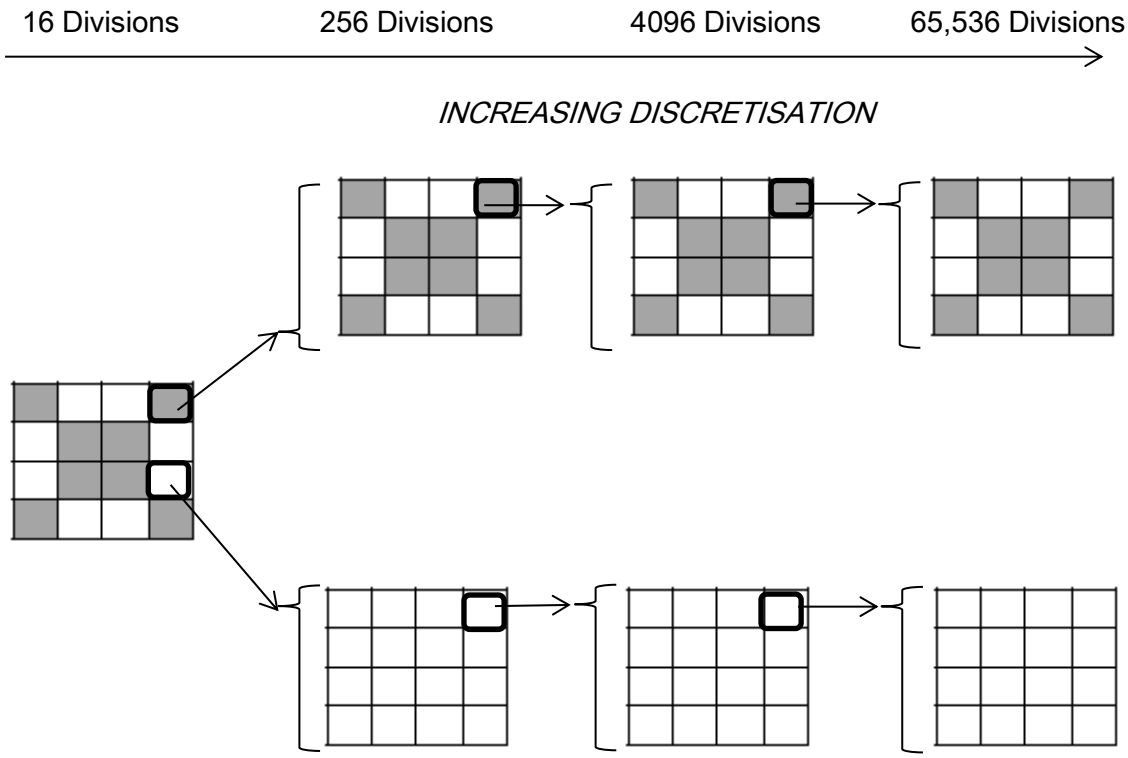


Figure 42 Relationship between increasing discretisation and entropy of a distribution.

It is apparent that increasing the level of discretisation produces a proportionate increase in the value of entropy for that arrangement however the relative distance to maximal entropy (its disequilibrium) remains unchanged.

If one now examines an alternate series of arrangements, wherein at each additional level of discretisation there is *further reduction of entropy* (or uncertainty) away from a possible maximum for that *sub-region* in a *fractal* manner that can be illustrated thus (figure 43);

Figure 43 Illustration of effects of increasing discretisation with fractal arrangement



A very different pattern of behaviour emerges (table 43, figure 44); specifically, when examined at increasing levels of discretisation the attained value for entropy moves increasingly further away from its corresponding point of equilibrium; i.e. the disequilibrium *increases.* The consequence of this is that the product of such entropies and their disequilibrium values, herein taken to be complexity also increase when entropy continues to be proportionately reduced away from possible maxima at ever finer degrees of examination. *Thus, complexity can be increased toward a maxima by the specification of increasingly apparent structure at ever finer levels of consideration* (figure 45).

Level	*Divisions*	H_0	*16*	*15*	*14*	*13*	*12*	*11*	*10*	*9*	*8*	*7*	*6*	*5*	*4*	*3*	*2*	*1*
1	16	4	4	3.91	3.81	3.7	3.6	3.5	3.3	3.2	3	2.8	2.6	2.32	2	1.58	1	0
2	256	8	8	7.81	7.61	7.4	7.2	6.9	6.6	6.3	6	5.6	5.2	4.64	4	3.17	2	0
3	4,096	12	12	11.7	11.4	11	11	10	10	9.5	9	8.4	7.8	6.97	6	4.75	3	0
4	65,536	16	16	15.6	15.2	15	14	14	13	13	12	11	10	9.29	8	6.34	4	0
5	1,048,576	20	20	19.5	19	19	18	17	17	16	15	14	13	11.6	10	7.92	5	0
6	16,777,216	24	24	23.4	22.8	22	22	21	20	19	18	17	16	13.9	12	9.51	6	0
7	268,435,456	28	28	27.3	26.7	26	25	24	23	22	21	20	18	16.3	14	11.1	7	0
8	4,294,967,296	32	32	31.3	30.5	30	29	28	27	25	24	22	21	18.6	16	12.7	8	0
9	68,719,476,736	36	36	35.2	34.3	33	32	31	30	29	27	25	23	20.9	18	14.3	9	0
10	1,099,511,627,776	40	40	39.1	38.1	37	36	35	33	32	30	28	26	23.2	20	15.8	10	0

Table 43 Effect of increasing resolution (discretisation) on the entropy of a distribution with a fractal pattern

Where again values in each column (16-1) are the H_1 for that distribution, characterised by how many squares n/16 are darkened at the most superficial level of the 4x4 grid; as one would expect for 16/16 the $H_1 = H_0$

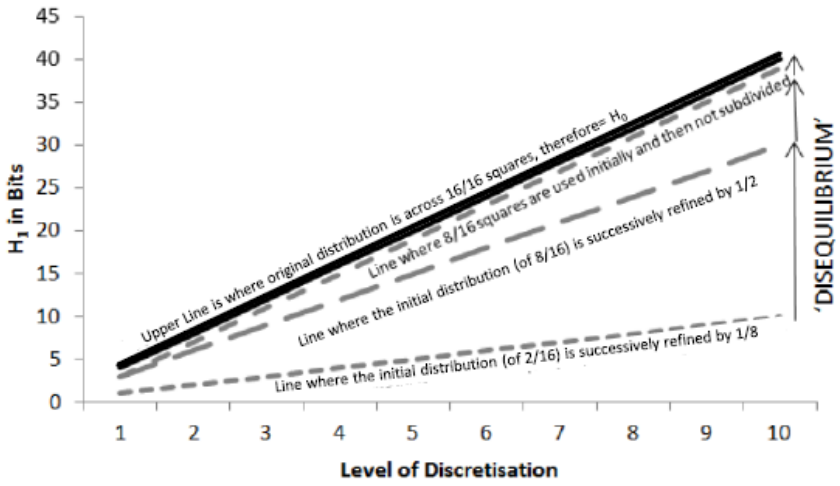


Figure 44 **Increasing disequilibrium arises with fractal arrangements with increasing levels of discretisation**

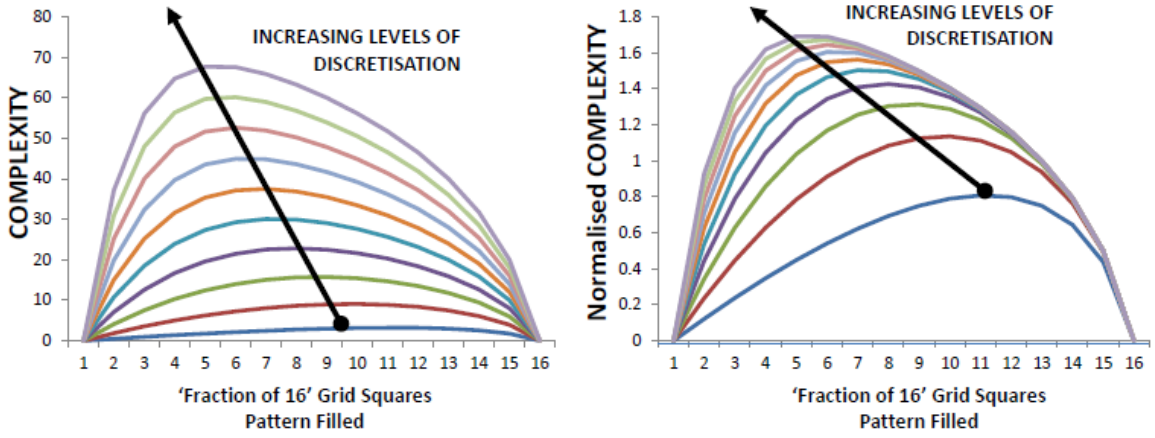


Figure 45 **Complexity increases towards a maxima with fractal arrangements**

The phenomena of increasing levels of structure becoming evident at higher resolution of evaluation or measurement is not a property found with classical Euclidean objects(1129). The term 'object' herein is applicable not just in reference to spatially extended physical items, but arrangements with temporal structure such as signal time-series. Such classical objects are directly amenable to measurement at a singular, optimal characteristic scale typically a degree finer than that at which the item exists and absolute values do not change with finer or coarser examination thereafter(1129). For example, a line of 1 metre will measure the same absolute value whether measured in metres, centimeters or millimetres.

Naturally occurring structures and systems however ubiquitously demonstrate a mismatch or inequality between the topological Euclidean dimension ($D\gamma$) in which they are embedded and the fractional or 'fractal' dimension (D) they appear to occupy as suggested by the gradient of change between the logarithmic size or magnitude of an item which arises with a logarithmic increase in the resolution of its measurement (798, 1129).

There are a variety of methods for establishing this ratio. The Hausdorf-Besicovitch topological covering procedure, alternately named the '*box counting*' procedure examines the fractal dimension (given as D_B) of a line or surface by examining how the minimum number of required boxes (N) changes as a function of square box-length (ε)(798); which is given by the relationship:

$$D_B = \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{\log(\frac{1}{\varepsilon})}$$

Objects where the change in the number of required boxes is a simple linear function of the change in box size will produce results where $D_B = D\gamma$ and can be considered non-fractal. Conversely, where $D_B > D\gamma$ it is typically non-integer and the object is fractal by nature; demonstrably possessing a higher dimensionality than the Euclidean space which contains it(798, 1129).

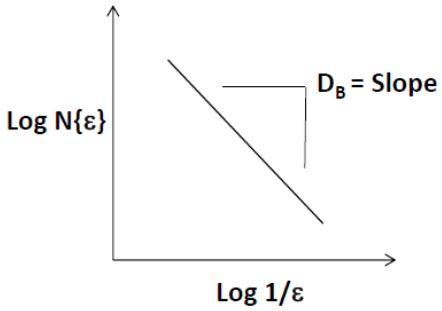


Figure 46 Elucidating The Presence of Fractional Dimension By Examining Behaviour Across Scales

The fractal dimensionality of natural systems are effectively derived by estimating the gradient of logarithmic changes between measurement output (i.e. Box number) and measurement scale (i.e. Box length) from least squares regression or similar(figure 46)(1130). Two important acknowledgments are that natural objects, in contrast to the perfect mathematical objects of the Mandelbrot Set (1129) and similar, are imperfect fractals existing between a range of scales and do occasionally feature relationships with different gradients, in such cases being considered multi-fractal(1131). Secondly, although the resulting fractional dimensions by their nature appear as *fractions*, they in turn reflect gradients of change on logarithmic scales – thus, apparently small changes in value can represent *very large qualitative changes* with respect to the system considered.

The value of this non-linear mathematical approach is that it offers a means to objectively quantify structural properties of natural objects which typically exist with differing features across a range of scales(1129, 1130).

In addition to possessing non-integer fractional dimensions naturally arising fractal structures are also typified by possessing irregular (non-Euclidean) shapes and a degree of self-similarity; such that the morphological appearance at one scale is very much akin to that another(798, 1129, 1130). The ubiquitously observed branching in many natural systems from trees to circulatory systems perfectly exemplify these properties.

Fractality of cerebral neuronal structure is very much evident having been identified by many investigators (1132-1134). Histological and neuroimaging changes suggest an increase in fractality accompanies natural neurodevelopmental increases in morphological complexity, cerebral gyration and neuronal maturation (798, 799, 1132). The very evolution of the cerebral neocortex appears to have been characterised by an increase in its structural fractality also (1085).

It is also noteworthy that the application of simple morphometrics (geometric size and volume) to cerebral structures do not relate as closely to changes in cerebral cognitive-motor function as the degree of fractality during development(799, 1135). Similarly, a lessening of fractional dimension of the cerebral components is seen in numerous neurodegenerative diseases in a manner which has *a closer association with clinical severity than standard volumetric analyses* (701, 1136-1143). For example, this has been seen in the context of Alzheimer's disease where cortical ribbon fractality offered superior association with cognitive performance over cortical volumetric analysis (1143) and similarly in the relevant structures of Amyotrophic Lateral Sclerosis (1138), Multiple Systems Atrophy (1142), Schizophrenia (1139) and most relevantly to our pursuits, Multiple Sclerosis (1140, 1141). In this latter setting, the fractal dimension of the grey and white matter structures is seen to start diminishing in otherwise normally appearing white (1141) and grey (1140) early in the condition.

Application of fractal techniques to suppression weighted sequences have also recently been demonstrated to offer a superior diagnostic classification sensitivity over more conventional MRI derived metrics in MS also (1144) and fractality applied to FLAIR sequences has offered an impressive method of automated discrimination and subsequent quantification of the extent of active and chronic lesion load in a manner of similar efficacy but importantly without recourse to the use of paramagnetic contrast agents (1145).

A methodological advantage of the cross-scale nature of fractal analysis is that it is *typically self-normalising with respect to volumetric differences between subjects* (799) and can also be readily applied to time series (of EEG and MEG) for example. The approaches possess some superiority over the linear methods based on spectral decomposition by Fourier which require an assumption of relative signal stationarity (where the mean signal value and its standard deviation are relatively invariant over time)(642, 712); this is not a feature of many real biological signals which like EEG are non-stationary (798).

The Higuchi fractal dimension (HFD) (1146, 1147) again works in manner akin to Hausdorff Box-counting and in this instance offers a non-integer value between 1 and 2 for the time-series in question; where HFD=1 would be achieved by a simple linear signal and HFD=2 would represent pure noise across the entire time-series (798). Practically, again owing to their cross-scale evaluation fractal signal analysis is considered to be less vulnerable to perturbation by artefact (798, 799)and the HFD of EEG has been seen to fall in response to loss of consciousness with sleep, sedation and anaesthesia (799, 1148).

The Higuchi Fractal Dimension of MEG (1149) and EEG (1150) time-series has also been observed to fall in the neurodegenerative context of Alzheimer's disease, be lower in association with the neurodevelopmental difficulties of Autism (1151) and serve as a reliable indicator of active and impending electrographic seizure activity in the context of epilepsy (1152). HFD and other fractal approaches have been effectively incorporated into EEG classifier systems which may discriminate between MS patients and healthy controls at a group level and diminished EEG fractality has also been observed in relation to MS disability, again compared to healthy controls (1153). However, a detailed exploration of EEG signal fractality in relation to MSCI remains outstanding.

Thus, the advent of fractal geometry ushered in by Benoit Mandelbrot(1154) has finally provided rigorous approaches to quantify the quintessential but previously intangible features of complex natural structures. Herein, acknowledging no singular consensus-carrying definition of complexity exists we have sought to make use of a conception which is at least operational; and for which it is demonstrable that fractal arrangements offer maximal complexity. Indeed, fractals have come to serve as a surrogate index of naturally occurring complexity in their own right (889, 1129, 1155, 1156).

Collectively therefore, the structural and early electrophysiological findings in neurodegeneration and particularly MS would suggest that the system of interest in relation to MSCI, namely the brain does undergo a measurable *loss of spatiotemporal complexity with respect to its architecture and operation* as a consequence of disease.

EMERGENCE

From the foregoing discussion of complexity, as gauged by our entropy-information theoretic based metric being maximised by spatial structures with fractal properties this can without any modification be further extended to apply across higher dimensions, including that of time.

In doing so we may thereby consider how spatially distributed phenomena change over time, namely their *dynamics*.

It is recognised (154, 155) that within dynamical systems various elements may feature at various positions within a given state space at any given time and as time proceeds the state of each element may change as determined by the general laws or rules underlying element behaviour within the system in question. It is familiar that element states may evolve toward particular values or positions within the state space even if they do not necessarily come to hold those values precisely; such areas or regions are described as *attractors*(154, 155).

In the case of simple systems (i.e. a swinging pendulum) there is evolution toward the point attractor which describes the position once the pendulum has come to rest. Other simple systems may evolve toward attractors with simple forms (tori, cycles and lines for example)(155).

In many complex systems, the attractor geometry within the dimensions of a given state space is seen to possess *fractal architecture* whilst nonetheless meeting the other specific criteria used to define attractor regions(154, 155).

These so-termed '*Strange Attractors*' are commonplace in natural complex dynamical systems and the brain is no exception(1157, 1158).

The relevance of such behaviour to our consideration of *emergence*, which may be directly quantified as the ratio of information produced by a system to that going in (885, 886);

$$Emergence = \frac{\boldsymbol{New}\ Information\ Generated\ By\ Interacting\ System}{Information\ Already\ Present\ Within\ Individual\ Subcomponents} \qquad \varepsilon = \frac{I_{Out}}{Iin}$$

which is effectively the *new information* the system produces from the 'raw' elements of information available at levels beneath which emergence arises, is highlighted by the following reflections.

If a spatially distributed arrangement has low relative entropy that is far from its thermodynamic equilibrium (i.e. a crystal), by our chosen metric of complexity this is also low and further if it does *not* change over time then no new entropy-information (different from that present at the start) will have arisen either; as such the low spatial complexity will be matched by a low temporal complexity and the potential degree of emergence will be zero.

Similarly, a spatially distributed arrangement with a high relative entropy (i.e. a gas) near its thermodynamic equilibrium again has a low complexity, however if this *distribution* remains unchanged over time (i.e. system level state information is essentially unchanged even if elements move) it too has low temporal complexity and thereby the potential degree of emergence will again be zero.

If we then come to consider a system of elements with a spatially distributed arrangement which is fractal in nature, from our foregoing discussion it is appreciated that the entropy or information contained therein is of high structural complexity. However, if this system remains unchanged over time (i.e. it is of minimal temporal complexity) or becomes *simpler* by undergoing self-organisation (wherein information is essentially lost) once more the potential degree of emergence is negligible.

Finally we come to consider that the *maximal degree of emergence* comes from a system wherein the elements not only adopt spatially distributed fractal arrangements (of consequently maximal spatial complexity) but where these change successively over time to confer maximal temporal complexity also.

From this several further general statements can be made.

Firstly, emergence can be compromised by either a loss of spatial or temporal complexity. It is fragile.

Secondly, emergence can continue to grow not simply over the change of one spatial arrangement into another but over a potentially unlimited number of spatial state changes provided there remains an *unbroken causal association* between the 'initial' information and that ultimately arising. The rules governing information generation do *not* have to be immediately evident within the state space in question.

Thirdly, there is no restriction against such information-generating sequences of state change (which lead to emergence) running in parallel or contributing to one another, increasing the potential degree of emergence further still.

Fourthly, the degree of emergence will not be uniform across all spatial and temporal scales of a given system; it will be highest at a particular spatial scale and a specific temporal resolution, determined by the complexities of each and the occurrence of segregating phenomena which interrupt (or break) the association of one state (or sequence) with another.

Finally, those systems displaying greater degrees of spatio-temporal complexity (*i.e. producing the most structured information over time*) are likely to display the *highest degrees of emergence*.

With these reflections in mind it follows that systems moving from spatio-temporally complex (information-generating) behaviour to simpler self-organising (information-losing) dynamics will by virtue *lose their capacity for emergence*. Furthermore, transient and possibly unwanted phenomena which break causal associations between elements in one state and the next will similarly limit capacity for emergence also.

A strange attractor in itself represents the dynamic behaviour of a series of system elements over an interval of time and within a given period the elements can adopt a large variety of different states within its boundaries(154). Nonetheless, for the duration of its existence the behaviour of the elements contributing to this fractal structure is tightly confined to some states *to the exclusion* of many, many others conferring higher spatio-temporal complexity than would characterised by simple dynamical behaviour alone.

The potential for emergence such a pattern of dynamic behaviour amongst information-generating elements can create in isolation is profoundly expanded by causally linking elements in one strange attractor state to those in another – either spatially by expanding the system in question or temporally by following one strange attractor state with another to which it is mechanistically linked.

Herein, the degree of emergence will be a combined function of the collective complexity of the elements within individual strange attractors (produced by their dynamic behaviour), the information they generate and how this grows when coupled to other systems of elements which similarly undergo strange attractor dynamics. It therefore also follows that the rate at which the information from one such attractor leads or indeed 'feeds' into another has a clear implication for the maximum degree of possible emergence per unit time.

Translating the application of these general conceptions across to the complex dynamical system in question, the brain, and its functions is no small task. Nonetheless, a growing body of literature suggests cerebral behaviour across the full range of macro, meso and microscopic cellular scales offers the desired levels of spatio-temporal complexity to confer high orders of emergent properties(307, 535, 642, 700, 708, 814, 867, 1159, 1160).

Although to direct visual inspection the human electroencephalogram demonstrates a well-established pattern of oscillations characterised by their frequency, topographical distribution and behavioural associations (644, 675); assessment of the cranial distribution, or landscape of the Global Field Potential (GFP) over the same millisecond timescale reveals a distinct pattern of activity, characterised by discrete alternating periods of apparent transient-stationarity of such distributions lasting ~60-125 milliseconds before they near-instantaneously transform or *collapse like an avalanche* into a new distribution which is held for a further short interval (848, 1161, 1162).

These quasi-stable GFP distributions linked by precipitous changes are termed '*microstates*'(848)(figure 47). On the rationale differences in potential landscapes between states is a reflection of their genesis by the activity of different neuronal assemblies they were considered to be representative of distinct aspects of cerebral processing(848, 1163). Furthermore abnormalities of microstate syntax and state duration compared to controls have been seen in a number of neuropsychiatric conditions, ranging from Schizophrenia (853, 1164) to overt delirium (1165).

That the vast cortical expanse of each cerebral hemisphere is not only arranged in a structurally hierarchical manner but into an assortment of functional networks (the components of which are coupled in their activity over time) has been the main landmark insight offered by fMRI and MEG techniques (535, 761, 762, 766).

These spatially distributed networks are considered to offer, by nature of the histo-architecturally specialised regions which comprise them high levels of task-specific information processing which can occur in parallel but nonetheless necessarily segregated and distinct to that within other such networks(535).

The recognition of prototypical microstate class distributions reliably shared by individuals(848) has been matched by the identification of a collection of resting state and task-positive networks which are similarly conserved (535, 766). Moreover, it has recently been demonstrated these are not independent phenomena but intrinsically related.

Microstates appear to be an electrographic correlate of specific functional network component activity(848, 1166). The temporal resolution of fMRI techniques would suggest all components of a particular network are contemporaneously active; conversely the high temporal resolution of EEG and MEG techniques suggests the individual sub-component regions are active in a *sequential* fashion (1167, 1168) which in turn has clear implications for the nature of the resultant information processing.

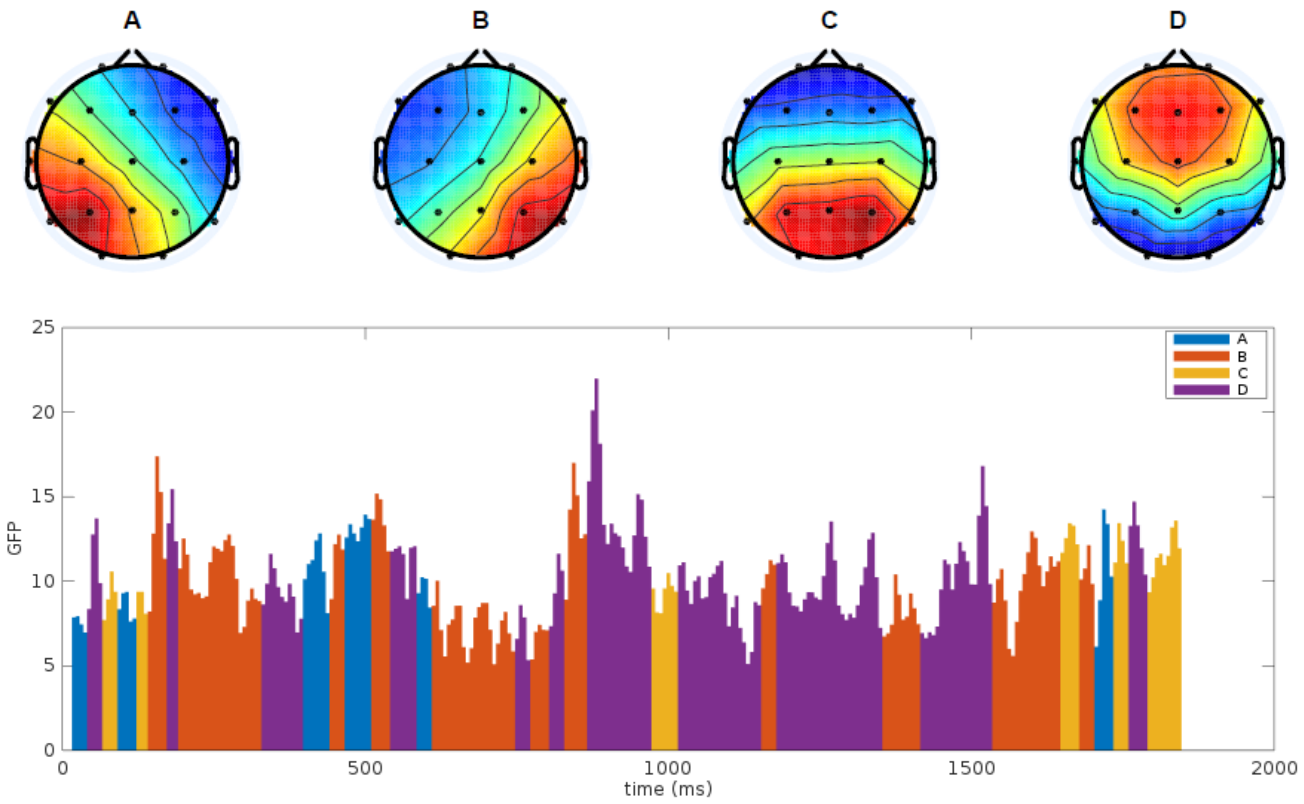


Figure 47 Cerebral Microstate Topographies

Evident in the scalp Global Field Potential, 4 key distributions(A-D) account for the majority of variance in the resting state EEG data and correspond to activity within specific Intrinsic Cortical Networks. This example came from data acquired on a patient with MS from the previous investigation; further work to extract and delineate this phenomena to enable state-adjusted connectivity in this disease setting is underway.

Image courtesy of Dr. D. Western.

Notably, that *any* transient stationarity of the scalp potential distribution is discernible *at all* points to an underlying synchrony or approximately coherent pattern of activity amongst contributing regions (848). This in turn is wholly compatible with the cognitive binding through synchrony model proposed by Singer (728, 735, 736, 1169) (1999) to underlie integration of information between brain regions and is already supported by a range of animal (307, 740, 1169) and human findings (738, 1170).

Whilst the *control* of differential engagement of the highest level task-negative (DMN) and task-positive (CEN) intrinsic cortical networks is seemingly afforded by a Salience

Network (and other local intrinsic interactions) exerting an 'informed' modulating influence respectively upon them(1048), and the capacity to segregate activity between and then subsequently integrate *coupling across* them is demonstrably critical to the highest forms of abstract cognitive processing including *imaginative creativity* (1171), one is left with the question of what *drives* the successively observed patterns of activation evident at the global scale?

Some insight may be offered by consideration of the dynamics of the very GFP microstates demonstrated to reflect momentary activity within the respective intrinsic cortical networks(848); as described the patterns of transient stationarity are punctuated by short intervals of collapse leading into new microstates(1162, 1172, 1173).

In healthy subjects the dynamic pattern of such events displays characteristics compatible with the system in question operating at a point near *criticality*(1172), which has come to adopt a *specific* physical and statistical meaning since the publication of Bak's Sandpile Avalance model(1174) some three decades ago.

The model itself (figure 48) considers the consequences of the successive sustained addition of single grains of sand to a consequently growing sandpile; it is empirically demonstrable that the pile itself will continue to grow until such points (where due to forces of gravity overcoming weightbearing capacity of the pile) a degree of collapse in the form an avalanche will arise(1175) *returning* the sandpile near to a more stable state(1176).

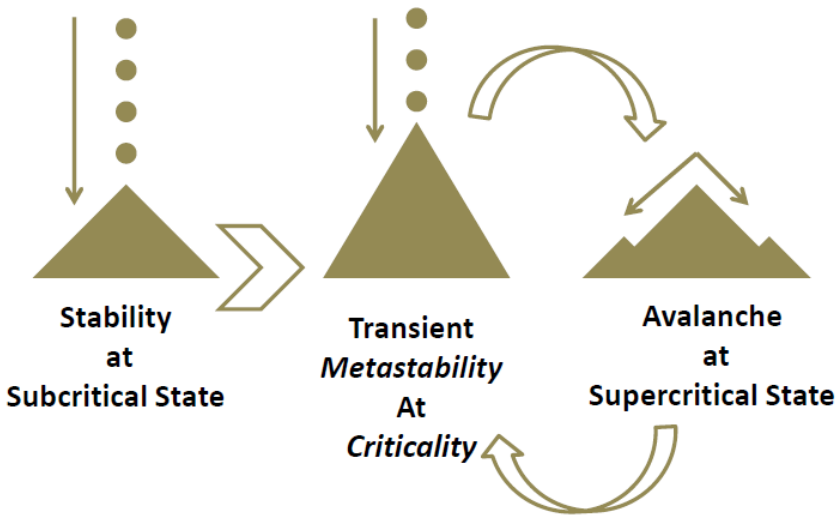


Figure 48 The Sand-pile Model Of Criticality

A sand-pile experiencing the steady continued addition of single sand grains will grow to a critical point whereby subsequent addition of grains makes an avalanche increasingly likely; the avalanche itself will return the system from a supercritical state back toward one prior to the point of criticality. The emergent relationship between avalanche size and likelihood is governed by a power law. Adapted from (1174-1176).

The term *critical* in this context effectively refers to the point of transition in the behavioural dynamics of the system between two different phases(1176) namely remaining stable and undergoing collapse by avalanche. A system that remains below this point of criticality, i.e. one that is subcritical will not display avalanching behaviour, conversely one that remained supercritical would simply avalanche to completion with both scenarios resulting in a terminal lack of dynamics. However, a system which continues to reside at this point of criticality will not only *continue* to display an oscillation between periods of transient stability coupled with intermittent avalanche but the relationship between avalanche magnitude and frequency which emerges will be accounted for by a power law of the form:

$$P(S) = kS^{-\alpha}$$

Wherein P(S) is the probability of an avalanche of size S, k is a proportionality constant and α an exponent which effectively characterises the relationship of decreasing likelihood of events of larger size. It is noteworthy that such power laws are seen to describe real-world occurrence of earthquakes, solar flares and adverse economic events; all of which represent emergent phenomena from complex adaptive systems(156, 863).

Furthermore within the brain at the micro scale, activity patterns from local field potential recordings, both in cortical slice preparation and from the primate cerebrum *in vivo* have demonstrated dynamical neuronal avalanching behaviour which similarly follows a quantifiable power law (1177, 1178). Not only is the direct enhancement of such avalanching dynamics by neuromodulators such as dopamine(1179) a remarkable observation but that such a pattern exists *at all* can be taken as a clear indication of integrated coupling(1180); for the pattern which would emerge from an uncoupled disorganised system would be more in keeping with a Poisson distribution and not a power law which implies a governing or underlying deterministic *systemic structure*(1177).

A further key feature of such critical dynamics and the associated power laws which arise is the consequence of *scale invariance*(700, 1176); whereby the exponent of the power law evident at the microscale can be seen to extend up into the macro (system level) scale; if appropriately captured(figure 49). Given the existence of such relationships and their demonstrable presence within the brain(1180) it is therefore not unreasonable to infer that critical dynamics at the microscale are the main driving influence of dynamics at higher scales – the very network switching likely arises from avalanching behaviour at the microscopic scale(722, 1176); which is biologically cogent; albeit one must acknowledge the exact network which is engaged will determine in a supervenient manner which neuronal populations are dominantly engaged at any one instant.

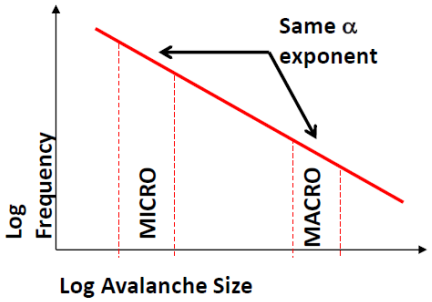


Figure 49 The Scale Invariance of Power Law Relationships in Critical Phenomena

Such relationships theoretically offer a way to evaluate the *micro*scopic integrity of a system by examining *macro*scopic behaviour.

Thus, as with our consideration of the property of entropy which effectively unites macroscale and microscale phenomena, an exploration of the nervous system's operation at the point of criticality may offer similar cross-scale insights and ability to interrogate cerebral health at the microscopic scale by exploring its macrostate dynamics.

A number of neural network modelling studies have demonstrated that such critical dynamics bestow a number of advantages with respect to conferring optimal information transmission and computational power in addition to rapid large scale responsiveness to comparatively small inputs (1181, 1182) and an effective resistance against 'locking' into a single state. A further important benefit to information processing is that of *segregation*. With the profound connectivity within the cerebrum (each neuron being coupled to every other by a mere handful of interceding links (700)) the active deselection or disengagement of neuronal groups becomes effectively *as important* as those which are selected or engaged. Current models of the positive psychiatric phenomena seen in the context of Schizophrenia(1183) and Lewy Body Disease(1184) being a consequence of inappropriate sensory-perceptive integration highlight the importance of mechanistic segregation in processing. That a global increase in functional connectivity across the cerebrum resulting in a loss of the normal distinct separation between the intrinsic cortical networks which underpin cognition is seen to accompany the controlled hallucinosis induced by Lysergic Diethyalmide (1185) further underscores the vital importance of segregated processing activity in ensuring only 'appropriate' integration occurs. That the neuropsychobehavioural phenotype of MS does *not* typically feature overtly apparent positive psychiatric phenomena(27) does not however diminish the relevance of a possible disturbance of segregated processing to its cognitive sequelae; the clinical manifestations of which would centre around accuracy in perceptual judgements, executive acts of selection and appropriately directed attention – all of which are indeed seen to be perturbed in the context of MSCI(29, 400, 448).

Whilst some persuasively argue for a central role of spatially extensive and suppressive hyperpolarising thalamocortical delta oscillations contributing to this in normal healthy *waking* (1186) (which further attests to the importance of *proportionate balance* across the EEG spectrum), the metastable switching from one dominantly active network component to the next in a *successive* manner *punctuated* by avalanches (848) similarly

offers a further mechanism of informatic segregation which may also sit alongside the non-linear selection-deselection mechanism emerging from cortico-striato-thalamic interactions(843, 951).

The term *self-organising* criticality is typically applied to systems which appear to spontaneously return to the point of criticality, or subcriticality after undergoing an avalanche event(1176, 1187); although exactly *how* the brain performs this is unclear(1176) it does clearly appear to do so and must therefore possess a balanced distributed mechanism for achieving such.

In the absence of such a mechanism being definitively identified one can nonetheless still characterise and to a degree quantify its nature by examining the relationship between the state or phase a system finds itself in (termed the *order*) and changes in putative *control* parameters which alter it(155, 1176). Visualised on a phase transition diagram (figure 50), rather sitting exactly at a critical *point*, the system may reside within a critical *region* whose bounds are effectively determined by a similarly limited range of control parameter settings, the deduction of which may offer insights as to the effective integrity of the system in question.

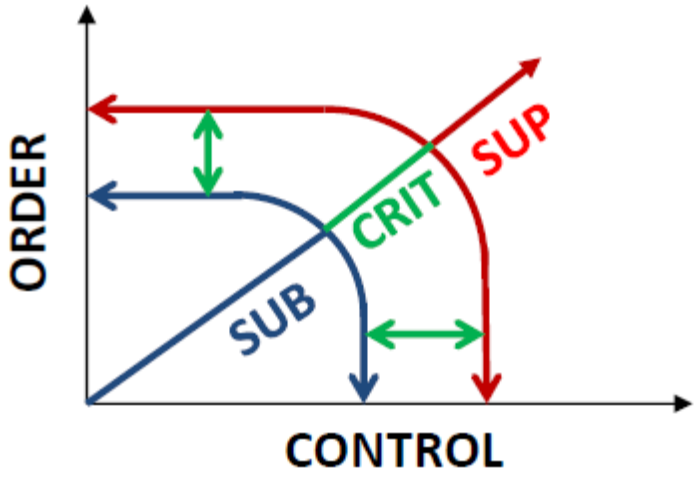


Figure 50 Phase Transition Diagram

Schematically Illustrating the Conceptual Relationship Between Order, Control Parameters and System Dynamics at varying degrees of Criticality.

Whilst the issue of criticality has drawn considerable attention in the field of epilepsy with respect to modelling seizure genesis as entry into an oscillatory runaway into a supercritical state of cerebral dynamics(1188), the importance of white matter integrity and in particular the specific conduction delays afforded by it have been modelled as a pivotal aspect of the collective control parameters underlying the very generation of natural oscillations seen on the EEG(683), which are themselves regarded in some quarters as a further manifestation of critical behaviour(700). It is noteworthy and likely non-coincidental that the that moment-to-moment fluctuations in microstate topography across the GFP of the scalp appear matched to oscillation in the activity of their

respective sources particularly at alpha band frequencies(1173) suggesting as one might anticipate to a degree, that the two phenomena are indeed intimately linked.

Ultimately therefore identifying a large scale signature of criticality may offer an opportunity to identify variance of such a 'control parameter', particularly in the setting of extensive white matter disease and resultant conduction perturbation of the kind seen in MS. This may offer another form of 'system-level' functional integrity metric, one which indexes its *dynamic* properties. Of note, whilst rapid phase transition events akin to the phase slips derived from Hilbert Transformation of band-passed EEG we explored previously have been shown to display a quantifiable power law relationship between spatial extent and likelihood within healthy controls(1155), the exploration of such phenomena in Multiple Sclerosis remains outstanding.

CONSCIOUSNESS

Across academia it is familiar to make judgements of ability with an approach akin to Guttman Scaling, wherein one supposes an underlying rank order of performance with questions or challenges of increasing difficulty and the transition point of competence is taken as a given value (866).

Therefore in our pursuit of an index of brain functional integrity it is not unreasonable to consider that developing a metric to quantify an emergent attribute of this complex adaptive system may offer greater yield than pursuing correlates of singular or even multiple lower level cognitive processes. In doing so we will be focussing upon the most complex property that this complex system gives rise to, that which likely requires greatest structural and functional integrity and consequently may be most sensitive to measurable decline.

This is not to consider even momentarily that cognitive faculties of verbal memory, information processing speed, arithmetic or executive function are simple. Although the modular taxonomy, external outputs and structural underpinnings for each have been macroscopically localised by fMRI and lesion studies(534, 535, 558, 697, 764, 766, 1038, 1189) the mechanisms of how neuronal assemblies might give rise to sentence construction and calculation are only nearly coming to light(1190). These abilities clearly represent emergent behaviours that arise from neuronal network interplay.

However with respect to their reification it is noteworthy they remain largely artificial psychological constructs and their assessment on tests comes with major caveats on inference as performance output is never going to be a *pure* function of the attribute in question. This position is in line with the Process Overlap model of cognitive performance outlined by Kovacs and Conway(1081) wherein psychometric output reflects engagement of different general executive and domain-specific mechanisms; notably this is also empirically supported by multicomponent analyses applied to outcomes of commonly deployed neuropsychometric tests(1191).

Furthermore, as discussed there is a spectrum of emergence; some phenomena arise from their generative systems in a weaker or stronger manner than others (156, 885, 886, 890, 1192). As direct quantification of the relative emergence of each cognitive faculty remains outstanding the attribute to focus upon is less clear. This said it may be helpful to apply the following criteria: 1) seeking as near a global emergent property as possible (in appreciation of the variable and disseminated nature of MS damage and its consequences) 2) more strongly emergent properties should be less replicable or amenable to simulation, and 3) ideally all patients who might participate in trials where this property is measured should possess it, ideally spontaneously. This last point is a reflection on the observed effect of premorbid numeracy or lack thereof on tests such as the PASAT(1193), a feature never truly corrected for.

When considering the vast repertoire of cerebral cognitive functions against these criteria, intuitively the most strongly emergent phenomena to pursue maybe that of *consciousness itself*.

Whilst it is recognised useful measurement instruments typically possess good properties of precision and replicability alongside an ability to predict outcomes against which their outputs are highly correlated (11, 201, 203, 866, 1194) it is also helpful albeit not essential for them to be coherent with some underlying principle model or theory (866, 1195). Constructing a measure of consciousness however demands the foundation of an effective *working model* before considering how this may be applied pragmatically to EEG and the resulting instrument subsequently to translational research in MS.

Consciousness is an emergent high level property of a system

Is it reasonable to suppose consciousness can be amenable to representational and not simply pragmatic approaches to measurement? Simpler elemental forms of qualia (unimodal sensory experiences) for example did yield the Weber-Fechner and Stephen's Psychophysical laws underlying the relationship between stimulus intensity and perceived magnitude(1196) suggesting at least certain forms of experience are amenable to albeit approximate and subjective quantification.

In the long history of measurement it has frequently been the case that very imperfect pragmatic systems which were nonetheless very useful preceded both more complete understanding of the measured property and a more representational method to quantify it (866, 1195).

It is also relevant to consider that the very practice of neurophysiology generally is based on application of knowledge of the relationship between the seemingly fundamental and indirectly observable phenomena of electricity and magnetism(642, 867). They remain characterised by constant behavioural associations in the form of natural laws, have near circular definitions based on their action on other things and nonetheless have been integral to human endeavour and progress over the past two centuries(867). In short, working but incomplete knowledge of measurable phenomena is no clear barrier to tremendous utility. The situation with respect to consciousness and

the scale of pragmatic challenges entailed are considered not dissimilar to those facing the dawn of Thermodynamics in the 19th century (897) and what consensus there is appears to rest upon *complexity* somehow being a fundamental component of it((1197).

The Integrated Information Theory of Consciousness

Of the many conceptual models(745, 891, 898, 899, 903, 904, 906, 911, 1198, 1199) advocated as at-least partial solutions to the problem of how conscious experience arises in the brain, the Integrated Information Theory (IIT) developed by Guilio Tononi *et al.*(744, 841, 884, 892-896, 1200, 1201) offers perhaps the clearest account and utilisable framework for empirical applications.

Whilst making collective sense of the relative contributions of the set of objectively established neural correlates of consciousness (NCC)(e.g. gamma oscillatory binding) has been a direct starting point for many previous approaches(1202), the inception of IIT has conversely commenced from appreciation of the quintessential attributes of first-person subjective experience itself described in a considered set of axiomatic truths regarding its nature(892, 894-896, 1200).

These key axioms of conscious experience attest to certain properties, which if one ascribes to the philosophical position of emergent monism (wherein consciousness arises from processes within the brain) must be causally determined by the nature of the underlying mechanisms in question(989).

The features such a mechanism or '*Physical Substrate of Consciousness*' (PSC) should have on the basis of the described axioms are outlined in a corollary series of ontological postulates; which are taken to represent a series of necessary and sufficient conditions to produce consciousness(841, 989).

Importantly, this collection of postulates outlines the properties of the PSC itself and it is then a further matter to consider how this exists or arises from the series or subset of the acknowledged NCC.

The founding *axioms* of IIT are effectively statements of universally familiar truths regarding *all* conscious experience; and are considered complete, consistent and independent of each other(892, 894, 895, 1200, 1202).

The first, whilst seeming 'obvious' is nonetheless critical for later considerations and is termed the axiom of ***intrinsic existence****;* that consciousness is real and exists from the *intrinsic perspective* of the individual (or system) under consideration(892, 989), in keeping with the Cartesian viewpoint of it being the most definite truth we can be sure of(1203).

The second axiom is that of ***composition***, acknowledging there is a clear *structure* or content of that which is experienced from the intrinsic perspective which features multiple phenomenological distinctions of various types(841, 892, 989).

Thirdly, such content is ***specific*****,** it is only one thing or collection of things and *not many possible others* and therefore is highly *i****nformative****(841, 892, 894, 896)*.

Next, the seemingly unified, unavoidable and consistently indivisible nature of experience is respected with the axiom of ***integration*****;** that the specific and structured nature of that intrinsically experienced is a form of highly integrated information, that is not experienced in a reduced form of its constituent parts as separate entities but always unified and *irreducible*(841, 892, 989, 1202).

Finally, the simple reflection that conscious experience at any one instant has definite contents which are included and much which is not leads to the axiom of ***exclusion***; consciousness has limits and therefore a boundary operating at a particular spatio-temporal scale or resolution(841, 892, 894).

These axioms provide key insights from consideration of undeniably complex phenomena. Each seemingly holds in all situations/cases and each also informs us as to a key property the PSC (and the system in which it is embedded, the NCC) must possess if it is to produce consciousness(841, 896).

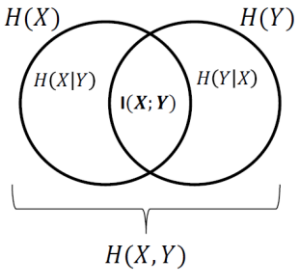
The postulate arising from the first 'most obvious' axiom, that of *intrinsic existence* is conversely perhaps the least straightforward but is nonetheless central to the wider IIT framework.

Intrinsic Existence necessarily requires a real system of elements to support it however this alone is insufficient unless such a system is able to have **causal relations** with the world around it (in keeping with Alexander's Dictum) and in order to facilitate intrinsic experience must possess some form of cause-effect relation within itself also(841).

For such '*cause-effect power'* (*cep*) to exist there must be a relationship between the 'causes' applied to a system and the range of effects which arise; systems insensitive to causes *or* incapable of eliciting effects *or* wherein no internal relationship between the two exists are considered to lack intrinsic existence(841, 896).

The principle of mutual information (MI) is a direct extension of Shannon's original work(1127) and was initially applied by Tononi and Edelman(744) as a mathematical basis for establishing the presence and quantification of cause-effect relationships within putative elements considered to contribute to the PSC.

The entropy-information shared between the states of any two separate variables (for instance time-series of activity from different components or the input/output properties of a singular entity) which each possess their own associated individual entropies (regarding range and uncertainty of possible states) can be established by the following widely accepted identity formulae(1204) (figure 51):



$$I(X;Y) \equiv H(X) - H(X|Y)$$

$$I(X;Y) \equiv H(Y) - H(Y|X)$$

$$I(X;Y) \equiv H(X) + H(Y) - H(X,Y)$$

$$I(X;Y) \equiv H(X,Y) - H(X|Y) - H(Y|X)$$

Figure 51 Mutual Information – Fundamental Identities and Venn Diagram Of Relationships

Wherein I is the mutual information shared between two variables, H is the entropy of a given variable or its entropy conditional on knowledge of another(1204).

In this context of assessing cause-effect relationships, the H(X) and H(Y) can be used to characterise the entropies of these variables respectively (i.e. H(Cause) and H(Effect)). If they are truly unrelated or uncoupled then H(Cause)+H(Effect) will simply equal the joint entropy of the whole system, i.e. H(Cause,Effect) and no mutual information will be present between them and as such, at least in the frame-work of IIT(989), no intrinsic cause-effect power will exist either.

In contrast, if I(Cause;Effect) is >0 an intrinsic information relationship between causes and effects will be present. Within the IIT framework only systems with such cause-effect power (cep) are considered to exist from an intrinsic perspective or at least contribute to and be relevant to the overall PSC(841, 896).

The derivation of *cause-effect information* has evolved over the three subsequent iterations of IIT(841, 892, 896, 1205), with the most recent formalism (in IIT v3.0)(841) being appreciably the most rigorously defined; nonetheless the conceptual basis of considering the information relationship between causes and effects remains at its heart.

In the current framework an element has cause-information (*ci)* only if it is selectively sensitive to (altered or affected by) certain inputs from a range of external possibilities(841). For example, if an element is selectively put into a state (e.g. 'ON') by detecting some types of external feature and not others, it can generate information about the external feature as *uncertainty* is reduced when the right causes provoke an 'ON' response. This is captured by the relative entropic distance (*D*) between the entropy of possible external states (which would be maximal i.e. H_0) and the distribution of possible states when we know the element is 'ON' *a priori*. If an element cannot reduce the uncertainty about the external states In question, it cannot therefore generate information about the cause of its current state(841, 896).

This *ci* is complemented by effect-information (*ei*); which similarly exists only if there is some information relationship (i.e. relative entropic distance *D*>0) between the distribution of possible effects produced by the element in question being in pushed into the 'ON' state (by the causes above) and the resulting external outcomes(841, 896). If the effects of the element make no difference to external outcomes, it is producing no effect-information.

The property of *cei* is an extension of I(Cause;Effect) and is taken as the *minimum* value of either *ci* or *ei*; which respects the 'information bottleneck' produced by whichever aspect conveys the lesser information forward(841, 896).

In summary, *intrinsic information* is generated by elements only if they are able to reduce uncertainty about external states by having selective causes *and* similarly lead to specific effects which make some *difference* to other external states as a result; if either of these requirements are absent and no cause-effect coupling exists the element has no *intrinsic causal existence* from its own perspective(841, 896, 1202) (figure 52).

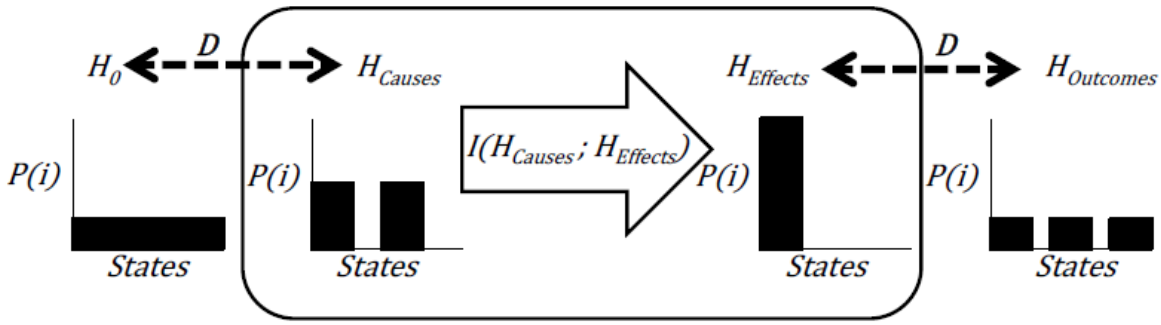


Figure 52 The Presence of Intrinsic Existence Is Dependent on The Presence of Coupled Cause And Effect Information within the elements in question

Adapted from (989); Herein P(i) represents a probability distribution of states which have an associated entropy, H. The statistical distance between two entropy distributions is taken as D. For cause-effect relationships to exist (and the 'element' in question to have ***intrinsic existence***) there must be cause information (D>0 between H_0 and H_{causes}), effect information (D>0 between $H_{effects}$ and $H_{outcomes}$) *and* mutual information ($I(H_{causes};H_{effects})$) between the two to yield ***cause-effect information*** *(cei).*

The nature and consequences arising from the *information emerging* from a system's cause-effect interaction is covered by the remaining axiom-postulate considerations.

The axiom of composition, wherein conscious experience is recognised as having a structured content filled with many phenomenological distinctions of various types led to the postulate that the system comprising the PSC should similarly possess elements capable of making such distinctions and therein to do so as an element or subset they equally should have an intrinsic cause-effect relationship with the whole system in which they are embedded if they are to contribute to and exist within it(841, 896, 1205).

In this framework therefore the PSC will/should comprise a number of '*elements*' each with cause-effect relationships with the others forming that particular system(896).

The elements causally coupled together in this fashion will possess a collective cause-effect repertoire (*CER*) and it is the pattern of causes and effects from this as a whole which is postulated to underlie the system's capacity to specify the contents (or information) within conscious experience and such discriminations or differentiation between states should similarly be a feature of the PSC(841).

Notably, *specification* involves *selection* of a particular cause-effect repertoire from a larger range of possibilities(895).

Importantly, the more precise the selection from an increasingly large range of possible states (i.e. the greater the reduction in uncertainty) the more information the contribution carries; as such, as seen this way making a phenomenological distinction (that which is experienced) involves specifying both what something *is* largely by saying what it is *not*(892, 894, 895).

This seemingly negative definition may seem an initially counterintuitive proposition yet is nonetheless congruent with the notion of information as the reduction of uncertainty(1127) by either specifying a selection and/or *excluding alternatives*.

This '*importance of the negative*' has led to the concern of 'silent units' being relevant to the PSC(1206). Namely, that elements such as a neuronal group or neurons themselves seemingly contribute to the distinction between states even if they are 'inert'.

A possible solution to this critique comes from considering that it is the information formed as a part of the cause-effect *process* which contributes to the intrinsic experience of the system; not the inputs onto the cause-effect system themselves *per se*(989).

A system of elements with a CER that can intrinsically differentiate a vast number of colours (for example) and therefore has a 'concept' of colour still has so even if the external inputs which might cause it to adopt certain states within its CER do not fire.

However, if the CER itself is lost, with loss of the intrinsic cause-effect relationships defining it, then the concept intrinsically experienced by the damaged system ceases to exist.

For example, an individual of normal vision placed into a purely monochromatic environment still (even without immediate recourse to mental imagery) would have a *concept of* and could *experience* other colours as the necessary CER within their PSC remains intact despite the monochromic surrounds to which it is exposed(841, 1200, 1202).

Conversely, patients rendered achromatopsic following injury to area V4 lose not only their colour perception, but their *concept* of colour also(841, 895, 1202). In the same fashion patients with damage at higher levels of the visual cortical hierarchy with prosopagnosia lose both the perception and *concept* of faces as direct consequences of losing the necessary CER which can generate information about such features (841, 989, 1202).

The PSC must therefore possess a CER across its elements capable of specifying *all* of the phenomenological distinctions the system is capable of experiencing from its intrinsic perspective(1202).

Further consideration of the CER possessed by the system of elements which comprise the PSC suggests that in keeping with the axiom of integration its CER and the information arising from it should be similarly *unified* into an *indivisible whole* akin to the manner in which it is intrinsically experienced(1202).

This ***irreducibility*** to component parts or subsets is both directly in concordance with the foregoing discussion of emergent phenomena(156, 890) (*itself the very purpose of pursuing consciousness or a biomarker thereof as an index of brain functional integrity herein*) and a significant but not insoluble problem with respect to its determination and quantification(1207, 1208).

From the consideration of mutual information to objectively gauge cause-effect relations with*in* elements and thereafter across systems it is possible to determine how the purview of possible past states of all the elements relates to the purview of all possible future states of those same elements and the information integrated therein *across the system*(896, 1205).

The method of partitioning analysis can be employed to systematically *'cut'* elements and subsets out of the larger whole(744) (by dividing systems into complementary subsets of the unified whole) thereby perturbing the CER of the remaining systems(989)(figure 53).

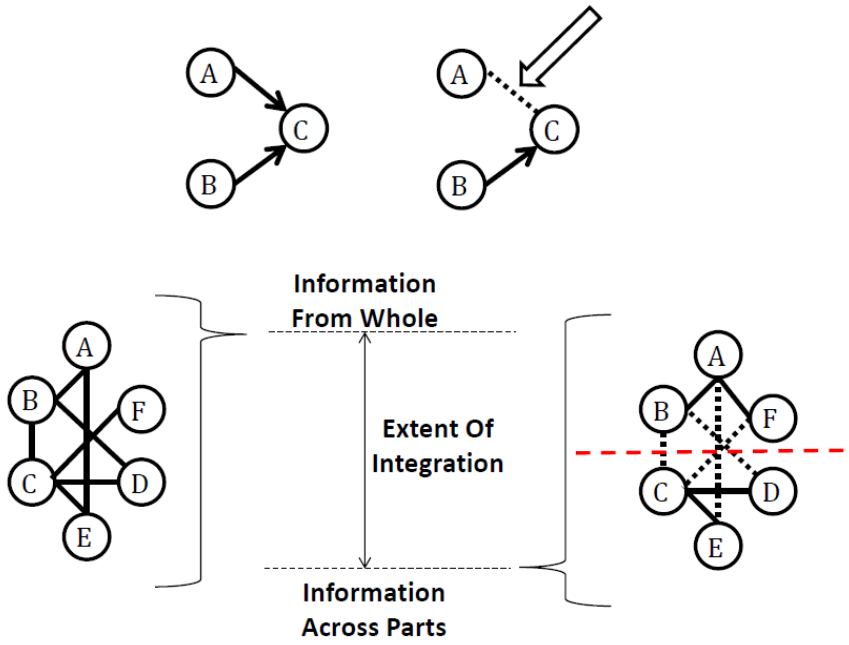


Figure 53 A Schematic Representation of Partitioning Analysis For Assessing the Extent of Integration

(as advocated by (744, 841, 896) In a simple arrangement of two nodes (A and B) contributing information to a third (C), the extent of integration can be quantified by examining the *loss* which arises when the contribution of one node is removed by partitioning it from the arrangement. This principle can be extended to more complex arrangements wherein the extent of integration is represented by the difference between the information which arises from the collective whole (ABCDEF) versus its bipartitioned elements (BAF and CED). The amount of information in each arrangement is not solely a product of the coupling but the logical integrative operations that occur *within nodes* as a function of the inputs upon them. The *minimal information partition* (MIP) is the smallest 'cut' which makes a difference out of all possible partitioning permutations(841, 896) and will continue to grow proportionately for increasingly integrated arrangements. The elegance of this approach is matched by the appreciably non-trivial challenge of its application to real-world settings(1207).

Accepting that the notions of *emergence* and *integration* are intimately linked to the general principle of the '*whole being greater than the sum of its parts'* a reasonable approach taken to assess the *degree of integration* and consequently the extent to which new information arises from interaction is to decompose the systems under consideration into parts and assess the relative effects (on the integrated outcomes) of doing so(744).

In the case of single elements the *cei* generated is taken as the minimum relative entropic distance shared between intrinsic causes and effects of an element in a particular state(989). Assessing the integrated information that arises when such elements are coupled to form higher order mechanisms with far more elaborate *CER* patterns (which specify ***concepts***) and which are thereafter bound into systems wherein the whole *CER* combines a vast array of concepts into a singular ***complex*** is theoretically amenable to such an approach of *part*itioning analysis(989). Therein by a manner akin to that used in the derivation of the original Neural Complexity (C_N) metric outlined by Edelman and Tononi(744) and those deployed in non-neurological settings to gauge the degree of integration within organic molecular network structures (1209) a system of elements can undergo partitioning, where it is effectively 'cut' into complementary

subsets which maintain their functional couplings within the subsets outside of those bisected by the partition.

This can either be a bidirectional partition or a unidirectional partition with respect to whether the flow of information in both directions between elements or only one is ablated.

Each subset will still possess its own *CER* for each state of its constituent elements and thereby produce a varying but quantifiable amount of *cei(841)*.

Equally, there is a value of the information that *would* be exchanged across such a bipartition were it not in place and able to be integrated into the whole(841).

The degree of integration of information within a mechanism (by the IIT framework(989)) is taken as the *minimum information partition;* specifically from consideration of *all possible* bipartitions across a given system it is the one which makes the 'least difference' to the overall *cei* of the *CER* and its value is taken as φ^{MIP}.

If the φ^{MIP} between two subsets is zero then there is no coupling between them and they do not intrinsically exist as a single entity; conversely, more integrated mechanisms will accordingly possess higher φ^{MIP} (989).

The value of φ^{MIP} is taken as a direct *index of irreducibility* as it reflects the smallest difference or decomposition one can make before what remains (the two complementary partitioned subsets) *equates to simply being a sum of their respective parts and nothing more*(841, 896, 1200).

This approach, applied to *mechanisms* to yield φ^{MIP} is applied in the same manner to higher order *systems* to yield Φ^{MAX} which is therefore an index of the degree of integration of the information of the *global, maximally irreducible cause-effect* (*MICE*) structure or **complex** emerging from the PSC at any instant(1202).

This specification of a complex having the *MICE* structure for a particular period offers some basis for the final postulate of exclusion; wherein processes which do *not* contribute to such CES i.e. they occur outwith it and make no difference (provide no information) to it do not contribute to the PSC and therefore have no representation in conscious experience(1202).

Conversely, processes making a direct influence on the CES of the PSC have representation within it but not in a manner that is separate or distinct from other parts of the complex i.e. activity is either definitely included as a part of an integrated whole or excluded entirely (not experienced)(1202).

The exclusion postulate is another component of IIT that has drawn criticism from others, particularly on the basis of its clarity and strength of argument for its justification, i.e. it is not wholly acceptable adequate to make claims on the basis of self-evidence (1206).

Nonetheless, from consideration of the temporo-spatial scale over which such *MICE* structures would likely occur in the cerebral setting it makes clear predictions about the 'granularity' or resolution of conscious experience consistent with that which is observed(893, 989, 1202).

Consider that whilst individual neuronal firing is clearly a fundamental aspect of cerebral processing, the time needed to integrate this into necessarily larger and more complex (irreducible) structures by mechanisms involving attentionally driven feedforward and feedback processing and the like is much longer, in the order of tens to several hundred milliseconds. Indeed, the p300 and similar cognitive evoked response latencies(549, 550, 662, 666) are a direct testament to this.

Similarly, the characteristic duration range of the aforementioned spatially (network) extensive microstates and phase shifts(965) also puts a higher-integrative '*frame-rate*' of experience(1210) on the order of 10s-100s of milliseconds and phenomena such as '*change blindness'* (1211) certainly point to a variable upper limit of the duration of MICE structures within the human brain.

The capacity for macro-scale interactions to produce higher degrees of integrated information with greater causal emergence in comparison to component structures at the micro-level has also been demonstrated empirically *in silico* (1212) which intuitively fits with the principle of neuronal groups integrating more than individual neurons as greater CEP results from their inter-group interactions. Also, at the lower levels of neuronal firing for example there is a higher degree of *degeneracy* (various causes produce the same resultant effects) which is clearly not so evident at higher levels of the central processing hierarchy(1202); this again contributes to higher CEP at such levels.

Whilst IIT has been described as the leading conceptual framework for approaching the '*hard problem* of consciousness' in modern neuroscience (1213) it is admittedly incomplete (841) and despite receiving praise for offering at least the basis of an empirically testable framework (897)it has also received some criticisms(1206), which are not simply related to the adopted approach(892) of beginning with phenomenology and 'working *back*' to neural correlates via a series of derived postulates about the PSC they should contain.

If one accepts the emergence of Φ begins from the very moment properties or variables within an assembly or system start to possess mutual information about each other which has a *causal* basis and this becomes integrated(892), by the very nature of this schema it brings with it an associated spectrum - at each end there will be minima which are non-zero. In essence, by application of its tenets one would or at least could attribute values of Φ (and therein *degrees of consciousness*) to physical systems not typically associated with 'consciousness', certainly in the classic clinical sense(893). The possibility of such '*funny instantiations'* whilst clearly disquieting for some (911, 1214) is clearly congruent with the emergent panpsychist perspective of others and either way no clear grounds for rejecting the model - given there would be many magnitudes of

difference between the Φ one would attribute to comparatively simple arrangements and that of the PSC within the NCC of the brain(893).

There are also valid concerns that the IIT framework may account for the necessary but not completely the sufficient conditions required to generate conscious experience (1197) or moreover that consciousness may not be quantitative in the manner suggested by the nature of deriving the *intensive* property of Φ (1215) which therefore calls into question whether meaningful quantitative comparisons between systems (e.g. individuals in different clinical states) might be indeed even be viable. Further, whilst some raise the question of why use of bipartitions and not tripartitions or indeed higher degrees of partitioning is required to derive Φ, there is no clear rationale given for why a switch to such a regimen would be required or ultimately yield significantly different outcomes(1214).

All this notwithstanding, significant challenges with the real-world derivation of Φ do exist(1207, 1208). Even if one accepts that on the basis of the Dual Aspect of Information that the *intrinsic information CES* held within a system of elements will have an exactly corresponding *extrinsic* physical entropy distribution observable to various types of external measurements; one is faced with the immediate realisation that by the described approach of partitioning to identify minimal integration partitions across *all* the purviews, of *all* elements, in *all* states is at least currently computationally intractable(841, 1202). Estimating Φ becomes exponentially more demanding with each additional element added to a given system. Current complete derivations can manage systems with less than only 20 or so nodes; this is very far off the billions comprising an average cerebrum(841, 897, 1202).

Some consider the intractability to be a reflection not solely of the large number of partitioning pertubations involved but also a hallmark of some inherent degree of uncomputability of consciousness(904, 906) which sits alongside its more computational features; such an argument for something '*extra*' alongside nonetheless complicated information processing was itself the basis of Penrose's previous Quantum Orchestrated Reduction hypothesis(904, 906).

There is also the difficulty posed by the continued evolution of the exact definition of Φ with successive iterations of the framework(841, 892, 896) and for all its attraction to many investigators no *canonical* version has been established despite growing consensus amongst strong advocates (893, 1207, 1213) of its centrality to the framework.

In parallel the translation of the *idea* of Φ into *metrics* meaningfully applicable to real-world neurophysiological systems has generated an evolving series of candidates the effectiveness and relative merits of which are still subject to debate(1207, 1216).

However, there is a growing convergence of empirical findings(748, 749, 1217-1219) to support the general IIT framework and in turn the rationale it offers for a methodology of measuring the disturbed ability of the brain's complex systems to produce emergent phenomena in the presence of disease related damage.

Empirical Support for Integrated Information Theory

The explanatory power of IIT in accounting for why certain brain states (e.g. seizure and anaesthesia) are associated with reduced consciousness despite having *greater* or *similar* electrical and metabolic activity compared to wakefulness and why consciousness is relatively preserved with injury to some regions (cerebellum) and not others (cortico-thalamic complex) (1202)is accompanied by a growing number of empirical findings that similarly support it to a degree.

Work has largely focussed on explaining the '*limiting cases*' and discerning structure-functional differences between them and the consequences of such differences on capacity for integrating information.

A series of studies examining the temporo-spatial electroencephalographic behaviour of the cortex in response to non-invasively delivered TMS stimulation in various clinical states of consciousness have been conducted (747, 748, 1217, 1219-1221).

The typical response elicited in healthy individuals in a state of quiet wakefulness is one which propagates from the site of simulation to many different areas over the subsequent 300 milliseconds before returning to its statistical baseline.

Such responses are considered a reflection of the *effective* cortico-cortical connectivity between cortical regions and notably the pattern of response is not markedly dissimilar between different regions of initial stimulation.

Conversely, during states of slow wave sleep (746) (and to a lesser degree in REM (1222)) the cortical EEG responses to stimulation are both shorter in duration and remain relatively localised without marked propagation to other areas. This has been interpreted to be congruent with a reduction in effective inter-cortical connectivity (746, 749, 1222) and such findings have more recently been independently replicated by others in Non-REM sleep (1223).

These findings are consistent with the changes in cortico-thalamic unit operation from tonic irregular activity to a bistable pattern comprised of oscillations between depolarised UP states and the markedly less excitable hyperpolarised DOWN states that constitute the delta oscillations of Slow Wave Sleep (700, 1224, 1225), which emerge in response to falling neuromodulatory influence to the cortex from acetylcholine and a selection of monoamines (1226).

Interestingly, in comparison to the subjective experience of falling asleep the transition to sleep from a neurophysiological perspective appears to not necessarily result from a sharp *global* phase change but instead such slow wave activity may emerge *locally* in distinct cortical regions and structures (which effectively go 'off-line') before such oscillation is seen more *globally* (1227, 1228). This has been observed both in rodents (1229) and humans (1230), wherein the case of the former having arisen in cases of sleep-deprivation such transient 'off-line' periods were associated with motor performance errors (1229, 1231).

The periods of hyperpolarisation greatly attenuate responsiveness and thereafter the capacity for onward propagation and thus integration(749).

As previously discussed there are a range of approaches to the notion and subsequent quantification of complexity(156, 890); our working model alongside the IIT framework adopts the statistically physical approach outlined by Lopez-Ruis *et al.,* (887, 888) however several approaches have focussed on quantifying the algorithmic complexity or more particularly the compressibility of information sets, such as variants of the lossless compression algorithm outlined by Lempel and Ziv (1122) (LZc) which has been successfully applied to data storage and transmission for many decades.

Application of the LZc to non-invasively acquired EEG studies from wake and NREM sleep states (1232) has demonstrated a transition to lower complexity signals on entry into sleep. This has been recapitulated by using a multi-dimensional EEG complexity battery featuring not only the LZc but also Amplitude Coalition Entropy of the most active channels and Synchrony Coalition Entropy of the synchronised channels on intracranial stereotactically placed intracerebral recordings from epilepsy surgery programme participants by the group of Schartner *et al.* (1233). This expands on similar findings by the same group demonstrating a loss of complexity by such measures applied to the non-invasive EEG of patients under propofol anaesthesia (1234). A particular insight offered by the stereo-EEG studies is that the global reduction in complexity, by each of their chosen metrics was also associated with some regional disparity, with drops in the complexity of parietal, occipital and temporal regions being more marked than those observed in frontal regions, in both sleep states of REM and NREM versus wakefulness (1233). This will be an important consideration in the pursuit and construction of global indices in diseases with a regionally heterogenous disease burden.

On fMRI BOLD studies the patterns of statistically-dependent association which are traditionally used to delineate and define resting state networks are seen to merge into undifferentiated and generally more homogeneous patterns of activity (at the BOLD timescale) with entry into (and deepening levels of) sleep (1228).

As an important aside, this pair of observations also offers a striking example of the caution required in interpreting functional connectivity analyses; the inference of the statistically associated metabolic activity equating to connectivity is the very foundation of network abstraction in such modalities(534, 535, 761) and yet, in a clinical state electrophysiologically characterised by reduced effectivity connectivity it ‘paradoxically’ appears to increase.

Outside the domain of sleep, such TMS-EEG studies have similarly been conducted in patients under various forms of sedation and general anaesthesia (748, 1217) with similar impairments of propagation and reduced duration of response.

With the capacity to induce larger, more distant activity taken as a *surrogate* of *integrative* potential and the temporal diversity of activity considered reflective of the ability of the underlying cortex to generate *differential* patterns of response a *single*

metric based on the information compressibility from a binarised form of the EEG response has been developed(749).

This Pertubation Complexity Index (PCI)(749), so named because it examines the complexity of the EEG responses following the perturbation of TMS stimulation again relies on the application of the Lempel Ziv lossless compressibility algorithm to encode the pattern of associated EEG evoked potentials, which is then normalised to yield values between 0 and 1.

The PCI metric has drawn some criticism in that at best, whilst perhaps congruent with the IIT framework it provides only indirect support and indeed may simply be measuring the excess entropy of the evoked response(1206); however in countering this it is notable that such an entropy is in itself a metric which by definition quantifies information(1235).

Nonetheless, PCI has been derived in all of the above limiting case states of wakefulness, sleep states, sedation, anaesthesia and extreme clinical states of persistent vegative state and Locked In Syndrome(749, 1217-1219); wherein it reliably served to dissociate between them in a manner predicted by IIT and consistent with Laurey's clinical spectrum of conscious states(907).

Importantly, it was also able to distinguish between those patients in the hinterland of Minimally Conscious State who subsequently would and would *not* emerge back to higher levels of consciousness(749).

More recently the PCI has been applied to the differential states elicited by various anaesthetic agents such as conventional anaesthetics associated with loss of consciousness and the more dissociative agent of ketamine; therein the PCI was effectively able to demonstrate different responses consistent with at least a form of consciousness being preserved in subjects receiving ketamine (748).

The same Lempel Ziv approach applied to fMRI BOLD activity patterns from an admittedly small (n=6) cohort of healthy individuals following presentation of visual noise and semantically meangingful stimuli was also able to offer some significant discrimination, producing higher values in response to processing of meaningful stimuli, suggesting it seemingly produced greater information integration across the cerebrum (1236).

There is also suggestion that IIT is vulnerable to 'Fading' and 'Dancing Qualia' arguments from the school of Philosophy of Mind (911). The former is a variant of the ('Trigger's Broom') scenario whereby one asks if neuronal elements were gradually replaced sequentially by non-neuronal equivalents would qualia gradually fade or disappear with the loss of a critical neuron or element? Only persistence of qualia with ongoing computation would apparently be congruent with the model. The latter argument targets the identity between particular qualia and the activity of certain neuronal groups; would qualia appear to 'dance' in and out of experience with switching of such groups? the dependence of phosphene generation (by application of TMS to the occipital cortex) on the phase of local alpha oscillation (813) and the early awake intracranial topography

work of Penfield and Jasper(1237) would suggest this is likely the case and neither offer major challenge to IIT.

The model is incomplete as is our knowledge of NCCs and the solution to the Binding problem(741, 833, 1202). The proposition that synchronous activity between spatially disparate neurons is essential to generating unified percepts and integrated processing is increasingly well established (729, 731, 732, 735, 736, 741, 1170, 1238, 1239). That this occurs through linking oscillatory activity in the higher frequency gamma bands is also supported (729, 733, 856, 1240) and that these in turn are modulated by regional oscillations at lower frequency bands in a multiplexed fashion is increasingly recognised(727, 813, 819). However the actual '*how*' and '*why*' such activity leads to a bound integrated outcome is unclear and mysterious. Some groups have posited binding ultimately occurs as a result of quantum level phenomena such as molecular coherence and wave function collapse(903-906). Penrose(904, 906) with support from Hameroff built the original 'Orchestrated Objective Reduction' model on the premise something *beyond* simple computation was occurring in neurons and these quantum phenomena within the microtubular infrastructural components of neurons across the brain contributed to qualitative conscious experience (by binding through quantum coherence). Non acceptance of this idea has paralleled the lack of empirical support. Newer models, stimulated by the recognition that *only* quantum level effects (such as the maintenance of ions in a decoherent wave-like state) can account for the behavioural properties of neuronal ion channels (namely with respect to the rapid transit through and high specifity of their pores) (1241), have suggested quantum effects may *still* contribute to binding through coherence or similar interactions in the Zero Point Field(1242) and others suggest this may occur directly within the waves of the brain's electromagnetic field itself (1243). Pertubation of the EM field by endogenous neuronal activity encodes exactly the same information from a physical perspective and has been demonstrated to modulate spatially disparate neuronal activity in a reciprocal fashion allowing mutual influence (1244). Of note, this is a property already therapeutically exploited by transcranial direct current stimulation (1245).

The intrinsic relationship between the EM field, neuronal ionic dynamics and resultant activity suggests such ideas are not readily testable and additionally there are many who consider attempts to explain consciousness with physical field and quantum explanations as simply trying to 'account for one mystery with another' (1197) and hold a number of objections (1242).

Furthermore despite their fascinating conjecture, not only does uncertainty about the relevance of such ideas exist but they are clearly outwith the scope and resolution of scalp electroencephalography. Indeed, whilst there are good arguments as to why consciousness is effectively a fundamental and emergent physical property of matter (867, 1246) in keeping with the '*Duties of a Doctor*' outlined by the General Medical Council(1247) we shall instead make the care of patients our first concern and maintain focus on construction of something useful rather than labour fruitlessly toward a complete solution of the so-termed *Hard Problem* of Consciousness(897).

Operational utility is therefore the primary aim.

It is notable that the IIT model does not sit in a mutually exclusive manner against other conceptual frameworks, such as the longer established Global Workspace Model of Conscious processing developed by Baars *et al.,* (833, 1198) which offers a mechanistic account based on the highly re-entrant flow of information around the aforementioned loop systems. Indeed, whilst the model offered by Baars describes a putative *mechanism* (the *how*), Tononi's IIT explains the nature of the *result* and *why* it produces qualia. The models are thus wholly complimentary.

Notably that whilst sceptics object to IIT being a solution to the Hard Problem of Consciousness, they nonetheless agree it may address the '*Pretty Hard Problem' of Consciousness*; namely offering a means to predict which physical systems can give rise to this emergent property(1206).

The absence of a complete model should not dissuade us from attempts at constructing useful metrics. Rough but nonetheless useful notions of 'length' preceded concrete representational theory of this concept by millennia (866, 1195).

Towards An Applicable Conceptual Framework :

The Entropy-Complexity-Integration Relationship

In the same manner that the Joint Entropy of two discrete random variables can be derived by application of the Shannon Entropy formula to the joint probability distribution of the two variables in question by :

$$H(X,Y) = -\sum_{x}\sum_{y} P(x,y)\log_2[P(x,y)]$$

When more than two such variables are under consideration this simply expands to:

$$H(X_1,\cdots,X_n) = -\sum_{x}\cdots\sum_{n} P(x_1,\cdots,x_n)\log_2[P(x_1,\cdots,x_n)]$$

The previously explored relationship between entropy, disequilibrium and the resultant complexity can therefore still be applied to arrangements with greater than two spatial dimensions, as we had initially considered for illustrative purposes.

Indeed, dimensions under contemplation can be expanded to those of the familiar three spatial and one temporal H(X,Y,Z,t) or indeed further still, wherein other properties could be considered, such as the activity status (*'on/off'*), '*charge*' or even '*colour*' for example and would be captured as H(X,Y,Z,t,*activity,charge,colour*).

One can therefore see that with additional properties considered in the joint entropy of our higher dimensional system, the opportunity for 'increasing disequilibrium' and therefore conferring or achieving increased complexity as a result exists.

By example, the complexity achieved from a three dimensional spatial arrangement that changed over time and had varying patterns of activity, 'colour' or some other property that changed also would yield very high levels of complexity, compared to one which was spatially homogeneous, of uniform activity or pattern and generally varied comparatively little as a function of time.

Notably, the same justifications given for fractal arrangements representing maxima of complexity given earlier in the discussion hold equally in such higher dimensional arrangements.

From the identities of Mutual Information it is accepted that H(X,Y)= H(X) + H(Y) if the variables X and Y are statistically completely independent of each other.

The introduction of any statistical dependence between such variables will lead to the existence of shared mutual information between them and H(X,Y) will reduce.

Therefore, in keeping with the aforementioned physical notion of complexity explored previously given that H(X;Y) with I(X;Y)>0 is *always* less than H(X,Y) when I(X;Y)=0 it will also have greater disequilibrium as a consequence of being more distant from H_0 (maximal entropy) which would effectively be at the same point for both systems of H(X,Y), namely that with shared mutual information and that without.

Therefore, quantitatively the relative entropic distance between $H(X,Y)^{I(X;Y)>0}$ and $H(X,Y)^{I(X;Y)=0}$ is directly related to the amount of mutual information which arises as a consequence of the causal and statistically dependent relationships occurring therein (figure 54).

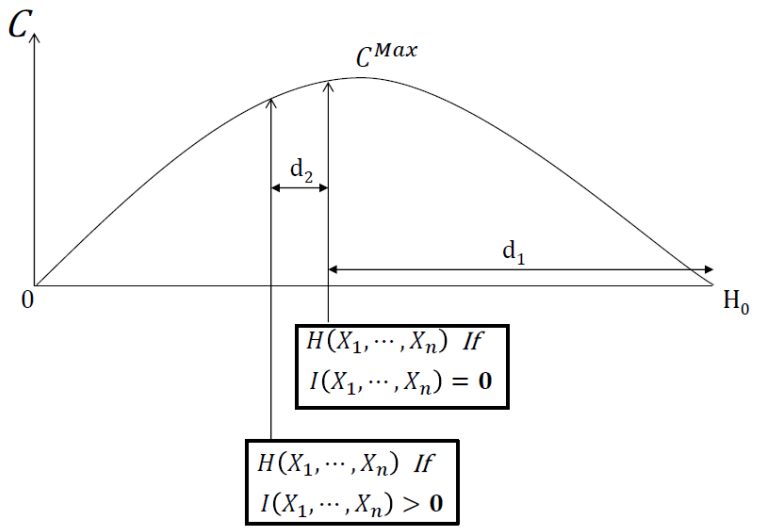


Figure 54 The Effect of Increasing Mutual Information On The Complexity of A System

If the number of variables (X_n) is held fixed and similarly the possible entropy of each also held fixed the only means of increasing mutual information (given that it can never exceed $H(X_1,\ldots,X_n)$ is by integration of information between elements (by overlapping and connecting statistically dependent interactions).

From our considerations of integrated information (Φ) this is regarded and qualified by how *irreducible* it is to contributory parts. Indeed, it is a property which arises in relation to the happenings of the system considered within the dimensions of $X_1,\ldots,X_n$ yet it *is not fully reducible* to components the within the dimensional limits of $X_1,\ldots,X_n$; an extra dimension (X_{n+1}) is required to account for it; Φ is therefore *truly emergent as a higher dimensional physical property of the system-space under consideration* and can also therefore be meaningfully represented as orthogonally related to the other key properties of Entropy (H) and Complexity (C) (figure 55).

Akin to C, Φ is a non-negative property which will have two minima defined by the absence of all information (C=0 and $\Phi=0$ when H=0) and when all information present is completely *unintegrated* ($\Phi=0$ when $H(X_1,\ldots,X_n)=H(X_1)+,\ldots,H(X_n)$ and thus $I(X_1;\ldots;X_n)=0$).

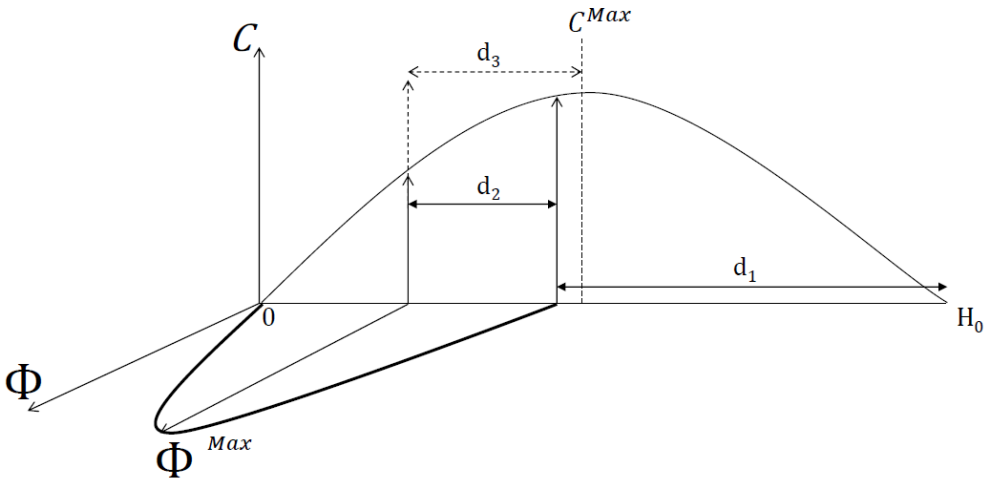


Figure 55 The Entropy-Complexity-Integration Relationship

It also follows a maximum for Φ will be achieved under very specific deducible conditions; such as when there is considerable information present and this is partly but not completely integrated to a high degree (in a maximally irreducibly fashion).

Also from our consideration of the accompanying C we can also make significant inference about the nature of the structures (across dimensions of $X_1;\ldots;X_n$) conducive to high Φ.

C^{Max} is the maxima of complexity achieved by purely fractal structures, which by their nature extend across scales. At the scale under consideration if the system described by $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)=0}$ has a less than purely fractal structure i.e. it is *off*-fractal it will sit to the left of the C^{Max} line on the entropy-complexity spectrum.

Again given that $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)>0} < H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)=0}$ the system will sit even further to the left of the putative C^{Max} line.

Therefore we can reasonably infer two key properties exist if this is the case; firstly the '*structure*' specified by $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)>0}$ will be '*off-fractal*', in that it may have fractal-*like* appearances across some but not all scales, hence bestowing considerable complexity and capacity for specialised and sophisticated information representation where it exists.

Secondly, fractal-like structure should be complemented by the presence of non-random structural motifs (across $X_1,\ldots,X_n$ dimensions, therefore *not necessarily* just within three of space) which *directly contribute* to the integration of information which is the driver of increasing distance between $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)>0}$ and $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)=0}$ and equally the difference in Complexity associated with these points.

The increased capacity for integration is the benefit of a trade-off from moving away from indeed a more complex structure.

The first structural motif would be the presence of re-entrant interactions; both objectively demonstrated to facilitate integration of information *in silico*(841, 895, 1248) and as discussed identified nearly ubiquitously throughout the cerebrum.

The second motif would be the non-random arrangement of the system in question into specialised distinct functional clusters interconnected by a relative minority of longer range connections through central areas acting as hubs; i.e. *small worldness.*

The presence of small worldness and fractal topography within the same structure is neither mutually exclusive or indeed without precedent in the field of telecommunications(769).

Notably whilst short path average path lengths could be achieved with the addition of further components into a fractal structure to achieve the associated metabolic and spatial cost would be prohibitive in a system such as the vertebrate brain and the effects on clustering (functional specialisation) and overall capacity for Φ remain a matter of speculation.

In our consideration of how maximum Φ is achieved we can also appreciate the fundamental limits or bounds the extrinsic entropy and complexity of the system place upon it.

Indeed the upper bound of Φ is set at a point where for $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)>0}$ an optimum level of integration is perfectly balanced against the wider entropy of the system in question.

Continued increments in $I(X_1;\ldots;X_n)$ past this point (i.e. increases in d_2) effectively reduce the entropy in the wider system also $H(X_1,\ldots,X_n)$, with a resultant fall in the information generated (Φ) as it becomes further integrated past this point.

The physical interpretation of this is revealed by considering a system which is overly-integrated (wherein all components have the same information about each other

because they are all fully coupled and collectively doing the same thing) will have a low Φ.

Conversely, a system wherein elements are generating lots of information/entropy but this is poorly integrated (due to reduced interaction by uncoupling or disconnection) will similarly produce less Φ.

The optimum Φ is achieved only when the system in question can both generate lots of different information (by maintaining diverse functional specialisation) and simultaneously sufficiently integrate this into higher order information without the features underlying this latter faculty compromising those of the former.

It was this recognition of the importance of *balanced integration and capacity for differentiation* (the act of informatively selecting one 'thing' from many) that drove the original conception of 'Neural Complexity' applied to the partitioning analysis of systems(744).

Importantly the model advocated here also offers explanatory insight into the consequences of *neurological status* upon Φ and therefore conscious experience (figure 56).

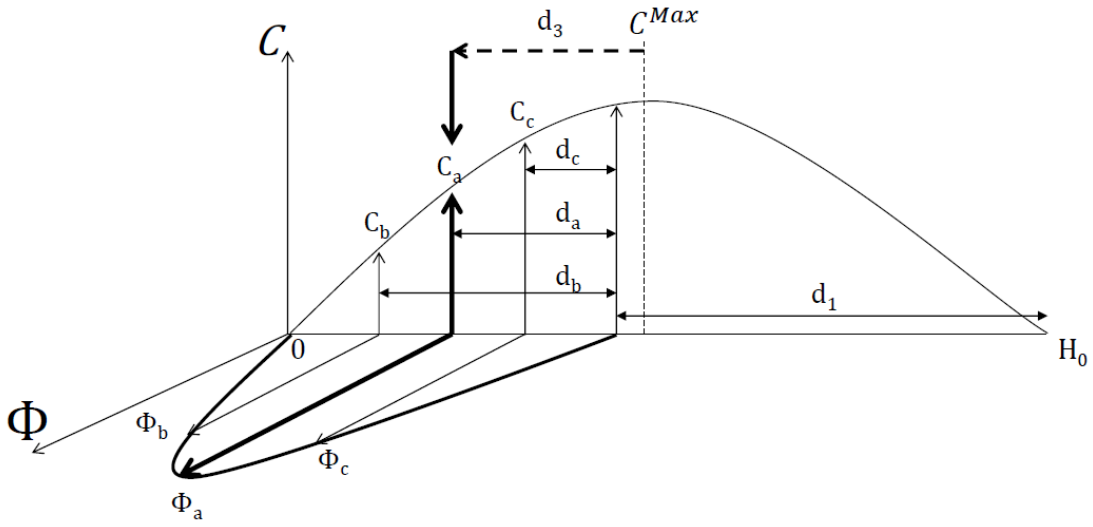


Figure 56 The Dynamic Effect on Phi of Varying Complexity or Mutual Information

The optima of Φ (at d_a, C_a, $Φ_a$**)** is one limiting case highlighting the relevance of both balanced integration-differentiation and the Off-Fractal-Small World-Re-entrant system required to achieve it.

In the case of excessive integration (highlighted by **d_b, C_b,$Φ_b$** in the figure) there is travel toward the origin (reduction) for both the extrinsic complexity of the system and the intrinsic Φ arising from it (as capacity for differentiation is attenuated).

The real-world clinical correlate of such an occurrence would be a generalised seizure for example- all regions doing the same (integrated) minimally differentiating activity;

therefore low Φ(896, 1200). The complexity of the structure (which includes its activity) would be greatly reduced also, as is typically seen on direct visual inspection of the accompanying EEG(644, 674).

A worse situation still arises when there is minimal activity of any kind (minimal entropy to be integrated and minimal integration of that which is present also) and such patterns of low C and low Φ 'would be' likely encountered in states of profound coma and traumatic brain injury for example if the model is correct.

Tononi *et al.*(749), offer a further example of *why* consciousness is lost during states of sleep and anaesthesia. In such conditions the EEG is typified by a loss of entropy (1249, 1250)(and complexity(1251, 1252)) and this is accepted to be a consequence of neuromodulation altering the responsiveness of the cortex; interregional cortico-cortical communication over the cortical surface is greatly attenuated and the effective connectivity between regions (746, 1222) and their resultant capacity to integrate information is abolished.

The system loses complexity, integrative function and differentiation ability and with it Φ falls and consciousness does not emerge(749).

The presence or absence of such a conducive structure-functional topography has also been used to account for why consciousness arises from activity within some structures (i.e. the cortico-thalamic complex) and not others such as the predominantly parallel system of the cerebellum(841, 989). Partitioning analyses applied to greatly simplified versions of these structures which nonetheless possess their predominant structural motifs of small-world re-entrance (cortico-thalamic) and parallel streams (cerebellum) strongly support the capacity of greater Φ arising from the cortico-thalamic complex(841, 895, 1078, 1253); which is clinically congruent with conscious experience being far more dependent on this structure than that of the cerebellum(898); the loss of which has surprisingly lesser effect (sequelae of CCAS notwithstanding(1254)).

A further important feature of the model is that it recognises consciousness as being a non-binary property with a **quantifiable spectrum of possibilities**.

The relevance of this model to our primary line of enquiry can be appreciated by consideration of more *subtle* perturbation of these key parameters in comparison to such 'dramatic' limiting case examples.

If we consider a system that undergoes damage which impairs its small-world architecture, *or* its re-entrant processing capacity, *or* some of its fractal-like connectivity, *or* indeed some uncertain combination of all these properties then this will contribute to a decrease in the difference/distance between $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)>0}$ and $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)=0}$ and therefore result in lower Φ.

Counterintuitively, the overall complexity change associated with such damage may be *negative or positive* depending on whether the system is respectively either generally

undergoing collapse or if loss of certain elements results in a relatively more fractal structure, which one could consider to be far less likely.

Loss of elements will also cause a general reduction of possible entropy with similar reductions in Φ even if the balance between $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)>0}$ and $H(X_1,\ldots,X_n)^{I(X1;\ldots;Xn)=0}$ is maintained.

Finally if such balance is itself not maintained, Φ again as with the all the pertubations outlined above would be reduced.

It is worth considering, that as with so many of the other more classically appreciated cognitive changes accompanying neurodegeneration, early reductions in Φ may lie beyond, or beneath the awareness of the individuals affected.

One can therefore appreciate that Φ and C are both intimately linked, scaled on a spectrum and extremely vulnerable to the entire range of structure-functional perturbations encountered in neurodegeneration and particularly MS.

The translation of such ideas into a practicable system of measurement unsurprisingly is not straightforward.

Consensus agreement on the very definition of C itself remains outstanding and yet the formulation offered here has direct physical relevance to real-world systems whilst offering a 'structural' interpretation of entropy-information.

Its quantification will depend both on the exact method used to derive the entropy in question; how many properties go into consideration of $H(X_1,\ldots,X_n)$ and in what modality (?EEG) and at what spatiotemporal resolution do we consider them?

Our choice of entropy quantification will have an equally important bearing on the value of H_0.

Furthermore the 'optimal' method for quantifying the disequilibrium and the other relative entropic distances when defining the *cei*, φ^{MIP} and Φ^{Max} used in IIT similarly remains without broad consensus albeit variations of the Kullback-Liebler Divergence and Earth Mover's Distance (Wasserstein Metric) have served adequately well so far(841, 989).

Nonetheless, it appears at least feasible that a configuration of attainable EEG-derived metrics conceptually based on the properties of Φ and C may function to quantifiably capture their presence in the real world systems (brains) and thereby detect disease-related perturbations.

Recognising that variation between expected (on the basis of concept) and realised outcomes on a chosen scale may result from methodological factors influencing how metrics are acquired rather than a flaw in the model of their basis *per se* has stimulated the initial selection of several properties which from the foregoing model should be intrinsically linked and driven by the same underlying factor.

This may help overcome or at least account for some of the anticipated variance from the conceptual model.

The scaling and effectiveness of the resulting measurement system can be developed by inclusion of typical EEG samples from real clinical examples limiting cases of the nature described above, in addition to inclusion of healthy control and MS subject datasets.

The inclusion of such extremes will aid interpretation of findings particularly with respect to the orientation of responses and what may constitute a clinically meaningful change – at least on the larger scale initially.

Important Insights from Disequilibrium ('D3')

From our foregoing consideration of complexity as a product of the Shannon entropy H_1 and its disequilibrium from the maximal H_0 it was not unreasonably asserted that C_{MAX} would be realised by arrangements with fractal properties; achieving the most *structure* within the confines of the dimensions in which the given arrangement is embedded.

Three important considerations follow from this and the relationship of C_{MAX} to Φ_{MAX}.

Firstly, within a *perfect*ly fractal system, whilst they may be generated by the iterative application of an algorithmically simple procedure, rule or law which governs branching or similar; beyond this there is no clear requirement for the elements within such constructs to have *causal interactions* with each other albeit they may share a degree of mutual information owing to the nature of having a shared law leading to their genesis.

For example, if we consider the dense Cambrian layer of a beautiful tree; the iterative branching produces an arrangement which has a fractal character limited across the spatial scales over which branching is observed. The resulting structure is therefore appreciably very complex and the absence of a significant causal interaction between one site and another (barring accidents and disease, chopping off one branch has no significant effect on another) is no barrier to this complexity.

The presence of causal interactions between different regions, with resultant reduction in entropy that would accompany them suggests that indeed, the most complex arrangements are those of a *perfectly* fractal nature wherein the parts come to be arranged by an original organising principle but subsequently have *no causal interaction* upon each other; and this applies whether one is considering a system with a purely spatial arrangement or one which additionally or principally varies over time.

One might also not unreasonably argue that a system which is multi-fractal (with different patterns evident at distinct scales(1131)) is perhaps more complex than one with a 'singular' type of fractal pattern to it and therefore question how examples of the

former (forests, communication networks and not least the cerebrum) could be considered *less complex* than geometric and arithmetic examples of the latter with classic fractal constructs of the Mandlebrot Set, Sierpinski Cube and Menger Sponge(1129). The resolution to this is once again the appreciation of scale. The multi-fractal examples clearly exist and operate over a finite range of scales typically defined by the lengths of shortest and longest branches; they are therefore fractal to a point and within a finite range(1129, 1131). Conversely, more perfect fractals (figure 57) by their nature display ever greater amounts of inner structure with ever closer examination with independence from scale being a defining feature(1129).

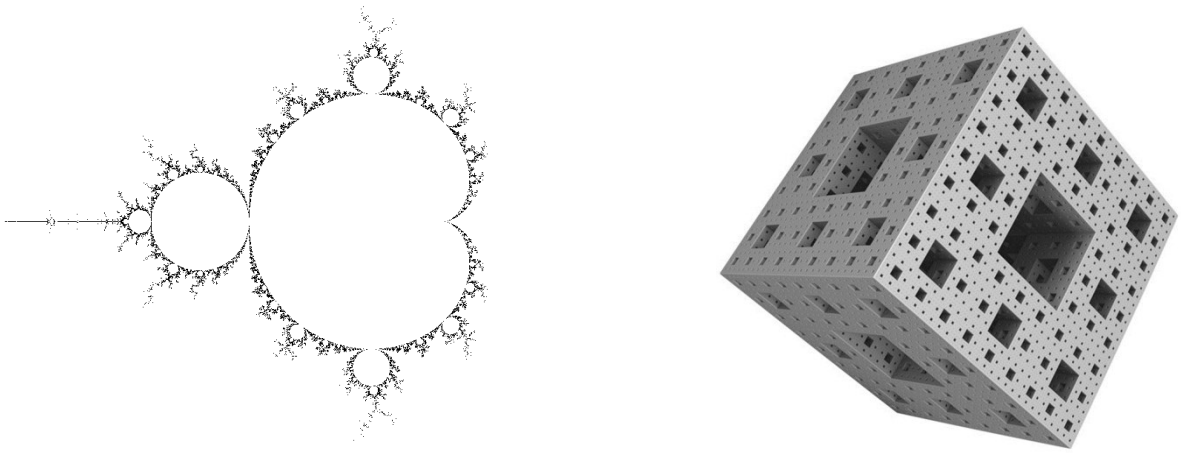


Figure 57 Examples of Perfect Fractals

The Mandlebrot Set (left, from (1255)) and Menger Sponge (right, from (1256)).

Thus, a first limit on *physically attainable complexity* comes from the very limits on scale over which real-world arrangements with fractal-like properties can actually exist.

A second limitation on complexity comes from *purpose*. In contrast to the beautiful Cambrian layer envisaged above, for effective functioning of communication networks whether telegraphic or cerebral in nature it is imperative that the elements contained within are able to exert *causal interactions* on each other. The average distance or path length between disparate elements in such networks has a clear bearing on their capacity to communicate in a temporally adequate fashion(684, 769).

It is demonstrable that endowing networks with fractal properties is one method of reducing such average path length or effective network diameter; in as much as continuing to generally add increasing numbers of nodes (N) ultimately serves to bring down the relative increase in average diameter (r) across the network as it grows in size. This is captured by the relationship (770):

$$\bar{r} \sim N^{\frac{1}{d_B}}$$

Where when applying the box-covering algorithm (1257) to network structures, the relationship between the number of boxes N_B required and their length L_B is governed by the fractal dimension d_B in the manner:

$$N_B \sim L_B^{-d_B}$$

However, precious resources of energy, space and structural material place fundamental evolutionary constraints (136, 1042) on the form of networks nature can realise; particularly within the cerebrum, which already utilises 20% of cardiac output (26) and faces the limiting geometry of the adult female pelvis (136, 898).

The solution to achieving short average path lengths in such fundamentally constrained networks is to adopt a degree of Small-World architecture (307, 535, 700, 762); wherein the service of certain nodes as highly interconnected hubs coupled by a relatively small number of long range couplings serves to markedly bring down the average path length without the requirement for all the extra elements that would be needed to do the same by a completely fractal arrangement. The relationship between average network diameter and the number of nodes in the small world setting is alternately captured by (770):

$$\bar{r} \sim ln\, N_0$$

Therefore a dichotomy between fractality and small-worldness may exist within the same networks (1258); however they are *not necessarily* completely mutually exclusive but may instead be complimentary and perhaps differentially more evident depending on which *scale or resolution the network in question is examined* (769, 770, 1258).

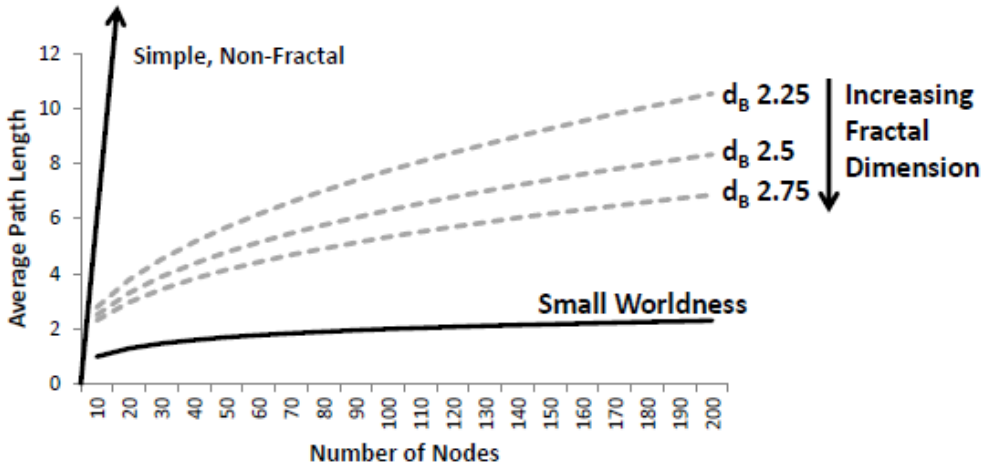


Figure 58 The Beneficial Reductions Of Average Path Length in Networks Possessing Fractal and Small World Architecture

Modelled from equations immediately above.

A comparison of network characteristic and its effect on average path length in different arrangements is illustrated above based on the given formulae (figure 58). Here data from a simple non-fractal network is also included for comparison (the modelled graph is effectively a purely linear chain-like arrangement of nodes with no further branching).

An important reconciliation of the dichotomy between fractal and small world network properties has been achieved by application of Renormalisation Group methodology by Rozenfeld *et al.* (770) to real world canonical examples of networks held up to possess both small worldness and fractality, such as the world wide web and protein interaction networks amongst others. This technique effectively examines system properties across a range of scales by iterative application of the box-counting methodology to increasingly super-ordinate networks at higher levels of evaluation wherein nodes that fall within specified radii are in essence collated or 'normalised' into a single super-node with each change in scale.

In the case of a fractal network assessed by the established box-counting technique; in keeping with the principle of increasing detail becoming evident with ever finer degrees of evaluation which characterises fractals, as one uses ever smaller box sizes in attempts to cover the network in question the number required to do so increases. A log-log plot of box size b against number of required boxes N_b produces a line, the slope of which is equal to the fractal dimension d_B of the network in question (770).

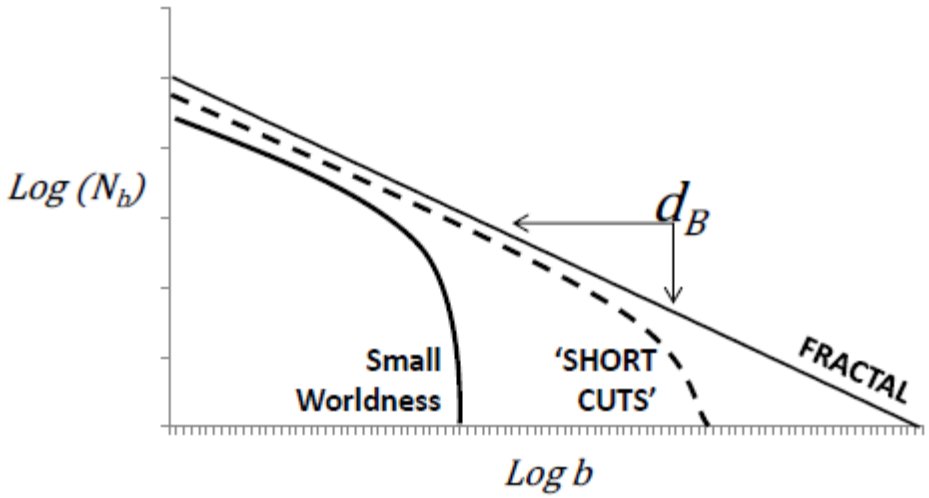


Figure 59 The Transition and Overlap Between Small Worldness and Fractality In Network Structures

Derived from the equations above and modelled after (770).

Application of the aforementioned renormalisation technique demonstrates that the introduction of some additional 'short cuts' by re-wiring perturbs the relationship between b and N_b beyond certain sizes of box and thus beyond a certain threshold in resolution of measurement the curve perturbation is consistent with the appearance of small world networks (769, 770) (figure 59). Hence, both may indeed appear to *simultaneously co-exist within the same network* with our judgement therefore being

heavily influenced on which side of the apparent transition point between small worldness and fractality our system of ascertainment lands us.

This offers some insight into why such a seemingly powerful natural organising principle *may not be evident when sought by functional connectivity studies* in the human brain; in addition to being highly dependent on the method of path length and clustering determinations, the small-worldness exists embedded within a network that is simultaneously fractal at certain scales which adds a further challenge to its clear ascertainment. This is not a trivial issue. The apparent inconsistency of findings relating to cerebral small-worldness in health and disease despite a very scientifically plausible foundation has been a growing cause of concern amongst investigators(771).

Furthermore, introduction of small-worldness into an arrangement is seen to greatly contribute to the *integrative* capacity of networks. Therefore, in the same way a reduction away from C_{MAX} arises due the inherent physical limitations of scale applied to an arrangement in question, systems needing to have causal interactions between their elements and integrate will have to make additional compromises away from C_{MAX} to do so in response to the natural constraints discussed.

Therefore, the complexity of a system achieving Φ_{MAX} will be therefore both less than C_{MAX} and less than the complexity of a hypothetical arrangement where all the same elements existed but had no causal interaction with each other (i.e. it only existed as parts *per se* not unified into a whole).

As such, a causally interacting system producing Φ may on measurement 'paradoxically' appear to be *less* complex than when it starts to distengrate depending on the nature in which couplings or contributing elements are being lost. Hence, a drop in Φ may conversely be associated with either a *gain or reduction* in complexity of the producing system if the driving pathology (or physiology) respectively removes either the capacity for causal interaction (by disconnection) or the generation of structured information (by loss of elements).

Such a clear separation of effects is unlikely *in vivo* and a balance between the two influences is likely. Given the quadratic nature of relationships connecting both Φ and complexity to an arrangement's entropy, there is the theoretical existence of two non-unique solutions for both properties. *If* however the overall framework is valid and the non-trivial matters of property measurement are resolved, it is appreciable that differing phenomena contributing to a loss of Φ may be characterised by their simultaneous effects on complexity also; i.e. those that break a system into parts (reduced Φ, increased complexity) versus those that break a system's parts *per se* (reduced Φ and complexity). Furthermore, examined in further detail the ratio of relative changes in Φ and complexity may similarly offer means to classify the driving pathology.

The third and final insight regarding the relationship between C_{MAX} and Φ_{MAX} comes with respect to the temporal dynamic characteristics of the system in question.

A maximally complex system would not simply have a purely fractal spatial arrangement of its elements; they would be complimented by a pattern of change over time that was similarly fractal in nature (i.e. more detail revealed when examined at ever greater temporal resolution).

Assessment of focal neuronal spiking, local field potential changes amidst neuronal assemblies and large scale electroencephalographic recordings support the existence of such a fractal quality to electrophysiological time-series recordings arising from activity at the micro-, meso- and macroscopic scales (700, 1259, 1260). Detailed *structured* activity is evidently nested within a range of frequencies, across timescales of microseconds to minutes or longer(867).

However, again certain fundamental biophysical constraints exist arising in conjunction with the purpose of system if it is to feature causal interactions which serve to integrate between elements.

Specifically, the dynamics of ion channel conductance, synaptic transmission, action potential initiation, neuronal motif processing and axonal conduction delays(683, 1101) all collectively contribute to an intrinsic upper limit on the rate and timescale over which maximal integration of information from disparate regions can occur. As such, in the same manner that the introduction of small-worldness alongside fractality represents a necessary compromise of spatial complexity to facilitate *integration*, the fractal temporal dynamics of neuronal network activity should feature a similar *concession* in temporal complexity to allow for the generation of maximally integrated information structures over time.

Therefore intermixed with a broad family of ongoing nested oscillations and spiking activity across a range of frequencies, at the temporal scale consistent with the highest degree of integration a departure to a distinct pattern of dynamics should be evident, one characterised *not* by stereotyped oscillation but by periods or states of apparent transient 'unchanging' stationarity, which allow for the aforementioned delays and produce maximally integrated activity before there is brief change and another part of the system is transiently dominant performing a different but again highly integrated task.

This *metastable* pattern and the duration of the stationary periods within it is a compromise in temporal complexity (away from a purely fractal pattern) to overcome inherent physiological time delays. As discussed already, a realisation of such metastability in the cerebrum is the existence of microstates evident on EEG as quasi-stationary distributions in the Global Field Potential across the scalp (848). These differing patterns appear to relate to function within characteristic Intrinsic Cortical Networks and undergo large scale avalanching patterns of change in a manner consistent with self-organised criticality(1172).

So finally, we can come to appreciate that the given complexity of an arrangement contributing to Φ_{MAX} represents a *'sweet spot' in vivo;* with an ideal spatial configuration representing balance between small-wordness and fractality and also temporal dynamics

which comprise optimal metastable behaviour against a background of fractally nested activity patterns arising from the spatially-extended system in question. Ultimately therefore, given the constraints imposed by nature(136, 1042), we should expect the system producing Φ_{MAX} to exist in an *off*-fractal spatio-temporal configuration of sufficient but imperfect complexity.

The proposition that complexity may either increase or decrease with an accompanying fall in Φ applies equally to the domain of temporal dynamics as well as spatial arrangement. As such, pathophysiological decrements of Φ may be differentially accompanied not by possible changes in spatial complexity but by temporal alterations which may offer both insights and a means to classify the nature of their contribution.

For example, a disease state affecting a system may not simply lead to a reduction in Φ (integrative capacity) by producing loss of elements or their uncoupling by disconnection, but also disturb the very criticality underlying the avalanching between metastable states and therein faculty for temporal integration also. The clinical manifestation of such a change would likely be characterised as a slowing of '*information processing speed'*.

Therefore, evaluation of Φ and the spatial and temporal complexities of the system in question which produce it, potentially offers a route to both gauge integrative function of the system and also discriminate between the causes of pathology limiting it.

Given that, for the reasons discussed the pathology underlying MSCI likely contributes in *many ways* to lost integrative function (figure 60), this multi-pronged approach based on a unified complimentary framework is not unreasonable in the first instance.

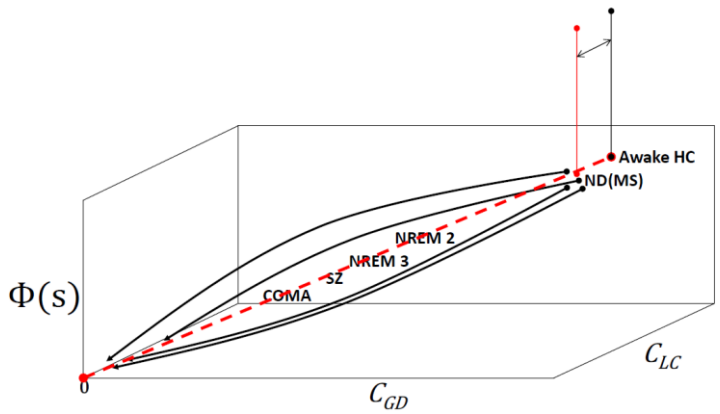


Figure 60 A Schematic of the Translation of The Complexity-Entropy-Integration Framework to A Clinically Applicable Model

Examining the Interrelationship between Local Channel Complexity (CLC), Complexity of Global System Dynamics (CGD) and capacity for Integrated Information. Wherein decline of the latter may accompany various trajectories of change in spatiotemporal complexity of the system in question as a result of clinical status. The changes associated with Neurodegenerative Disease (in the case of MS) may be subtle but potentially discernible on a scale calibrated to sufficient granularity of measurement.

Application of this framework to EEG

Evoked Potential techniques offer the output of a scaled metric whose latency has a direct representational relationship to the conduction velocity within pathways and this in turn indexes myelination(182), nonetheless there is a significant issue of the *discontinuity* which arises when responses can no longer be recorded(181, 312) yet clearly some functional capacity persists from an observable clinical perspective. Similarly, contemporaneous structural metrics of brain, spinal cord and optic nerve all offer numbers relating to area and volume which change over the course of the illness(157, 229, 523, 590) but nonetheless they too appear to have a range outside of which change is minimal and a comparable series of discontinuities arises(196, 1261). From a measurement perspective these floor and ceiling effects force the use of ordinal categories to capture absence of responses(181, 183) or limits the application of such indices to narrower phenotypic subsets, to the exclusion of potential trial participants.

Electroencephalography in contrast has no such discontinuities(644). By internationally established standard techniques(406) of 2 dimensional recording and display of brain wave time-series data there is *representation of the entire state-space of human conscious experience* from a clinical perspective(642, 644). Aside from the isoelectric (zero) output associated with absence of cerebral function, *all* other states have a described electroencephalographic pattern characterised by an assortment of archetypal oscillatory rhythms which vary in their frequency, behaviour and topographical distribution which are intermixed with a further litany of more transient well-recognised physiological phenomena(644). The EEG is determined by clinical state, maturation, a range of toxic and pharmacological agents and the presence of disease(675). Notably, the repertoire of EEG changes in response to disease is also surprisingly narrow, whereby in the broadest terms the underlying activity either slows down (frequently with an observed deterioration in cognitive performance) and/or discharges angrily in an uncontrolled manner often giving rise to clinical manifestations of seizure or myoclonus(644).

Whilst the generally *non-specific* nature of the EEG changes associated with encephalopathic brain dysfunction is often a source of diagnostic frustration to investigating clinicians it conversely speaks to a major advantage of the technique – namely its output is directly determined by cerebral function and its perturbation is an indirect consequence of various pathologies upon that basis(675, 922).

Furthermore the *continuity* of EEG changes is also seen within non-physiological states in a correlated manner as they vary in clinical severity, progress over time and reflect increasing doses of anaesthetic or metabolic toxin(644).

Indeed, whilst it has been possible to derive clinically useful EEG rating systems for gauging severity and prognosticating outcome in the specific aetiological cases of hypoxic-ischaemic encephalopathy and hepatic encephalopathy(644, 1262, 1263) and EEG derived tools (e.g. *Bispectral Index*) have become increasingly used to provide some indication as to depth of anaesthesia (with certain agents) (1264) to specifically minimize risk of operative awareness, the *promise* of EEG's continuity of changes in the setting of dementing illnesses appears as yet unrealised in the clinical setting despite a growing body of encouraging research.

Whilst it is possible to recognise that between earlier and later stages of Alzheimer's and Lewy Body disease the EEG is seen to become increasingly dominated by slower (delta and theta activity)(1265), both of which are themselves significantly different from records of healthy aged matched controls, translating such observations into a scale or screening tool of any utility has not occurred. Vascular dementia features a similarly perturbed EEG(560, 1266, 1267) whilst conversely that of several phenotypes of Fronto-temporal dementia appears relatively preserved on conventional inspection until comparatively late in the course of disease(644) similarly beyond such time that it would currently offer any use. In some contrast EEG has found some use in the diagnostic investigation of Prion diseases(1268, 1269). These invariably fatal and precipitously rapid neurodegenerative diseases feature a general sequence of changes as patients move from clinically early to terminal stages generally characterised by increasing dominance of slow activity and the presence of periodic complex discharges; interestingly the prion species in question (Sporadic or Variant) and the patient's own genotype appears to modify this pattern, again on conventional visual inspection(1268, 1269). The pattern of a shift in spectral dominance in favour of slower activity at the expense of faster oscillations is also described in Huntington's Disease(1270, 1271), the purer tauopathies of progressive supranuclear palsy and corticobasal degeneration (1272) and similarly in the context of Normal Pressure Hydrocephalus (1273), wherein EEG characteristics based on analysis of the frequency-power spectrum have demonstrated even some capacity to prognosticate on the outcome of surgical intervention to correct the perturbed CSF dynamics underpinning this disorder(1274).

In each of these conditions, and indeed several other acquired non-degenerative pathologies such as delirium(1275, 1276) the transition from a normal balance of faster to slower frequency activity in health to increasingly predominant slower oscillatory patterns has been objectively indexed by quantitative EEG techniques (QEEG) typically exploring the shift in the power spectrum by examining the power ratio between higher and lower frequency bands; typically in the form of delta-theta:alpha-beta ratios(655, 709, 1270, 1277-1282). In many conditions a pathological shift favouring a greater power distribution in the slower bands at the relative expense of the faster rhythms has been observed in a manner that relates to clinically evident cerebral dysfunction(654, 709,

715, 1165, 1266, 1267, 1270, 1273, 1278-1281, 1283, 1284). Of particular note, such a shift has also been identified in Multiple Sclerosis(554, 1285).

The significance of such changes can be immediately appreciated by further application of the notion of complexity explored herein to such perturbations of the normal quantitative balance of EEG spectra.

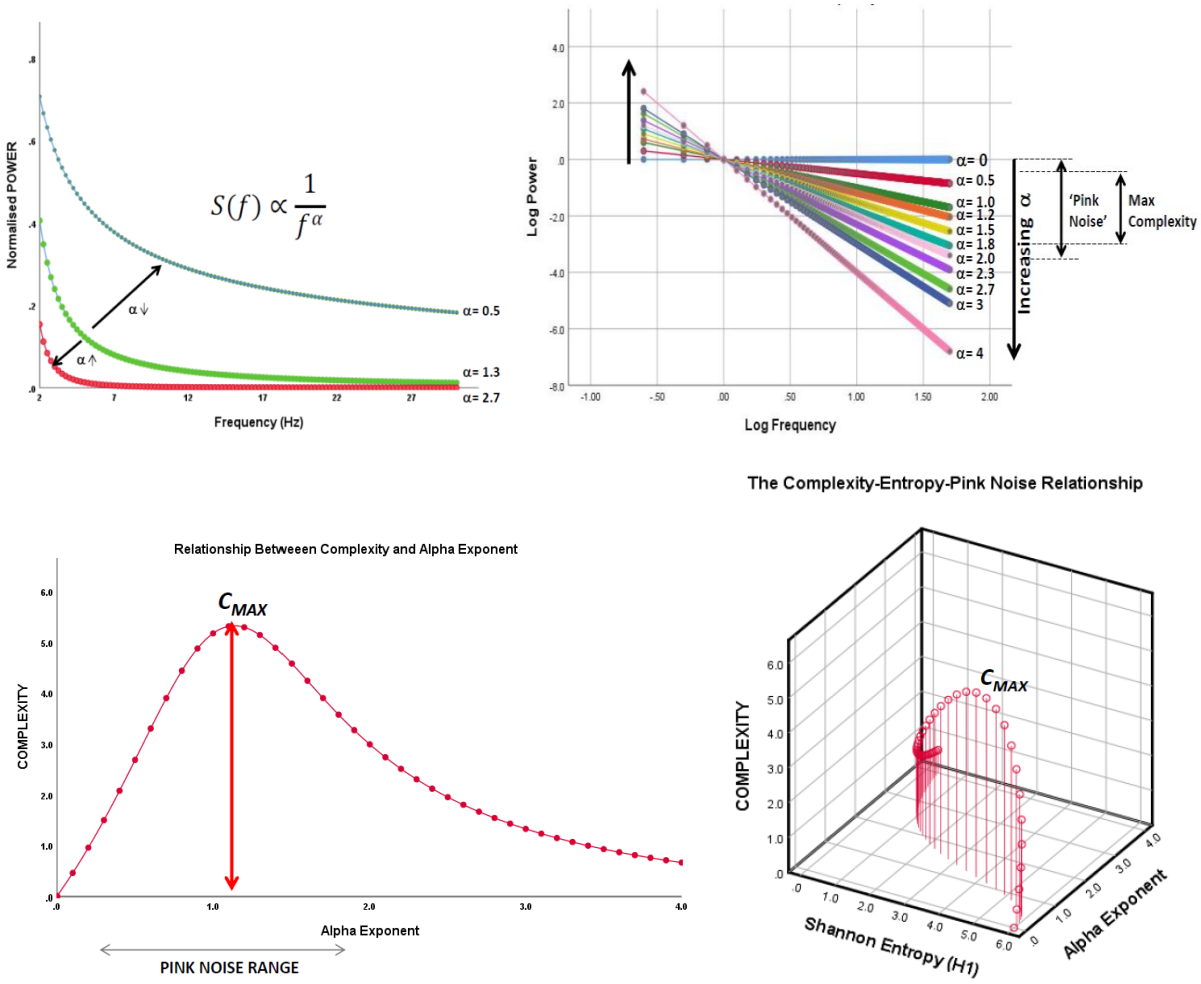
The power density spectrum of conventional healthy EEG is apparently(1286) governed by a power law relating the amount of energy per Hertz by a relationship such as:

$$S(f) = \frac{1}{f^{\alpha}}$$

Where *S(f)* is the power per Hz at any given frequency f distributed in a manner characterised as 'pink noise' where the exponent α lies between 0 and 2; this itself is a signature of many effective complex systems featuring oscillatory dynamics both within and outside the field of neuroscience(700). One acknowledges that in mammals this power spectrum has an additional peak in the middle of the alpha band (1286), that similarly has been seen to both arise at marginally slower frequencies and at lower normalised levels of power in patients with MS versus healthy controls(554).

Nonetheless, deriving Shannon Entropy (H_1) values for such distributions and subsequently values for Disequilibrium from H_0 enables derivation of Complexity and direct evaluation of how this property changes as a function of the α exponent governing the EEG power spectrum; this is not an unfeasible comparison since the normalisation of power arises as a direct action of calculating entropy and disequilibrium (figure 61).

Figure 61 The Modelled Relationship Between EEG Power Spectral Density and Complexity



(A; top left) *The power spectrum displays a distribution characteristic of Pink Noise in dynamical systems wherein power per Hz at any given frequency (**S(f)**) closely follows the relationship S(f) α 1/f^{α}. The exponent α effectively characterises the distribution.* ***(B top right)*** *a range of power spectra with varying α realised on a log-log plot.* ***(C bottom left)*** *The relationship between Complexity and α exponent; notably a peak is achieved within the range classically ascribed to Pink Noise.* ***(D bottom right)*** *The three-way interdependence between Shannon Entropy, Complexity and α exponent. The pattern of altered power spectral balance (increased delta/theta:alpha-beta ratios) seen in many neurodegenerative diseases is congruent with both an increased α exponent and resultant fall away from maximal complexity.*

It is evident that again a singular maximum of complexity arises for a particular α exponent and most notably this arises directly within the range which typifies pink noise activity.

It is further evident that disturbances of the kind seen on QEEG studies in cerebral disease, including MS (554, 555, 815), and AD (1279) which would be characterised by a left-ward shift in the power distribution and hence a rise in α exponent would further directly result in a *non-linear decline* of system complexity as considered here. *The result is to effectively place a lower ceiling of constraint on the informational structures which can be held therein*.

Thus, the described entropy-based approach to complexity employed here prior to further direct application does already appear to offer some significant explanatory

power (in a quantitative fashion) for a number of commonly identified empirical findings and phenomena. It also highlights that the natural balance of oscillatory EEG dynamics likely exists near a further optima or 'sweet spot' offering maximal complexity (and opportunity to achieve structured information therein).

However, the *gulf* from identifying abnormality associated with disease to clinical utility has not been crossed by EEG for a number of reasons.

Firstly there has been the anticipated heterogeneity in results between studies, which in themselves have been generally small and not readily comparable due to methodological variations(645). Secondly gold-standard confirmation of the disease under test by pathological confirmation is not widely available(Stam in (644)); in the context of where a dominant proportion of patients will have mixed-aetiology dementia this is significant(1287). Thirdly, the number of longitudinal studies is particularly small and the number of arguably sufficient length even smaller (also Stam in (644)). Finally, even though a growing number of studies utilising higher levels of abstraction above the conventional visual inspection have demonstrated capacity to discriminate between health and disease and also between disease types and occasionally severity, the reliability and natural variance of such metrics remains wholly undemonstrated and largescale consensus on the best method of their ascertainment remains absent (645).

These six challenges to quantitative topographic EEG analysis were first clearly outlined 3 decades ago (646)and all remain largely unsolved due to the combined effects of predominant effort focussed on exploring application prior to validation, prior limitations in computational analysis for the necessarily large data-series and the significant shift in focus onto structural imaging over the same period.

Nonetheless, the developments toward EEG utility in Alzheimer's are generally encouraging, speaking to the value of considering multiple parameters over singular output characteristics to differentiate a clinical state(680, 1280). However these examples appear particularly *tuned* to the effects of deficit within particular neurotransmitter systems, namely that of acetylcholine(680). Historically insufficiency of this has been recognised to directly underpin many cognitive deficits in Alzheimer's disease and been an effective focus for symptomatic treatment(26). Similarly whilst even greater cholinergic deficits are present in Lewy Body Dementia(1288) this is not a general theme of dementias *per se* including Multiple Sclerosis and explains the lack of *consistent* therapeutic benefit of pro-cholinergic agents in this context(38, 587).

From the foregoing discussion it therefore remains reasonable to pursue a system of multi-parametric EEG analysis tuned not to a specific transmitter deficit, pattern of disconnection or altered dynamics but to the continuous spectrum of human consciousness; more explicitly the continuum of capacity for qualia which in the described model of IIT is a direct consequence of a brain's faculty for globally integrating information. Also such an approach may also not be as vulnerable to the challenge conferred by the Multiple Realizability(893) of consciousness. By examining three related but distinct properties different patterns of change may be accompanied by unique signatures enabling disentanglement of adaptive and maladaptive responses. For

example whilst electrophysiological connectivity, fMRI activation and cognitive potentials all appear to display a curvilinear rise and subsequent fall with disease advancement that possibly precludes their use in a linear sense (as 'low' values may possibly appear at either end of the spectrum confounding simple correlational analysis) a multi-dimensional metric may allow us to overcome such a challenge.

One readily acknowledges the necessary trend to define measures in fundamental terms given the vulnerability of physical systems which themselves superseded anthropological standards(866) however in this case it remains that as Protagoras observed *'Man is the Measure of all things'* (1289) and it is against a large number of humans that we shall have to calibrate our scale. The advocated model may offer metrics which can 'travel', being applicable to a very wide array of brain states and pathologies outwith the confines of Multiple Sclerosis. **I**n the first instance therefore some initial empirical support for the model is required as a necessary prelude to any larger scale attempts at derivation and calibration. To this end a collection of EEG data from a variety of brain states and levels of consciousness, taken to reflect 'limiting case' examples of the kind discussed herein were collated with a view to exploring the relationship to complexity (indexed by fractal dimension), mutual information (indexed by a recently described metric of Φ*) and criticality of system dynamics captured by examining the avalanching-behaviour of phase slip events in the alpha band.

METHOD

To establish *proof-of-concept* empirical support for the model outlined above a retrospective cohort of EEG data attained to International Federation of Clinical Neurophysiology standards in the 10:20 montage was collated. This included 20 datasets from patients undergoing prolonged recording as part of evaluation for Non-Epileptic Attack Disorder (NEAD)(4 of whom had possible overlap with Epilepsy, either Temporal Lobe (TLE) or Idiopathic Generalised (IGE)) which provided data from epochs of wake, rapid eye movement (REM) and slow wave sleep(SWS). These recordings were visually inspected and selected by a qualified physiologist with sleep staging expertise and considered to be *electrophysiologically normal* examples of each respective sleep state. Where concurrent epilepsy was a possibility no sequences with epileptiform abnormality or electrographic seizure were included in the analysis and this also applied to additionally selected EEG recordings from patients with coma due to a range of aetiologies. These were included simply as initial exemplars of *severe* encephalopathic dysfunction with clinically suggested profound impairment of consciousness. All of these locally sourced recordings (of which 20 minute raw recordings of each state were provided) had already been conducted and reported upon as part of routine clinical care, and in keeping with the preceding permission granted by NBT Research & Innovation Department were irreversibly anonymised after the point of selection by the physiologist prior to abstract processing; for the purpose of initial model building only salient clinical and demographic information from the clinical referral accompanied the data. The filter settings were 0.5-70Hz with a sampling rate of 250Hz for all clinical recordings, a summary of which is included in tables 44 & 45 below.

Patient	Age(y)	Sex	Indication	Medication
1	30	Female	NEAD	Clobazam
2	43	Female	NEAD	
3	31	Female	NEAD	
4	31	Female	NEAD ?TLE	
5	35	Female	NEAD	Lamotrigine
6	27	Female	NEAD	Keppra
7	62	Female	NEAD	Fluoxetine, Tegratol, diazepam
8	36	Female	NEAD	Venlaflaxine
9	18	Female	NEAD/Epilepsy	Carbamazepine
10	63	Female	NEAD/Epilepsy (IGE)	Sodium valproate
11	45	Male	NEAD	Clonazepam, mitazapine
12	36	Male	NEAD	
13	34	Female	?TLE	Keppra, lamotrigine
14	59	Male	NEAD	
15	74	Female	NEAD	
16	60	Male	Epilepsy	Keppra, phenytoin
17	59	Male	NEAD	
18	56	Male	NEAD	
19	54	Male	NEAD	
20	47	Male	NEAD	

Table 44 Demographic Characteristics of Clinical Cohort providing matched slow wave sleep (SWS), Rapid Eye Movement and Wake EEG samples

Age (y)	Gender	COMA Aetiology
66	Male	Traumatic Brain Injury
66	Male	Traumatic Brain Injury
30	Male	Traumatic Brain Injury
28	Female	Subdural Haemorrhage
69	Male	Subdural Haemorrhage
42	Female	Subarachnoid Haemorrhage
60	Female	Uncertain
26	Male	Post-Status
19	Male	Post-Status
68	Male	Traumatic Brain Injury
71	Male	Subarachnoid Haemorrhage
24	Male	Fulminant Demyelination
67	Female	Traumatic Brain Injury
44	Female	Uncertain Aetiology
67	Female	Post Anoxia with Myoclonia
56	Female	Toxic encephalopathy
32	Male	Uncertain Aetiology

Table 45 Demographic and Aetiological Mix of Subjects on whom EEG within Coma was analysed

Also incorporated was a collection of open-access (1290, 1291)healthy control resting state (2 minutes eyes open and 2 minute eyes closed) EEG data sampled at 160Hz from 109 adult subjects collected as part of the Brain Computer Interface Research and Development Program at the Wadsworth Center, New York State Department of Health in New York, the data was anonymised and without further demographic classifiers however this work was sponsored by the National Institutes of Health (references EB00635 and EB00856). Notably, for the properties examined there were generally no concerning statistical discrepancies between the healthy control set and the locally attained waking NEAD recordings.

The previously attained EEG dataset from 30 subjects with MS, with resting state samplings in the eyes open and eyes closed condition from both pre and post cognitive testing with the MACFIMS battery were analysed also. Further subgroup analysis of high and low severity of MSCI was achieved by performing a median-split of the cohort with respect to the number of tests failed on the MACFIMS battery by population criteria given more robust performance of this metric in comparison to the MSQ in this small cohort.

In addition to initial direct visual inspection of recording quality, for consistency all signals were additionally passed through the recently described Harvard Automated Pre-processing Pipeline for EEG(781) (HAPPE) in keeping with the terms of the accompanying GNU license. This has been developed to offer standardised and automated artefact handling in addition to technical reports on the quality of data remaining for analysis post-processing (781). Herein all data were initially high-pass filtered at 1Hz prior to Independent Component Analysis for possible artefact identification the output of which is then handled (i.e. removed or incorporated) (781).The HAPPE pipeline is subsequently able to reconstruct or reject remaining signal for the intended time-frequency analysis(781). Automated component rejection is achieved through the Multiple Artifact Rejection Algorithm (MARA), a machine-learning algorithm which having been trained against a large electrophysiological dataset demonstrates high accuracy in evaluating the ICA-derived components suspected of being artefact(781). A Minimum of 30 seconds of continuous artefact-free signal (after data-cleaning and acceptable objective quality rating by the HAPPE) from all available channels was set as entry requirement into the next stages of metric abstraction in post-processing. This did result in a notable but non-prohibitive attrition of some data-series.

Derivation of Higuchi Fractal Dimension was attained using the established algorithm as described and also applied by Smits (1147). In this case broad band EEG between 1-48Hz was examined over repeated 2 second intervals of at least 30 seconds of artefact-free data to yield reliable averages.

1. The original EEG time-series y(t) constitutes *N* points;

   Such that $y(t)[y(1), y(2), \dots, y(N)]$

2. The Higuchi Algorithm then generates a series of new time series derived from this original sequence by *down sampling y(t)* every *k* samples (until *k* reaches a maxima of k_{max}):

   $$y_k^m: y(m), y(m+k), y(m+2k), \dots, y(m+int\left(\frac{N-m}{k}\right)k)$$

   Wherein *m* is the first sampling.

3. For each resultant 'curve' sequence which arises (y_k^m) its length $L_m(k)$ is given by:

   $$L_m(k) = \frac{1}{k}\left[\frac{N-1}{int\left(\frac{N-m}{k}\right)k}\left(\sum_{i=1}^{int\left(\frac{(N-m}{k}\right)} |y(m+ik) - y(m+(i-1)k)|\right)\right]$$

4. This is calculated for all values of *k* to k_{max}.
5. For each value of *k* the length of curve is derived by averaging across the collected sets of $L_m(k)$ values:

   $$L(k) = \frac{1}{k}\sum_{m=1}^{k} L_m(k)$$

6. The fractal dimension is subsequently given by:

   $$L(k) \sim k^{-FD}$$

7. Applied experimentally, the slope of the relationship between log($L(k)$) and log (1/k) can be derived by least-squares linear best fit regression and equates to FD.

2. Derivation of Φ*. This recently outlined metric purportedly offers an index of some *degree* of integration by considering the reduction of information *shared* across a system when it is decomposed, or evaluated as numerous *fully separate* parts; this is termed the 'atomic partition'. Importantly it is therefore distinct from the both the **Φ** concept and bipartitioning methodology outlined in the formal IIT framework(841, 896), which has been equally limited in its application by issues relating to computational tractability.

The **Φ***approach effectively examines the mutual information between the time-series of channel A in the present (A') and that channel at a set time (tau) in the past (A) and additionally between that same present channel (A') and *every other channel* at the past time point (B,….,S for our 19 Channel system). In the 'whole' unpartitioned system only that mutual information which is non-redundant in each interaction is added – i.e. that

information does not overlap or replicate information duplicated by interactions between other past-present pairs.

For example, if A->A' contains mutual information that is entirely duplicated by the interaction of B->A' then that information is wholly redundant and *does not count* as being additionally informative.

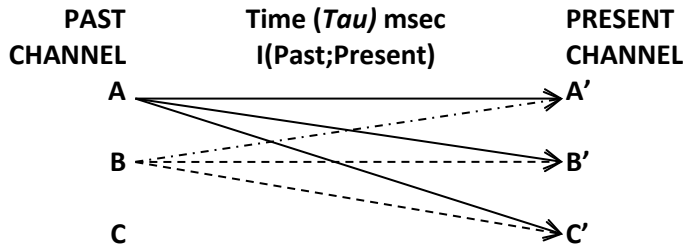


Figure 62 Phi Star Explained

In The Partitioned State I(A;A'), I(A;B') and I(A;C') contain no redundant information; In the Integrated 'whole' state I(A;A') may *share* information with I(B;A') (or other pairings) which introduces a degree of redundancy. The overall *sharing and redundancy which arises when interactions are considered at the system level* compared to wholly separate parts is the basis of the index Φ*.

In the state of atomic partition there will be no considered redundancy and the mutual information between each pairing (A->A') will effectively be taken as the individual capacity of what is happening at channel X in the past to *inform us* about what is happening at another channel in the present *in isolation (as they are considered as if they were in genuine separation).*

In going from an atomically partitioned arrangement to one where collective interaction introduces informatic redundancy across individual cross-channel-over-time interactions (i.e. *at the system level uncertainty is reduced about the relationship of present and past states)* one can infer information is shared and take the magnitude of the collective redundancy which arises as the value for Φ*.

Methodologically, the mutual information and component entropies to quantify information are generally derived in the manner outlined above with redundancy quantified by the mismatch decoding approach outlined by Oizumi *et al.,*(1208) ;

Wherein taking X^{t-T} and X^{t} to represent *multi*variate Gaussian variables (where T, or *tau* is the temporal separation in time *t*), as per above the mutual information between them $I(X^{t-T}; X^{t})$ is calculable from:

$$I(X^{t-T}; X^{t}) = \frac{1}{2}\log\frac{|\sum(X^{t-T})|}{|\sum(X^{t-T}|X^{t})|}$$

Where $\sum(X^{t-T}|X^{t})$ is a covariance matrix of the conditional distribution $p(X^{t-T}|X^{t})$ (1208)and this is applied to the data-series of the whole and partitioned states in question, with sequential analysis of two second epochs and output averaged over the total 30 second window to yield the Φ* used for further comparison against the clinical

variables. In the first instance this was applied to the broad band frequency range of 1-48Hz and examined against increasing values of tau from 15 to 500 milliseconds. Specifically, peak values and integrals for the attained Φ* over the 500 millisecond windows were examined.

3. Quantification of Phase Slip Dynamics. As in the previous work phase slips were identified by taking the first derivative of instantaneous frequency after band-pass filtering of the raw EEG signal. In this case the number of synchronously concurrent phase-slips at separate channels was taken as the metric of event size. Event likelihoods were derived on the frequency of phase slips per second and at least 30 seconds of artefact-free EEG was examined per subject to derive estimates. The relative paucity of larger events suggested an upper limit to the event size of interest when deriving metrics of critical dynamics. An original intention had been to derive a zeta function of the exponent of the decay in relation between phase-slip size and likelihood of occurrence, however the differing upper limit of event size between groups and the numeric constraint on event size posed by the limited number of channels suggested exploration of the ratio between likelihood of small (1 channel only) phase slips and large (4 channel) events would serve as a meaningful proxy in the first instance.

RESULTS

Higuchi Fractal Dimension. The HFD analysis provided a range of values (table 46) consistent with those observed in the literature (1147). Whilst the average HFD displayed a strong capacity to discriminate between levels of consciousness *no* apparent difference between MS and Non-MS subjects in the different waking state conditions was evident (see figure 63) nor was any association with indices of MSCI severity.

HFD	Coma	SWS	REM	WAKE	HCEC	HCEO	MSEOPR	MSEOPO	MSECPR	MSECPO
N	14	15	20	18	83	68	24	22	17	15
Range	0.401	0.250	0.241	0.169	0.184	0.255	0.105	0.139	0.151	0.104
Minimum	1.424	1.473	1.601	1.712	1.731	1.688	1.799	1.769	1.749	1.785
Maximum	1.825	1.723	1.842	1.881	1.915	1.943	1.904	1.908	1.899	1.889
Mean	1.534	1.557	1.705	1.818	1.833	1.811	1.848	1.841	1.833	1.831
Std. Error	0.033	0.019	0.013	0.010	0.005	0.007	0.006	0.009	0.010	0.009
Std. Deviation	0.124	0.075	0.057	0.043	0.046	0.059	0.029	0.041	0.039	0.034
Variance	0.015	0.006	0.003	0.002	0.002	0.003	0.001	0.002	0.002	0.001

Table 46 Descriptive Statistics for Higuchi Fractal Dimension by Group

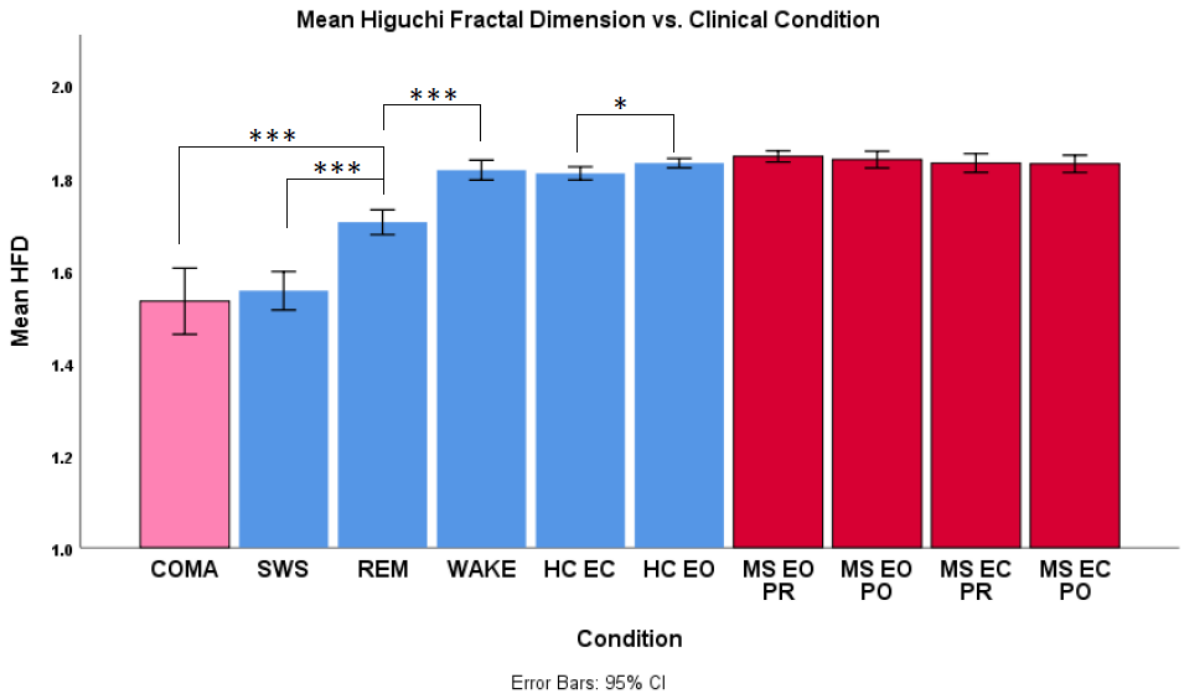


Figure 63 Mean Higuchi Fractal Dimension by Clinical Condition

Φ* Metric. The Φ∗ metric applied to the broad band (1-48Hz) produced a range of values for each sampling condition (table 47, figure 64) which again demonstrated a strong capacity to discriminate between level of consciousness but no consistent difference when comparing between MS and non-MS subjects in the waking state. This was the case both with respect to the *peak* or *maximal* value for Φ∗ and its value at successive intervals over the subsequent 500msec interval as demonstrated by figure 65 below when considering the matched samplings between different sleep states in the NEAD cohort.

Taking the inverse of Φ∗, i.e. $\Phi*^{-1}$, offered a metric which positively scaled with level of consciousness. A plot of average HFD against $\Phi*^{-1}$(figure 66) highlights the strong but non-linear correlation between these properties (Rs .728 p<.001); wherein entry into clinical states associated with consciousness is seemingly marked by an inflexion point in the relationship between the two properties; i.e. complexity appears to increase accompanied by slowing in the relative climb of $\Phi*^{-1}$. Whilst superimposition of the MS subjects onto this relationship (figure 67) encouragingly supports they are in the domain associated with consciousness it did not offer any discrimination from non-MS subjects by those metrics.

To further elucidate possible relationships between Φ∗ and MSCI, rather than focussing solely on the peak Φ∗ value across the broad band, the metrics were derived for classical bandwidths of theta, alpha, low alpha, high alpha and beta. In addition to maximal values, the integral of Φ∗ across a 500msec time window was derived for the pre and post, eyes open/closed conditions of the MS cohort.

Whilst differences between eyes open and eyes closed were evident no significant pre/post changes were identified at the group level (figures 68 & 69). However, significant relationships with the severity of MSCI became apparent particularly in the *post* testing samples(table 48) despite minimal apparent change in the *group level mean*.

Rather than a purely statistical artefact this may possibly be accounted for by a *differential dynamic* change in $\Phi*$ in the alpha and theta bandwidths in response to cognitive exertion; indeed it would appear that the $\Phi*$ interestingly appears to *increase* within those bandwidths in those subjects with a greater severity of MSCI (figure 70). These findings only border on statistical significance and thus must be interpreted with caution. Nonetheless, given the principle of how this metric operates, effectively the shared mutual information from across all channels (above that from all channels considered separately) between *present* and *past* states increases in response to cognitive exertion in a manner that relates to impaired performance over many indicators of MSCI. As elsewhere, no consistent relationship with cognitive fatigue rating on the NFI scale was evident; again the utility of such an instrument in such small groups and the validity of the construct it measures is an increasingly open question.

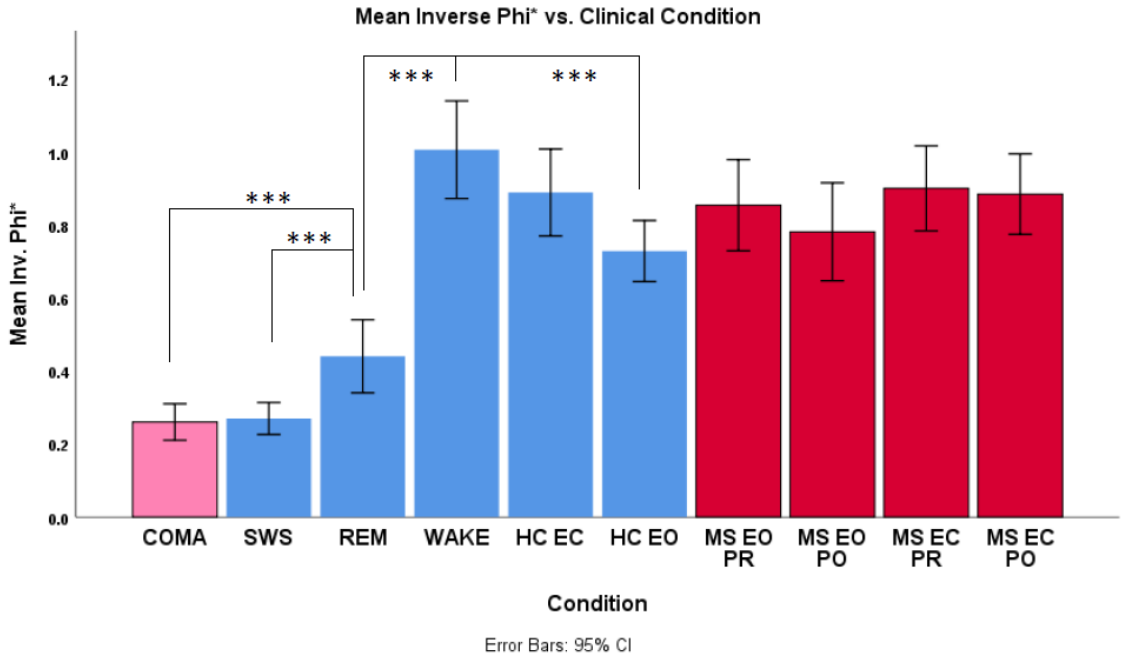
This *increased sharing of information between present and past states* is effectively produced by greater similarity between present and past states over the time intervals in question; because they are exhibiting *less dynamic change over time* – there is less temporal complexity of their dynamics. Attrition of the dataset with respect to the eyes closed MS group following application of the HAPPE limited the subsequent exploration therein but similar patterns again emerged. This pattern also accounts for why greater maximal broadband $\Phi*$ is seen in association with lower levels of consciousness; a system that is displaying minimal dynamics will share more mutual information (from the entropy perspective) with itself over a given time interval. These findings are therefore consistent with the advocated model but suggest $\Phi*$ is a *fundamentally different property* from Φ *proper* as described in the formulation of IIT and whilst informative cannot therefore serve as a surrogate of it.

MI	Coma	SWS	REM	WAKE	HCEC	HCEO	MSEOPR	MSEOPO	MSECPR	MSECPO
N	14	15	20	18	83	68	24	22	17	15
Range	0.282	0.287	0.818	0.860	1.867	2.065	1.119	1.352	0.904	0.703
Minimum	0.150	0.184	0.253	0.616	0.303	0.334	0.389	0.268	0.501	0.479
Maximum	0.432	0.471	1.071	1.475	2.171	2.400	1.508	1.620	1.404	1.181
Mean	0.261	0.270	0.441	1.007	0.729	0.890	0.855	0.782	0.901	0.885
Std. Error	0.023	0.020	0.048	0.063	0.042	0.060	0.060	0.065	0.055	0.051
Std. Deviation	0.086	0.079	0.214	0.269	0.384	0.493	0.296	0.303	0.227	0.199
Variance	0.007	0.006	0.046	0.072	0.148	0.243	0.088	0.092	0.051	0.040

Table 47 Descriptive Statistics for Peak Inverse Phi

Figure 64 Average Maximum Φ* Value vs. Clinical Sampling Condition

(* p<.001 on Independent Samples T-Test)**



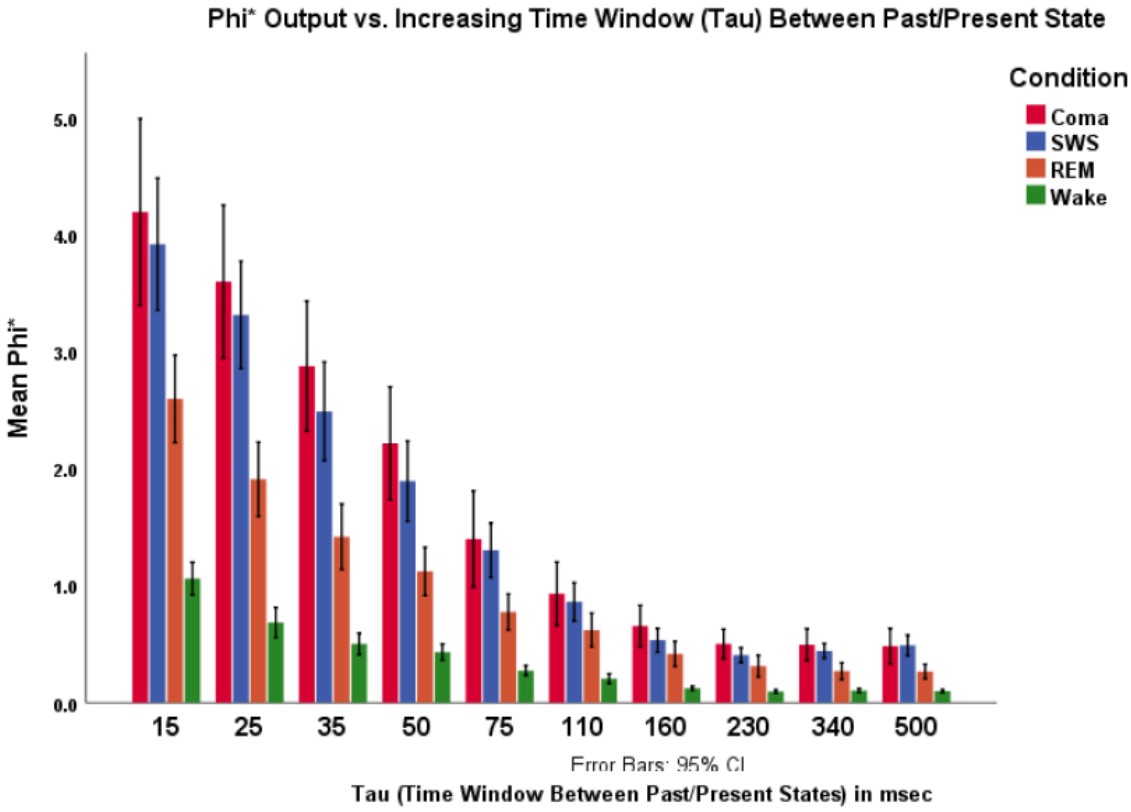


Figure 65 Average Φ* Value with Increasing Time Window (Tau) Between Past/Present States In Relation to Level of Consciousness with the Same Individuals (and additionally Coma Subjects)

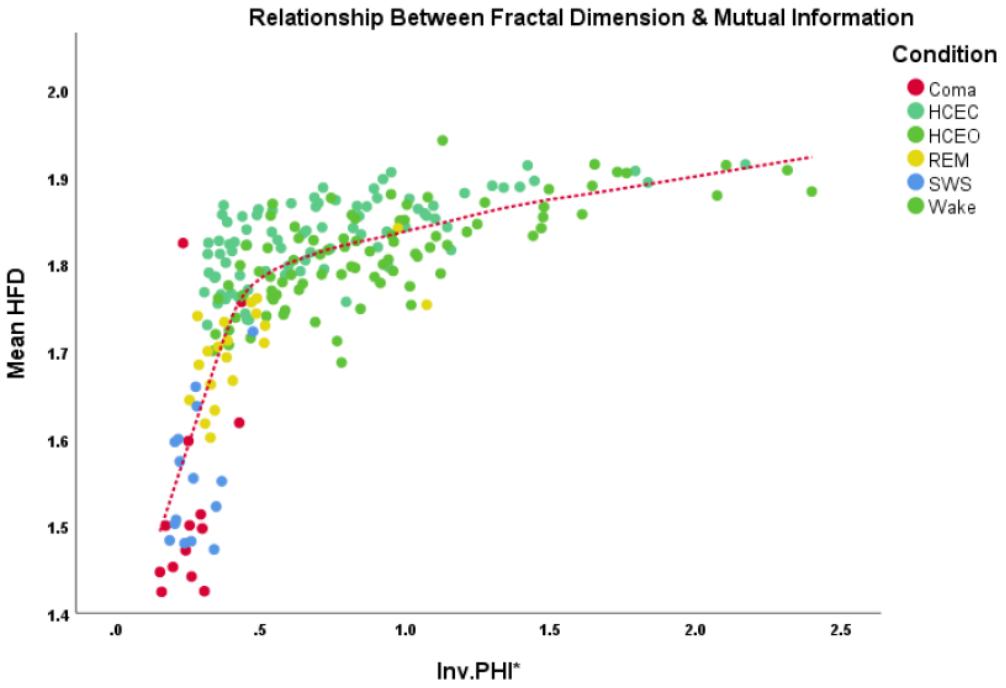


Figure 66 Relationship Between Fractal Dimension & Mutual Information at System Level Measured by taking the inverse of the peak value of Φ* applied to the Broad Band EEG (1-48Hz) (Rs =.728 p<0.001)

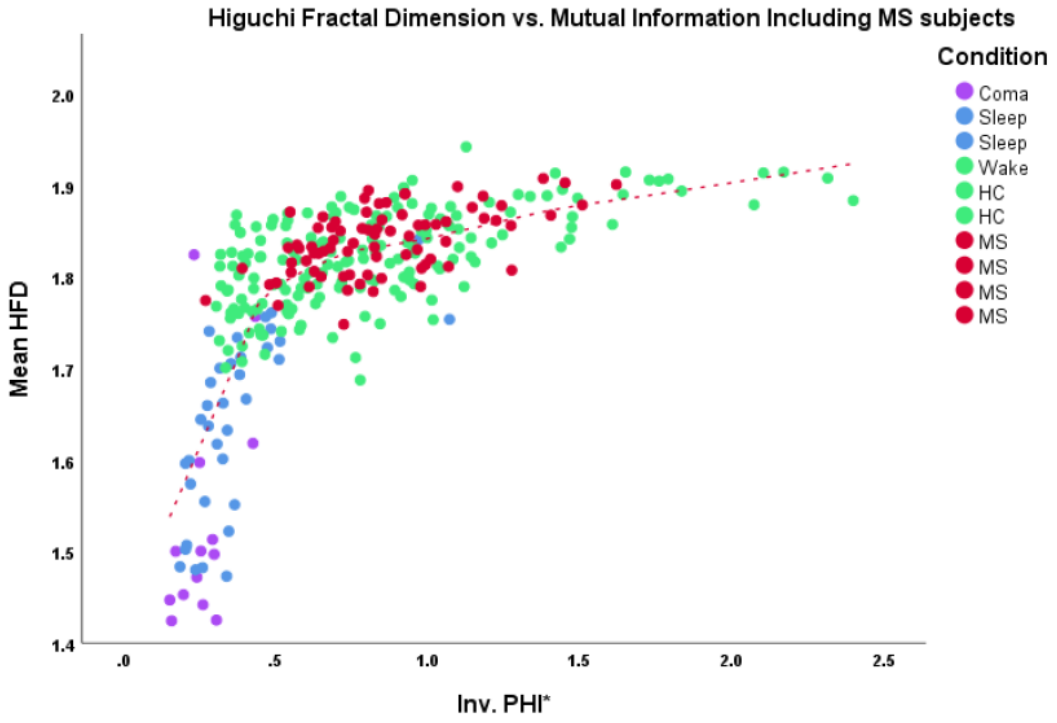


Figure 67 Relationship Between Fractal Dimension & Mutual Information at System Level Measured by taking the inverse of the peak value of Φ* applied to the Broad Band EEG (1-48Hz); Including MS Subjects

N=24 PRE N=22 POST			PRE			POST			POST		
			MSQ	MAC.FAILS (Population)	MAC.FAILS (Self)	MSQ	MAC.FAILS (Population)	MAC.FAILS (Self)	PASAT 3'	PASAT 2'	SDMT
Peak Value	Broad Phi*	*rs*	.121	-.013	.113	.08	.225	.374	-.244	-.385	-.068
		p	.574	.951	.599	.725	.314	.086	.275	.077	.762
	Theta Phi*	*rs*	.208	-.057	-.029	.065	.169	.003	-.08	-.188	-.375
		p	.33	.792	.893	.774	.452	.988	.723	.401	.085
	Alpha Phi*	*rs*	.07	.118	.204	-.076	.451*	.434*	-.48*	-.491*	-.304
		p	.747	.584	.339	.736	.035	.043	.024	.02	.167
	Hi Alpha Phi*	*rs*	.116	.089	.152	-.025	.358	.339	-.371	-.424*	-.267
		p	.59	.68	.479	.911	.102	.123	.09	.049	.230
	Lo Alpha Phi*	*rs*	.158	-.079	.143	-.196	.435*	.341	-.478	-.391	-.317
		p	.46	.713	.504	.382	.043	.12	.024	.072	.151
	Beta Phi*	*rs*	.206	-.235	-.19	-.097	.314	.158	-.563*	-.453*	-.483
		p	.334	.268	.375	.669	.155	.483	.006	.034	.023
Integral 500ms	Broad Phi*	*rs*	.237	-.051	.092	.25	.102	.182	-.433*	-.429*	-.055
		p	.266	.814	.668	.262	.651	.419	.044	.046	.807
	Theta Phi*	*rs*	.134	-.06	.037	-.186	.474*	.402	-.307	-.400	-.458*
		p	.533	.781	.862	.408	.026	.064	.164	.065	.032
	Alpha Phi*	*rs*	.062	.063	.188	-.095	.451*	.356	-.475*	-.423*	-.371
		p	.774	.769	.379	.673	.035	.104	.025	.049	.089
	Hi Alpha Phi*	*rs*	.064	.044	.158	-.125	.435*	.366	-.459*	-.415	-.376
		p	.765	.838	.461	.58	.043	.094	.032	.055	.085
	Lo Alpha Phi*	*rs*	.003	-.124	.132	-.278	.257	.178	-.328	-.250	-.354
		p	.99	.565	.539	.21	.248	.428	.136	.262	.106
	Beta Phi*	*rs*	.227	-.101	-.056	.133	-.041	-.205	-.433*	-.324	-.427*
		p	.286	.64	.796	.556	.855	.36	.044	.141	.048
	θ+α+β integrals	*rs*	.168	-.005	.124	-.075	.425*	.309	-.475*	-.464*	-.427*
		p	.433	.981	.563	.74	.048	.162	.026	.029	.048

Table 48 Correlation Matrix Between Φ* and Indices of MSCI within the MS Cohort in the Eyes Open Condition Values of Φ* and its integral over 500msec for each bandwidth were examined.

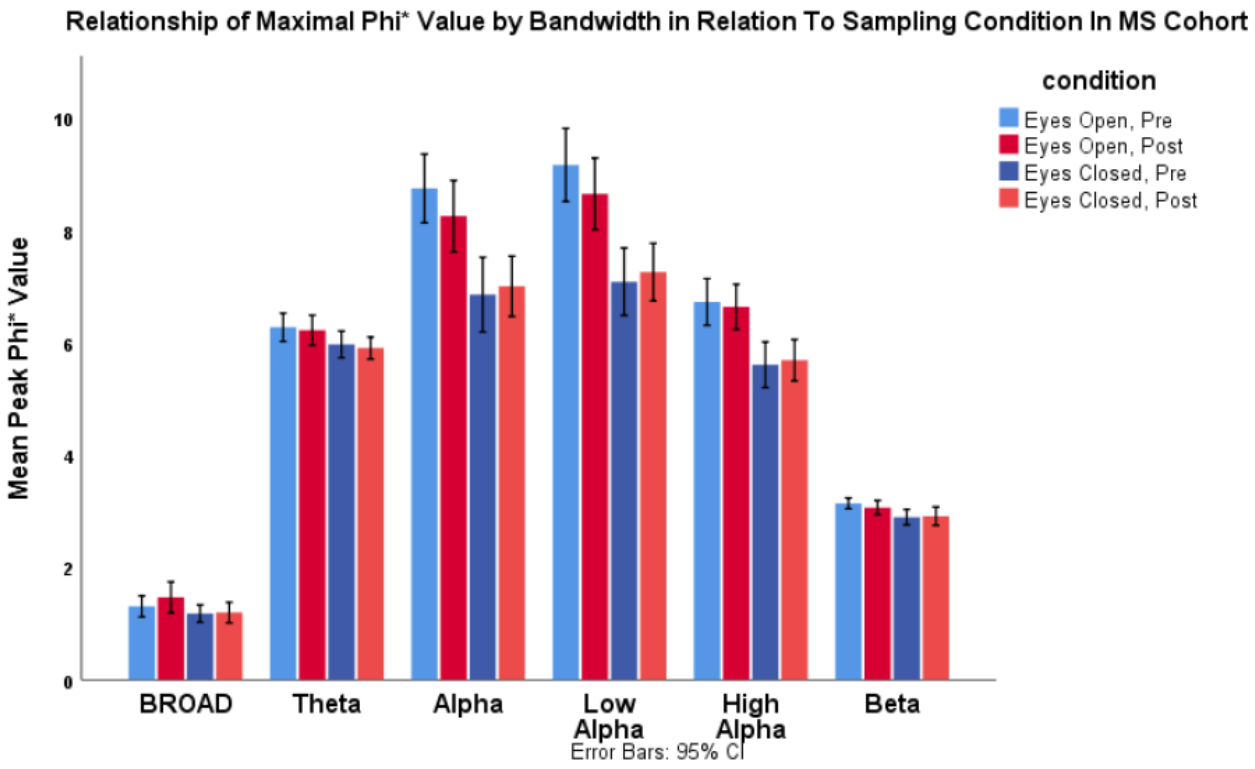


Figure 68 **Peak Value of Φ* by bandwidth of interest and MS Sampling Condition**

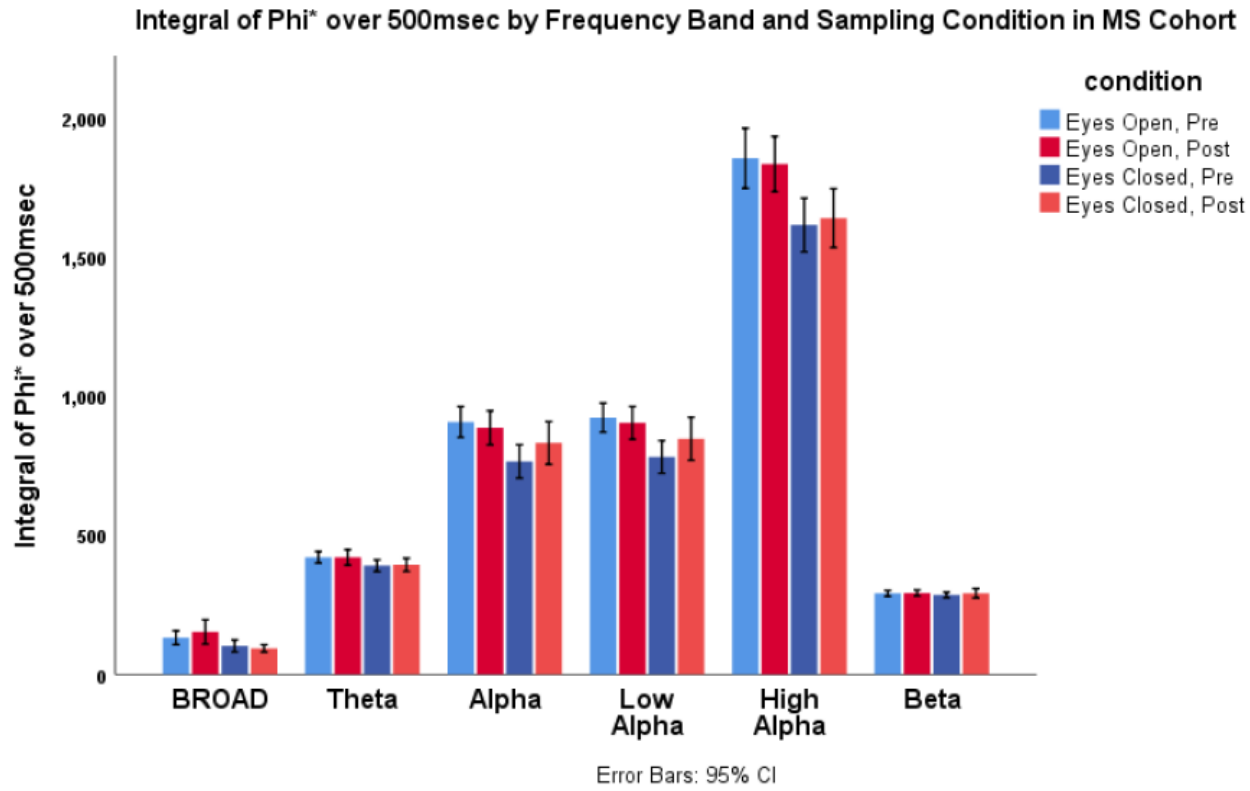


Figure 69 **Integral of Φ* over 500msec by bandwidth of interest and MS Sampling Condition**

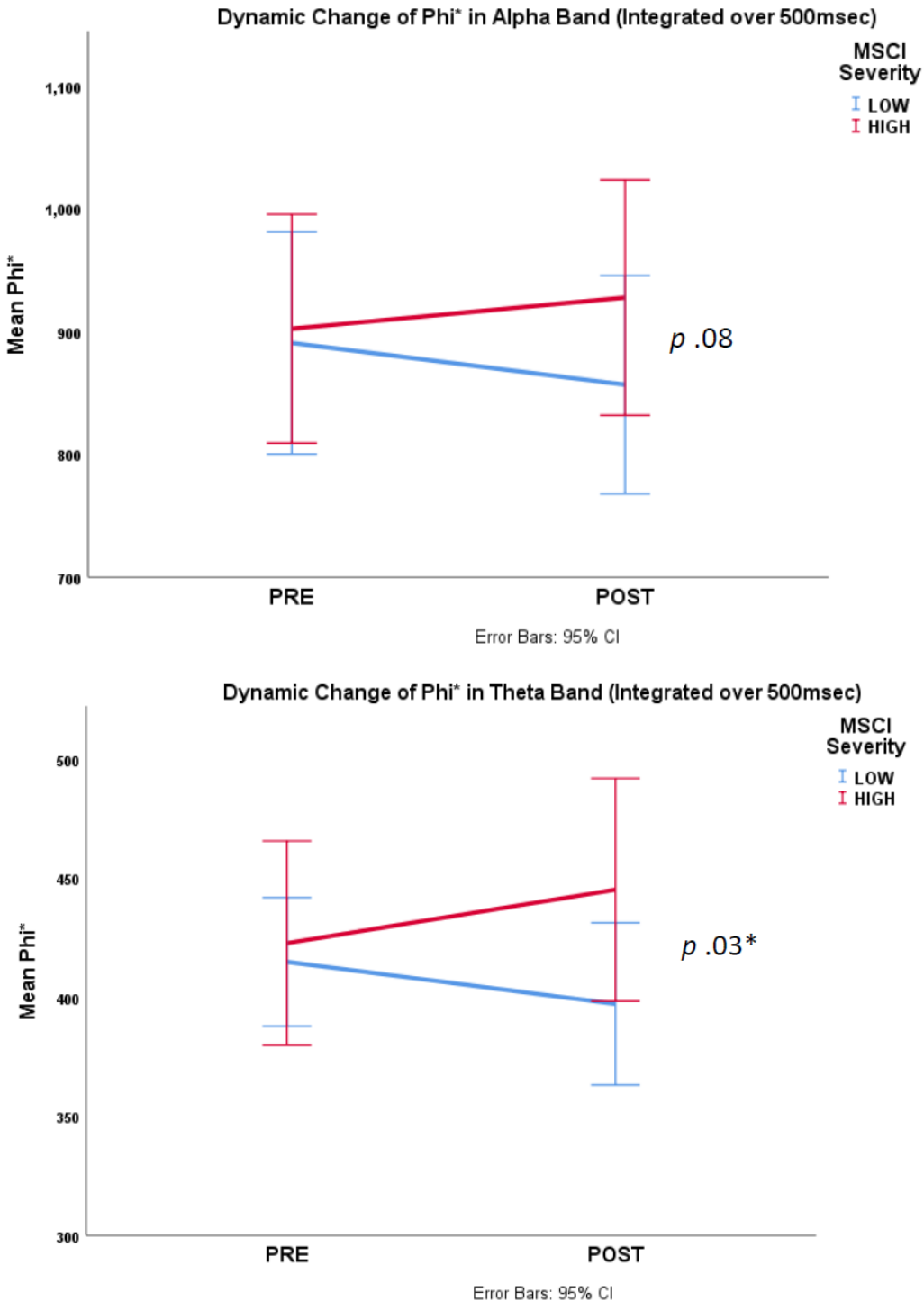


Figure 70 Possible Differential Dynamic Change in Φ* in the Alpha and Theta Bandwidths between groups of differing severity of MSCI Following Cognitive Exertion

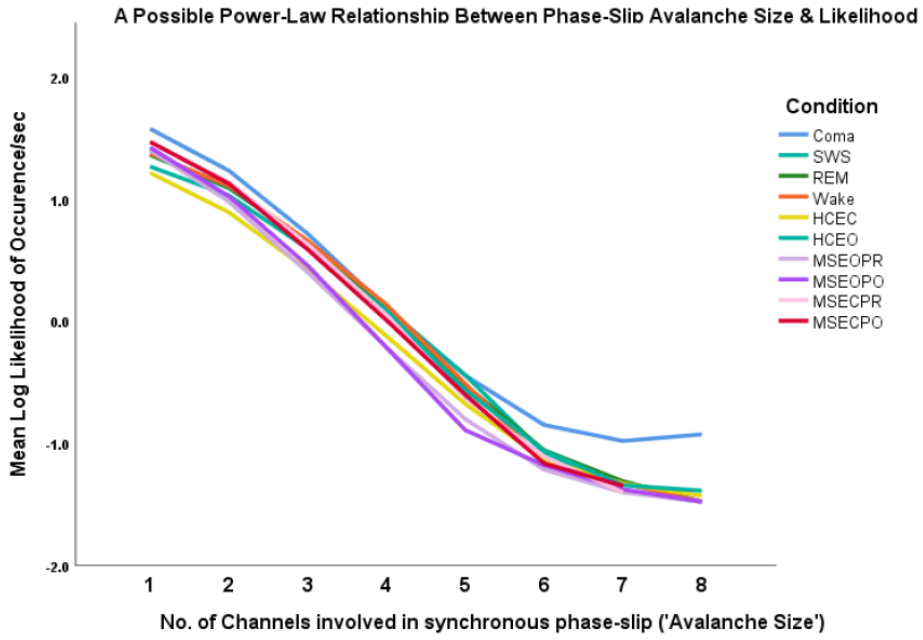
Relationship between Phase-Slip Extent and Likelihood. In *all* subjects yielding data of acceptable quality after the HAPPE an inverse relationship between the number of channels involved in a phase-slip event and the likelihood of such an event was seen (figure 71, table 49). Allowing for the limited number of channels and their positioning which therefore has a direct bearing on the resolution of judging event size, the data *do* suggest the possible existence of a power-law pointing to the dynamics of such events being driven by self-organized criticality within the cerebrum.

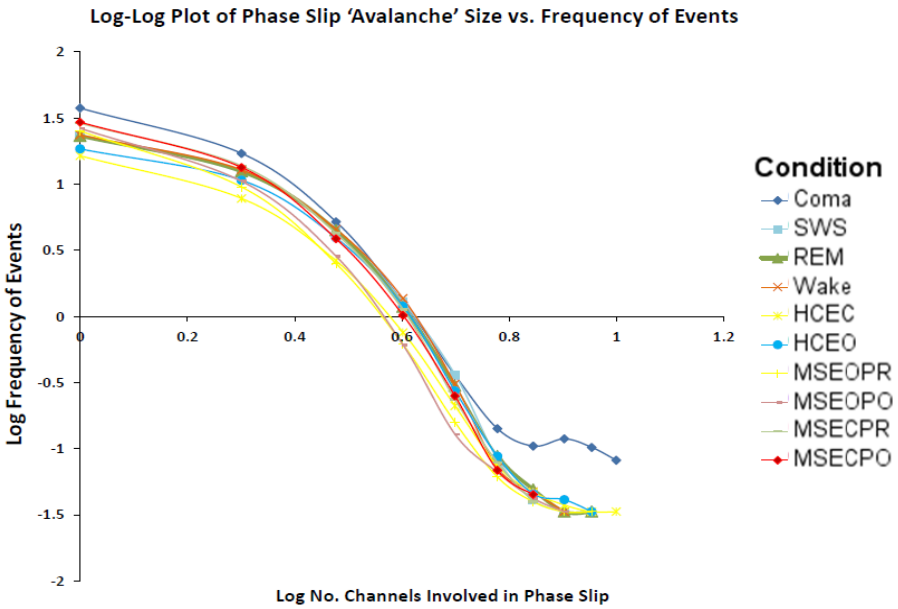
Examining the ratio of frequency between events (hereafter termed phase-slip *avalanches*) affecting single channels and a larger number of channels as a surrogate index of such criticality demonstrated significant discriminatory difference between all MS and Non-MS/Non-Coma subjects in all states (figure 72). Of note, within the MS cohort this index of criticality was also seen to significantly inversely correlate with the severity of MSCI; particularly in the *post* testing eyes open condition(table 50). Again, the attrition of eyes closed samples by the HAPPE procedure rendered analysis therein unreliable.

However, as with the $\Phi*$ testing, a *dynamic* change in the property may underlie the strengthening of such associations in the post-test condition; such that the group level mean changes minimally owing to a statistical balance between increase of criticality in the less impaired and a further decrease in those more impaired, leading to the appearance of stronger correlations with cognitive performance scores. However, the magnitude of the dynamic change posited did not achieve statistical significance (figure 73) in this instance and artefactual findings from multiple comparisons remain an alternate explanation; hence larger scale enquiry is planned.

Figure 71 Existence of A Possible Power-Law Relationship Suggesting Self-Organised Criticality of Phase Slip Dynamics

(top: log-likelihood vs. Event Size, Bottom log-log plot)





	Coma	SWS	REM	Wake	HCEC	HCEO	MSEOPR	MSEOPO	MSECPR	MSECPO
N	14	15	20	18	83	68	24	22	17	15
Range	0.1003	0.0590	0.0528	0.0439	0.1106	0.1041	0.0606	0.0406	0.0490	0.0346
Minimum	0.0103	0.0320	0.0325	0.0393	0.0107	0.0252	0.0071	0.0095	0.0172	0.0180
Maximum	0.1106	0.0910	0.0853	0.0832	0.1213	0.1293	0.0677	0.0501	0.0661	0.0526
Mean	0.0447	0.0575	0.0523	0.0595	0.0520	0.0686	0.0275	0.0258	0.0410	0.0365
S.E. Mean	0.0086	0.0038	0.0028	0.0033	0.0026	0.0028	0.0027	0.0025	0.0032	0.0030
Std.Deviation	0.0321	0.0149	0.0127	0.0141	0.0240	0.0229	0.0132	0.0119	0.0131	0.0114
Variance	0.0010	0.0000	0.0000	0.0000	0.0010	0.0010	0.0000	0.0000	0.0000	0.0000

Table 49 Descriptive Statistics for Small:Large Phase Slip Event Ratio

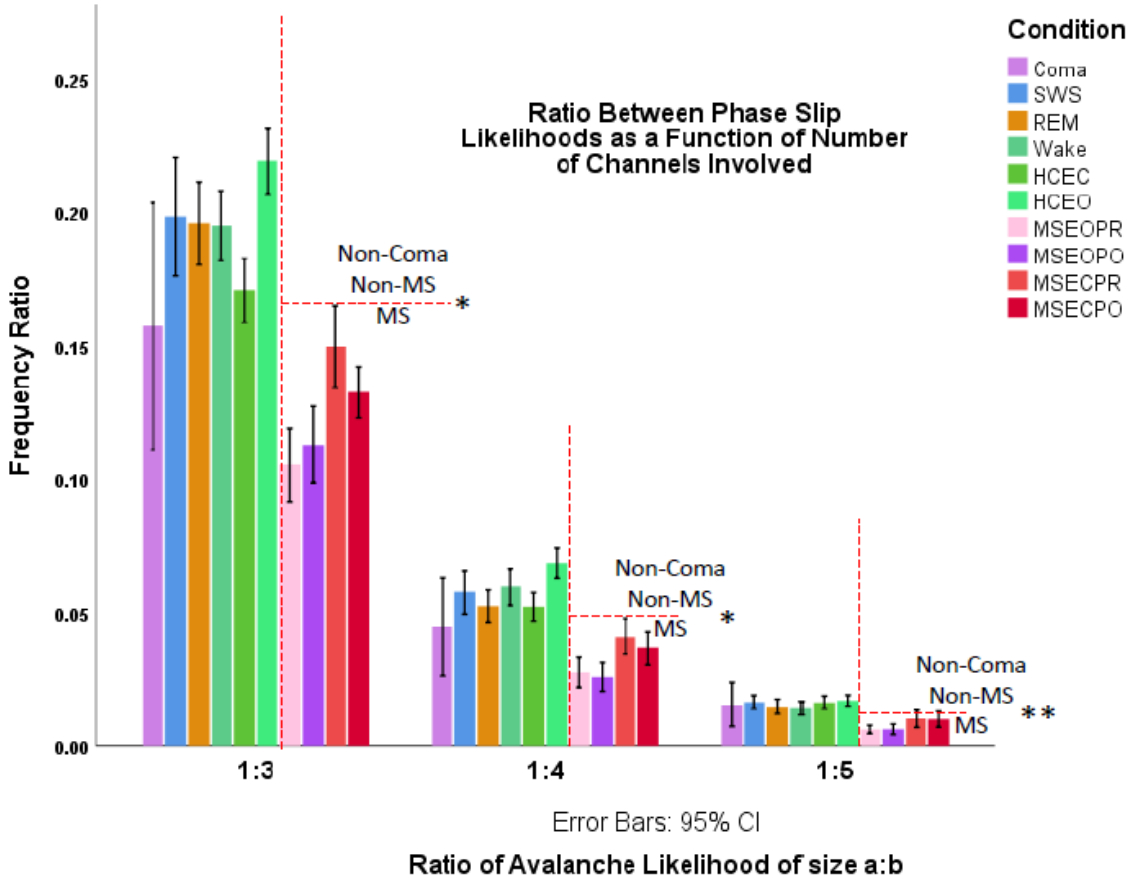


Figure 72 Ratio of Likelihood of Small to Large Phase-Slip Avalanches by Clinical State and Sampling Condition

Of note, the *minimal level of significance* in the difference between the MS and Non-MS awake subjects is demonstrated (as judged on a comparison of means by unpaired samples T-Test, * p<.05, ** p<.01).

		Eyes Open		Eyes Closed	
		Pre	Post	Pre	Post
MSQ	r_s	.01	.226	.409	.364
	p	.961	.311	.103	.182
MACFIMS Fails	r_s	-.374	**-.488***	**-.497***	.305
(Population Crit.)	*p*	.072	**.021**	**.042**	.268
MACFIMS Fails	r_s	-.379	**-0.49***	**-.648****	-.072
(Self Criteria)	*p*	.068	**.021**	**.005**	.8
Cognitive Fatigue	r_s	**-.445***	-.292	-.256	.252
Rating	*p*	**.029**	.187	.322	.365
	N	24	22	17	15

Table 50 Relationships of Phase Slip Size Ratio to Indicators of MSCI

Rs= Spearman's Rank Correlation, *= p<0.05, **=p<.01.

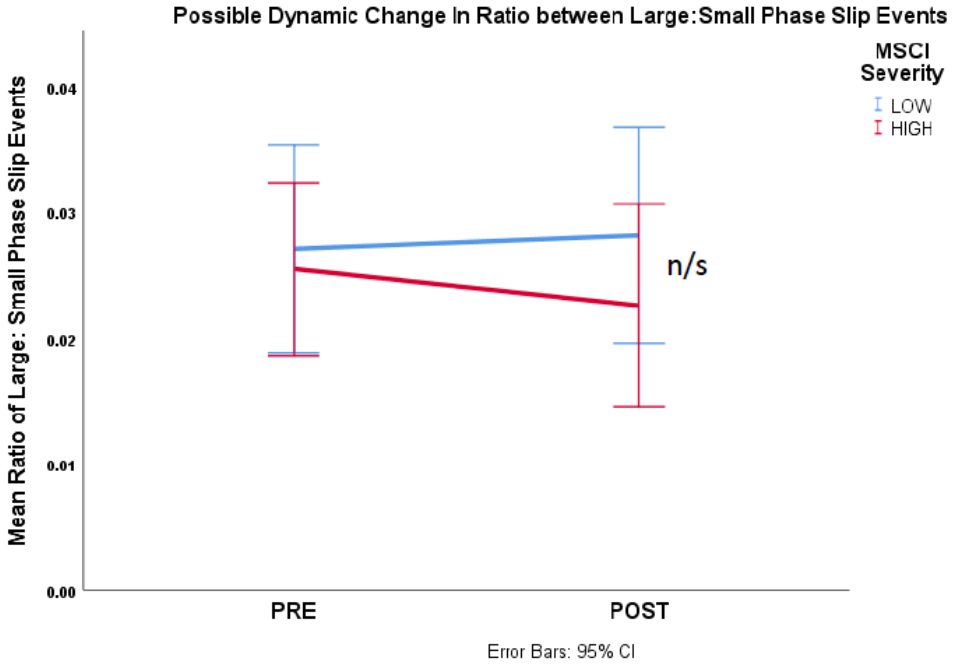


Figure 73 **Possible Differential Dynamic Change in Criticality of Phase-Slip Avalanching in response to cognitive exertion between groups of high and low severity MSCI**

DISCUSSION

Following a deeper consideration of the pathophysiological consequences of the disseminated immunological attack and subsequent neurodegeneration which characterises Multiple Sclerosis the conceptual position that MSCI represents a *disintegration* of cerebral processing was reached.

Recognising this as a *higher-level* consequence than the advocated model of *disconnection* suggested by some neuroimaging studies, coupled with the appreciation that the physical substrate underlying virtually *all* cognitive faculties is *integration* within and between broadly distributed and profoundly convergent and divergent re-entrant loop circuits offered an explanation as to why attempts to gauge MSCI on the basis of more *elemental* features such as connectivity have not offered a sufficient account of MSCI or a validated biomarker with which to gauge it.

Furthermore, the closer association of grey matter structural decline, both cortical and subcortical, with MSCI suggests it is the particular loss of integrative function afforded therein which is particularly relevant to both physical and cognitive disability.

Acknowledging that cognitive processes are therefore a truly *emergent* property which arise from such collective interactions but are *irreducible* to the components, or subsets of the components which produce them in a manner generally characteristic of complex adaptive systems stimulated pursuit of metrics which may index system-level functional integrity more globally.

Of all the many cognitive processes, perceptual capacities and behavioural outputs which arise from cerebral function, it is arguable that none is so clearly emergent to such a high degree as the production of unified conscious experience, namely *qualia.*

Therefore, a putative neurophysiological biomarker system causally-linked to the capacity for generating these phenomena may in turn provide a metric of the ability of the system in question to produce emergent output from its integrative functioning, including the less-emergent cognitive faculties we also seek to measure and preserve from neurodegeneration.

Whilst philosophical debate about the very existence of qualia and its tractability to exposition continues alongside scientific uncertainty around the exact relationship between the set of neural correlates of consciousness and the physical substrate of consciousness, the Integrated Information Theory of Consciousness continues to evolve with growing empirical support which is a testament to both its conceptual elegance and capacity for producing scaled quantifiable measurements.

Faced with the challenge of attempting to measure seemingly intangible and abstract properties using a modality which brings with it a great many degrees of freedom with respect to methodological acquisition, herein we have sought to construct a framework ultimately based on a fundamental natural property, namely entropy. Of the many varied approaches of capturing complexity, we have employed a tractable formulation which

both fits with our notion of that property representing *structure* and by turns has direct applicability to real physical systems.

Furthermore, it has been demonstrated that arrangements with fractional dimension beyond the Euclidean dimensions in which they are embedded are a direct route to increasing complexity and in turn the fractal dimension of an arrangement offers a means to quantify its complexity; whether it is a spatial arrangement (such as a network) or the temporal sequence of a time-series signal.

Armed with this notion of complexity, the loss of fractality seen in MS cerebral architecture and the shift in 1/f pink-noise of the EEG power spectrum also seen in this condition (and others with left-shift toward increasing slow:fast ratios) can both be interpreted as a *loss of structure* (independent of low-level properties such as volumes and amplitudes respectively).

The founding of complexity in terms of entropy also enables direct elucidation of the relationship to the further property of mutual information considered as the shared entropy between distinct variables or sources thereof. Earlier conceptualisations posited increasing levels of consciousness were associated with an optimal balance of mutual information between disparate regions; with excessive shared MI being associated with a loss of consciousness and conversely a reduction in the former (suggesting loss of balance the other way) has also been observed in neurodegenerative diseases including MS (557).

However, there is a fundamental difference between information which is simply *shared* (as captured by MI) and that which is *integrated*. This property Φ, in keeping with the notion of emergence is quantified by its qualitative irreducibility into simpler elements; and it is this irreducibility which suggests that it too (like the applied notion of complexity) must have an orthogonal relationship to the property of entropy upon which it is also fundamentally based.

The unified tripartite entropy-complexity-integration framework which arises from a synthetic fusion of the preceding physical notion of entropy-complexity outlined by Luiz-Pascal *et al.* and the IIT model outlined by Tononi *et al.,* offers four main advantages.

Firstly, it can possibly be applied to all systems; is not restricted to one aspect of cerebral functioning, or one disease area or even one type of ‘brain’. Secondly, by identifying the features associated with maxima of complexity (fractals) it offers an account of why such arrangements are ubiquitous within the spatial architecture and electrophysiological activity of the brain – and also why certain features such as small worldness and metastability arise as absolutely necessary requirements to overcome fundamentally inherent natural constraints on the communication and integration of information.

Thirdly, when faced with adaptive changes (again a characteristic general feature of complex systems) in the face of disease, which are recognised to occur and confound mono-parametric measurements of connectivity and ERP response in the context of MS,

application of a tripartite framework offers a means to directly disentangle such effects and detect change. For example, a capacity to integrate information may be maintained in the face of disease but arguably this may come at the expense of some corollary change in either spatial or temporal complexity of the associated processing, and the advocated tripartite model would be *sensitive* to this.

Fourthly, for at least complexity and mutual information methods are available to cross the bridge from theoretical property to empirical measurement using EEG data. The adequate derivation of Φ, in a manner compatible with its conception is far less tractable which is regrettable given it being the key quantity of interest in this context of attempting to measure the extent of disintegration due to MS.

This notwithstanding, novel methods to non-invasively quantify Φ on EEG datasets are beginning to emerge and our team has already begun to explore the feasibility of applying the required technique of partitioning analysis to EEG time-series. Currently, given the vast number of permutations which arise from bi-partitioning even reduced montage data-processing for even short epochs of a *single subject* runs over extended periods (days). In the absence of significant methodological short cuts or computational advances the use of such a derivation as a real-time clinical metric is questionable; however the common practice of off-line processing of MRI data and similar in translational research suggests such use in that setting would still be very viable.

Therefore, as a pilot exploration of the general utility of such a framework and its relevance to MSCI we sought to establish some initial empirical proof-of-concept validation of the model prior to any larger exploration and commitment of human and computational resource.

At this theoretical stage it was felt reasonable to conserve time and avert any patient burden by utilising convenience sampling of retrospectively acquired data attained to ICFN standards in real-world clinical settings and make use of externally available healthy control datasets in addition to the MS subject EEG acquired previously to facilitate between-groups analysis.

Caveats around the heterogeneous case mix of those subjects with varying levels of coma and severe encephalopathy are duly acknowledged in addition to further reservations that may arise from using NEAD subjects as a proxy for healthy controls; this said, the recordings of such subjects were reported as electro-physiologically normal by specialist review and the diagnostic category typically mandates detailed neurological evaluation at the diagnostic stage. Further, the EEG samplings were staged by trained personnel in accordance with contemporary American Academy of Sleep Medicine standards and considered typical of respectively associated conscious states. The use of concurrent anticonvulsant medications by such subjects is a further point of note. Future exploration and model validation will seek the use of prospectively acquired healthy control subjects free of such potential confounds, the effects of which may indeed be subtle.

Furthermore there is an acknowledged incomplete matching with respect to the healthy control and MS cohort used herein which would appropriately be addressed in future explorations. The relative congruence of findings between the wake group of NEAD patients and the healthy control cohort however is encouraging that findings between those two sets are reasonably generalizable.

The use of phase slip events within channels and their concurrent occurrence across channels to identify avalanching behaviour mandated the use of tighter artefact thresholds be applied to all examined datasets (for consistency) than had been applied previously to solely the MS cohort in prior work. This led to an appreciable but non-prohibitive degree of attrition across the datasets but at this stage of model testing in time-series mainly acquired for clinical grounds this was felt requisite to enable meaningful comparison.

The average Higuchi Fractal Dimension of EEG signals demonstrated a significantly positive association with gross changes in the level of consciousness, however it did not significantly differ between MS and Non-MS subjects awake who were awake. Further examination of the 'flatness' of the distribution of HFD across channels (taken as the ratio between the geometric and arithmetic means) was the same between MS and Non-MS awake subjects and there was no suggestion of particular differences of this metric between such groups at individual channels or regions *per se.*

It is noteworthy, that the scale of change which may be clinically significant with this metric (given its logarithmic basis) may be particularly small – for example a 0.02 difference in HFD effectively distinguished subjects with Alzheimer's disease from Healthy controls in work by other investigators (1147).

The magnitude of such difference stands in sharp comparison to the difference across levels of consciousness seen here; clearly the ascent to consciousness represents a quantum leap in the degree of complexity of EEG dynamics. The effect of age on HFD has been described to show a peak toward mid adulthood with advancing maturation prior to a subsequent natural decline after 60 years (1147); as with all our metrics going forward adjustment for age will be imperative for what is arguably the strongest confounder in MS research and translational endeavour.

Similarly, the behaviour of the Φ*metric outlined by Oizumi (1208) behaved similarly in terms of significantly discriminating between different levels of consciousness but not betwixt awake MS and Non-MS subjects. Indeed, simply taking the reciprocal of their advocated measure (Φ^{*-1}) offers a strong positive relationship with conscious state.

The formulation the authors described was based on the notion that Φ*represents the difference of information when examining the mutual information between a present and past state of a unified system (held within the EEG time-series of all channels) and when they are considered separately as individual channels(figure 74). This is taken to represent a form of 'atomic partitioning' which is far more tractable than the more conventional Φ calculations.

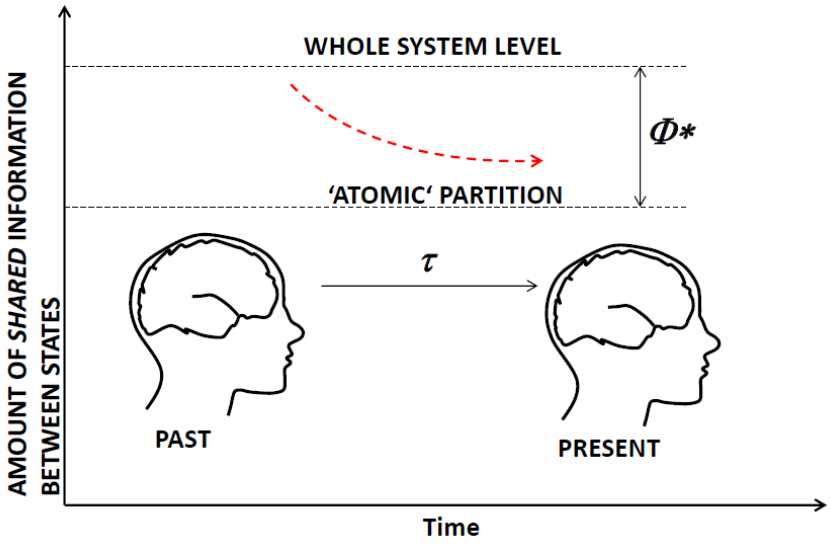


Figure 74 A Schematic Representation of Φ*

This metric quantifies the degree of redundancy or shared mutual information between past and present states (separated by time τ) which arises when systems are considered as a collective whole above that when considered as separate parts in the atomic partition condition. Our observations demonstrate that with increasing temporal separation between past and present states Φ* naturally falls (red dashed line); *notably less dynamic change of activity at the system level would result in greater Φ* shared over the temporal window in question.*

Whilst the method has the further advantage over several other described **Φ** formulations in providing only positive outputs and values which do not exceed the maximum entropy of the system in question, the only empirical support for its application to date comes from intracranial stereo-tactic EEG recordings in Macaque primates (1208). To our knowledge its application to conventional human scalp EEG is undescribed.

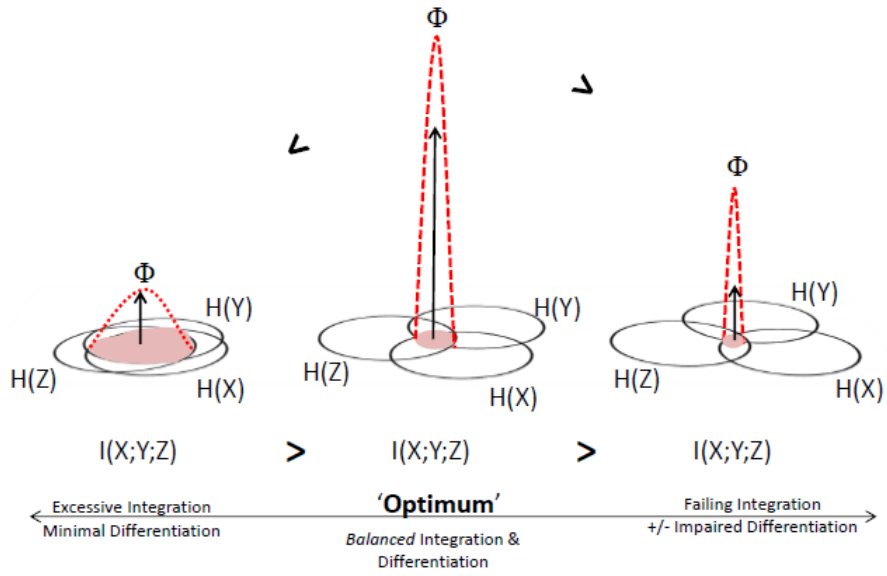


Figure 75 Why Increased Mutual Information Can Be Associated With Lower Conscious States

Examination of the relationship between HFD and Φ^{*-1} reveals one that is both strongly correlated and non-linear with an inflexion point which appears associated with the entry into wakeful consciousness. The behaviour of these surrogates of complexity and mutual information respectively, in relationship to conscious state taken as a clinical surrogate of Φ (proper) is so far wholly congruent with the outlined complexity-entropy-integration relationship; or at least the region left of the theoretical *Cmax*.

The result of Φ*and thus the form of system level Mutual Information it represents being greater in the less conscious individuals within our cohort might initially be interpreted as *not* supporting the model; this however is resolved by reconsidering that with increased '*sharing*' of information (for each source to have more information about the others) they will ultimately become of the same information and as such the *overall entropy* will diminish; as such increasing MI should accompany a falling complexity, as is empirically observed here.

Our findings demonstrate that whilst related there is more to Φ than the Mutual Information or form thereof captured by Φ*; indeed what is required is a way to assess the *structure* of the shared information, possibly in much the same way a examining the disequilibrium of an entropy distribution offered a means to quantify its structural complexity. Whether a product of the system level mutual information Φ* and its disequilibrium from the overall entropy of said system would provide a metric of Φ which sufficiently matched the output from a partitioning approach remains to be seen but is nonetheless readily testable and a focus of our ongoing work. Notably, this would also provide only positive values, display a maxima for a point of balance between the system's mutual information and its entropy and similarly be reduced either by excessive increases in the former or a reduction of the latter(see figure 75).

Both the HFD and Φ* have been applied to the broad-band frequency range between 1-48Hz in a deliberate attempt to interrogate the wider EEG signal structure of the system rather than remain confined to a particular narrow-band frequency range given the foregoing discussion on the appreciable importance of cross-frequency interactions. However, some initial exploration of within bandwidth behaviour has generated findings which further support the proposition that worsening MSCI is associated with *a loss of complexity in processing activity over short time intervals* (present states are more similar to past ones) which is also dynamically exacerbated by cognitive exertion.

Whilst the HFD provides an index of the complexity of oscillatory structure and local dynamics of EEG time series from individual channels, as described in the foregoing discussion a loss of complexity in temporal dynamics may arise possibly independent of a compromise of spatial complexity and thus still parallel an embarrassment of integration and capacity for emergent properties.

The association of average phase slip rates in the fronto-temporal regions of MS patients with performance on information processing speed tasks which we observed in the preceding work, and the description by Thatcher *et al.,* (721, 725, 803) of an association of between the spatial extent of such phase slips with cognitive performance in other contexts suggested these events may also serve as an index of global dynamics.

With similar transient phenomena such as large scale microstate transitions in global field power demonstrating an inverse power-law distribution between the size and frequency of such events typical of self-organised critical behaviour (a recognised general signature of complex dynamical systems) we sought to both ascertain whether or not the apparent avalanching behaviour of phase shifts displayed such dynamics and whether these were perturbed in the context of MS. Characteristic distributions of exponentially decreasing phase-slip avalanche frequency with increasing size were evident, yielding findings consistent with power-law distributions and underlying critical dynamics in the examined alpha band in all examined datasets.

Of note, in the context of both MS and the mixed-aetiology coma/encephalopathy subjects there was a greater frequency of singlet phase shifts relative to other subjects, which was accompanied by a relative paucity of larger scale avalanches. This was to the extent that zeta functions were not considered reliable for comparison against the MS subjects due to a relative absence of avalanches typically involving greater than 4 channels in any one instant. Even over this shortened interval of the power-law distribution between phase-slip avalanche size and likelihood, the MS groups consistently displayed far steeper exponential drop-off in the balance between smaller and larger avalanches in a manner suggestive of perturbed criticality of their dynamics due to alteration of an aforementioned 'control parameter'. Furthermore, a significant association with the overall severity of MSCI and the extent of perturbed avalanche dynamics was evident.

A biological interpretation of these findings would suggest the absence of larger phase-slip avalanches within the MS cohorts attests to a failure of cortical recruitment via the thalamus and its cortical projections. The instantaneous, zero-phase lag switching

occurring synchronously between disparate cortical regions does suggest they are mediated by a common source. It should also be noted that the phase-slip events are not simply slower *per se*, infact conversely, *singlet* slips are possibly more common but it is the very criticality of the mechanism driving them and recruiting them which is disturbed. Alternate models for phase slip generation are posited; with some authors arguing they are driven by direct long-range cortico-cortical coupling and serve either as a direct means to achieve the critical synchrony between disparate sources or potentially contribute to the equally important *segregation* of information by switching large cortical territories out of involvement in relevant processing (965); these are not mutually exclusive models and the empirical findings here and elsewhere suggest future focus upon them is warranted.

Notably the absence of significant findings with respect to MS and the HFD metrics in this cohort whilst interesting does not suggest application of the larger model is inappropriate. For as demonstrated with respect to the effect on complexity of shifting the power balance between slower and faster rhythms which similarly follow a power-law distribution, the same applies with respect to an adverse shift of the avalanche size-frequency distribution resulting in a quantifiable loss of complexity in temporal dynamics. Thus, going forwards, as envisaged at the outset measurement of large scale temporal dynamical complexity alongside metrics of local spatio-temporal dynamics and integrated information will be vitally important in capturing and most importantly *interpreting* patterns within different groups. A further advantage of the general model is that different aetiologies of cerebral dysfunction may demonstrate distinct patterns of failing complexity as they lose integrative function.

Exploration of the utility of Integrated Information Theory and its metrics in the setting of Multiple Sclerosis is warranted. In the first instance however an attempt to recapitulate the empirical support for the advocated framework in prospectively acquired matched healthy control subjects and aetiologically homogeneous and confirmed cerebral pathologies (i.e. hypoxic ischaemic encephalopathy, prion disease and seizure phenomena) may aid further elucidation of the continuous *spectrum* the model suggests and importantly characterise vulnerabilities to artefact and measurement tolerances in the approach to analysis.

CONCLUSION

MSCI likely represents a syndrome of *disintegration* consequent of the pathological structural loss and disconnection which characterise the disease. The disseminated and heterogeneous nature of injury in the condition producing a *diaschisis* suggests focus on individual disease facets, morphometrics, cognitive subsystems or their connections in isolation fundamentally cannot effectively index *higher-dimensional emergent properties which arise from the whole clearly being far more than the sum of its parts.*

A framework based on a physical quantification of complexity and the elegant and empirically testable IIT theory of Tononi *et al*. has been outlined and empirically tested with results suggestive of early promise and need for further exploration in the domain of MSCI.

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FUTURE DIRECTIONS

'Developing a measurement system is not something to be done on a rainy Sunday afternoon. If done properly it may take years.'

Henrica de Vet(866).

Our endeavours so far have not been wholly discouraging albeit they have introduced a number of significant but nonetheless anticipated challenges that will need to be overcome in pursuit of those metrics desired for future inquiry.

As a group we remain committed to the pursuit of means by which translational research can be accelerated. However, clinical service with the BrAMS team has demonstrated the very real life-threatening danger(1292) that can accompany the application of therapeutics even once necessary licenses and regulatory approvals have been granted. One must therefore be sharply conscious of the fact that whilst translational studies remain powered to detect clinical effectiveness on the selected primary outcome measures(613, 1293) conversely they are not powered in their design to necessarily pick up possible but infrequent safety issues. Therefore, a reasonable argument in favour of using metrics which demand larger sample recruitment in translational studies for prolonged periods of time is that almost by default they bring with them more (and equally, if not more important) safety information than shorter, smaller scale studies employing more sensitive measures.

This said, given our consideration of cognition and its primary assessment by low-tech pencil-and-paper tests one cannot help but wonder where the MS community of patients and care providers would be now if focus had fallen on cognitive disability rather than physical debility at the outset of natural history studies(13-16, 21, 22, 148, 151, 214, 215) and the subsequent translational research that followed originally.

It has taken the neurology community nearly a quarter of a century to come a point of majority acceptance that disease modification makes a difference to longer term outcomes(218) in addition to suppressing clinical relapse events(1294) early on. This painfully slow transition from scientifically reasonable doubt, to equipoise to near-but-incomplete consensus(100, 579, 1295) has demanded the conduct of a large scale risk-sharing(217, 218) scheme within the National Health Service and large scale innovative propensity-matching based longitudinal cohort analyses from international data(1296) to make convincing arguments which (with findings from the pivotal phase III trials) now influence much of modern MS practice in the United Kingdom. With the rates of sustained physical disability accrual on the EDSS scale being a key temporal determinant of how long such efforts take(148, 169, 213), it is not unreasonable to ask the hypothetical question of whether such prolonged equivocation and health economic resistance would have arisen had phase III studies demonstrated cognitively preserving benefits (even independent of relapses) that were associated with sustained employment and participation?

Many of the questions raised about Multi-modal evoked potentials herein are partly in the process of being answered by other investigators internationally (181-183, 188, 190, 191, 726) and also more locally in the form of the Assessment of Bone-Marrow Derived Cellular Therapy in PMS (ACTIMUS) (393) study being conducted at the North Bristol NHS Trust with the assistance of the Grey Walter Neurophysiology Department. The longitudinal application of a near identical evoked potential battery in the setting of a double-blind cross over study of autologous bone marrow within a progressive cohort using the same clinical and radiological outcome metrics(393) may ultimately offer both some validation of the cross-modality integration of biomarker outcomes delineated from the work in our second investigation and also attest as to whether or not the observed rate of neurophysiological decline observed here is also seen in a larger cohort during their placebo year-long interval; and also if a therapeutic benefit is seen in response to the intervention whether or not this is reflected in the evoked potential responses which are serving as the primary outcome measure.

The issue of short interval evoked potential response variability (i.e. over days and weeks) has not clearly been examined since the inception of our work by investigators elsewhere, nor it would appear since the early 1980s; indeed whilst the work of Nuwer(387) cites an annual change it is not clear if shorter interval data is available. This remains an open question that would possibly require only a small number of enthusiastic patients over a sequential series of alternate-weekly visits for several months to effectively answer.

Also, if we can feel confident in removing visual and brainstem evoked potentials from our battery and remain focussed on the long tract studies given their clearer, weighted association with the clinically relevant mobility outcomes we will be able to significantly shorten our testing battery time from its current two hour average.

Within the team there has also been discussion of how this may be shortened still further by possible exploitation of so-termed long-loop responses; whereby the

presentation of a run of single stimuli (to support averaging) to either the common peroneal nerve adjacent to the knee or the median nerve at the wrist leads to a series of characteristic responses recorded from the muscles they innervate (the tibialis anterior or abductor pollicis brevis respectively) (1297). The responses arriving in sequence are the M response from direct anterograde conduction along the stimulated motor nerves, followed by a H-response (equivalent to the short-loop reflexes which are the substrate of clinical deep tendon reflexes(656)) and subsequently a longer-latency response produced by a relay of conduction of the stimuli along the sensory afferents, then up the dorsal columns, across the medial lemniscus to the sensory cortex prior to excitation of the motor cortex and thereafter a descending propagation along the corticospinal tract and subsequently the anterior horn cell axons to the respective muscle groups(1297). The H-response latency offers a means to deduce the central conduction loop time and further still knowledge of concurrent SSEP and CMCT latencies affords an opportunity to derive central relay times between the sensory and motor pathways(1298). An immediately apparent advantage of such a procedure is that the afferent sensory and efferent motor tracts are effectively interrogated in condensed runs of stimulation over several minutes after a preparation time of quarter of an hour or so. It remains to be seen if interruption along any part of the loop renders the entire subsequent pattern of responses absent as one might anticipate; if so its use in the setting of Progressive MS particularly might be prohibitively constrained despite the elegance of the approach. However, some small scale exploration in the setting of MS has suggested promise (1298) and the capacity to interrogate sensorimotor coupling in a cheap logistically feasible manner has encouraged use in other neurodegenerative settings (1299). However, in line with the idea that evoked potentials might serve as a means of '*biologically staging'* the condition as discussed after our third investigation, the long loop responses might be a rapid way of demonstrating an absence of any significant demyelinative delays within the long tracts in early disease.

A feasibility study examining the practicability of this approach would be required alongside clinical disability ratings and performance of the standard MMEP battery as performed herein.

Resources of all types are however limited and given the rising primacy MSCI is receiving from patients and the MS healthcare community alike(599) (coupled with a general appetite for trial paradigms which do not exclude participation of patients rendered wheelchair dependent(604)) in the first instance we are planning to apply our efforts to the domain of MS related cognitive disturbance.

Therefore the two primary goals will be to proceed with further validation of the MSQ by objectively discerning its change and variability over time and most importantly its relationship to real-world outcomes, of the kind central to characterising efficacy in clinical trials(199, 235, 602). This will also provide a meaningful metric of MSCI against which putative candidate surrogate neurophysiological biomarkers of this nebulous but important property can be judged. With the MSQ based on the BICAMS battery one immediately has answers to the usual challenges of test standardisation and availability of norms(402, 407, 457, 578). The component tests also come with already composed

alternate forms(401, 402, 457); important for helping to at least partly mitigate practice effects. Whilst some data on test-retest reliability(1300) may be available for individual tests it remains to be seen how this will be manifest when incorporated into the MSQ approach and the receiver operating characteristics will further need to be clarified in more detail. The practicality of administration and deriving scores appears to be very feasible even by minimally-trained non-psychologist staff(401, 402), which is a major advantage given the great mismatch between the supply and demand on specialist psychologist staff in the MS field outwith tertiary centre environments in the United Kingdom. (585, 1301, 1302).

Much consideration has gone into how to best derive indices of such real-world function. From our discussion of health-economic impact(6, 599, 792, 1303) and quality of life(6, 418, 452, 525, 793, 1304), employment(449, 451, 791-793, 1304-1309) and general participation(167) are both critically important determinants and greatly undermined by MS. Two validated rating instruments, providing quantified output against which MSQ output can be compared have been identified, these being the Community Integration Questionnaire (CIQ)(1310, 1311) and the Work, Productivity, Activity Inventory (WPAI)(1312). Both have usefully been deployed in the settings of neurological disease and the WPAI has successfully been employed in demonstrating functional benefits from more aggressive Disease Modifying Therapies in Multiple Sclerosis(1313). Additionally, in light of the findings from Surmowski *et al.,(587, 1314-1319)* on the apparent mitigating effects of Cognitive Reserve against MS pathology contributing to MSCI going forward with our enquiries including some validated instrumental rating of this construct will be required in subsequent analyses. The Cognitive Reserve Index Questionnaire CRiQ(1320) instrument appears to have a growing evidence base supporting its use and there are no reasons to suspect its application in the setting of MS would be any less valid than other neurocognitive syndromes explored to date.

As we have herein, assessments of important covariates, contributors and possible confounders will need to be formally undertaken alongside any assessment of cognitive performance. The previously utilised instruments of the DASS, NFI-MS and the Epworth/PSQI Sleep Scales have yielded useful information and proven very practical to deliver. One nevertheless remains mindful of the issues surrounding the very measurement properties of these scales and the abstract and somewhat intangible nature of those constructs they purport to measure; particularly with respect to fatigue(11, 1321).

Whilst fatigue remains a great unaddressed issue in MS(30), despite its ubiquitous prevalence and frequent primacy in driving employment loss(792, 1306) and reduced quality of life(30), as a research target the lack of valid regulatory-accepted outcome measures to gauge it is a central prohibitive factor in conducting much needed translational enquiry. Our group has considered the merits and explored the feasibility of approaching this important and almost neglected issue. The resulting perspective is that the challenge of dealing with such a nebulous subjective construct should be met with the rigour of seeking to measure its objective clinical correlate, namely *stamina* as tested by performance on sustained cognitive or motor tasks and identify possible

biomarkers of such change therein. A therapeutic improvement in stamina, should intuitively lead to a corresponding decrement in fatigue. The strong association of the latter with affective disturbance(167, 994) suggests that even focussing on the objective and measurable is not likely to provide a satisfactory solution to a problem which appears to truly be '*many things to many people*'.

Also, as fatigue does at least seem to have some significant relation to the integrity and efficiency of cerebral networks(852, 1321) and cortico-striatal loops(846), it is not unreasonable to consider that the ability to therapeutically improve these would similarly be met by reductions in fatigue. Therefore, achieving a cognitive metric and valid biomarkers thereof which do ultimately facilitate the arrival of cognitively enhancing drugs may simultaneously deliver benefits for fatigue symptoms as well.

This is not an unreasonable supposition. Agents with distinct modes of action in separate focussed lines of enquiry have demonstrated the ability to both improve fatigue symptoms and performance on focussed neuropsychometric tests (31, 33, 34, 1322-1326). However, the cognitive evaluations are on very small groups, using heterogenous outcomes with no validated tether to real world function and have in some cases yielded findings inconsistent with earlier work(464).These agents have short duration effects for both symptoms which persist only during the course of their administration; however such responses do provide empirical support for the notion that interventions leading to more persistent improvements in cognition (by restoration or repair of the neuro-glial parenchyma) should similarly bestow more sustained amelioration of fatigue.

If we accept such a paradigm where MSCI arises from embarrassed cerebral network functional integrity (and importantly the *dynamics* therein) and fatigue is a corollary subjective phenomena which accompanies the associated loss of such network efficiency in the general sense(1321) (itself a recognisable dynamically changeable property relating to use) then focussing our efforts on that domain which is most amenable to objective measurement will not necessarily constituent a full-scale abandonment of the desire to '*help*' the other problems but rather perhaps represent the quickest route to aiding *both*. Notably, the same '*dual effect*' would also *not* necessarily hold if one were to remain dominantly focussed on spinal cord based surrogate metrics.

Therefore, with this in mind future efforts will focus on the cognitive domain in primacy. The approach envisaged is to take a necessary series of effectively parallel but complimentary steps to arrive at the desired point of having both a deployable cognitive outcome metric for clinical use and a neurophysiological surrogate of it also suitable for translational signal studies.

Before elaborating on the shape such a journey to that desired destination might take, there is the critical need to appreciate that such efforts as those described herein and those planned do not exist in isolation and study design should endeavour to be adaptive to contemporary developments as they arise elsewhere. For example, over the time course of these locally conducted enquiries other groups have successfully explored neuro-filament light chains as molecular biomarkers of neurodegeneration within the thecal space of patients with MS(243, 274, 626-628, 1327). The dependence on attaining

cerebrospinal fluid appeared to be a major obstacle limiting the wholescale application of this indicator to translational and routine clinical practice, albeit one acknowledges examples of CSF sampling in several studies has been undertaken for axonal components such as neuro-filament and other immunological signatures including oligoclonal bands(280, 286, 1328). However, the requirement for CSF sampling by a procedure which is both painful and near universally disliked by patients, may be avoidable with the very recent demonstration of effective and sensitive assays to serum assays for such neuro-filament elements(627). Such developments have only recently been described however the potential utility of a blood-test based index of active neuronal damage could be profound and it would not be unreasonable to explore the rate of cognitive change, brain atrophy and electrophysiological decline with the output of such assays.

Whilst the promise and logistical attraction of such a biomarker is immediately apparent, its advent does not by any means render our pursuit redundant for the simple reason that we are not simply seeking a marker which can demonstrate attenuated decline but equally *recovery* as we move into an era where this is a serious primary objective(396, 1329) of clinical trials. Furthermore, interpreting the significance of our own and other novel candidate biomarkers, will all depend on the availability of ecologically valid clinical outcomes with which they can be compared to achieve validation.

Therefore, to empirically establish the tripartite relationships and nature of interaction between our candidate neurophysiological biomarkers, our candidate cognitive outcome and real world function *two* simultaneous efforts are proposed.

Firstly, if there is to ever be wide adoption of a electroencephalographical biomarker of cognitive function an effort is required to both confirm the validity of the proposed framework which underpins it and also achieve a consensus on the optimal methods of its ascertainment; both with respect to technical signal acquisition and subsequent mathematical derivation.

The challenges of higher level EEG based abstractions were articulated three decades ago by Entevenon(646) following review of the first pioneering explorations of functional brain connectivity analysis using EEG and remain almost equally resolved today(645).

Nonetheless, accepted international standards for the conduct of clinical EEG already exist(406, 679) and moreover have been in place for decades(922). Moreover, being now two decades into the era of digital EEG with similarly internationally utilised data formats there already exists a vast quantity of high quality, real world recording from the broadest possible range of disordered neurological states and health. *If* for the purposes of logistic simplicity we accept the standard clinical acquisition (or at least a subset of) as a starting point; acknowledging however the variety of possible recording montages described in the experimental literature(645), then we can begin from a point where sufficient raw data is already in existence. Without dismissing that higher density EEG recordings may undoubtedly offer more information *per se* (642, 749, 1219), the extra yield beyond specialist visual inspection really is a function of the nature of particular abstractions applied and for connectivity analyses particularly (649), owing to spatial filtering and deformation of charge distribution by the cranium and its contents

(642), for all intents and purposes there is an insurmountable limit to the spatial resolution of scalp EEG which has been inherently appreciated from the outset and cannot be overcome by the addition of ever greater recording points(642, 644, 707). Furthermore, we are not seeking *all* possible information; we are seeking *enough to be useful.*

In discussion of an 'optimal' EEG biomarker, one is considering not only the general approach and its theoretical basis. Even if we accept standard 10:20 recording positions, filter settings and reference position, the act of translating a particular conception through to a scaled numerical value derived from EEG signals requires identification of the frequency bandwidth of interest and both the duration and number of epochs taken for consideration and which channels (all available or some in particular) will be utilised(646). There then follows the selection of montage type and the ubiquitous challenge of artefact handling (642, 675); standard clinical interpretation relies on such rejections being made by the reporting neurophysiologist through visual pattern recognition and application of experience-based heuristics (675). Herein, attempts at standardised automated artefact handling by independent component analysis and rejection have been explored with some benefit alongside direct visual selection by the operator. However, if there is to be large scale EEG acquisition without processing being dependent on visual inspection by trained specialists the artefact handling will need to be *extremely* reliable and may ultimately benefit from the growing advances in machine learning being applied to EEG (1330-1332), an approach which would be very well suited to such a task but possibly come with significant additional (but by no means insurmountable) computational demands.

After all such selections for the best choices for the options outlined above are established to arrive at remaining raw signal for abstract processing, one is then faced with identifying the most effective method of subsequently doing so.

This is a large but achievable task wherein the challenge arises dually from both the range of methods available (645, 716) and the exponentially greater number of permutations introduced by the necessary selection of processing parameters; for example the choice of embedding dimension for synchronisation likelihood calculations when employed as a connectivity metric between time-series data (556) or the windowing applied to Fourier outputs for coherence analysis(720). Parameter choice can understandably greatly influence output.

Whilst the necessary development of a robust underpinning theoretical framework and published experience of others will help select certain avenues (out of the vast range of possibilities available) and useful processing parameters, the number of options available (any, one or none of which may lead to the desired outcome) remains non-trivial.

Even applied to the same time series data, the behaviour with respect to range and variability of output will likely be distinct for different methods and parameter choices thereof.

This heterogenous variance amongst a putatively very large number of variables, where for each what could be classified as a clinically important difference for detection remains absolutely unknown, makes performance of an accurate conventional prospective power calculation for a subsequent clinical validation study all but impossible in the first instance.

Proceeding to further prospective EEG data collection without either prior or *simultaneous* narrowing down the choice of possible candidate neurophysiological metrics and deriving the aforementioned measurement properties would greatly increase the likelihood of both type I and II error. This would respectively be due to the necessary performance of excessive multiple comparisons analysis and the risk any further collection would be insufficiently powered to detect effects which may be subtle but nonetheless important; given that MSCI and cognition are apparently non-linear emergent phenomena.

The solution to this '*problem of permutations*' is to expand upon the approach provisionally adopted with our last line of enquiry; namely to utilise a very sizeable collection of EEG records retrospectively acquired to clinical standards, from across the broadest possible range of persons and functional brain states. This would be complimented by a large collection of healthy subject data also.

Thence going forwards in an efficient automated fashion one would process *all* candidate outcome metrics, somewhat heuristically refined but with a range of testable parameter settings and using various combinations of electrode channels and further still using various epoch durations, frequency band choices and so forth. The output from an amply populated repository would be accompanied by standard confidence intervals for mean values of each metric which could be regularly assessed and if deemed excessive met with continued *even* population of the dataset until high confidence was ideally finally achieved.

As per the reasons and framework outlined in the final chapter herein, those candidate electrophysiological metrics and their parameter settings which most effectively associated with and discriminated between 'hard' clinically relevant states of consciousness (as landmarks on the spectrum of the brain's capacity to integrate information) would then be taken forward (with their established measurement properties such as variance) for examination in the necessary prospective work.

One might not unreasonably question how such a calibration exercise might run contemporaneously alongside any prospective exploration involving further data collection.

The answer comes from considering the nature of the proposed endeavour.

In line with the steps of outcome measure development outlined by regulatory authorities (208, 235) and taken sequentially herein with respect to multimodal evoked potentials in Progressive MS, prospective work seeking to evaluate the utility of a

clinical cognitive outcome (the MSQ) should demonstrate association with meaningful outcomes both cross-sectionally and in relation to longitudinal change over time.

If the MSQ is to be adopted it must be supported by empirical evidence from a sizeable and typical cohort of patients. The number of subjects required to assess the cross-sectional association of MSQ with the described real-world metrics of occupation and participation will appreciably be influenced by the desired confidence and level of significance intended which is as yet undecided by the group. However a provisional calculation based on the MSQ findings (in the fourth investigation described herein) is that a reasonably powered study would require somewhere between 180-250 patients to participate in such a task.

It is envisaged that recruitment would proceed prospectively in a serial fashion directly from routine outpatient clinic attendances and that whilst the MSQ and occupation/participation metrics would constitute the primary outcomes under examination, easy-to-acquire contemporaneous EEG recording would serve to offer important secondary outcomes when processed in the most optimal fashion suggested by the large scale automated analyses outlined above.

From our own explorations described in the last two sections, we already possess *several* methods of EEG analysis which *may* after necessary refinement offer meaningful associations against the MSQ and so one would not be collecting the EEG in the absence of reasonable processing methods. One would simply have the anticipation that an even more optimal processing strategy could be identified as prospective data collection proceeded which would then also be applied to explore its associations both with MSCI and real-world outcomes.

Given the centrality of neuroimaging by MR to the modern understanding and management of MS(10, 57, 196, 197, 226, 248, 266, 299, 624, 1333-1335) it will be important to assess the relationship of both the clinical MSQ outcome and the neurophysiological candidates to be conventional structural metrics, those related to disease burden (that both apparent and 'normal appearing'(268, 873, 1336) and also if and where possible structural-functional connectivity assessed by the appreciable range of available means(706).

If fruitful, on the bases of such initial endeavours once armed with sufficient information to shape longitudinal design one would then be in a position to proceed with exploration of these clinical and neurophysiological metrics over time to gauge short, medium and longer term variability and patterns of decline over periods both consistent with current disease modifying therapy trials (1-2 years) and those of shorter duration (months) which may be useful for demonstrating benefit of symptomatic interventions. The predictive utility of both the clinical MSQ outcome and the neurophysiological candidates at anticipating significant changes in real world functioning (i.e. loss of employment, difficulties with therapy concordance, loss of cognitive independence) will also be open to exploration and compared with similar prognostic capacity of neuroimaging.

This latter opportunity is not wholly tangential to the primary objectives. As discussed elsewhere there is a growing move to set '*No Evidence of Disease Activity*' (NEDA) as a treatment target in the application of Disease Modifying Therapy(117, 1337). As yet, cognitive assessment (for the described reasons that motivated our development of the MSQ) is not routinely applied or included within the NEDA criteria, which have been constantly under revision at least for the period of the works herein. Nonetheless, there is recent work demonstrating that inclusion of cognitive indices into NEDA significantly reduces attainment of this (1337). Two immediate realisations of this follow. Firstly, that MSCI may progress in the absence of other clinical relapse, EDSS change or novel apparent abnormality on standard radiological metrics used to make judgements of NEDA; but fortunately some attempt to operationalise a definition for 'isolated cognitive relapses' has been made (1338). Secondly, that institution of an accepted and reliable clinical cognitive metric may well have implications for DMT decision making(599). A very important caveat here is the very real risk of conflating clinical cognitive deterioration due to mechanisms of inflammatory relapse and similar deterioration due to mechanisms of progression. Pursuing the latter with potent immunomodulatory therapy does not come with the same risk/benefit balance for patients as the former(178, 179) and even in cases of agents having demonstrated evidence of some modest benefit in progressive phenotypes of disease(1339), such positive outcomes seem to particularly emanate from patients with para-clinical evidence of a significant inflammatory component(280). Concerns about such a conflation are not unfounded as evidenced by the both the resistance to using NEDA outside of research settings and also the drive to reconsider the place of *progressive* EDSS changes and atrophic brain change in future NEDA criterion.

In summary, the planned investigations outlined above may offer a possible route to empirically build upon the initial steps already taken toward the overarching objective of developing the necessary measurement instruments.

XI

CONCLUSIONS

i. The serious clinical consequences of MS represent the collision of two complex adaptive systems, leading to the collapse of neurological function(59). It is perhaps the complexity of both systems that underlies the lack of predictability of the disease itself, the pathophysiological outcomes from it and also the response to putative treatments.

ii. All of the currently licensed disease modifying therapies are immunomodulatory(25) in nature with evidence strongly supporting a dominant role for autoimmunity in the pathogenesis of the disease and ability to modify such behaviour as being markedly beneficial from the earliest clinical stages of the disease. Only modest, but nonetheless present therapeutic gains from immunomodulation have just recently been seen for the first time in the context of the later Progressive stage of the disease(96). More recently still has there finally been the recognition that intervention earlier on with immunomodulation can certainly attenuate the timing and likelihood of reaching progressive milestones(1296). Whilst encouraging, our patients still face a clinical landscape that offers very little if anything to those in the progressive phase whether they arrived by the primary or secondary phenotypic routes, an arbitrary clinical distinction that has possibly served no significant purpose and since the inception of this work is now being moved away from. The absence of *major* benefit from monotherapy immunosuppression in progressive disease, even of the strongly suppressive kind is a signal that chimes with the pathology seen at post mortem in these patients – it is qualitatively different to that seen earlier in the disease both in distribution and complement of culprit cells(1). Translational research in the progressive phenotype has been and remains largely under-represented in clinical trials(179) in a manner that stands in sharp contrast to the proportion of misery, disability and wider health economic burden that it confers(6, 1303). Whilst many confounding factors may account for this disparity, measurement properties of the main clinical outcome measure accepted by regulatory bodies remain a significant prohibitive factor. With a view to

possibly aid in acceleration of translational research, particularly at the phase I/II phase our group has sought to identify candidate biomarkers of MS related disability which ultimately facilitate more practicable phase I/II therapeutic 'signal' studies of candidate therapeutics.

iii. Neurophysiological evaluation by means of evoked potential analysis has been an established para-clinical tool to aid the diagnosis of MS for nearly half a century(182) and a range of methodological approaches have been adopted in research settings. Herein, we have explored the utility of long tract and cranial evoked potentials as part of Multi-modality batteries to possibly serve as candidate biomarkers of clinical disability in the setting of Primary Progressive Multiple Sclerosis. Against accepted outcomes of the EDSS and widely used MSFC, composite MMEP scores displayed a good positive association with physical disability ratings in cross-sectional analysis. The relative independence of such association from the particular method of quantifying evoked potential abnormality was an encouraging convergence of findings.

iv. A further novel cross-sectional analysis of MMEP with subsequent conventional and contemporaneously emerging structural metrics derived from MRI, similarly re-enforced the established performance of the MMEP composite scores in reflecting disability but also suggested a degree of superiority in their association over gross structural integrity, particularly of the brain. Over time the centrality of myelopathy in driving progressive disability accrual has been increasingly appreciated(196) however analysis of our cohort suggested the strongest possible handle for developing a surrogate of physical disability may arise from proportionately weighting the importance of the long tract neurophysiology indices with the structural metrics. To fully develop this idea a larger dataset would be required and the challenges relating to dataset attrition; particularly from an imaging perspective were clearly highlighted by our group's endeavours. The informative strength of composite indices appears to stand in sharp relief to their vulnerability to loss of their contributing components.

v. Evaluating the performance of the MMEP metrics longitudinally over a period of three years did demonstrate a pattern of decline which appeared to parallel and to a degree foreshadow physical declines to come, with effects most marked on consideration at the group level. The trends of individual MMEP variation over time at first glance appears less encouraging however this should not be wholly unexpected given that marked fluctuation in real world performance is itself a well-recognised characteristic of the disease(335) but nonetheless an inherent challenge to developing measurement approaches. An important and outstanding limitation is the relative paucity of information on evoked potential variability over various time intervals that would be relevant in considering any future trial design. Our endeavours are in the growing company of related works by others internationally in the field, particularly

with the advent of increasing neuroprotective(521) and remyelinative therapies(74) and the promise of such demonstrated in non-human MS-like models of disease using evoked potential techniques(301). This issue of EP variability is therefore more than a simple reflection; it is a problem which can and needs to be addressed going forward. This notwithstanding, a possibly useful application of MMEP may be to provide some form of chrono-biological *staging* of the condition, given both their demonstrated predictive value at suggesting future progression and their particularly superior sensitivity to subclinical and currently-subradiological disease. Such attributes may also prove useful for the purpose of enriching clinical trial recruitment for those patients most at risk of hitting confirmed disability progression endpoints within logistically achievable trial durations and thereby both potentially shorten and reduce the required number of participants into trials of putative therapeutics.

vi. The demonstrated limitation of MMEP with respect to cognition is not a minor one. The consequence and prevalence of MSCI is substantial and again appears to have been largely overlooked by much of the translational endeavour in the MS field up until quite recently; despite having been recognised as a characteristic feature of the disease in the original description of '*Sclerose en Plaque*' by Jean Martin Charcot(1340) over a century ago and being a clear contributor to much of the suffering, occupational loss and dependence that accompanies the disease(29).

vii. The lack of direct therapeutics for the domain of cognition in MS is partly attributable to methodological challenges and in particular a lack of widely accepted cognitive outcome measures considered ecologically valid through having meaningful relations to real world functional outcomes. Although performance on some singular domain tests have subsequently demonstrated a relationship to occupational status(577), a composite index sensitive to the underlying construct of MSCI and sensitively adjusted to known premorbid factors known to both modify the effect of MS pathology and raw performance on cognition was outstanding. To go forward with any subsequent exploration of candidate biomarkers of cognitive faculties implied a need for efforts to define such a composite. Given the vast array of techniques, permutations and calibrations available of neurophysiological techniques in particular, interpreting their respective meaning required a reduction of dimensions considered by the arrays of cognitive batteries used to clinically capture elements of MSCI and reduce risk of subsequent type I error. To this end the MSQ, derived by principal components analysis of performance on the MACFIMS battery adjusted for an estimate of pre-morbid intelligence appeared to meaningfully capture the severity of MSCI into a singular scaled index. The relationship of this to real world outcomes remains to be established and is planned in imminent future work; also the test-re-test variability and acceptable re-testing intervals similarly need to be established. Nonetheless, it

was felt the MSQ would provide a useful metric of MSCI for subsequent exploratory pilot studies of neurophysiological candidates as cognitive biomarkers.

viii. On the initial premise that MSCI is effectively a large-scale 'disconnection' syndrome our group pursued a series of EEG derived connectivity indices in a typical and phenotypically mixed cohort of MS patients and explored the relationship with cognitive impairment alongside evaluation of a range of local processing, global dynamic and more classical cognitive evoked potential indices. Whilst initially moderately encouraging a number of significant challenges were identified. Firstly, the conceptual model and subsequently the mono-metric approach to analysis may be inherently insufficient for tackling the problem at hand. Secondly, methodologically the range of choices available to investigators exploring abstract EEG analysis is extremely broad and agreed consensus remains outstanding on which is optimal. This in turn is a likely source of the sometimes confounding findings reported and the very real risk of error particularly when almost by necessity investigators are having to perform multiple comparisons analysis at the pilot exploratory phase.

ix. With these findings and subsequent issues in mind a re-consideration of the problem on its own terms was undertaken.

Through recognising the brain as a complex system and focussing on the defining feature thereof, namely the generation of emergent phenomena, by working towards a neurophysiological surrogate of such emergence we may in turn possess a global index of brain functional integrity. Contemplation of the functional neuroanatomy perturbed by MS pathology strongly supports the position that far beyond a disconnection syndrome, MSCI is in fact more an issue of disintegration in the fullest sense. Consequently seeking metrics capable of gauging integrative capacity and calibrating them against relative extremes of brain function characterised by the associated degree of emergent phenomena (consciousness) seemed a logical step. The initial pilot exploration provided sufficient empirical support to suggest further developmental inquiry is warranted and that the relationship between complexity, entropy and integrated information may offer both metrics and insights into the pathophysiology of Multiple Sclerosis and its cognitive sequelae, particularly with respect to the importance of large scale cerebral dynamics.

x. Taking the promise and lessons of these works forward would require several possibly overlapping pieces of work in the immediate first instance. A study of evoked potential variability is required, however practically this need only focus on Motor and Sensory long tract studies to all limbs and ideally this would be coupled with MRI imaging of the brain and spine to further develop the cross-modality approach described herein.

xi. The relationship of MSQ to real world functioning with respect to occupation, dependence and possibly even driving safety also needs exploration and this planned work could readily be applied alongside the aforementioned EP-MRI work.

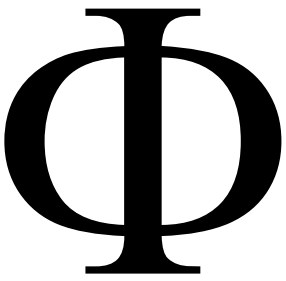
xii. With respect to further exploration of EEG derived metrics; the first challenge here is establishing the optimal approach to multi-property analysis; either by achieving consensus with other groups and/or calibrating metrics of interest with a very large data-series across a range of functional brain states.

xiii. Once a meaningful cognitive composite outcome (the MSQ) and the ideal candidate EEG metric (calibrated to degree of emergent consciousness) has been established, further development by cross-sectional and longitudinal observational work, akin to that we performed with MMEP can then performed as a hopeful prologue to future direct translational enquiry in this most challenging and equally debilitating disease.

xiv. The difficulties inherent to research in the MS field pale in comparison to the adversity faced by our patients and their loved ones on a daily basis over the course of many years; their necessary courage is truly inspiring and in response to their suffering we simply must go forward.

xv. By respecting the natural organising principles of the central nervous system we may come to protect and ultimately restore its beauty; wherein the ability to *measure* an individual's capacity for phenomenal *Qualia* may provide the necessary tool to identify means of preserving their truly unique *Quintessence.*

L.J.W.C.

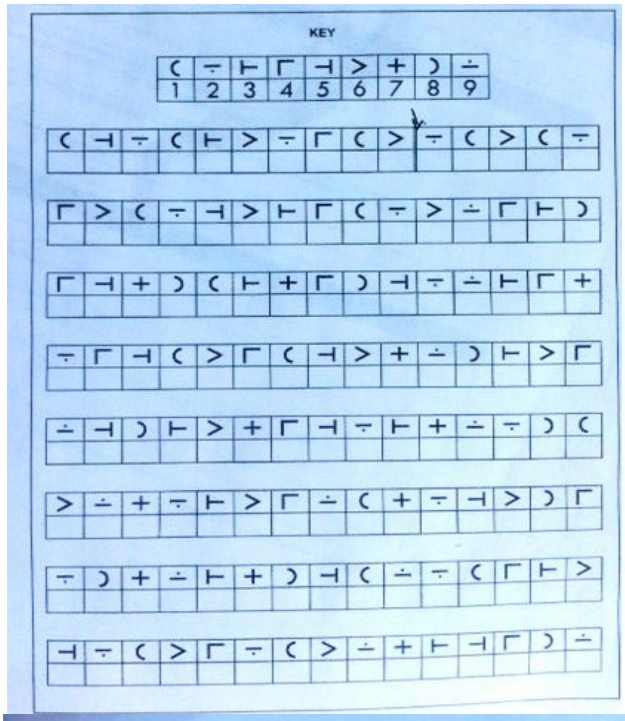


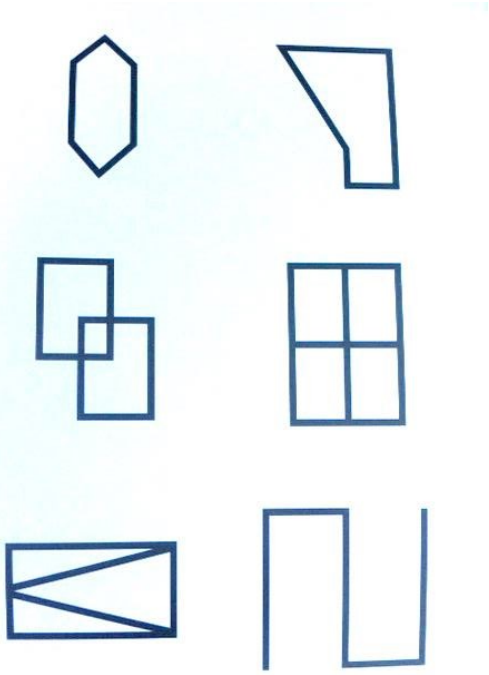
APPENDIX

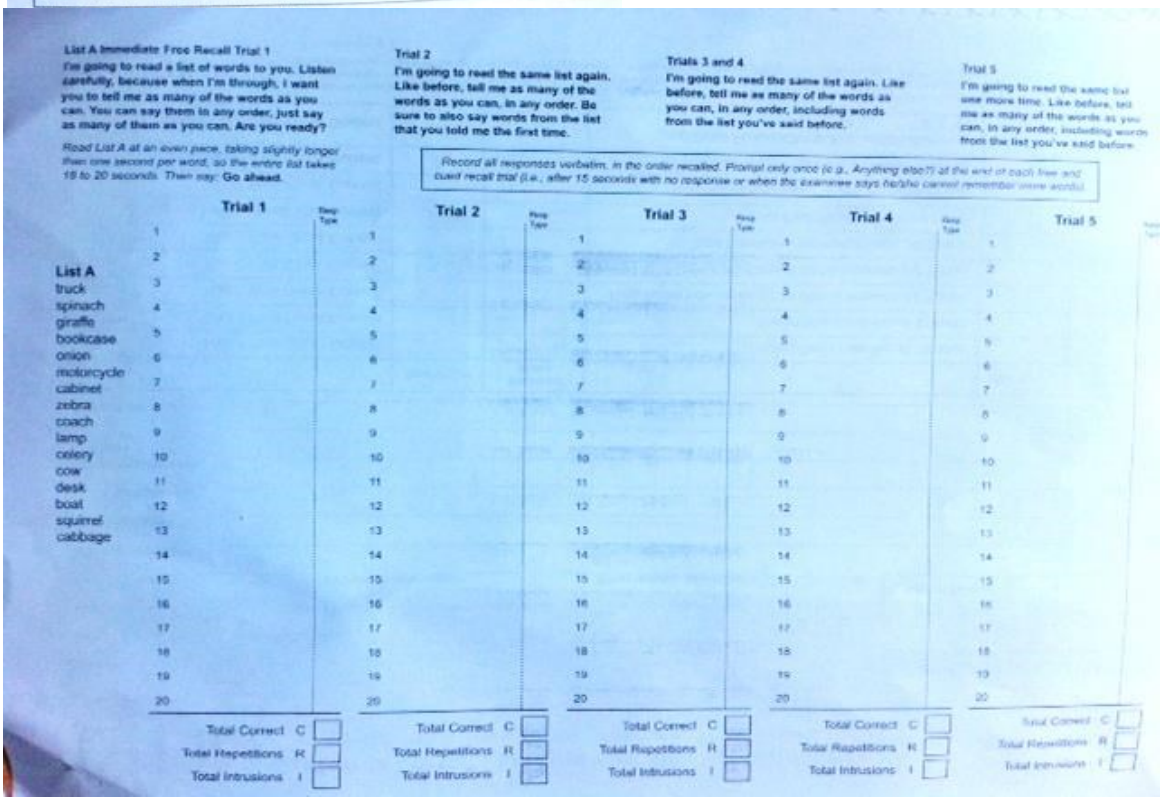
PSYCHOMETRIC TESTING MATERIALS, DISABILITY RATING SYSTEMS, & ADMINISTERED QUESTIONNAIRES

All subject to copyright and contained herein solely for purpose of illustrative example.

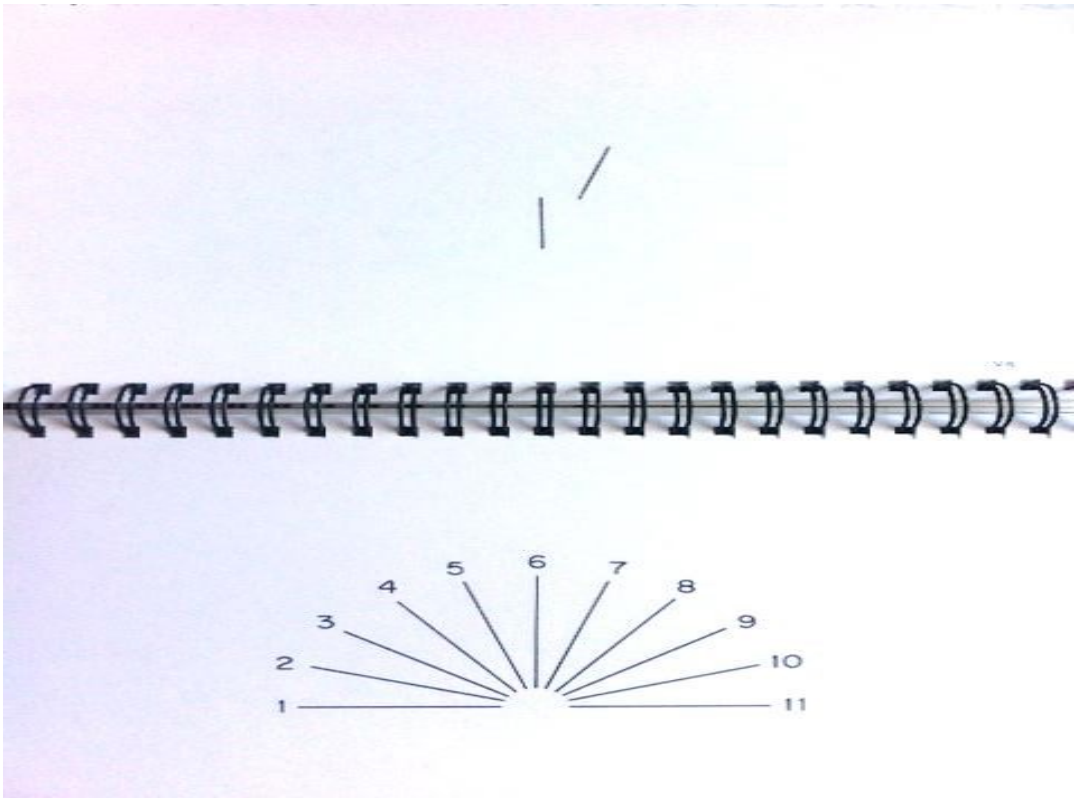
SMDT, BVMT-R and CVLT-2 Exemplars; Overleaf JLO and DKEFS Sorting Cards








Diana
Jane
Mike
John
Maria
Steve


1
2
3
4
5
6
7
8
9
10
11

DASS 21

NAME _____ DATE _____

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you over the past week. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all - NEVER

1 Applied to me to some degree, or some of the time - SOMETIMES

2 Applied to me to a considerable degree, or a good part of time - OFTEN

3 Applied to me very much, or most of the time - ALMOST ALWAYS

		N	S	O	AA	FOR OFFICE USE D	A	S
1	I found it hard to wind down	0	1	2	3			
2	I was aware of dryness of my mouth	0	1	2	3			
3	I couldn't seem to experience any positive feeling at all	0	1	2	3			
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3			
5	I found it difficult to work up the initiative to do things	0	1	2	3			
6	I tended to over-react to situations	0	1	2	3			
7	I experienced trembling (eg, in the hands)	0	1	2	3			
8	I felt that I was using a lot of nervous energy	0	1	2	3			
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3			
10	I felt that I had nothing to look forward to	0	1	2	3			
11	I found myself getting agitated	0	1	2	3			
12	I found it difficult to relax	0	1	2	3			
13	I felt down-hearted and blue	0	1	2	3			
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3			
15	I felt I was close to panic	0	1	2	3			
16	I was unable to become enthusiastic about anything	0	1	2	3			
17	I felt I wasn't worth much as a person	0	1	2	3			
18	I felt that I was rather touchy	0	1	2	3			
19	I was aware of the action of my heart in the absence of physicalexertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3			
20	I felt scared without any good reason	0	1	2	3			
21	I felt that life was meaningless	0	1	2	3			
	TOTALS							

NFI-MS Neurological Fatigue Index

Date:

Name: _____ Age: _____ Sex: _____

Instructions: For each statement, tick ✓the box which best sums up your response as to how you have been feeling in the past two weeks.

1. I can become tired easily

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

2. Sometimes I lose my body strength

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

3. My limbs can become very heavy

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

4. My body can't keep up with what I want to do

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

5. The longer I do something the more difficult it becomes

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

6. Sometimes I have no option but to simply stop what I've been doing

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

7. I usually get tired on most days

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

8. I can become weak even if I've not been doing anything

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

9. Sometimes I really have to concentrate on what are usually simple things

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

10. I have problems with my speech when I'm tired

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

11. My coordination gets worse as the day goes on

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

Instructions: For each statement, tick ✓the box which best sums up your response as to how you have been feeling in the past two weeks.

12. Mental effort really takes it out of me

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

13. I need to rest in the day

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

14. I need to sleep in the day

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

15. Sleep in the day can really help me

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

16. Resting allows me to carry on

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

17. I try to get everything done in the morning

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

18. I try to rest or sleep beforehand, if I know I've got to do something that requires a lot of effort

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

19. I get a feeling as if I have not slept for a couple of nights

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

20. I yawn a lot

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

21. I sometimes wake in the night for no reason

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

22. When I awake in the morning I feel unrefreshed

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

23. Often in the morning, I don't feel like getting out of bed

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

GUIDELINES FOR EDSS AND FUNCTIONAL SYSTEM SCORING
BrAMS Departmental EDSS Rating Sheets

Note 1

A score of 1 in the Functional Systems implies that the patient is not aware of the deficit and that the deficit or sign does not interfere with normal daily activities (with the exceptions of optic, bowel/bladder and cerebral functional systems).

Note 2

EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory and the precise step in the scale is defined by the Functional Systems (FS) scores(s). EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation and usual equivalents in Functional Systems scores are provided.

Note 3

EDSS should not change by 1.0 step unless there is a change in the same direction of at least one step in at least one FS. Each step (e.g. 3.0 to 3.5) is still part of the EDSS scale equivalent i.e. progression from 3.0 to 3.5 should be equivalent to the EDSS score of 3.

Note 4

Usually the EDSS score will not be lower than the score of any individual FS score.

(a) For calculation of the EDSS, the score from the visual function system is to be converted as follows:

6 = 4
5 = 3
4 = 3
3 = 2
2 = 2
1 = 1

(b)

Bowel and Bladder FS Score	Converted Bowel and Bladder FS Score
6	5
5	4
4	3
3	3
2	2
1	1

Note 5

"Signs only" is noted in the source document when the examination reveals signs of which the patient is unaware.

Note 6

Symptoms which are not MS related will not be taken into consideration for assessments but should be noted. In case of doubt, the examining physician should assume a relation to MS.

Note 7

EDSS grades 6.0 and 6.5 contain both a description of assistance required and walking range. In general the descriptions given at these EDSS scores are valid. However, the following exceptions are made:

If a patient is able to walk considerably longer than 100m (> 120m) with 2 sticks, crutches or braces, he is in category 6.0.

If a patient is able to walk more than 5m and less than 120m with 2 sticks, crutches or braces, he is grade 6.5.

Note 8

To ensure unbiased EDSS assessments in controlled clinical trials, the EDSS rater should not inquire about the patient's condition, except as necessary to perform the EDSS assessment. Patients must be observed to walk the required distance.

N.B. Assistance by another person is equivalent to bilateral assistance.

If a patient is able to walk more than or equal to 50m with one stick, crutch or brace, the EDSS score is 6.0.

Use of an ankle foot orthotic device is not considered unilateral assistance.

If he needs assistance by another person and is not able to walk more than 50m with 1 stick, crutch or brace he is also in grade 6.5.

INSTRUCTIONS FOR VISUAL SYSTEM ASSESSMENT

The visual acuity score is based upon the line on the Snellen chart at 6 metres for which the patient makes no more than one error.

Test best corrected vision. If glasses are not available use a pin hole and test visual acuity with a standard Snellen chart at 5m. Not MS related vision deficits do not influence the visual function score but should be noted in the source document. To score 1 visual acuity will be 6/6 but patient reports monocular visual blurring and then describes a scotoma on Amsler chart or equivalent with either eye or visual acuity is less than 6/6, but better than 20/30.

Functional System Scoring Guidelines

Score 1 of optic disc pallor or a deficit in colour vision is present, but only if the visual acuity if better than 20/30. If visual acuity if 20/30 or worse, then the FS score is not affected by disc pallor or colour vision deficits. Testing for optic disc pallor and colour vision is optional.

Visual Acuity

6/6 = 20/20
6/9 = 20/30
6/12 = 20/40
6/18 = 20/60
6/24 = 20/80
6/36 = 20/120
6/60 = 20/200
1/60 = 4/200

FS – VISUAL (OPTIC) FUNCTIONS

Visual Acuity (corrected) OD (right eye) : _____

OS (left eye) : _____

		No	Signs Only	Moderate	Marked
Fields	OD				
	OS				
		No	Mild	Large	
Scotoma	OD				
	OS				
		No	Yes		
Pallor optional	OD				
	OS				

Definitions

Fields :

0 Normal
1 Signs Only : deficits present only on formal (confrontational) testing
2 Moderate : patient aware of deficit, but incomplete hemianopia on examination
3 Marked : complete homonymous hemianopia or equivalent

Scotoma :

0 None
1 Mild : detectable only on formal (confrontational) testing
2 Large : spontaneously reported by patient.

Disc Pallor :

0 Not present
1 Present

Functional Systems Score – Visual (Optic) Functions

- ❑ 0 = normal
- ❑ 1 = mild scotoma and/or disc pallor and/or visual acuity (corrected) of worse eye less than 20/20 (1.0) but better than 20/30 (0.67).
- ❑ 2 = worse eye with maximal visual acuity (corrected) of 20/30 to 20/59 (0.67-0.34).
- ❑ 3 = worse eye with large scotoma and/or moderate decrease in fields and/or maximal visual acuity (corrected) of 20/60 to 20/99 (0.33 – 0.21).
- ❑ 4 = worse eye with marked decrease of fields and/or maximal visual acuity (corrected) of 20/100 to 20/200 (0.2 – 0.1); grade 3 plus maximal acuity of better eye of 20/60 (0.33) or less.
- ❑ 5 = worse eye with maximal visual acuity (corrected) less than 20/200 (0.1); grade 4 plus maximal acuity of better eye of 20/60 (0.33) or less.
- ❑ 6 = grade 5 plus maximal visual acuity of better eye of 20/60 (0.33) or less.

FS – BRAINSTEM FUNCTIONS

Cranial Nerve Examination

	None	Signs Only	Mild Disability	Moderate Disability	Severe Disability = Marked	Complete loss of function
EOM Impaired						
Nystagmus						
Trigeminal Damage						
Facial Weakness						
Hearing Loss						
Dysarthria						
Dysphagia						
Slow Tongue RAM						
Other Bulbar Signs						

Definitions

EXTERNAL OCULAR MOVEMENTS

0 None
1 Signs only: Subtle and barely clinically detectable, EOM weakness. Patient does not complain of blurry vision, diplopia or discomfort.
2 Mild : Subtle and barely detectable EOM weakness of which patient is aware. Obvious incomplete paralysis of any eye movement – patient not aware.
3 Moderate : Obvious incomplete paralysis of any eye movement of which patient is aware; or Complete loss of movement in one direction of gaze in either eye.
4 Marked : Complete loss of movement in more than one direction of gaze in either eye.

NYSTAGMUS

0 None
1 Signs only or mild : Gaze evoked nystagmus below the limits of "moderate". (FS score of 1).
2 Moderate : Sustained nystagmus on 30° (horizontal or vertical gaze, but not in primary position). Patient may or may not be aware.
3 Severe : Sustained nystagmus in primary position or coarse persistent nystagmus in any direction that interferes with visual acuity, complete internuclear ophthalmoplegia with sustained nystagmus of abducting eye, oscillopsia.

TRIGEMINAL DAMAGE

0 None
1 Signs only
2 Mild : Clinically detectable numbness of which patient aware.
3 Moderate : Impaired discrimination of sharp/dull in one, two or three trigeminal branches. Trigeminal neuralgia (at least one attack in past 24 hours).
4 Marked : Unable to discriminate sharp/dull or complete loss of sensation in entire distribution of one or both trigeminal nerves.

FACIAL WEAKNESS

0 None
1 Signs only
2 Mild : Clinically detectable facial weakness of which patient is aware.

3 Moderate : Incomplete facial palsy e.g. weakness of eye closure requiring patching overnight or weakness of mouth closure resulting in drooling.
4 Marked : Complete unilateral or bilateral facial palsy with lagophthalmos or difficulty with liquids.

HEARING LOSS

0 None
1 Signs only Hears finger rub in one or both sides and has lateralised Weber test, but does not complain of hearing problem.
2 Mild As in 1, but aware of hearing problem.
3 Moderate : Cannot hear finger rub and/or misses several whispered numbers, on one or both sides.
4 Marked : Misses all or nearly all whispered numbers.

DYSARTHRIA

0 None
1 Signs Only
2 Mild : Detectable, patient aware.
3 Moderate : Obvious, impairs comprehensibility.
4 Marked : Incomprehensible.
5 Inability to speak.

DYSPHAGIA

0 None
1 Signs only
2 Mild : Difficulty with thin liquids.
3 Moderate : Difficulty with liquids and solid food.
4 Marked : Sustained difficulty with swallowing, required pureed diet.
5 Inability to swallow.

OTHER BULBAR FUNCTIONS

0 Normal
1 Signs only
2 Mild disability : Clinically detectable deficit of which patient is usually aware
3 Moderate disability
4 Marked disability

Functional Systems Score – Brainstem Functions

- 0 = normal
- 1 = signs only
- 2a = moderate nystagmus and/or moderate EOM impairment – 2b = other mild disability
- 3a = severe nystagmus – 3b = marked extraocular weakness – 3c = moderate disability of other cranial nerves
- 4a = marked dysarthria – 4b other marked disability
- 5 = inability to swallow or speak

FS – PYRAMIDAL FUNCTIONS

Definitions

Reflexes : indicate differences between R and L by "<" and ">"
0 = absent; 1 = weak; 2 = normal=
3 = exaggerated;
4 – non-sustained clonus (a few beats of clonus); 5 = sustained clonus.

Plantar Response
0 = flexor; 1 = neutral or equivocal; 2 = extensor

Limb Strength – BMRC Rating Scale: Note – the weakest muscle in each group defines the score for that group. Each movement should be tested, but only pathological findings must be noted.

0 = no activity; 1 = visible contraction without visible movement joint;
2 = visible movements only on the plane of gravity; 3 = movements against gravity possible but not against resistance; 4 = movements against resistance possible but impaired; 5 = normal strength.

Limb Spasticity
0 = normal; 1 = mild, barely increased spastic tonus after rapid flexion of an extremity;
2= moderate, can be overcome, full range of motion possible; 3 = severe, barely surmountable increased spastic tonus after rapid flexion of an extremity, full range of movement not possible;4 = contractured.

Gait Spasticity :
0 = normal; 1 = barely perceptible;
2 = evident, minor interference with function;
3 = permanent shuffling, major interference with function.

Overall Motor Performance :
0 = normal; 1 = abnormal weakness (as compared to peers) in performing more demanding tasks eg when walking longer distances, but no reduction in limb strength on formal (confrontational) testing;
2 = reduction in strength of individual muscle groups at confrontational testing.

Motor Examination					
Reflexes				R	L
		Biceps			
		Radial			
		Triceps			
		Knee			
		Ankle			
Plantar Responses		Flex	Neutral	Ext	
	R				
	L				
Limb Strength				R	L
		Shoulder			
		Elbow Flexors			
		Elbow Extensors			
		Hand/Finger Flexors			
		Hand/Finger Extensors			
		Hip Flexion			
		Knee Flexion			
		Knee Extensors			
		Plantar Flexion (Foot/Toe)			
		Dorsiflexion (Foot/Toe)			
Limb Spasticity		Arm			
		Leg			
		Gait Spasticity			

Functional Systems Score – Pyramidal Functions

- 0= normal;
- 1 = abnormal signs without disability;
- 2 = minimal disability, patient complains about fatigability or reduced performance in motor tasks (motor performance Grade 1) and or BMRC grade 4 in one or two muscle groups.
- 3a = mild to moderate paraparesis or hemiparesis, usually BMRC Grade 4 in more than two muscle groups or BMRC Grade 3 in one or two muscle groups, movements against gravity are possible;
- 3b = severe monoparesis, BMRC Grade 2 or less in one muscle group;
- 4a = marked paraparesis or hemiparesis, usually BMRC Grade 2 in two limbs;
- 4b = moderate quadriparesis, BMRC Grade 3 in three or more limbs;
- 4c = monoplegia, BMRC Grade 0 or 1 in one limb;
- 5a = paraplegia, BMRC Grade 0 or 1 in all muscle groups of lower limbs;
- 5b = hemiplegia
- 5c = marked quadriparesis, BMRC Grade 2 or less in three or more limbs;
- 6 = quadriplegia; BMRC Grade 0 or 1 in all muscle groups of upper and lower limbs.

FS – CEREBELLAR FUNCTIONS

Instructions : includes finger-nose test, rapid alternating movements (finger tapping and supination/pronation of hand, toe tapping and alternation side to side placements of foot or imitating bicycle in the air), heel-knee-shin test, gait.

Definitions

LIMB ATAXIA

0 None
1 Signs only
2 Mild : Tremor or clumsy movements seen easily, minor interference with function.
3 Moderate : Tremor or clumsy movements interfere with function in all spheres.
4 Severe : Most functions are very difficult.

TRUNCAL ATAXIA

0 None
1 Signs only
2 Mild : Swaying with eyes closed.
3 Moderate : Swaying with eyes open.
4 Severe : Unable to sit without assistance.

GAIT ATAXIA

0 None
1 Signs only
2 Mild : Problems with balance realised by patient and/or significant other.
3 Moderate : Abnormal balance on ordinary walking.
4 Severe : Unable to walk more than a few steps unassisted, or requires support by another person or assistive device because of ataxia.

Note : The presence of severe ataxia alone, (without severe truncal ataxia and severe ataxia in three or four limbs), results in a grade of 3 in the cerebellar functional system. If weakness interferes with testing of ataxia, score the patient's actual performance, but also indicate the possible role of weakness by marking an X after the Cerebellar FS score..

			None	Signs Only	Mild Abnormality	Moderate Abnormality	Severe Abnormality	Complete loss of function
Ataxia	Dysmetria Intention Tremor	RU						
		LU						
		RL						
		LL						
	Trunk EO							
	Trunk EC							
RAM Impairment		RU						
		LU						
		RL						
		LL						
Resting Tremor		RU						
		LU						
		RL						
		LL						
Gait Ataxia EO								
EC								
Straight Line EO								
Walking EC								
Romberg's Test								
Other _____								

ROMBERG'S TEST

0 Normal
1 Mild : Mild instability with eyes closed.
2 Moderate : Not stable with eyes closed.
3 Severe: Not stable with eyes open.

TANDEM WALKING

0 Normal.
1 Impaired.
2 Not possible.

Functional Systems Score – Cerebellar Functions

- ❑ 0 = normal
- ❑ 1 = abnormal signs without disability
- ❑ 2 = mild ataxia and/or moderate station ataxia (Romberg) and/or tandem walking not possible.
- ❑ 3a = moderate truncal ataxia
- ❑ 3b = moderate limb ataxia
- ❑ 3c = moderate or severe gait ataxia
- ❑ 4 = severe truncal gait ataxia and severe ataxia in three or four limbs
- ❑ 5 = unable to perform co-ordinated movements due to ataxia
- ❑ X = is use when weakness (grade 3 or more on pyramidal) or sensory defects interferes with testing.

FS – SENSORY FUNCTIONS

Definitions

SUPERFICIAL SENSATION – TOUCH/PAIN

0 Normal
1 Signs Only : Slight decrease in sensation (temperature or figure writing of which patient is not aware).
2 Mild : Patient is aware of impaired light touch or pain, but able to discriminate sharp/dull.
3 Moderate : Impaired discrimination of sharp/dull.
4 Marked: No discrimination of sharp/dull and/or unable to feel light touch.
5 Complete loss Anaesthesia.

VIBRATORY SENSATION

Note : use most distal joint to determine score

0 Normal
1 Mild : Graded tuning fork 5 – 7 of 8 (alternatively, detects more than 10 seconds but less than examiner).
2 Moderate : Graded tuning fork 1 – 4 of 8 (alternatively, detects more than 2 seconds but less than 11 seconds).
3 Marked : Complete loss of vibration sense.

POSITION SENSE

0 Normal
1 Mild : 1 –2 incorrect responses on testing. Only distal joints affected.
2 Moderate : Does not correctly perceive movement of fingers or toes. Proximal joints affected.
3 Marked: Astasia, no perception of movement.

		None	Mild Abnormality	Moderate Abnormality	Marked Abnormality	Complete loss of function
Superficial Sensation Touch/Pain	RU					
	LU					
	RL					
	LL					
Vibratory Sensation	RU					
	LU					
	RL					
	LL					
Position Sense	RU					
	LU					
	RL					
	LL					

LHERMITTE'S SIGN/PARAESTHESIAE

Does not contribute to FS Score

0 Negative
1 Positive

Functional Systems Score – Sensory Functions

- ❑ 0 = normal
- ❑ 1 = mild vibration or figure writing or temperature decrease only, in 1 or 2 limbs
- ❑ 2a = mild decrease in touch or pain or position sense and/or moderate decrease in vibration in 1 or 2 limbs
- ❑ 2b = mild vibratory (figure writing or temperature) decrease alone in 3 or 4 limbs
- ❑ 3a = moderate decrease in touch or pain or position sense, or marked reduction of vibration in 1 or 2 limbs
- ❑ 3b = mild decrease in touch or pain and or moderate decrease in all proprioceptive tests in 3 or 4 limbs
- ❑ 4a = marked decrease in touch or pain or loss of proprioception, alone or combined in 1 or 2 limbs
- ❑ 4b - moderate decrease in touch or pain and/or marked proprioceptive decrease in more than 2 limbs.
- ❑ 5a = loss (essentially) of sensation in 1or 2 limbs.
- ❑ 5b = moderate decrease in touch or pain and or marked reduction in proprioception for most of the body below the head
- ❑ 5c = sensation lost below the head

FS – BOWEL AND BLADDER FUNCTIONS

Definitions

BLADDER

Urinary hesitancy and retention

0 None
1 Mild : No major impact on lifestyle.
2 Moderate : Urinary retention, frequent UTIs.
3 Severe: Requires catheterisation.
4 Loss of Function : Overflow incontinence.

Urinary urgency and incontinence

0 None
1 Mild : No major impact on lifestyle.
2 Moderate : Rare incontinence occurring no more than once a week, must wear pads.
3 Severe : Frequent incontinence occurring from several times a week to more than once a day, must wear urinal or pads.
4 Loss of Function : Loss of bladder control.

BLADDER CATHETERISATION

0 None
1 Intermittent self-catheterisation
2 Constant catheterisation

BOWEL

0 None
1 Mild : No incontinence, no major impact on lifestyle, mild constipation.
2 Moderate : Must wear pads or alter lifestyle to be near lavatory.
3 Severe : In need of enemata or manual measures to evacuate bowels.
4 Complete loss of function

Functional Systems Scoring Guidelines

Patients who require intermittent catheterisation 1-2 times per day should receive an FS score of 3. Patients who require intermittent catheterisation 3 or more times per day should receive an FS score of 4.

	Bladder Function				
	None	Mild (rare) Abnormality	Moderate Abnormality	Severe Abnormality	Complete loss of function
Hesitancy					
Urgency					
Retention					
Incontinence					
	NO	Interm. 1-2	Interm. >2	constant	
Catheterisation					

	Bowel Dysfunction				
	None	Mild (rare) Abnormality	Moderate Abnormality	Severe Abnormality	Complete loss of function

	Sexual Dysfunction (Optional)				
	None	Mild (rare) Abnormality	Moderate Abnormality	Severe Abnormality	Complete loss of function

Functional Systems Score – Bowel and Bladder Functions

- 0 = normal
- 1 = mild urinary hesitancy, urgency or retention and/or constipation.
- 2 = moderate urinary hesitancy/retention and/or moderate urinary urgency/incontinence and/or moderate bowel dysfunction.
- 3 = frequent urinary incontinence or intermittent self catheterisation, needs enemata or manual measures to evacuate bowels
- 4 = in need of almost constant catheterisation
- 5 = loss of bladder or bowel function ; external or indwelling catheter
- 6 = loss of bladder and bowel function

FS – CEREBRAL FUNCTIONS

Definitions

MOOD ALTERATION:
0 None
1 Patient complains of depression or is considered depressed or euphoric by the investigator or "significant other".

Depression and euphoria are documented on the scoring sheet, but are not taken into consideration for FS and EDSS calculation.

DECREASE IN MENTATION :
0 None
1 Signs : Not apparent to patient and/or significant other.
2 Mild : Difficulties apparent to patient and "significant other" such as impaired ability to follow a rapid course of association and of surveying complex matters, impaired judgment in certain demanding situations, able to handle the daily routine but no tolerance for additional stressors, intermittently symptomatic to even normal levels of stress, reduced performance, tendency toward negligence due to obliviousness, fatigue or decreased ambition, However, not apparent while taking the history or performing the routine neurological examination.
3 Moderate : Definite abnormalities on formal mental status testing, but still orientated to time, place and person.
4 Marked : Not orientated in 1 or 2 spheres of time, place and person. Marked effect on lifestyle.

5 Dementia or Chronic Brain Syndrome : Confusion and/or complete disorientation.

	Mental Status Examination				
	None	Mild	Marked		
Depression					
Euphoria					
Fatigue					
	None	Mild	Moderate	Marked	Dementia
Decrease of Mentation					

FATIGUE
0 None
1 Mild : Does not interfere with daily activities.
2 Moderate : Interferes, but does not limit daily activities for more than 50%.
3 Severe : Significant limitation in daily activities (>50% reduction).

N.B. Because fatigue is difficult to evaluate objectively, in some studies it does not contribute to the Cerebral FS or EDSS step. Please adhere to the study's specific instruction.

Functional Systems Score – Cerebral Functions

- 0 = normal
- 1a = mood alteration only (does not affect EDSS score)
- 1b = mild fatigue, signs only decrease in mentation
- 2 = mild decrease in mentation, moderate or severe fatigue
- 3 = moderate decrease in mentation
- 4 = marked decrease in mentation (chronic brain syndrome – moderate)
- 5 = dementia

AMBULATION

Unrestricted ambulation means the patient is able to walk a distance without assistance that is regarded as normal, compared with healthy individuals of similar age and physical condition. In this case, the EDSS step can be anything between 0 and 0.5, depending on the FS scores.

Fully ambulatory means at least 500m of ambulation without assistance, but not unrestricted. The EDSS step can be anything between 2.0 and 5.0, depending on the FS scores. In this case, the pyramidal and/or cerebellar FS must be ≥2 to reflect this "restriction" of ambulation.

If ambulation is <500 metres, the EDSS step must be ≥4.5 depending on the walking ranges provided by the ambulation score (see page 13) and combination of FS scores. EDSS steps 5.5 to 8.0 are exclusively defined by the ability to ambulate and type of assistance required, or the ability to use a wheelchair.

If assistance is needed, the definitions of EDSS steps 6.0 or 6.5 include both a description of the type of assistance required when walking and the walking range. Assistance by another person is equivalent to bilateral assistance.

NOTE

The ambulation score represents both a description of walking range and the type of assistance required for ambulation. The score replaces the former use of several check boxes, but does NOT introduce new definitions. The use of wheelchair can now be scored on the scoring sheet. Please indicate the reported distance and time for the patient in the appropriate field on the scoring sheet, followed by the type of assistance and the walking distance measured during the assessment.

DISTANCE AND TIME REPORTED BY PATIENT

Maximal unassisted walking distance reported by patient (in metres) without rest or assistance and time required to walk max. distance according to patient (in minutes).

Reported distance able to walk in metres _____

Time Needed _____

ASSISTANCE

- 0 = Without help or assistance **(allowing the use of an ankle foot orthotic device, without any other type of assistive device)**
- 1 = Unilateral assistance : **one stick/crutch/brace/FES (unless able to walk 100m without)**
- 2 = Bilateral assistance : **two sticks/crutches/braces or assistance by another person**
- 3 = Wheelchair

DISTANCE

Unassisted : observe the patient walking unassisted for a minimum distance of 500 metres and measure the time needed if possible.

Actual distance walked in metres _____ (obligatory for>or equal to 500m if possible)

(Time Needed _____)

Assisted : observe the patient walking with the assistive device or help by another person for a minimum distance of 130 metres, if possible.

Actual distance walked in metres _____ (obligatory for>or equal to 130m if possible)

(Time Needed _____)

Having performed a complete neurological evaluation on this patient, I find him/her to have an EDSS score of _____

_____
Signature

-----/-----/---
DD/MM/YY

Kurtzke Expanded Disability Status Scale

0.0	Normal neurological exam (all grade 0 in FS).
1.0	No disability, minimal signs in one FS (one FS grade 1).
1.5	No disability, minimal signs in more than one FS (more than one FS grade 1).
2.0	Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5	Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0	Moderate disability in one FS (one FS grade 3, others 0 or 1) though fully ambulatory; or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory.
3.5	Fully ambulatory but with moderate disability in one FS (one FS grade 3) and mild disability in one or two FS (one/two FS grade 2) and others 0 or 1; or fully ambulatory with two FS grade 3 (others 0 or 1): or fully ambulatory with five FS grade 2 (others 0 or 1).
4.0	Ambulatory without aid or rest for ≥500 metres; up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps.
4.5	Ambulatory without aid or rest for ≥300 metres; up and about much of the day, characterised by relatively severe disability usually consisting of one FS grade 4 and combination of lesser grades exceeding limits of previous steps.
5.0	Ambulatory without aid or rest for ≥200m; usual FS equivalents are at least one grade 5 or combinations of lesser grades usually exceeding specifications for step 4.5).
5.5	Ambulatory without aid or rest for ≥100m.
6.0	Unilateral assistance (cane, crutch, brace) required to walk at least 100m with or without resting.
6.5	Constant bilateral assistance (canes, crutches, braces) required to walk at least 20m without resting.
7.0	Unable to walk five metres even with aid, essentially restricted to wheelchair; wheels self and transfers alone, up and about in wheelchair some 12 hours a day.
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transferring and wheeling self; may require motorised wheelchair.
8.0	Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day, retains many self-care functions; generally has effective use of arms.
8.5	Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self care functions.
9.0	Helpless bed patient; can communicate and eat.
9.5	Totally helpless bed patient, unable to communicate effectively or eat/swallow.
10.0	Death due to MS.

Ambulation Score

0 = Unrestricted

1 = Fully ambulatory

2 = ≥300m but <500m, without help or assistance (EDSS 4.5 or 5.0)

3 = ≥200m but <300m, without help or assistance (EDSS 5.0)

4 = ≥100m but <200m, without help or assistance (EDSS 5.5)

5 = Walking range <100m, without assistance (EDSS 6.0)

6 = Unilateral assistance, ≥50m (EDSS 6.0)

7 = Bilateral assistance, ≥120m (EDSS 6.0).

8 = Unilateral assistance, <50m (EDSS 6.5).

9 = Bilateral assistance, ≥5m, but <120m (EDSS 6.5)

10 = Uses wheelchair without help; unable to walk 5m even with aid, essentially restricted to wheelchair, wheels self and transfers alone; up and about in wheelchair come 12 hours a day (EDSS 7.0).

11 = Uses wheelchair with help; unable to take more than a few steps; restricted to wheelchair; may need some help in transferring and in wheeling self (EDSS 7.5).

12 = Essentially restricted to bed or chair or perambulated in wheelchair, but out of bed most of day; retains many self-care functions; generally has effective use of arms (EDSS 8.0).

LOWER EXTREMITY FUNCTION: TIMED 25-FOOT WALK

Subject ID Number | Subject Initials | Visit Date: Day | Month | Year

TIMED 25-FOOT WALK

Did patient wear an AFO? ☐ Yes ☐ No

Was assistive device used? ☐ Yes ☐ No

Assistive device used *(mark one)*:

☐ Unilateral Assistance ☐ Cane ☐ Crutch

☐ Bilateral Assistance ☐ Cane ☐ Crutch ☐ Walker/Rollator

Trial 1

Time for 25-Foot Walk ☐☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*: Specify:

☐ Unable to complete trial due to physical limitations ➡

☐ Other ➡

Trial 2

Time for 25-Foot Walk ☐☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*: Specify:

☐ Unable to complete trial due to physical limitations ➡

☐ Other ➡

Did it take more than two attempts to get two successful trials? ☐ Yes ☐ No

If yes, please specify reasons(s) for more than two attempted trials:

UPPER EXTREMITY FUNCTION: NINE-HOLE PEG TEST (9-HPT)

Subject ID Number | Subject Initials | Visit Date: Day / Month / Year

9-HOLE PEG TEST

DOMINANT HAND *(Check one):* ☐ Right ☐ Left

DOMINANT HAND

Trial 1

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Trial 2

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Did it take more than two attempts to get two sucessful trials? ☐ Yes ☐ No
If Yes, please specify reason(s) for more than two attempted trials:

NON-DOMINANT HAND

Trial 1

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Trial 2

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Did it take more than two attempts to get two sucessful trials? ☐ Yes ☐ No
If Yes, please specify reason(s) for more than two attempted trials:

COGNITIVE FUNCTION: PASAT - PRACTICE ITEMS	
Subject ID Number ___ ___ ___ Subject Initials ___ ___ ___	Visit Date: Day ___ ___ Month ___ ___ Year ___ ___ ___ ___

PASAT Practice Items

RATE #1
(3 sec.)

9 + 1 10___	3 4___	5 8___	2 7___	6 8___	4 10___	9 13___	7 16___	1 8___	4 5___
9 + 1 10___	3 4___	5 8___	2 7___	6 8___	4 10___	9 13___	7 16___	1 8___	4 5___
9 + 1 10___	3 4___	5 8___	2 7___	6 8___	4 10___	9 13___	7 16___	1 8___	4 5___

PASAT Practice Items

RATE #2
(2 sec.)

3 + 8 11___	2 10___	7 9___	9 16___	1 10___	8 9___	5 13___	2 7___	6 8___	4 10___
3 + 8 11___	2 10___	7 9___	9 16___	1 10___	8 9___	5 13___	2 7___	6 8___	4 10___
3 + 8 11___	2 10___	7 9___	9 16___	1 10___	8 9___	5 13___	2 7___	6 8___	4 10___

Materials	Start	Discontinue	Score
TOPF[IX] Word Card	Ages 16-90: Item 1	After 5 consecutive scores of 0	Score 0 or 1 point for each response.

Additional Prompts

If, at any time during administration, you are unsure which word the examinee is reading, say, **Point to the word you are reading.**

If the examinee's rate of reading is too rapid for accurate scoring, say, **You are going too fast for me to keep up. Please read the words more slowly.**

If the examinee's response is unclear, say, **Say it again.**

If the examinee asks what to do if he or she makes a mistake, say, **You can try it again.** If the examinee self-corrects his or her initial response, award credit appropriately. Examinees may correct their initial response(s) at any time during administration.

If the examinee provides multiple responses to an item, score only the intended response. If it is not clear which one is the intended response, say, **You pronounced the word more than one way. Which one did you mean?**

Give no further assistance except to remind the examinee to continue until told to stop (if necessary) or to redirect the examinee to the appropriate word or column.

Item		Score	
1. two	(TOO)	0	1
2. address	address (uh-DRESS) or (ah-DRESS) or (AD-dress)	0	1
3. whole	(HOHL)	0	1
4. eye	(I)	0	1
5. again	(ah-GAIN) or (ah-GEHN) or (uh-GAIN) or (uh-GEHN)	0	1
6. enough	(ee-NUHF) or (uh-NUHF) or (in-NUHF)	0	1
7. already	(awl-RED-ee)	0	1
8. cough	(KAWF) or (kof)	0	1
9. fuel	(FYOOL)	0	1
10. climb	(KLIM)	0	1
11. most	(MOHST)	0	1
12. excitement	(ik-SITE-munt) or (eck-SITE-munt)	0	1
13. mosquito	(muh-SKEE-toh)	0	1
14. decorate	decorate (DEK-oh-rate) or (DEK-uh-rate)	0	1
15. fierce	(fee-us) or (feerss)	0	1
16. plumb	(PLUM)	0	1
17. knead	(NEED)	0	1
18. vengeance	(VEN-jnss) or (VEN-junss)	0	1

Item		Score	
19. gnat	(NAT)	0	1
20. prestigious	(pre-STIJ-us) or (pre-STEEJ-us)	0	1
21. amphitheatre	(AM(p)-fih-thee-uh-ter) or (AM(p)-fih-theeta)	0	1
22. lacuna	(la-KOO-nuh)	0	1
23. iridescent	(ihr-ih-DESS-unt) or (ihr-uh-DESS-unt)	0	1
24. lieu	(LOO) or (l(y)oo)	0	1
25. wily	(WI-lee)	0	1
26. aesthetic	(ess-THET-ik) or (ees-THET-ik)	0	1
27. equestrian	(eh-KWESS-tree-un) or (ih-KWESS-tree-un)	0	1
28. porpoise	(POR-poyz; Scots) or (PAW-pus) or (POR-pus)	0	1
29. subtle	(SUH-tuhl)	0	1
30. palatable	(PAL-ah-tuh-bul) or (PAL-uh-tuh-bul)	0	1
31. homily	(HOM-ih-lay) or (HOM-ih-lee)	0	1
32. ogre	(OH-gur)	0	1
33. liaison	(lee-AY-zon(g)) or (lee-AH-zn)	0	1
34. xenophobia	(zen-oh-FO-bee-uh) or (zen-uh-FO-bee-uh)	0	1
35. piquant	(PEE-kuhnt) or (PEE-kwant)	0	1
36. menagerie	(meh-NA-juh-ree)	0	1

Additional Prompts

If, at any time during administration, you are unsure which word the examinee is reading, say, **Point to the word you are reading**.

If the examinee's rate of reading is too rapid for accurate scoring, say, **You are going too fast for me to keep up. Please read the words more slowly**.

If the examinee's response is unclear, say, **Say it again**.

If the examinee asks what to do if he or she makes a mistake, say, **You can try it again**. If the examinee self-corrects his or her initial response, award credit appropriately. Examinees may correct their initial response(s) at any time during administration.

If the examinee provides multiple responses to an item, score only the intended response. If it is not clear which one is the intended response, say, **You pronounced the word more than one way. Which one did you mean?**

Give no further assistance except to remind the examinee to continue until told to stop (if necessary) or to redirect the examinee to the appropriate word or column.

Item		Score	
37. umbrage	(UHM-brihj)	0	1
38. fecund	(FE-cund) or (FEE-cund)	0	1
39. scurrilous	(SKUH-ruh-lus) or (SKUR-ih-lus) or (SKUR-uh-lus) or (SKUH-rih-lus)	0	1
40. heinous	heinous (HE-nus) or (HEE-nus) or (HAY-nuss)	0	1
41. obfuscate	(OB-fuh-skat)	0	1
42. plethora	(PLETH-oh-rah) or (PLETH-eh-rah)	0	1
43. exigency	(eks-IH-jen-say) or (eks-IH-jen-see)	0	1
44. lascivious	(lah-SIH-vee-uhs) or (luh-SIH-vee-uhs)	0	1
45. picot	(PEE-koh)	0	1
46. cretonne	(kre-TON) or (KRE-ton)	0	1
47. vicissitude	(vi-SI-si-tyood)	0	1
48. ethereal	(ih-THEE-ree-ul) or (ih-THEER-ee-ul)	0	1
49. uxorious	(uhk-SOHR-ee-uhs) or (uhg-SOHR-ee-uhs)	0	1
50. lugubrious	(loo-GOOB-ree-uss) or (luh-GOOB-bree-uss)	0	1
51. misogyny	(meh-SAW-jeh-nee) or (mih-SAW-jin-ay)	0	1
52. perspicuity	(per-spuh-KYEW-ih-tay) or (per-speh-KYEW-uh-tee) or (per-speh-KYEW-uh-tay)	0	1
53. ubiquitous	(you-BIC-wih-tiss) or (you-BIC-wuh-tiss) or (you-BIC-wuh-tuss)	0	1

Item		Score	
54. dichotomy	(dye-KAW-toh-may) or (dye-KAW-toh-mee)	0	1
55. facetious	(fuh-SEE-shuhss) or (fah-SEE-shuss)	0	1
56. treatise	(TREE-tiss) or (TREET-iss) or (TREE-tiz) or (TREET-iz)	0	1
57. paradigm	(PAH-rah-dime)	0	1
58. macabre	(mah-KABR) or (mah-KAAB)	0	1
59. anechoic	(ah-nih-KOH-ihk)	0	1
60. acquiesce	(ah-kwee-EHSS)	0	1
61. dilettante	(DILL-ih-tan-tay) or (DILL-uh-tahnt)	0	1
62. eyrir	(AY-rihr)	0	1
63. hyperbole	(hy-PER-bu-lay) or (hy-PUR-bu-lay)	0	1
64. vertiginous	(ver-TIH-juh-nuhss) or (ver-TIDJ-in-iss)	0	1
65. hegemony	(HEH-geh-mon-ee) or (heh-GEM-o-nee) or (heh-JEM-o-nee)	0	1
66. insouciant	(in-SOO-see-(y)ant) or (ihn-SOO-see-unt) or (in-SOO-see-(y)unt)	0	1
67. vide	(VI-day) or (VI-dee) or (VEE-day)	0	1
68. ceilidh	(KAY-lee)	0	1
69. vivace	(vee-VAH-chay) or (vee-VAH-chee)	0	1
70. chthonic	(THON-ik)	0	1

Test of Premorbid Functioning
Total Raw Score (Max = 70) ____

Patient's Name: _____ Date: ____/____/____
month day year

ID#: _____ Test#: 1 2 3 4

MODIFIED FATIGUE IMPACT SCALE (MFIS)

Following is a list of statements that describe how fatigue may affect a person. Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time. In medical conditions like MS, feelings of fatigue can occur more often and have a greater impact than usual. Please read each statement carefully, and then circle the one number that best indicates how often fatigue has affected you in this way during the past 4 weeks. (If you need help in marking your responses, tell the interviewer the number of the best response.) Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

Because of my fatigue during the past 4 weeks....

		Never	Rarely	Sometimes	Often	Almost always
1.	I have been less alert.	0	1	2	3	4
2.	I have had difficulty paying attention for long periods of time.	0	1	2	3	4
3.	I have been unable to think clearly.	0	1	2	3	4
4.	I have been clumsy and uncoordinated.	0	1	2	3	4
5.	I have been forgetful.	0	1	2	3	4
6.	I have had to pace myself in my physical activities.	0	1	2	3	4
7.	I have been less motivated to do anything that requires physical effort.	0	1	2	3	4

Because of my fatigue
during the past 4 weeks....

		Never	Rarely	Sometimes	Often	Almost always
8.	I have been less motivated to participate in social activities.	0	1	2	3	4
9.	I have been limited in my ability to do things away from home.	0	1	2	3	4
10.	I have had trouble maintaining physical effort for long periods.	0	1	2	3	4
11.	I have had difficulty making decisions.	0	1	2	3	4
12.	I have been less motivated to do anything that requires thinking.	0	1	2	3	4
13.	my muscles have felt weak.	0	1	2	3	4
14.	I have been physically uncomfortable.	0	1	2	3	4
15.	I have had trouble finishing tasks that require thinking.	0	1	2	3	4
16.	I have had difficulty organizing my thoughts when doing things at home or at work.	0	1	2	3	4
17.	I have been less able to complete tasks that require physical effort.	0	1	2	3	4
18.	my thinking has been slowed down.	0	1	2	3	4
19.	I have had trouble concentrating.	0	1	2	3	4

		Never	Rarely	Sometimes	Often	Almost always
20.	I have limited my physical activities.	0	1	2	3	4
21.	I have needed to rest more often or for longer periods.	0	1	2	3	4

MODIFIED FATIGUE IMPACT SCALE - 5-ITEM VERSION (MFIS-5)

Following is a list of statements that describe how fatigue may affect a person. Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time. In medical conditions like MS, feelings of fatigue can occur more often and have a greater impact than usual. Please read each statement carefully, and then circle the one number that best indicates how often fatigue has affected you in this way during the past 4 weeks. (If you need help in marking your responses, tell the interviewer the number of the best response.) Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

Because of my fatigue
during the past 4 weeks....

		Never	Rarely	Sometimes	Often	Almost always
1.	I have been less alert.	0	1	2	3	4
2.	I have been limited in my ability to do things away from home.	0	1	2	3	4
3.	I have had trouble maintaining physical effort for long periods.	0	1	2	3	4
4.	I have been less able to complete tasks that require physical effort.	0	1	2	3	4
5.	I have had trouble concentrating.	0	1	2	3	4

PERCEIVED DEFICITS QUESTIONNAIRE (PDQ)

INSTRUCTIONS

Everyone at some point experiences problems with memory, attention, or concentration, but these problems may occur more frequently for individuals with neurologic diseases like MS. The following questions describe several situations in which a person may encounter problems with memory, attention or concentration. If you are marking your own answers, please circle the appropriate response (0, 1, 2,...) based on your cognitive function during the past 4 weeks. If you need help in marking your responses, tell the interviewer the number of the best response. Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

During the past 4 weeks, how often did you....

		Never	Rarely	Some-times	Often	Almost always
1.	lose your train of thought when speaking?	0	1	2	3	4
2.	have difficulty remembering the names of people, even ones you have met several times?	0	1	2	3	4
3.	forget what you came into the room for?	0	1	2	3	4
4.	have trouble getting things organized?	0	1	2	3	4
5.	have trouble concentrating on what people are saying during a conversation?	0	1	2	3	4
6.	forget if you had already done something?	0	1	2	3	4
7.	miss appointments and meetings you had scheduled?	0	1	2	3	4

During the past 4 weeks,
how often did you....

		Never	Rarely	Some-times	Often	Almost always
8.	have difficulty planning what to do in the day?	0	1	2	3	4
9.	have trouble concentrating on things like watching a television program or reading a book?	0	1	2	3	4
10.	forget what you did the night before?	0	1	2	3	4
11.	forget the date unless you looked it up?	0	1	2	3	4
12.	have trouble getting started, even if you had a lot of things to do?	0	1	2	3	4
13.	find your mind drifting?	0	1	2	3	4
14.	forget what you talked about after a telephone conversation?	0	1	2	3	4
15.	forget to do things like turn off the stove or turn on your alarm clock?	0	1	2	3	4
16.	feel like your mind went totally blank?	0	1	2	3	4
17.	have trouble holding phone numbers in your head, even for a few seconds?	0	1	2	3	4
18.	forget what you did last weekend?	0	1	2	3	4
19.	forget to take your medication?	0	1	2	3	4
20.	have trouble making decisions?	0	1	2	3	4

PERCEIVED DEFICITS QUESTIONNAIRE - 5-ITEM VERSION (PDQ-5)

During the past 4 weeks,
how often did you....

		Never	Rarely	Some-times	Often	Almost always
1.	have trouble getting things organized?	0	1	2	3	4
2.	have trouble concentrating on things like watching a television program or reading a book?	0	1	2	3	4
3.	forget the date unless you looked it up?	0	1	2	3	4
4.	forget what you talked about after a telephone conversation?	0	1	2	3	4

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