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Assessing brain and cognitive function in multiple sclerosis cross-sectionally and longitudinally with multimodal methods: insights from high-density electrophysiology, neuropsychological examination and magnetic resonance imaging

A dissertation submitted to the University of Dublin
for the degree of Doctor of Philosophy

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Hanni Kiiski

15th July 2013

A summary of the thesis

Cognitive impairment (CI) affects up to 65% of multiple sclerosis (MS) patients and can occur in the absence of physical disability (Hoffmann, Tittgemeyer, & von Cramon, 2007). MS is the most common chronic inflammatory demyelinating disease affecting the central nervous system among young adults in Western countries leading to severe disability, as there is no cure (Filippi, 2011). During early disease course, 85% of MS patients present with alternating periods of incapacitating relapses and stable remissions (relapsing-remitting MS, RRMS). This later develops into the secondary-progressive (SPMS) phase, characterised by less relapses but increasing disability. A minority (15%) of patients have a progressive accumulation of disability without relapses (primary-progressive, PPMS)(Rejdak, Jackson, & Giovannoni, 2010). CI in MS occurs most often for information processing speed, attention and working memory (Rogers & Panegyres, 2007). CI in MS is to some degree related to concurrent brain changes such as lesions (R. H. Benedict & Zivadinov, 2011). CI may significantly limit the independence and quality of life of MS patients (Amato, et al., 1995). Thus it is crucial to create innovative methods to support the early detection, efficient monitoring and treatment of the CI in MS.

Electroencephalography (EEG) records the ongoing electrical voltage changes on the scalp caused by multiple underlying neuronal network interactions. The P3b event-related potentials (ERPs) are averaged responses of the brain to detect improbable events in a train of frequent stimuli. Another P3 ERP, the P3a, is elicited by unexpected events in the train of attended frequent and infrequent stimuli. The P3 ERPs index information processing speed, attention and working memory, the cognitive areas most affected in MS (Leocani, Gonzalez-Rosa, & Comi, 2010; Polich, 2007). The P3 ERPs are favourable auxiliary measures compared with the gold standard neuropsychological (NP) examination and magnetic resonance imaging (MRI), to detect and monitor cognitive and brain function in MS. The advantages of cognitive-indexing electrophysiological measures include high temporal resolution, objectiveness, cost-effectiveness, easy administration, portability. In addition, they have the added benefit of not being affected by practice effects, education, or physical disability. P3 ERP activities are typically reported as the size and timing of the brain's response (i.e. amplitude and latency), or as frequency ranges of electrical oscillations; such as *theta* (4-8 Hz) and *alpha* (8-13 Hz) (Polich, 2007). Most previous research has found prolonged P3 ERP latencies and reduced P3 ERP amplitudes in MS (Magnano, Aiello, & Piras, 2006), however, longitudinal research is scarce (e.g. Pokryszko-Dragan, et al., 2009). These findings are inconclusive due to numerous methodological limitations.

The main focus of this thesis is to test the hypotheses that MS patients, particularly progressive MS patients, show delayed P3 ERP latencies, decreased P3 ERP amplitudes and reduced source activities both cross-sectionally, and that these changes would become greater over the 24-month follow-up period. The changes in P3 ERP activites are hypothesized to be associated to poor neuropsychological performance and pathological brain changes. In order to probe visual function in MS, another aim was to examine the utility of a novel method, Visual Evoked Spread Spectrum Analysis (VESPA) (Lalor, Pearlmutter, Reilly, McDarby, & Foxe, 2006). MS patients and healthy controls participated in the study once a year over a 3-year period. Each year they completed high-density EEG session comprising of visual and auditory P3b and P3a ERP tasks and VESPA, which was complemented with a physical examination and the Paced Auditory Serial Addition Test (PASAT, a difficult task on attention and working memory) (Tombaugh, 2006). One baseline study also included MRI and a comprehensive NP test battery.

The cross-sectional studies revealed MS patients to have more delayed latencies and reduced amplitudes in the visual modality in both the early sensory and cognitive-indexing late P3b components. Similarly, MS patients showed delayed P3a latencies, reduced P3a amplitudes and atypical topographical distribution of the activations. PASAT score correlated with auditory P3b latency and visual P3a amplitude. RRMS and SPMS patients differed in visual P3a latency, and PPMS patients had decreased P3b amplitudes compared to SPMS. In addition, genetic factors were shown to be related to CI in MS. Moreover, the source P3 EEG activities in MS patients showed a widespread decrease in P3b-related theta power, and a greater reduction in alpha power at posterior sites. The MS patients with CI presented with similar theta power reduction. The subsequent studies investigated the P3 ERPs longitudinally. The visual P3b and P3a amplitudes and auditory P3a amplitude showed greater decrease over the 12-month period in MS. Similarly visual P3b latencies of MS patients increased gradually over the 24-month period. The 12-month change in the P3a ERPs was associated with the change in PASAT score. Multimodal baseline analysis revealed the NP tests measuring information processing speed, attention and working memory to be associated with the visual P3 latencies. The visual P3a latencies and amplitudes were related to lesion loads in widespread brain regions. The VESPA P100 latency was delayed in MS over the 24-month period. In conclusion, the cognitive-indexing electrophysiological measures offer complementary information on the brain functions related to cognitive function in MS, not available in the MRI and NP. The VESPA method was deemed a reproducible measure to probe visual dysfunction in MS. The electrophysiological measures have utility in clinical research, and potential for use in clinical practice.

Publications arising from the thesis

Journal papers

Kiiski, H., Reilly, R.B., Lonergan, R., Kelly, S., O'Brien, M., Kinsella, K., Bramham, J., Burke, E.T., O'Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N, & Whelan, R. (2012). Only Low Frequency Event-Related EEG Activity is Compromised in Multiple Sclerosis: Insights From an Independent Component Clustering Analysis. *PLoS ONE, 7(9)*: e45536. (Chapter 4, Appendix 6)

Kiiski, H., Reilly, R. B., Lonergan, R., Kelly, S., O'Brien, M., Kinsella, K., Bramham, J., Burke, T., O'Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N., & Whelan, R. (2011). Change in PASAT performance correlates with change in P3 ERP amplitude over a 12-month period in multiple sclerosis patients. *Journal of the Neurological Sciences, 305(1-2)*, 45-52. (Chapter 5, Appendix 7)

Kiiski, H., Whelan, R., Lonergan, R., Nolan, H., Kinsella, K., Hutchinson, M., Tubridy, N., Reilly, R.B. (2010). Preliminary Evidence for Correlation Between PASAT Performance and P3a and P3b amplitudes in progressive multiple sclerosis. *European Journal of Neurology, 18(5)*, 792-795. (Chapter 4, Appendix 5)

Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Hutchinson, M., Tubridy, N., & Reilly, R.B. (2010). Impaired information processing speed and attention allocation in multiple sclerosis patients versus controls: A high-density EEG study. *Journal of the Neurological Sciences, 293*, 45-50. (Chapter 4, Appendix 4)

Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Bramham, J., O'Brien, M., Reilly, R.B., Hutchinson, M., & Tubridy, N. (2010). A high-density ERP study reveals latency, amplitude, and topographical differences in multiple sclerosis patients versus controls. *Clinical Neurophysiology, 121*, 1420-1426. (Chapter 4, Appendix 3)

Conference papers and peer-reviewed abstracts

Kiiski, H., Reilly, R. B., Lonergan, R., Kelly, S., O'Brien, M., Kinsella, K., Bramham, J., Burke, T., O'Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N., & Whelan, R. (2011). Change in PASAT Performance Correlates with Change in P3 ERP Amplitude over a 12-Month Period in Multiple Sclerosis Patients, Poster, Proceedings of the 47th Irish Neurological Association meeting 2011, Sligo Ireland, May 12-13 2011. (Chapter 5)

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Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Bramham, J., O'Brien, M., Reilly, R.B., Hutchinson, M., & Tubridy, N. (2010). A High-density ERP Study Reveals Latency, Amplitude, and Topographical Differences in Multiple Sclerosis Patients versus Controls, Commented poster, Proceedings of the 16th Annual Bioengineering in Ireland, Malahide Ireland, January 2010. (Chapter 4)

Whelan, R. Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Bramham, J., O'Brien, M.C., Reilly, R.B., Hutchinson, M., Tubridy, N. (2009). ERP abnormalities in multiple sclerosis patients: A high-density EEG study, Irish Journal Of Medical Science, 179, 3, S106. (Chapter 4)

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Glossary of terms

AA	= two A alleles of oligoadenylate synthetase 1
AG	= one A allele and one G allele of oligoadenylate synthetase 1
BAI	= Beck anxiety inventory
BDI-II	= Beck depression inventory-II
BESA	= brain electrical source analysis / four-shell spherical head model
BRB	= the brief repeatable battery
BVMT-R	= the brief visuospatial memory test revised
CI	= cognitive impairment
CI-MS	= cognitively impaired multiple sclerosis
CIS	= clinically isolated syndrome
COWAT	= controlled word association test
CSF	= cerebrospinal fluid
CVLT-II	= the California verbal learning test-II
DAT	= dopamine transporter
DMT	= disease-modifying therapy
EDSS	= Kurtzke expanded disability status scale
EEG	= electroencephalography
EMG	= electromyogram / electromyographical
ERP	= event-related potential
ERSP	= event-related spectral perturbation
ET	= essential tremor
FASTER	= the Fully Automated Statistical Thresholding for EEG Artifact Rejection
FDR	= false discovery rate
FFT	= fast Fourier transform
fMRI	= functional magnetic resonance imaging
HRSD	= 21-item Hamilton rating scale for depression
IC	= independent component
ICA	= independent component analysis
IFN	= interferon
ITC	= inter-trial coherence
MACFIMS	= the minimal assessment of cognitive function in multiple sclerosis
MMN	= mismatch negativity
MRI	= magnetic resonance imaging
ms	= millisecond

MS	= multiple sclerosis
MSFC	= multiple sclerosis functional composite
non-CI-MS	= non-cognitively multiple sclerosis
OAS1	= oligoadelynate synthetase 1
ON	= optic neuritis
PASAT	= the paced auditory serial addition test
PCA	= principal component(s) analysis
PD	= Parkinson's disease
PET	= positron emission tomography
PPMS	= primary-progressive multiple sclerosis
RNAseL	= ribonuclease L
ROI	= region of interest
RRMS	= relapsing-remitting multiple sclerosis
SDMT	= symbol digit modalities test
SNP	= single nucleotide polymorphism
SNR	= signal-to-noise ratio
SNST	= Stroop neuropsychological screening test
SOC	= the sockings of Cambridge test
SPM	= statistical parametric mapping
SPMS	= secondary-progressive multiple sclerosis
SRT	= selective reminding test
tVEP	= transient visual evoked potential
V1	= primary visual cortex
VEP	= visual evoked potential
VESPA	= visual evoked spread spectrum analysis

Chapter 1 - Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease affecting the central nervous system of young adults in Western countries. In most cases it leads to severe and irreversible clinical disability (Filippi & Rocca, 2011; Rejdak, Jackson, & Giovannoni, 2010). Globally 1.3 people are estimated to be affected by MS, (WHO, 2008) whilst more than 7000 people live with MS in Ireland. MS was estimated to cost the Irish state over €65 million in 2004 (McHugh, 2007). MS is a complex disorder with variable symptoms and pathological heterogeneity. The disease is thought to act on genetically susceptible individuals who have been exposed to a set of environmental factors, which triggers an autoimmune attack on the myelin sheath of central nervous system axons. Some studies also suggest MS represents a primary neurodegenerative disease due to the failure of anti-inflammatory strategies to prevent disease progression (Rejdak, Jackson, & Giovannoni, 2010).

Earliest descriptions of this debilitating condition focused on the apparent and predominant physical symptoms (Rejdak, Jackson, & Giovannoni, 2010). For decades, these symptoms have defined the nature of MS but in recent years, there has been an increased interest in the possible cognitive disturbances particularly in relation to attention and memory. The cognitive dysfunction present in the relatively large proportion of MS cases (up to 65%) is not always recognised by practicing clinical professionals. Hence, more in depth research needs to be conducted, with the results communicated to clinical professionals and the wider population. Often the impact of cognitive difficulties on everyday life can be hard to see at first, but these deficits become more evident in the later stages of the disease. In particular, individuals with MS may have difficulty concentrating, remembering important information and making decisions. Nevertheless, these subtle cognitive impairments can significantly limit the independence and quality of life for those suffering from MS. For example individuals with MS may be forced to leave full-time employment as most modern jobs present significant challenges on cognitive processes. MS may also have an adverse impact on their social life and mental health. In economical terms, the exclusion of young individuals from the work force adds indirectly to the costs of MS for the state. This is unfortunate as it may be possible to maintain a career with MS, allowing for minor adjustments to work conditions and with appropriate treatment. The issue will become more urgent in the future as the incidence of MS is expected to increase (Koutsouraki, Costa, & Baloyannis, 2010). Therefore, it is crucial to detect cognitive dysfunctions at an early stage, when it is still possible to attempt to treat these deficits and to help MS patients to better manage their life. An increased understanding of cognitive functions in MS through scientific research, will allow earlier detection and

monitoring of cognitive deficits, with the potential for improved treatment options. It also offers MS patients more options to plan for the future and seek appropriate treatment options, as they are aware of the effects of MS on their cognitive functions. It is hoped that active multidisciplinary knowledge transfer would create innovative methods and technology to provide ways to diagnose, monitor and treat the cognitive dysfunction in MS. This would assist independent living, and ensure a better quality of life, appropriate treatment and support for MS patients.

Cognitive functions are commonly detected with neuropsychological examination, which involves performing numerous tests that present challenges to various cognitive areas. The subsequent test scores are then compared to the normative data available. These neuropsychological test results together with behavioural observations are utilised in order to obtain a comprehensive account of a patient's cognitive functioning. The neuropsychological examination is the gold standard used to detect and monitor cognitive functions. However these measures are limited by various interfering factors. Due to the practice effects inherent in most neuropsychological tests, they can be reliably repeated only at certain intervals, typically one year apart from each other. They also risk being influenced by subjective factors, as the experience and skills of the individual performing the tests may affect the results. This is not an issue when fully trained and professional clinical neuropsychologists who have the necessary skillset and understanding of the properties and limitations of the psychometrics underlying the tests to conduct the neuropsychological assessment. However, these in-person sessions are time-consuming and expensive and thus, they should only be reserved for patients in need of a comprehensive neuropsychological assessment. Nevertheless, this issue has sometimes led to other, non-trained clinical personnel with limited knowledge of the nature of neuropsychological assessment conducting these tests. Hence, the results may not be a true reflection of the person's cognitive level. Furthermore, the neuropsychological tasks can be affected by the mental state of the subject, as depression, anxiety and fatigue have all been reported to affect the cognitive performance of MS patients (Arnett, et al., 1999; Demaree, Gaudino, & DeLuca, 2003; Diamond, Johnson, Kaufman, & Graves, 2008; Gilchrist & Creed, 1994; Litvan, et al., 1988; Thornton & Raz, 1997). The performance in neuropsychological tests can also be affected by demographic factors such as age and education, and by physical disability such as dysfunctions in speech and motor abilities (Magnano, et al., 2006). Another limitation is the loss of data due to the subject's reluctance to complete the challenging tasks (Tombaugh, 2006). Furthermore, the neuropsychological assessment does not provide information on the underlying brain activations and therefore the results need to be examined in adjunction with neurological and/or brain imaging results.

Electroencephalography (EEG) is a measure of electrical changes in the brain, which present as spikes that are transient and seemingly random events and rhythms (Kaiser, 2005). The measured electrophysiological activities can be associated with cognitive functions when the subject performs a cognitively demanding task during the EEG recording. The resulting task related electrophysiological activities are called the event-related potentials (Polich, 2007). There is a need to complement the information obtained from the conventional neuropsychological examination, due to its limitations listed earlier in the chapter. Including cognitive-indexing electrophysiological measures to the assessment procedure of CI in MS would provide a comprehensive picture of the cognitive difficulties and their underlying brain functions. Moreover, MS patients often decline to perform some of the most challenging neuropsychological tasks causing them distress, or are unable to complete them due to hand movement and/or speech impairments. Therefore, the electrophysiological measures may provide a means to offer additional information on the brain functions related to cognitive impairment in MS in a more objective, reliable and stress-free manner.

With the anticipated future advances in electrophysiological technologies, the EEG methods have the potential to be adapted as part of the clinical practices. Similar to the present EEG methods that are widely utilised to determine the nature of epileptic seizures, sleep disorders and visual dysfunction. Conventionally utilised event-related potential measures, comprise of numerous challenges, including, most importantly, the limited coverage of the scalp area and outdated signal processing methods. In the present research project high-density EEG array with electrode coverage over the entire scalp area is employed, which enables the utilisation of highly sophisticated EEG data analysis methods. Thus, this provides a more thorough insight into the electrophysiological responses related to cognitive functions in MS over previous research in this area. The main aim of this research is to determine if electrophysiological measures have the potential to serve as objective, sensitive and reliable ways to measure and monitor brain activations related to cognitive functions in MS.

Despite the recent enthusiasm over brain imaging technologies, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), offering high spatial resolution and the ability to detect cellular activity in subcortical structures of the brain, EEG measures still have various insuperable advantages. Most importantly, EEG provides higher temporal resolution than brain imaging methods. Furthermore, EEG is a reflection of the summation of cortical postsynaptic potentials and signal conduction along the axons, which are expected to be adversely affected in MS due to demyelination. Yet recent brain imaging methods merely measure the cell metabolism of neurons (blood flow, blood volume,

oxygenation) (Kaiser, 2005). In addition to these benefits, EEG technology also offers psychological and physical convenience. It is less expensive and portable in comparison to other neuroimaging methods, and has a long history of investigation spanning over 80 years (Millett, 2001). Measuring EEG involves wearing a simple cap or string of electrodes and it is the preferred method for young children along with neurological and psychiatric populations. On the other hand, these novel methods proposed in the present research require considerable computational power, skilled staff to analyse the acquired data and the high-density EEG can take from 30 - 60 minutes to set-up. It is anticipated that these obstacles will be reduced as new, more advanced technology, an increase in computational power, and user-interfaces designed for clinical environments, are developed and become available. Furthermore, the future of EEG looks promising as advances in telemetry and dry electrodes have the potential to eliminate the inconveniences related to measuring EEG. When considering the cost, EEG equipment is significantly more affordable than brain imaging technologies. In recent years, simultaneous EEG and fMRI (co-registration) has enjoyed popularity as it can combine the strengths of both approaches. However at the moment it is still not yet economically viable for clinical research and/or clinical practice.

There is also a demand for systematic, thorough studies which utilise large, representative groups and novel methods, to investigate the different aspects of cognitive functions in MS and its relationship to the brain function or dysfunction, which is not common in the research of MS or several neurodegenerative conditions. Therefore, the main impact of this research is to provide insight and novel information about the brain functions related to cognitive processing in MS both cross-sectional and longitudinal study design, and to find associations between the different measures of cognitive and brain functions. As the emergence of a unified cognitive moment relies on the coordination of scattered mosaics of functionally specialized brain regions (Varela, Lachaux, Rodriguez, & Martinerie, 2001), this research aims to locate and match these pieces together in MS by using the electrophysiological, brain imaging and neuropsychological measurements. It is hoped these methods and the information they provide could be applied in the study of other neurological conditions.

One of the main objectives of this thesis is to determine if the proposed novel electrophysiological methods can be applied as potential tools to detect and monitor the changes associated with the dysfunctions in cognitive and brain functions in general. The realisation that MS patients often present with cognitive deficits is a relatively new approach in the study of MS. Furthermore, the advanced electrophysiological methods offer opportunities that are likely to interest key professionals in clinical settings. The cognitive-

indexing electrophysiological measures are not routinely conducted in clinical settings. Hence as the methods become more fine-tuned and advanced, there is a potential for trained technicians to conduct the tests, as part of the clinical practice. This will add essential information on the brain and cognitive functioning to the clinical profile of each MS patient. These electrophysiological measures can potentially act as screening tests for cognitive function that direct the MS patient with signs of subtle cognitive deficits to a comprehensive neuropsychological assessment. They could also complement the neuropsychological findings and act as an objective means of confirming the findings from MS patients with depression, motor and speech impairments and other confounding factors interfering with the conventional neuropsychological examinations. These cognitive-indexing electrophysiological measures may also provide a useful tool in clinical research, when recruiting potential study participants to studies focusing on cognitive impairment in MS. Moreover, they may benefit clinical research by stratifying the risk in potential study candidates prior to their entry into clinical trials of drug therapies. In addition, they offer an objective way to screen subjects in order to reduce variability in trials and ultimately decrease the size, length and cost of clinical trials.

The increased understanding of the nature of cognitive dysfunction present in MS, and its progression related to disease activity and physical disability enhances the opportunities to predict the progression of cognitive deficits in MS. With early detection, a thorough understanding of the cognitive deficits and their underlying brain functions would offer a means to evaluate the appropriate treatment option for each patient. Once the specific nature and brain functions related to cognitive impairment in MS are delineated, it is possible to tailor drug therapies to take into account the effect that disease-modifying drugs have on cognitive functions. It is hoped new drug therapies can be designed to not adversely affect, and possibly improve, the cognitive functions in MS. In addition, neuropsychological rehabilitation has been proposed as an optional means to treat the cognitive impairments in MS, but the research is scarce and provides contradictory evidence on its effectiveness (Chiaravalloti, et al., 2005; O'Brien, Chiaravalloti, Goverover, & Deluca, 2008).

The research conducted is novel and unique in many respects. Firstly, high-density EEG is rarely utilised in clinical research involving electrophysiological studies of neurological conditions. Therefore, the previous cognitive-indexing electrophysiological studies in MS have lacked a thorough understanding of the brain and subtle cognitive functions in MS and the specific nature of these abnormalities. More importantly, only very few studies have longitudinally examined the electrical activities related to cognitive functions in MS, spanning

only up to a 2-year period (Flechter, et al., 2007; Gerschlager, et al., 2000a; Pliskin, et al., 1996; Pokryszko-Dragan, et al., 2009). The studies conducted in this thesis employed P3b and P3a event-related potential (ERP) paradigms. The P3b ERPs are averaged responses of the brain to detect improbable events in a train of frequent stimuli. Furthermore, the P3a is elicited by unexpected events in the train of attended frequent and infrequent stimuli. The P3b and P3a ERPs are thought to index cognitive function (Leocani, Gonzalez-Rosa, & Comi, 2010; Polich, 2007). These two cognitive-indexing electrophysiological paradigms utilised in the present project, serve as a new innovative way to complement the assessment of brain and cognitive function in MS.

This research project involved a multiprofessional network of researchers and experienced clinical practitioners from the Trinity Centre for Bioengineering, the Department of Neurology in St. Vincent's University Hospital and the University College Dublin Psychology Department, which made it possible to investigate the cognitive functions in MS in a comprehensive way. The facilities included state-of-the-art research facilities of high-density EEG system (Biosemi) with novel signal processing methods, a 2T MRI scanner and a comprehensive neuropsychological test battery tailored for the MS population.

The research conducted as part of this thesis aims to define the nature and progression of brain and cognitive dysfunctions in MS. The EEG methodology has been proposed as a potential biomarker for diagnosis and monitoring of cognitive and brain function in neurological disorders (Klassen, et al., 2011; Ziemann, Wahl, Hattingen, & Tumani, 2011). Previous EEG research performed in MS patients normally adopts a cross-sectional design (Magnano, et al., 2006). For longitudinal studies, the cognitive functions of MS patients have usually included either neuropsychological or MRI measures, excluding electrophysiological methods (e.g. Camp, et al., 2005b). The focus of this research is on cognitive-indexing electrophysiological measures and advanced analysis methods, to function as secondary outcome measures for detecting and monitoring brain function related to cognitive impairment in MS. Previous research has found MS patients to have delayed and reduced electrophysiological responses (Magnano, et al., 2006). In addition, deficits in certain cognitive functions including information processing speed, attention and memory have been found in MS patients (Denney, Gallagher, & Lynch, 2011; Denney, Sworowski, & Lynch, 2005; Lengenfelder, et al., 2006a; Parmenter, Shucard, Benedict, & Shucard, 2006; Paul, Beatty, Schneider, Blanco, & Hames, 1998a), of which the P3 event-related potential tasks applied during the EEG recording are thought to index. The cognitive deficits have been shown to increase over time in MS patients affected by subtle cognitive disturbances, at the early stages

of the disease course (Kujala, et al., 1997). These adverse changes are theorized to be caused by brain changes in MS, such as demyelinating lesions in widespread areas of the cerebral cortex and brain, in particular the cortical atrophy (Benedict & Zivadinov, 2011).

The present research involved various studies. The cross-sectional studies investigated the electrophysiological responses to the P3 event-related potential tasks in MS patients and in different MS subgroups, based on the disease course or the genotype information available. The influence of genetic factors on brain and cognitive functions in MS is not conclusive yet and more candidate genotypes, such as the proposed OAS1 genotype related to disease activity, need to be tested. Moreover, the electrophysiological P3 activations were studied at their estimated sources by using a novel EEG analysis method, the independent component clustering. The longitudinal analyses were conducted with the data acquired at baseline, 12-months and 24-months. Early findings from the multimodal analysis of the EEG, MRI and neuropsychological data are also included. The thesis also comprises a baseline and a 3-year follow-up study of the utilisation of Visual Evoked Spread Spectrum Analysis (VESPA) to probe and monitor visual dysfunction, commonly observed in MS. All studies comprised a comparable group of healthy volunteers, to guarantee that the differences and changes observed were not merely fluctuations in the healthy range of brain and cognitive functioning.

The principal objective of the research is to examine if the proposed electrophysiological methods can be applied as potential tools to detect and monitor the changes associated with the dysfunctions in cognitive and brain functions in MS. A number of general hypotheses were posed based on previous research. It is hypothesized that MS patients (especially progressive MS and those with OAS1 genotype related to increased disease activity) have reduced and delayed scalp-based and source P3 EEG activations and poorer performance in the neuropsychological task the Paced Auditory Serial Addition Test (PASAT), compared to healthy controls. It was hypothesized that the P3 EEG activations would correlate with the PASAT scores. Furthermore, MS patients were hypothesized to show greater prolongation and decrease of the P3 EEG activations, and a reduction in the PASAT score, compared to healthy controls over the 12-month and 24-month follow-up periods. In the multimodal investigation, the delayed and reduced P3 EEG activations were hypothesized to be associated with poor neuropsychological test scores and increased lesion load. Finally, the VESPA-like P100 components were hypothesized to be reduced and delayed at baseline in MS patients in comparison to healthy controls and show gradual delay in latency and reduction in amplitude, over the 24-month period.

The thesis is organised into a number of chapters. Chapter 2-Literature Review describes in detail the disease features and cognitive deficits of MS. This is followed by a description of the methods utilised to detect and monitor brain and cognitive function in MS. These include the conventional methods such as the neuropsychological examination, MRI and VEP, and the promising, new methods including EEG, P3 ERPs and VESPA. The thesis objectives are detailed at the end of Chapter 2-Literature Review. Chapter 3-General Methods depicts the study procedure and measures utilised in the research including the electrophysiological measures of the EEG, the P3 ERP and the VESPA, in addition to the MRI method, psychological measures, clinical assessment measures and genetic measures. Chapter 4 - Cross-sectional P3 ERP studies details the studies examining the scalp-based and source P3b and P3a ERP activations, and the PASAT performance, at baseline in MS patients and in different MS subgroups based on their subtype, cognitive functioning or OAS1 genotype. In Chapter 5 the P3 ERP activations and the PASAT performance in MS patients are examined over a 12-month and 24-month period. Chapter 6 comprises the cross-sectional investigation of the associations between high-density P3 ERP, MRI and neuropsychological performance. Chapter 7 delineates the cross-sectional and longitudinal analysis of the utility of the VESPA to probe visual dysfunction in MS. Chapter 8 discusses the main findings of the research in relation to previous literature. In addition, it considers the utility and impact of the electrophysiological measures to detect and monitor brain and cognitive function in MS and neurological conditions and future directions and implications.

Chapter 2 - Literature Review

This chapter will provide a comprehensive overview of multiple sclerosis and cognitive disturbances characteristic to it. In addition, the methods utilised to measure brain and cognitive function in multiple sclerosis, and the main findings acquired with these measures, are presented in detail. Firstly, the conventional measures of neuropsychological examination, MRI and VEP are described, followed by a depiction of the new, innovative measures including the EEG, the P3 ERPs and the VESPA. The explicit thesis objectives are detailed at the end of the chapter.

2.1. Multiple sclerosis

Multiple sclerosis (MS) is a complex disorder with pathological heterogeneity, requiring genetically susceptible individuals to be exposed to a set of environmental factors, which trigger an autoimmune attack on the myelin sheath of central nervous system axons. Some studies suggest MS represents a primary neurogenerative disease due to the failure of anti-inflammatory strategies to prevent disease progression. MS is the most common inflammatory demyelinating disease of the central nervous system, and the most common disabling neurological condition in young adults (Rejdak, et al., 2010).

2.1.1. Epidemiology, pathogenesis and etiology of multiple sclerosis

Globally, 1.3 million people are estimated to be affected by MS, although the figure is believed to be higher as some countries do not record data (WHO, 2008). In Ireland, more than 7,000 people live with MS, and MS has been estimated to have cost the Irish state over €65 million in 2004 (McHugh 2007). The incidence of MS has increased in recent years (Koutsouraki, et al., 2010). Nearly 70% of patients present with symptoms between ages 20-40, and the disease onset rarely occurs earlier than 10 years or after 60 years of age. The average age of onset is approximately 30 years (Rejdak, et al., 2010). Females are more likely to develop MS than males, with the female to male ratio being around 2.0 (Pugliatti, et al., 2006). In MS, the median time to death is approximately 30 years from disease onset and the average reduction in life expectancy is nearly 10 years compared to the general population (Bronnum-Hansen, Koch-Henriksen, & Stenager, 2004).

The main pathogenesis of MS has long been thought to be an autoimmune disorder of the central nervous system by T lymphocytes auto reactive for myelin proteins (Hafler, et al., 2005; Mars, Saikali, Liblau, & Arbour, 2011; McFarland & Martin, 2007). Nevertheless, many other immunopathological processes also play a role. Most importantly, these include primary apoptosis of oligodendrocytes (Barnett & Prineas, 2004), dysfunction of regulatory T cells (Zozulya & Wiendl, 2008), or B cell mediated autoimmunity (Franciotta, Salvetti, Lolli, Serafini, & Aloisi, 2008; Meinl, Krumbholz, & Hohlfeld, 2006). Notably, axonal degeneration already occurs in the initial stages of MS (Ferguson, Matyszak, Esiri, & Perry, 1997; Kuhlmann, Lingfeld, Bitsch, Schuchardt, & Bruck, 2002; Trapp, et al., 1998) and it is regarded as the dominating pathologic correlate of the irreversible, and over time progressive neurologic impairment in MS (Lassmann, 2010). Furthermore, the relationship between inflammation and neurodegeneration is intensely debated (Trapp & Nave, 2008). On one hand, it has been shown that axonal and neuronal degeneration occur predominantly in active demyelinating lesions (Peterson, Bo, Mork, Chang, & Trapp, 2001) with a highly significant association between inflammation and axonal injury throughout all stages of MS (Frischer, et al., 2009). On the other hand, there is convincing evidence of immune-mediated axonal damage in the absence of directly co-localized active demyelination, indicating that the pathogenesis of axonal degeneration is at least in part independent from demyelination (Bitsch, Schuchardt, Bunkowski, Kuhlmann, & Bruck, 2000; DeLuca, Williams, Evangelou, Ebers, & Esiri, 2006).

The etiology of MS needs further clarification. The general consensus formulated in recent years states that MS is an autoimmune disease triggered by environmental agents, which act in genetically susceptible people (Rejdak, et al., 2010). Therefore, the possible cause of MS involves both environmental exposure and genetic susceptibility. Environmental factors suggested to cause MS include infections, such as Epstein-Barr virus (Levin, Munger, O'Reilly, Falk, & Ascherio, 2010; Levin, et al., 2003), low sunlight, Vitamin D deficiency, diet (Ascherio, Munger, & Simon, 2010; Pierrot-Deseilligny & Souberbielle, 2010), smoking (Hawkes, 2007), air pollutants, toxins, and stress (Hernan, et al., 2005; Jafari & Hintzen, 2011; Marrie, 2004). Interestingly, MS prevalence varies according to the latitude gradient; it is greatest at the extremes of latitude in both northern and southern hemispheres. Furthermore, MS is more common in subjects with Northern and Western European ancestry (Poser, 1994; Rejdak, et al., 2010). However, a recent meta-analysis reported a disappearance of this latitude gradient (Koch-Henriksen & Sorensen, 2011).

Migration studies have shown that the risk of developing MS has been correlated with the place where early life was experienced (Compston & Coles, 2008). On the other hand, there

are ethnic groups that appear to be resistant to MS in spite of living in areas of relatively high prevalence rate, such as Maoris in New Zealand and Samis in the Nordic countries (Pugliatti, et al., 2006; Rejdak, et al., 2010), indicating hereditary causes to MS. MS is 20 times more likely to occur in first-degree relatives than in the general population. The clinical concordance rate in monozygotic twins has been estimated to be as high as 30% relative to rates of less than 5% in dizygotic twins (Dyment, Ebers, & Sadovnick, 2004). Genetic factors associated with increased risk of MS include polymorphisms in MCH class II alleles (Dyment, et al., 2004; International Multiple Sclerosis Genetics Consortium, 2007), the interleukin-7 receptor alpha gene (IL7RA), the interleukin-2 receptor alpha gene (IL2RA (=CD25)) (International Multiple Sclerosis Genetics Consortium, 2007), apolipoprotein E (ApoE) (Kantarci, de Andrade, & Weinshenker, 2002), C-type lectin family 16 member A (CLEC16A), 60S ribosomal protein L5 (RPL5), EV15 locus and CD40, and rs703842 on chromosome 12 (Oksenberg, Baranzini, Sawcer, & Hauser, 2008).

2.1.1.1. Oligoadenylate synthetase 1 and disease activity in multiple sclerosis

Furthermore, oligoadenylate synthetase 1 (OAS1) -genotypes have been associated with the different levels of disease activity in MS. A number of MS patients in the present research project were tested for their OAS1 -genotype, which is linked to type 1 interferons (IFNs). IFNs are innate immune cytokines that activate the JAK/Stat signalling pathway to induction of IFN-stimulated genes. The 2',5' -OAS family is central to the type 1 interferon (IFN) pathway for viruses whose replication includes production of double-stranded RNA. One member of this family of proteins is the OAS1, which induces ribonuclease L (RNAseL), resulting in degradation of viral RNA, inhibition of virus replication, and promotion of cellular apoptosis (Mullan, et al., 2005). Several OAS1 polymorphisms have been reported; one located at the exon 7 splice-acceptor site results in alternative splicing of the OAS1 mRNA. The G allele conserves the splice site, generating a p46 enzyme isoform, whereas the A allele ablates the splice site, resulting in a dual-function antiviral/proapoptotic p48 isoform and a novel p52 isoform (Bonnevie-Nielsen, et al., 2005). These alleles have been linked with altered enzyme activity, with OAS1 enzyme activity varying in a dose-dependent manner across the GG, AG and AA genotypes, with the G allele conferring high and the A allele low activity.

Differential OAS1 enzyme activity dependent on OAS1 genotype may determine, in part, the degree of antiviral or proapoptotic effectiveness of endogenous interferons, possibly influencing susceptibility to MS. The GG genotype (associated with higher enzyme activity) occurs at a lower frequency in MS patients compared to a control population, while the AA

genotype (associated with lower activity of OAS1) is evident at a higher frequency in MS patients (M. O'Brien, et al., 2010). Furthermore, the patients possessing only the A allele (AA) have decreased OAS1 disease (anti-inflammatory) activity, in comparison to those with the G allele (AG or GG) (M. O'Brien, et al., 2010). These results emphasise the role of the innate immune response in the etiology of MS. Thus, the reduced enzyme activity (when OAS1 activates RNAseL) caused by the presence of the A allele, does not effectively lead to the degradation of RNA, and the suppression of virus replication (M. O'Brien, et al., 2010). This may lead to infection, and subsequently trigger an autoimmune cascade of events (Grigoriadis & Hadjigeorgiou, 2006). It has also been speculated that the high frequency of the A allele observed in MS patients may indicate a tendency for apoptosis of anti-inflammatory cells, leading to excessive and prolonged inflammatory responses (M. O'Brien, et al., 2010).

2.1.2. The disease course of multiple sclerosis

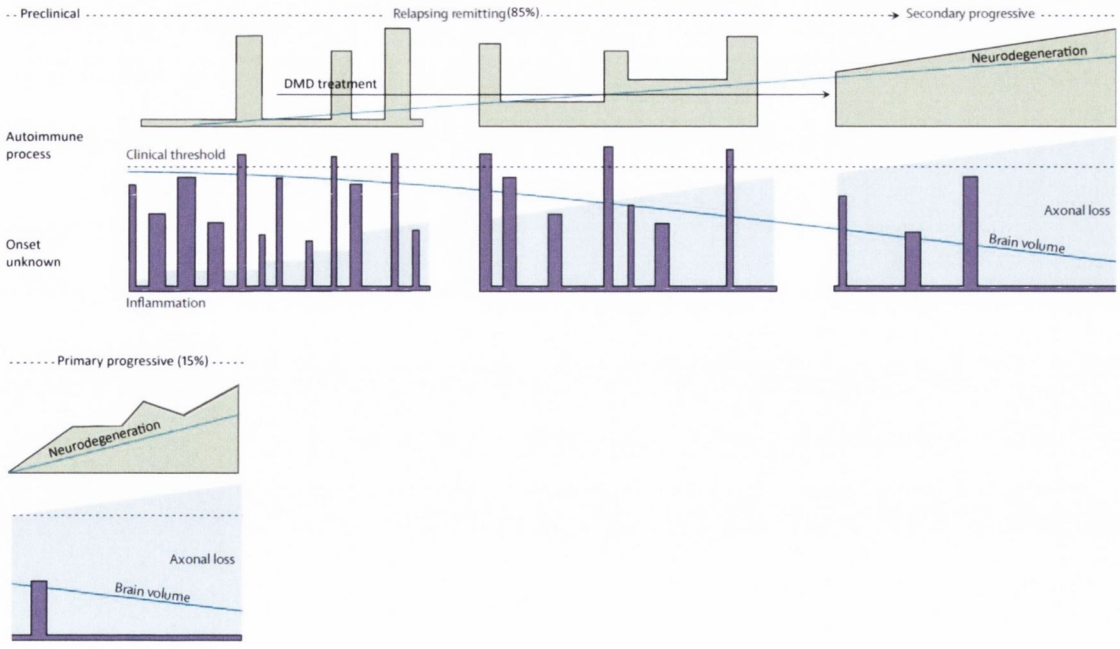
There is a wide interpatient variability of MS disease progression but several classifications of MS subtypes have been proposed, the most commonly used subtype classification was introduced by Lublin and Reingold (Lublin & Reingold, 1996), see Figure 2.1). At onset, approximately 85% of patients are categorised into the relapsing-remitting subtype (RRMS) (Lublin & Reingold, 1996; Rejdak, et al., 2010). RRMS is defined as a disease course that has relapses requiring clinical care, which are followed by a complete or partial recovery, and periods of stable course between the attacks, i.e. remission (Lublin & Reingold, 1996). Relapse in MS was originally defined as a focal disturbance of function, affecting white matter tract, and it is thought to last at least 24 hours, preceded by over 30 days of clinical stability (Schumacher, Beebe, Kibler, Kurland, Kurtzke, McDowell F, et al., 1968). Yet, more recently there has been discussion into regarding a minimum of 48 hours of symptom duration, coupled with changes in functional measures such as EDSS to the definition (Vollmer, 2007). The risk of a clinically manifesting relapse is significantly increased due to infections. There is some, but not conclusive, evidence that live vaccination, postpartum period of pregnancy, and stressful life events also increase the relapse risk (Compston & Coles, 2008; Rejdak, et al., 2010).

Generally, patients with the RRMS disease course eventually turn into the second phase of the disease called the secondary-progressive stage (SPMS). SPMS patients experience progression of the disease with fewer relapses and less complete recovery, resulting in accumulating disability over time. The disease progression will also occur between and without any relapses (Lublin & Reingold, 1996). At individual level, it is difficult to determine the transition from

RRMS to SPMS, but it is estimated that the transition begins at around 40 years of age (Confavreux & Vukusic, 2006). Moreover, it is anticipated that approximately 65% of the RRMS patients have entered the SPMS phase later than 25 years from its onset (Runmarker & Andersen, 1993). Roughly 10-15% of MS patients (Thompson, et al., 1997), who do not develop the RRMS disease course at onset, are classified to have primary-progressive subtype (PPMS). It is characterised by a gradual, nearly continuous decline over time with minor fluctuations, but with an absence of relapses (Lublin & Reingold, 1996).

In most patients (80%), clinically isolated syndrome (CIS) is the earliest indication of MS, in which they present with an acute episode affecting one or more sites. Moreover, other small subtype groups of MS have been proposed. Small numbers of patients have benign MS, in which the patient remains fully functional in all neurologic systems 15 years after disease onset (Kantarci & Weinshenker, 2005). Finally, progressive relapsing MS is the rarest subtype, defined as progressive disease from onset, but with distinct, acute relapses with or without recovery (Lublin & Reingold, 1996). CIS, benign and progressive relapsing MS subtype classifications were not included in the present study, which focused on the most common subtypes; RRMS, SPMS and PPMS.

Figure 2.1. The course and disease stages of the most common clinical subtypes of MS (adapted after (Compston, & Coles, 2008; Ziemann, et al., 2011).



Note. DMD treatment = disease-modifying drug treatment.

2.1.3. Brain changes in multiple sclerosis

Generally, brain changes due to MS are thought to be predominantly in the white matter, but small lesions are also found in the grey matter cortices and the deep grey matter structures. Macroscopic lesions can be observed in the spinal cord (50%), optic nerves (25%), brainstem/cerebellum (20%) and periventricular white matter (5%) (Rejdak, et al., 2010); see Table 2.1). The lesions are the result of a focal loss of myelin, with a relative preservation of axons and astrocytic gliosis. The pathological features of the lesions differ, and are influenced by location, age and possible regeneration. Extensive cortical demyelination is typically more predominant in patients with a progressive disease course (Rejdak, et al., 2010). Whole brain atrophy occurs at a faster rate in untreated MS patients (decrease in brain volume 0.5-1% per year) compared to healthy controls (0.1-0.3%), with the speed of the atrophy being independent of disease stage (De Stefano, et al., 2010; Filippi, et al., 2004). The brain atrophy measures are clinically relevant, as they are moderately associated with clinical disability (Miller, Barkhof, Frank, Parker, & Thompson, 2002; Minneboo, et al., 2008). However, the whole brain atrophy measures lack of specificity for location, and for the exact processes occurring in the tissue, i.e. grey matter versus white matter degeneration.

There is extensive evidence for grey matter demyelination, neuronal loss and atrophy (Kidd, et al., 1999; Kutzelnigg, et al., 2005; Peterson, et al., 2001). Grey matter atrophy is present in all MS stages and subtypes, although in the SPMS disease course atrophy rate is higher (Fisher, Lee, Nakamura, & Rudick, 2008; Tiberio, et al., 2005), and it is related to the level of clinical disability (Dalton, et al., 2004; Fisher, et al., 2008). Nevertheless, the lesion load and lesion volumes measured with T1- and T2-weighted MRI are at best only modestly correlated with clinical disability (Brex, et al., 2002; Molyneux, et al., 1998; Sahraian, Radue, Haller, & Kappos, 2010). The weak correlation may be due to many factors, for example frequent lesion location in non-eloquent brain regions in MS (Ziemann, et al., 2011). Some of the new imaging technologies proposed for the detection and monitoring of brain changes in MS include contrast magnetization transfer ratio (MTR), diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) (Ziemann, et al., 2011).

Table 2.1. Symptoms of multiple sclerosis by brain areas most affected in multiple sclerosis (adapted after Compston & Coles, 2008)

Brain area	Symptoms
cerebrum	cognitive impairment hemisensory and motor affective (mainly depression) epilepsy (rare) focal cortical deficits (rare)
optic nerve	unilateral painful loss of vision
cerebellum & cerebellar pathways	tremor clumsiness&poor balance
brainstem	diplopia, oscillopsia vertigo impaired swallowing impaired speech and emotional liability paroxysmal symptoms
spinal cord	weakness stiffness and painful spasms bladder dysfunction erectile impotence constipation
other	pain fatigue temperature sensitivity and exercise intolerance

2.1.4. The symptoms and the diagnosis of multiple sclerosis

MS is a highly unpredictable disease. The symptoms are extremely diverse, and MS has a highly variable clinical course. Most of the patients present with deficits in motor, sensory, visual and autonomic systems, but many other symptoms and signs can occur. The clinical presentation varies greatly both at onset and over time, which is a reflection of the number of brain areas affected by demyelinating plaques (see Table 2.1., Compston & Coles, 2008). Only a few of the clinical presentations are disease-specific, but especially characteristic are the Lhermitte's symptom (a sensation of electrical current running down the spine), and Uhthoff phenomena (transient worsening of symptoms when core temperature increases, such as after a hot bath or exercise) (Compston & Coles, 2008). Furthermore, depression and fatigue are common among MS patients (Bakshi, 2003; Demaree, et al., 2003). Fatigue is usually induced by activity, gets worse as the day progresses, aggravates MS symptoms and is alleviated by a short period of rest. The clinical presentation varies greatly both at onset, and with time which is a reflection of the number of brain areas affected by demyelinating plaques (Bakshi, 2003).

Factors considered to be good prognostic signs include female sex, onset of MS early in life, an initial presentation of optic neuritis or sensory symptoms, full recovery from the first attack, a long period of time between the first and second attacks, and a low baseline lesion load on MRI (Rejdak, et al., 2010).

Diagnosing MS can be challenging as patients present with a variety of symptoms, which are for the most part nonspecific to MS, and considered minor or transient at onset. This leads to challenges in differential diagnosing, as well as a considerable delay between the first presenting symptoms of MS, and the actual investigation and diagnosis of the condition. There are no definitive tests for diagnosing MS. The revised 2010 McDonald criteria (see Table 2.2., Polman, et al., 2011) is widely used to diagnose MS, only after other possible disease states have first been excluded. The MS diagnosis in revised 2010 McDonald criteria is based on clinical presentation, brain imaging and laboratory data. It requires at least two documented clinical deteriorations of the condition which are separated by at least 30 days. In the absence of clinical symptoms, MRI can sometimes be used solely to diagnose multiple sclerosis. The diagnosis based on MRI requires two distinct gadolinium-enhancing lesions in MR images with at least 3 months between images, or two distinct T2 lesions on MR images separated by 30 days. The validity of MRI as a diagnosing method has been confirmed by studies reporting more than 80% of patients having initially abnormal MRI to be converted to a clinically definite MS over a 20-year period (Fisniku, et al., 2008). Furthermore, additional tests, such as cerebrospinal fluid (CSF) analysis, and visually evoked potentials (VEPs) are not required by diagnostic criteria, but they are often completed with older patients, patients that have progressive onset, and where MRI is unavailable and/or non-diagnostic. Asymmetrically delayed VEPs imply for demyelinating lesion(s) within the optic nerve (Polman, et al., 2011).

The diagnosis of MS after the first attack may become increasingly important, as there is accumulating proof that early disease-modifying therapy will delay or slow the progressive phase of the disease (Goodin & Bates, 2009).

Table 2.2. The revised 2010 McDonald diagnostic criteria for multiple sclerosis (Polman, et al., 2011).

Clinical Presentation	Additional data required for MS diagnosis
Two or more attacks, objective clinical evidence of two or more lesions	None
Two or more attacks; objective clinical evidence of one lesion	Dissemination in space, demonstrated by: • One or more T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord); or • Await further clinical attack implicating a different CNS site
One attack; objective clinical evidence of two or more lesions	Dissemination in time, demonstrated by: • Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or • A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or • Await a second clinical attack
One attack; objective clinical evidence of one lesion (clinically isolated syndrome)	Dissemination in space and time, demonstrated by: • For: DIS • More than one T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord); or • Await for a second clinical attack implicatin a different CNS site; and • For DIT: • Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or • A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or • Await a second clinical attack
Insidious neurological progression suggestive of MS (PPMS)	One year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria: 1. Evidence for DIS in the brain based on one or more T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. Evidence for DIS in the spinal cord based on two or more T2 lesions in the cord 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

Note. PPMS = primary-progressive MS patients, CNS = central nervous system, MRI = magnetic resonance imaging, DIS = dissemination in space, DIT = dissemination in time, CSF = cerebrospinal fluid, IgG = immunoglobulin G

2.1.5. Treatment of multiple sclerosis

At present MS cannot be cured. Nevertheless, there has been a surge of new treatments to MS in the past 20 years (Table 2.3). Current therapies can suppress or reduce relapses, and it is hoped, that treating patients early in the disease course may provide an opportunity to prevent relapses and delay transition from RRMS to SPMS (Vollmer, 2007). However, once the disease progression begins, it advances independently of relapses and the condition responds poorly to available treatments, particularly in the secondary-progressive disease course (Kremenchutzky, Rice, Baskerville, Wingerchuk, & Ebers, 2006). Therefore, symptomatic and physical therapies remain the most effective means to manage MS over the long-term (Rejdak, et al., 2010). The new trends in the area are the intention to provide appropriate treatment tailored specifically to each patient, to offer effective therapy for SPMS patients, and to discover new, user-friendly treatment options (e.g. oral tablets) (Gawronski, Rainka, Patel, & Gengo, 2010; Miller, et al., 2008).

Current treatments available for the early stages of MS are interferon β, glatiramer acetate, natalizumab, and mitoxantrone (Gawronski, et al., 2010). Interferon-β-1a and interferon-β-1b (Oliver, Kohli, & Kasper, 2011) and glatiramer acetate (Ruggieri, Avolio, Livrea, & Trojano, 2007) are the most effective treatments for RRMS as they have successfully been shown to decrease the frequency of relapses (by approximately 30%), and slow down disease progression and disability. Interferon β is available as interferon β-1a or interferon β-1b. Interferon β-1a is administered once a week, by using an intramuscular or 3 times a week by a subcutaneous formulation, while interferon β-1b is available as a subcutaneous formulation given every other day (Gawronski, et al., 2010). The exact mechanism of interferon β is still unknown, but it is proposed that interferon β works by suppressing T-helper cell response, causing reduced T-cell migration across the blood-brain barrier (Kim, 2009; Menge, et al., 2008). Common adverse effects of interferon therapy include flu-like symptoms after injection, depression, and liver enzyme level abnormalities (Gawronski, et al., 2010).

Glatiramer acetate is another first-line treatment for MS, which is administered daily as a subcutaneous injection. The underlying mechanism behind the beneficial effect of glatiramer acetate is not fully elucidated, but it is thought to be related to the alteration of T-cell activation and differentiation (Menge, et al., 2008). Its adverse effects include injection-site reactions (indurations, masses) and depression. However, it is a suitable choice for patients unable to tolerate flu-like symptoms or have a coexisting depressive disorder (Gawronski, et al., 2010). Natalizumab is a humanized monoclonal antibody that antagonizes α4-integrin of

the adhesion molecule very late activation antigen (VLA)-4 on leukocytes. Inhibition of VLA-4 is responsible for blockade of T cells across the blood-brain barrier (Gawronski, et al., 2010; Menge, et al., 2008; Thompson, Noyes, Dorsey, Schwid, & Holloway, 2008). Natalizumab has been found to be very effective at decreasing relapse rates and slowing disease progression (Gawronski, et al., 2010). Nevertheless, natalizumab is considered to be a second-line treatment for patients non-responsive or intolerant to first-line therapies as it is reported to carry the risk of developing progressive multifocal leukoencephalopathy (PML), which is a serious and potentially fatal opportunistic infection caused by the JC polyomavirus (Menge, et al., 2008). The common adverse reactions include infusion and hypersensitivity reactions, infections (respiratory, urinary tracts), depression, headache, fatigue, diarrhoea, cholelithiasis and arthralgia (Gawronski, et al., 2010).

Another current treatment option is mitoxantrone, which is an immunosuppressive agent. It works by intercalating with DNA strands causing breaks, as well as inhibiting DNA repair through topoisomerase II (Menge, et al., 2008; Morrissey, Le Page, & Edan, 2005). Mitoxantrone affects rapidly dividing cells, and thus has secondary effects on the immune system, including antigen presentation, proinflammatory cytokine expression, and decreased leukocyte migration (Menge, et al., 2008). It is not a first-line agent because of its cardiotoxicity at cumulative doses above 100 mg/m2, causing it to have a limit on lifetime cumulative exposure (Gawronski, et al., 2010). Another serious adverse effect of the therapy is that it can cause severe bone marrow suppression (Morrissey, et al., 2005). Mitoxantrone is recommended to be used as a second-line treatment in patients with a very active relapsing disease which other therapies have not had a sufficient effect (Menge, et al., 2008; Morrissey, et al., 2005).

Immunomodulators, such as fingolimod, and monoclonal antibodies are the new emerging therapies for MS (Gawronski, et al., 2010; Jones & Coles, 2010), which are still being investigated to determine their value in its treatment. One of the major advantages these drugs offer, is that some of them are available in oral formulations. Undoubtedly, oral drugs are much more discreet, and have the potential to help to further expand therapeutic options, and improve compliance issues with current disease-modifying regimens (Gawronski, et al., 2010). The most investigated immunomodulators include fingolimod, laquinimod, teriflunomide, dimethyl fumarate and cladribine. Cladribine is an immunomodulatory purine analog that possesses lymphocytotoxic activity, acting by damaging DNA and thereby, causing selective cell death. Cladribine can be administered in oral, intravenous and subcutaneous formulations (Brousil, Roberts, & Schlein, 2006; Gawronski, et al., 2010). It appears to be

useful for progressive MS patients as it has been shown to reduce lesions and it is generally well tolerated (Gawronski, et al., 2010). Fingolimod, on the other hand, is an orally administered immunomodulator that acts on the sphingosine-1-phosphate receptor, which is responsible for lymphocyte release from lymhoid organs (Gawronski, et al., 2010; Menge, et al., 2008), decreasing overall numbers in circulation (Mehling, et al., 2008; Menge, et al., 2008). Nonetheless, it does not inhibit lymphocyte recruitment or affect T or B cells in peripheral organs, and therefore, it does not cause immunosuppression or lead to increased rates of infection or malignancies (Foster, et al., 2007; Mehling, et al., 2008; Menge, et al., 2008). Research into fingolimod has found it reduces inflammatory activity and lesions (O'Connor, et al., 2009), and lowers annual relapse rates (Gawronski, et al., 2010). Laquinimod is an orally administered immunomodulator, which is proposed to work by affecting the T-helper 1-T-helper 2 cytokine shift (Comi, et al., 2008; Weiner, 2008). The research on the effectiveness of laquinimod has reported varying results; some have indicated it reduces active lesions, and be generally well tolerated, reduces or stops relapses, there are no changes in disability status , with few adverse effects (Comi, et al., 2008; Polman, et al., 2005). Teriflunomide is an orally administered immunomodulatoy agent thought to possess anti-inflammatory and antiproliferative properties useful for the treatment of MS. It blocks pyrimidine synthesis in rapidly dividing cells such as T cells and B cells (O'Connor, et al., 2006; Tallantyre, Evangelou, & Constantinescu, 2008). It has found to reduce active lesions, lower annual relapse rates, affect disability progression rates, but it has a number of adverse effects also (O'Connor, et al., 2006). Dimethyl fumarate is an orally administered immunomodulatory agent shown to induce T-helper 2-like cytokines causing apoptosis in activated T cells. It also causes down-regulation of intracellular adhesion molecules, leading to reduced migration of lymphcytes into the central nervous system (Gawronski, et al., 2010). It has been reported to decrease the number of lesions with stable or improved EDSS scores and ambulation index, although many gastrointestinal complaints were also recorded (Schimrigk, et al., 2006).

Monoclonal antibodies include daclizumab, alemtuzumab and rituximab (Gawronski, et al., 2010; Jones & Coles, 2010), which are biologic agents that exclusively recognize specific target antigens. They cause immune responses such as cellular apoptosis or inhibition of ligand-receptor binding. Thus, causing concern over the adverse effect profile, which may include serious infections (Gawronski, et al., 2010). Alemtuzumab acts by targeting CD52 on lymphocytes and monocytes, thus causing extended reduction in the number of circulating T cells (Gawronski, et al., 2010). It has been reported to suppress inflammation, but to cause multiple adverse effects (infusion reactions), to be more effective in early stages of MS, lower annual relapse rates, and reduce the risk of disability (Coles, Deans, & Compston, 2004; Coles,

et al., 2008). Daclizumab antagonizes the α subunit of interleukin-2 on activated lymphocytes, inhibiting CD25-IL-2 complex formation (Kim, 2009; Oh, et al., 2009). Interleukin-2 is responsible for upregulation of the immune system, apoptosis of T cells , and inhibition of T-helper 17 cells. It has been suggested that the CD25-IL-2 complex in MS patients is abnormal, and daclizumab may be effective by inhibiting abnormal lymphocyte complexed from becoming activated (Kim, 2009; Oh, et al., 2009). It has shown to reduce lesions, improve EDSS scores, yet it has resulted in many adverse effects (systemic immune responses etc)(Bielekova, et al., 2009). Rituximab causes cytotoxicity by selectively depleting CD20+ pre-B cells and B cells (Petereit, Moeller-Hartmann, Reske, & Rubbert, 2008). After rituximab treatment trials, lesions and relapse rates were decreased, but there were many infusion reactions (Hauser, et al., 2008).

It is crucial to begin treatment early in the MS disease course as irreversible axonal damage often begins before the clinical onset of MS, and treatment is less beneficial in later disease stages (Goodin & Bates, 2009). In clinical practice, the choice of the most appropriate disease-modifying treatment for any particular individual with MS is usually a complex one, involving many patient factors, in addition to the relative efficacy and side-effects profiles of the treatment options. Unfortunately, some patients fail to respond to therapies and at present, it is not possible to predict who will respond to a specific therapy (Rejdak, et al., 2010). There are no clear guidelines for the switching of procedures in clinical practice. At present, it is unclear whether a wash-out period is needed for interferon β and glatiramer acetate treatments, as for example, mitoxantrone requires a wash-out period of 3 months. The duration of the wash-out period should be based on the drug used, the duration of treatment and the possible sequelae of the treatment (Kappos, et al., 2007).

Table 2.3. The current and emerging disease-modifying therapies for MS.

Drug	RRMS	SPMS	PPMS
Interferon β-1a	+		
Interferon β-1b	+		
Glatiramer acetate	+		
Natalizumab	+		
Mitoxantrone	+	+	
Cladribine		+	+
Fingolimod	+	+	
Laquinimod	+	+	
Teriflunomide	+	+	
Dimethyl fumarate	+		
Alemtuzumab	+		
Daclizumab	+	+	
Rituximab	+		+

2.1.6. Cognitive impairment in multiple sclerosis

Cognitive impairment (CI) affects up to 65% of MS patients, and can occur in the absence of physical disability (Hoffmann, et al., 2007). Previous research has identified deficits in cognitive function of individuals with MS in various areas, most importantly in: attention (Paul, Beatty, Schneider, Blanco, & Hames, 1998b); information processing speed (Denney, et al., 2011; Denney, et al., 2005); working memory(Lengenfelder, et al., 2006b; Parmenter, Shucard, Benedict, & Shucard, 2006); executive functions (Foong, et al., 1997); learning (DeLuca, et al., 2006); and verbal fluency (Huijbregts, et al., 2004). Cognitive dysfunction can present early in the disease course, with 20-40% of benign MS patients or clinically isolated syndrome (CIS) patients showing signs of cognitive impairment (Amato, Zipoli, & Portaccio, 2006; Glanz, et al., 2007). Cognitive function is thought to become increasingly compromised once patients transition from relapsing-remitting to the secondary-progressive course (Benedict, et al., 2004; Comi, et al., 1995; Filippi, et al., 1994), particularly with the simultaneous degeneration of the cerebral grey matter (Fisher, et al., 2008). Disease duration and disability level are usually poorly correlated with CI in MS (Amato, et al., 2006).

Cognitive dysfunction is a crucial part, in addition to the disability level and MS disease course, which determine the impact MS has on the quality of life of the patient. The extent of cognitive dysfunction, high disability level and progressive disease course were reported to predict independently, the handicap a patient experiences in their work and social activities (Amato, et al., 1995). Nonetheless, it must be noted that cognitive function in MS can be affected by depression, anxiety, fatigue and physical disability (Arnett, et al., 1999; Demaree, et al., 2003; Diamond, et al., 2008; Gilchrist & Creed, 1994; Litvan, et al., 1988; Thornton & Raz, 1997). The nature of the association between depression and cognitive decline in MS is unclear, as depression may cause cognitive dysfunction, or it may result from an underlying disease process that also causes the cognitive deficits (Feinstein, 2006). The present study considered these factors by including corresponding questionnaires and tests in the research procedure.

2.1.6.1. Longitudinal assessment of cognitive impairment in multiple sclerosis

The longitudinal studies which include healthy volunteers as a control group (Amato, et al., 1995; Amato, Ponziani, Siracusa, & Sorbi, 2001; Huijbregts, Kalkers, de Sonneville, de Groot, & Polman, 2006; Jennekens-Schinkel, Laboyrie, Lanser, & van der Velde, 1990; Kujala, et al., 1997), have reported a progressive deterioration of various cognitive domains in MS. In a

study spanning over 10 years (Amato, et al., 1995; Amato, et al., 2001), impairments in verbal memory, abstract reasoning and linguistic processes were observed in MS patients at baseline, and during the following two re-testing sessions. Deficits in attention and short-term spatial memory also emerged after 10 years. Learning impairments were observed in MS patients after 4-years (Jennekens-Schinkel, et al., 1990). Other studies reported MS patients to have impaired performance in measures relating to the speed of information processing over a 3-year period (Denney, Lynch, & Pannenter, 2008; Huijbregts, et al., 2006). Furthermore, MS patients showed deficits after a 2-year period in verbal memory performance and patients with confirmed disability progression had dysfunctions in attention and executive functions (Duque, et al., 2008). The early, mild cognitive deficits were found to be predictive of future cognitive status as initially, the cognitively mildly deteriorated MS group had widespread deficits after 3 years. While the cognitive performance of MS patients classified as cognitively preserved at baseline was similar to the controls, remaining stable over a 3-year period (Kujala, et al., 1997). Studies not including a control group have identified deficits in information processing after 8 years (Bergendal, Fredrikson, & Almkvist, 2007), in verbal long-term memory and in general intellectual function after 8.5 years (Piras, et al., 2003), or no significant change after one year (Hohol, et al., 1997).

Furthermore, an interesting and reasonably robust finding is that initially, the cognitively mildly deteriorated MS groups demonstrated progressive cognitive decline in various neuropsychological tests. Whereas initially the cognitively preserved patients remained more or less stable over a 2-10 year time period (Bergendal, et al., 2007; Camp, et al., 2005a; Kujala, et al., 1997). This may be due to the differences in the degree of lesion load and increasing atrophy. Likewise, some MS patients may have had significant atrophy or lesion load at baseline and thus already show mild cognitive decline (Huijbregts, et al., 2006).

Cross-sectional studies have reported progressive MS subtypes to have more cognitive decline than RRMS (Gaudino, Chiaravalloti, DeLuca, & Diamond, 2001; Huijbregts, et al., 2004; Potagas, et al., 2008). Huijbregts et al.(2006) investigated the differences between subtypes over a 2-year period and reported progressive MS subtypes to show a lack of improvement in processing speed, involving visuo-spatial ability or working memory compared to healthy controls. A study that did not include a control group, reported more deterioration of SPMS in visual information processing over an 8 year period, relative to RRMS (Bergendal, et al., 2007). Denney, et al. (2008) studied CI in only PPMS patients and observed the processing speed to have a significantly greater decline, in comparison to healthy controls over a 3-year period. Other longitudinal studies (Amato, et al., 2001; Kujala, et al., 1997) examining CI in MS have

not compared subtypes with each other, due to the typical clinical progression of MS from RRMS to SPMS, over time.

2.1.6.2. Factors affecting cognitive performance in multiple sclerosis

A variety of factors affect cognitive function in MS. Age is related to cognitive performance in MS, but not to any greater degree than in healthy controls (Bodling, Denney, & Lynch, 2009). However, in a longitudinal study of early-onset MS patients, age was associated with an increased decline of cognitive performance (Amato, et al., 2001). Besides aging, some studies indicate the male gender to be a risk factor for progression in cognitive disability (Beatty & Aupperle, 2002; Savettieri, et al., 2004). Even though depression, subjectively reported fatigue and disease duration is expected to be highly associated with cognitive deterioration, these conditions have only a limited or weak correlation with cognitive functioning in MS (Arnett, Barwick, & Beeney, 2008; Morrow, Weinstock-Guttman, Munschauer, Hojnacki, & Benedict, 2009; Patti, et al., 2009). This may be due to the heterogeneous course of cognitive impairment in MS (Benedict & Zivadinov, 2011). Cognition is also affected by health behaviours, of which smoking has been suggested to be associated with cognitive decline (Staff, Lucchinetti, & Keegan, 2009). Health behaviours are most likely to be related to personality traits (Chapman, Duberstein, & Lyness, 2007), and the role and interactions of these factors require further investigation.

2.1.6.3. Cognitive impairment in multiple sclerosis and genetic factors

More recently genetic factors have been linked to the cognitive function in MS. The relationship of APOE ε4 genotype and CI in MS has been extensively investigated but results have been contradictory (Ghaffar, Reis, Pennell, O'Connor, & Feinstein, 2010; Oliveri, et al., 1999; Parmenter, Denney, Lynch, Middleton, & Harlon, 2007; Shi, Zhao, Vollmer, Tyry, & Kuniyoshi, 2008; van der Walt, et al., 2009). Some studies have implicated MS APOE ε4 carriers to have increased risk of brain tissue loss (Enzinger, et al., 2004; Ghaffar, et al., 2010; Horakova, et al., 2010; Koutsis, et al., 2009; Koutsis, et al., 2007; Oliveri, et al., 1999; Savettieri, et al., 2004), and cognitive decline (Parmenter, et al., 2007). On the other hand, studies that have been carefully controlled (Ghaffar, et al., 2010; Portaccio, et al., 2009; van der Walt, et al., 2009) and/or with large sample size (Savettieri, et al., 2004) have failed to show APOE ε4 allele status to be a substantial risk factor for cognitive impairment in MS. The relationship of other genetic factors, most notably, human leukocyte antigen (HLA), brain-derived neurotrophic factor (BDNF) and myeloperoxidase gene, and cognitive function in MS have shown similar

mixed results (Cerasa, et al., 2010; Jensen, et al., 2010; Manna, et al., 2006; Weinstock-Guttman, et al., 2011; Zivadinov, et al., 2007). HLA class II region on the short arm of chromosome 6 has been confirmed to be a genetic factor for susceptibility to MS, but no link with cognitive function measured with only Symbol Digit Modalities Test (SDMT) could be established (Jensen, et al., 2010). Genetic variations in BDNF (neurotrophin common in adult brain and produced by immune cells), in specific Met66 allele status in BDNF rs6265 genotype, were associated with grey matter volume, and there was a trend with the PASAT Z-score (Zivadinov, et al., 2007). Moreover, Val66Met polymorphism of BDNF contributes to some of the variability in working memory in MS (Cerasa, et al., 2010). There is also some evidence to support that the C allele of the rs2030324 BDNF single nucleotide polymorphism (SNP), may be associated with protection against visual cognitive processing deficits (Weinstock-Guttman, et al., 2011). Other SNPs which have been associated with an increased risk of MS, such as polymorphisms of interleukin 2 receptor α (IL2RA), interleukin 7 receptor (IL7R) and C-type lectin family 16 member A (CLEC16A) (Oksenberg, et al., 2008). Yet, none have been linked to cognitive deficits in MS (Jensen, et al., 2010).

2.1.6.4. Protective factors of cognitive impairment in multiple sclerosis

Brain MRI measures and cognitive performance in patients with MS are most often strongly correlated. However, only one-third to one-half of that variance is explained. Therefore, it is unusual that cognitive impairment is not consistently found in MS, and that it is not more strongly correlated with brain MRI measures. One suggestion is that some individuals with MS have a larger cognitive reserve, i.e. behavioural adaptation or compensation that enables improved cognitive performance in the setting of cerebral disease (Benedict & Zivadinov, 2011). The concept of cognitive reserve has been widely used in research literature on Alzheimer's disease, to explain why a person with high intelligence can sustain more cerebral injury, before presenting with cognitive dysfunction (Stern, 2006). The hypothesis of cognitive reserve has been supported in the MS research as well, as correlation between atrophy and cognitive performance was shown to be insignificant with high-level cognitive reserve MS patients, whereas it was robust in low intelligence MS patients (Sumowski, Chiaravalloti, Wylie, & Deluca, 2009). Also, reports of increased cerebral activity in response to cognitive task during functional MRI, which is correlated with improved cognitive performance, are in favour of the cognitive reserve hypothesis (Chiaravalloti, et al., 2005). Consequently, it is likely that the correlation between cognitive dysfunction and brain atrophy is mediated by cognitive reserve (Sumowski, et al., 2009). Longitudinal studies assessing patients with SDMT and PASAT twice over a 5-year period, reported a substantial decline in patients with low cognitive

reserve. However, no remarkable change in the cognitive functions of patients with high cognitive reserve was reported, particularly for SDMT (Benedict, Morrow, Weinstock Guttman, Cookfair, & Schretlen, 2010). In conclusion, high premorbid intelligence appears to protect against the progression of cognitive impairment in MS.

2.1.6.5. Treatment for cognitive dysfunction in multiple sclerosis

Cognitive rehabilitation and pharmacological therapy are the most commonly applied treatments for cognitive impairment. Previous research has not supported cognitive rehabilitation as an effective method (O'Brien, et al., 2008). It must be noted, however, that these types of clinical research are challenging to conduct with double-blind and placebo-controlled designs, and they do not experience similar levels of support from the industry as pharmacological treatments (Benedict & Zivadinov, 2011). However, one small-sample study (Chiaravalloti, et al., 2005) revealed that visual imagery strategies improve verbal memory in MS patients. Therefore, it provides preliminary evidence for the general intuitive assumption that increasing brain activity can slow neural, and cognitive, deterioration (Benedict & Zivadinov, 2011).

Pharmacological interventions for cognitive impairment in MS include the disease-modifying therapies (DMTs) discussed earlier, and symptomatic medications that target cognitive symptoms more specifically. Evidence exists to show that DMTs can be effective in reducing the deterioration of cognitive impairment. Interferon β-1a showed beneficial effects on cognitive function in RRMS patients (Fischer, et al., 2000; Patti, Amato, et al., 2010). Likewise, another encouraging result was found in a study on clinically isolated syndrome patients, with early onset treatment of interferon β-1b who performed better in the PASAT after a 3-year period (Kappos, et al., 2007). It has been suggested that treatment with interferon β-1a may have dose-dependent cognitive benefits in mildly disabled patients with RRMS, supporting the early initiation of high-dose interferon β-1a treatment (Patti, Amato, et al., 2010). The beneficial effects of DMTs (interferon β, glatiramer acetate) in the short-term, may occur due to the anti-inflammatory effects of the therapy, whereas in the long-term it is due to the protective effects on tissue damage in the brain (Patti, Leone, & D'Amico, 2010) . Specifically, DMTs may reduce or even prevent the progression of cognitive impairment by containing the development of new cerebral lesions or by reducing the progression of brain atrophy (Patti, Leone, et al., 2010).

Research on the effect of symptomatic medication on cognitive functions in MS has centred mainly on mental processing speed and episodic memory (Benedict & Zivadinov, 2011). Most

studies have lacked sufficient numbers (i.e. over 50) of treated MS patients and have had other design problems, such as not including reliable and comprehensive neuropsychological measures and/or not including healthy controls. Positive effects of an acetylcholinesterase inhibitor, donepezil, on memory was found in a pilot study (Krupp, et al., 2004), but when the sample size was increased and methodological constraints corrected the results were negative (Krupp, et al., 2011). A study on similar acetylcholinesterase inhibitor, rivastigmine, did not replicate positive effects on cognitive performance (Shaygannejad, Janghorbani, Ashtari, Zanjani, & Zakizade, 2008). Modafinil was found to have beneficial effects on performance in tests of processing speed or executive function, but due to weak methodology these results would need to be replicated (Benedict & Zivadinov, 2011). Beneficial effects on cognitive performance were reported in a small-sample study after single doses of stimulant methylphenidate (Harel, Appleboim, Lavie, & Achiron, 2009). There is mixed evidence on the usefulness of other stimulants. One small-sample study portrayed beneficial effects on cognitive functions with L-amphetamine (Benedict, et al., 2008), whereas there were no significant effects on attention or on the speed of mental processing in a large-sample study (Morrow, Kaushik, et al., 2009). When a consequent retrospective study (Sumowski, et al., 2011) comparing the same MS patients based on the status of their memory impairment was conducted, positive outcome on cognitive performance especially in visual memory was evident. However, the neuropharmacological mechanisms behind the positive results require further study (Sumowski, et al., 2011). Furthermore, the effect of potassium-channel blocker 4-aminopyridine on cognitive functions in MS was investigated in a preliminary study with 20 subjects, but indicated negative results (Smits, et al., 1994). Potassium-channel blockers have the potential to facilitate neuronal function, as they can correct the abnormal potassium currents that result in impaired action potential duration and amplitude in demyelinated axons (Benedict & Zivadinov, 2011). The lack of positive results may be due to methodological limitations, as potassium-channel blockers have been shown to have positive effects on fatigue and motor function (Bever, et al., 1996; Rossini, et al., 2001). A small-sample study, of only 16 subjects, reported negative results on the effects of amantadine on cognitive function in MS, although there was a trend for a benefit from SDMT (Geisler, et al., 1996).

Most previous studies examining the effect of pharmacological treatment on cognitive performance in MS, have only utilised one or a couple of neuropsychological tests to assess the change in cognitive function. In particular, only 3 have included electrophysiological measures, such as P300 (Flechter, et al., 2007; Gerschlager, et al., 2000b; Pliskin, et al., 1996). One of the electrophysiological studies reported a significant reduction in both P300 amplitude and in latency after one-year of treatment with interferon β-1b (Flechter, et al.,

2007). Another study reported P300 latencies to be increased after one-year of interferon β-1b, but the differences disappeared after the exclusion of 3 patients with P300 latency, 2 standard errors from the P300 mean (Gerschlager, et al., 2000a). Yet, in the other study there were no significant changes in P300 latency after 2 years of interferon β-1b treatment (Pliskin, et al., 1996). These research studies are the only ones examining P300 longitudinally, and therefore the effect of disease progression (and age) cannot be excluded from the results. It is possible that interferon β-1b does not have an effect on cognitive function in MS. These effects are difficult to measure without research on non-pharmacological with a longitudinal study designs. These studies have major methodological limitations, such as small sample sizes, omission of healthy control groups and progressive MS patient groups, also low-density EEG recording methods and data analysis.

2.2. Conventional methods to detect and monitor brain function, cognitive impairment and visual dysfunction in multiple sclerosis

2.4.1. Neuropsychological methods

In the past, there has been a great variety in the range of different neuropsychological tests that have been utilised to measure cognitive function in MS. Furthermore, many attempts have been made to develop an optimum battery of neuropsychological tests most sensitive to cognitive impairment in MS. These batteries have been criticised for being either too brief or including tests that assess cognitive functions intact in MS (Benedict, et al., 2002). Currently, Brief Repeatable Battery (BRB) for MS (Rao, Leo, Ellington, et al., 1991) is widely considered as the gold-standard assessment tool of cognitive function in MS (Camp, et al., 2005a; Sepulcre, et al., 2006) and its sensitivity has been proven in clinical research studies (Amato, et al., 2004; Christodoulou, MacAllister, McLinskey, & Krupp, 2008; Huijbregts, et al., 2006). It only takes approximately 45 minutes to administer and includes a Selective Reminding Test (SRT; Buschke & Fuld, 1974), a 7/24 Spatial Recall Test (Barbizet & Cany, 1968), a Controlled Oral Word Association Test (COWAT; Benton & Hamsher, 1989; Borkowski et al, 1967), a Paced Auditory Serial Addition test (PASAT; Gronwall, 1977), and the Symbol Digit Modalities Test (SDMT). The assets of BRB are rudimentary corrections for practice effects and the availability of several European translations and normative data (Portaccio, et al., 2009). However, the variation of clinical manifestations and cerebral pathology, possibly cause broad neuropsychological

compromise. In particular, this requires more comprehensive testing, especially the domains of higher executive function and spatial processing not included in BRB.

The Minimal Assessment of Cognitive Function in Multiple Sclerosis battery (MACFIMS; Benedict, et al., 2002) was developed to address these deficits by an expert panel, including members from the United States, Canada, the United Kingdom and Australia, convened by the Consortium of MS Centres. The MACFIMS is based on a thorough literature review, and the battery emphasises reliability, normative data and equivalent alternative forms. The main aim was to develop a test battery that would be sensitive to mild cognitive impairment, monitor the longitudinal course of the impairment, work with clinical treatment planning, be free of cultural and language biases and be relatively quick and easy to administer. MACFIMS takes approximately 90 minutes to complete and it includes most of the original tests from the BRB but the 7/24 Spatial Recall test is replaced by the Brief Visuospatial Memory Test-Revised (BVMTR; Benedict et al., 1996; Benedict manual 1997), the SRT with the California Verbal Learning Test-II (CVLT-II; Delis et al., manual 2000), and Delis-Kaplan Executive Functioning System Sorting Test (Delis et al., manual 2001). The Judgement of Line Orientation Test (Benton, 1994) has been added. The validity and reliability of MACFIMS have been demonstrated in previous studies (Benedict, Cookfair, et al., 2006; Duquin, Parmenter, & Benedict, 2008; Parmenter, et al., 2007). In the present research project, an adapted version of MACFIMS (see Table 3.3.) is employed to assess cognitive function of MS patients.

The neuropsychological test that has attracted the most interest when investigating CI in MS is the PASAT (Gronwall, 1977; Tombaugh, 2006). It is widely used to evaluate the cognitive status of MS patients. The PASAT is also the chosen task to measure cognitive performance in MS functional composite (MSFC; Cutter, et al., 1999), with the other two components measuring arm and leg function. The PASAT measures the attention processes, speed of information processing and the working memory (Tombaugh, 2006), which are known to be impaired in MS (Rogers & Panegyres, 2007). The PASAT is also a sensitive measure of the longitudinal changes in cognitive functions in MS (Huijbregts, et al., 2006; Rosti, Hamalainen, Koivisto, & Hokkanen, 2007). However, the PASAT has proven to be extremely challenging for many MS patients, and it is therefore very unpopular among patients which leads to loss of data. In turn it is disadvantageous in clinical trials in which repeated testing may increase the drop-out rate (Williams, O'Rourke, Hutchinson, & Tubridy, 2006).

In addition to the PASAT, the SDMT is another short neuropsychological test generally accepted as one of the most reliable and valid measures of cognitive status in MS (Benedict & Zivadinov, 2011). The SDMT is a 90 second measure of information processing speed, and many studies (e.g. Benedict & Zivadinov, 2007; Christodoulou, et al., 2003; Tekok-Kilic, et al., 2007) indicate that it is strongly correlated with brain pathology. The SDMT has been suggested to replace the PASAT in MFSC, but until now, there has not been sufficient information supporting this conclusion (Benedict & Zivadinov, 2011; Williams, et al., 2006).

Nonetheless, performance in the neuropsychological tests may be affected by various factors. First of all, the physical symptoms related to MS cause difficulties in completing some of the neuropsychological tests. For example, upper extremity motor dysfunction may interfere with performance on cognitive measures that require some aspects of manual dexterity (Parmenter, Testa, Schretlen, Weinstock-Guttman, & Benedict, 2010). Similarly, dysarthria or impaired oral agility appears to affect performance on measures dependent on rapid speech, such as the Controlled Oral Word Association Test (Arnett, Smith, Barwick, Benedict, & Ahlstrom, 2008). Depression is common in MS patients and has an impact on neuropsychological test performance (Arnett, et al., 1999; Demaree, et al., 2003; Diamond, et al., 2008; Gilchrist & Creed, 1994). Furthermore, the level of anxiety and practice effect (Barker-Collo, 2005) also affect MS patient's neuropsychological performance.

2.4.2. Magnetic resonance imaging (MRI)

Structural MRI is commonly utilised to determine the diagnosis of MS. Demyelinating lesions can especially be observed in the cerebral and spinal white matter, which satisfy the dissemination in space, which is standard for the diagnosis (Fazekas, et al., 1999). T1-weighted MRI is generally utilised to define anatomy and it is also able to detect abnormal signals in the white matter (represented as hypointensities reflecting chronic axonopathy), while detecting pathological changes is possible with T2-weighted MR images (Truyen, et al., 1996; Zivadinov & Bakshi, 2004).

A variety of studies have found associations between abnormal MRI findings and cognitive impairment in MS (Benedict & Zivadinov, 2011). Early work (Rao, Leo, Haughton, St Aubin-Faubert, & Bernardin, 1989) reported a strong correlation between the area of white matter lesions and cognitive dysfunction, which was replicated in subsequent studies. Demyelinating lesions are also found in the cerebral cortex (Geurts, Bo, et al., 2005; Geurts, Pouwels, et al., 2005), which have been associated with cognitive impairment (Bagnato, et al., 2010;

Roosendaal, et al., 2009). One study reported cortical lesions in the medial frontal and temporal regions to correlate with MACFIMS memory task scores, after the influence of white matter lesions was controlled (Bagnato, et al., 2010).

Lesion volume measures have not been found to be informative in pathological substrates of cognitive dysfunction in MS (e.g. edema, inflammation or axonal loss) (McDonald, Miller, & Barnes, 1992; Peterson, et al., 2001; Trapp, et al., 1998; Trapp, Ransohoff, & Rudick, 1999). Brain atrophy was shown to worsen over a 2-year period (Rudick, Fisher, Lee, Simon, & Jacobs, 1999). Similarly the brain:intracranial volume ratio was found to correlate with neuropsychological tests of nonverbal memory and mental processing speed (Hohol, et al., 1997). Two studies employing a linear regression approach with multiple MRI measures to determine the most robust or statistically relevant predictor of cognitive impairment (Benedict, et al., 2004; Christodoulou, et al., 2003) reported central atrophy to account for most of the variance. Moreover, another study (Benedict, et al., 2004) found third ventricle width to be a predictor of cognitive dysfunction, and thalamic volume measures to be nearly 17% lower in MS patients and strongly correlated with several MACFIMS variables. The relationship of cortical atrophy to cognitive function has been investigated. Cortical atrophy was clearly associated with various measures of memory and executive function (Benedict, Bakshi, et al., 2002). The study was replicated by two investigations using more reliable quantitative MRI methods for calculating cortical volume (Amato, et al., 2004; Benedict, Bruce, et al., 2006). This displayed cortical volume loss correlated with cognitive dysfunction, cortex volume was reduced and cognitive function impaired relative to healthy controls.

In MS, another area related to cognitive function (particularly to memory) is the hippocampus, especially mesial temporal cortex (Anderson, et al., 2010; Benedict, Ramasamy, Munschauer, Weinstock-Guttman, & Zivadinov, 2009; Sicotte, et al., 2008). MS patients had considerable volume loss in specific mesial temporal cortex structures (such as dentate gyrus, subiculum, entorhinal cortex, curnu ammonis). These volumes were shown to correlate with episodic memory tasks such as CVLT-II but not the PASAT, indicating dissociations between the effects of processing speed and memory (Sicotte, et al., 2008). This finding was later replicated (Benedict, et al., 2009).

The grey matter atrophy is also a relevant risk factor for cognitive impairment in MS. Loss of grey matter tissue is notably correlated with cognitive impairment. Multiple grey matter regions are compromised and are associated with cognitive impairment (Benedict & Zivadinov,

2011). Independent associations between subcortical volume loss, and cortical atrophy, and MS-related cognitive dysfunction were demonstrated in one study (Benedict, et al., 2009).

An MRI procedure, the functional MRI (fMRI), measures brain activity by detecting associated changes in blood flow. fMRI provides information regarding how brain areas and neural networks underlying cognitive processes are affected by MS (Caramia, Tinelli, Francia, & Pozzilli, 2010). In studies, working memory functions of MS patients are by far the most comprehensively evaluated cognitive domain utilising fMRI. Most studies have reported increased recruitment of the prefrontal cortex during working memory performance, in tests such as the PASAT (Chiaravalloti, et al., 2005; Mainero, Pantano, Caramia, & Pozzilli, 2006; Staffen, et al., 2002), the Sternberg task (Hillary, et al., 2003), and the n-back task (Colorado, Shukla, Zhou, Wolinsky, & Narayana, 2012), especially in the right hemisphere. The increased activity in the right prefrontal cortex is also related to the slower processing speed measured with the SDMT (Genova, Hillary, Wylie, Rypma, & DeLuca, 2009). Furthermore, a few studies have shown that the brain activity related to working memory functions (measured with the PASAT and the Stroop) is more dispersed throughout the brain in MS patients compared to the healthy controls (Mainero, et al., 2004; Rocca, et al., 2009; Staffen, et al., 2002). The findings of brain activity related to attention deficit is conflicting, as MS patients with most adversely affected cognitive function have shown decreased activation in prefrontal cortex (Nebel, et al., 2007), or no difference relative to healthy controls (Penner, Rausch, Kappos, Opwis, & Radu, 2003). Overall, these findings suggest two main patterns of functional cerebral activity during cognitive performance in MS. Firstly, the regions of cortical activation are similar in MS patients and healthy controls, but MS patients can show increased level of activation. Secondly, the responses seen in fMRI are frequently observed in MS patients in areas where no activations were found in healthy controls. These brain activity patterns can be explained to be due to compensatory cerebral reorganisation (as a result of neuroplasticity) and/or increased task effort (Caramia, et al., 2010; Chiaravalloti, & DeLuca, 2008). The fMRI has also been utilised to study cognitive fatigue in MS, and the brain's functional organisation with task-free resting state paradigms (Caramia, et al., 2010).

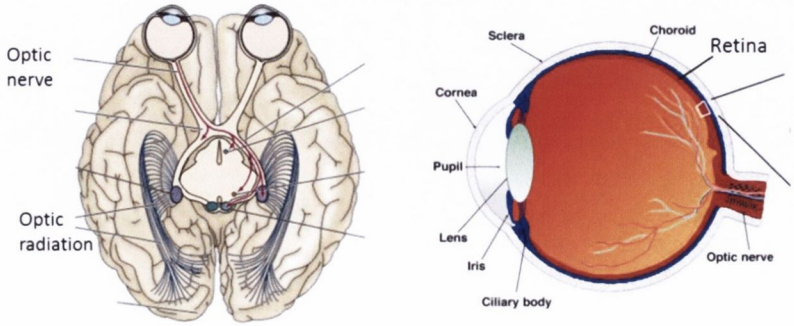
2.4.3. Visual evoked potentials (VEP)

Visual dysfunction is a common feature of MS as approximately 95% of MS cases have optic neuritis (ON) (i.e. swelling of the optic nerve). This causes patients to experience a decline in vision over a 7-10 day period with vision improving within 30 days of onset. Other visual dysfunctions in MS include nystagmus (involuntary eye movement), internuclear

ophthalmoplegia (impairment of horizontal eye movements) and gaze palsies (inability to move both eyes in the same direction). The type of visual dysfunction depends on the site of the demyelinating lesion within cerebral white matter (Frohman, Frohman, Zee, McColl, & Galetta, 2005). Visual dysfunction is common at the onset of MS and the Visual Evoked Potentials (VEPs) can be utilised as part of the clinical practice to confirm MS diagnosis (Polman, et al., 2011; Polman, et al., 2005). Their reliability in MS patients is moderate, although only few studies exist (Ziemann, et al., 2011). The VEP recordings remain the preferred test for detecting changes in optic nerve function subsequent to clinical and subclinical optic neuritis. MS patients with ON show a pathologically prolonged VEP P100 latency (Ziemann, et al., 2011). The diagnosis of MS is a lengthy procedure as no definitive test exists for its detection. Furthermore, current diagnosis criteria for MS (Polman, et al., 2011) stipulates that a patient must present with a specific combination of attacks and lesions which are detected using magnetic resonance imaging (MRI) or VEPs. A study conducted by Ko (2010) found that VEPs can be superior to MRI in the detection of such lesions, and therefore are of great importance in the diagnosis of MS.

The VEPs are visually evoked electrophysiological signals extracted from the electroencephalographic activity recorded from the scalp (Odom, et al., 2010). The VEP paradigm elicits a large positive peak around 100 ms after stimulus presentation, P100, preceded by a large trough. The P100 is the largest component over a large occipital area on the scalp, in other words over the visual processing areas on the cortex. As the visual cortex is primarily activated by the central visual field, the VEPs depend on the functionality of the patient's central vision at any level of the visual pathway including the eye, retina, optic nerve, optic radiations and occipital cortex (Figure 2.2).

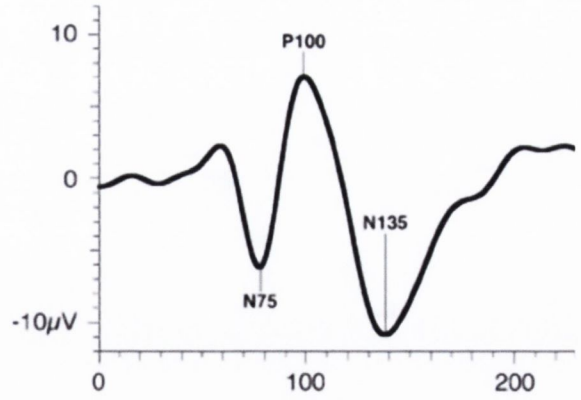
Figure 2.2. VEPs depend on the functionality of multiple parts of the visual pathway (figures from SA Medical (2010) and Kolb (2012)).



To obtain a VEP, a visual stimulus is presented to a subject and the electrical activity of the visual pathway is recorded via EEG. A transient VEP (tVEP) is recorded when the temporal frequency of the presented stimulus is low and consists of a number of discrete deflections. Responses can be evoked by either patterned stimuli (pattern stimuli) or un-patterned stimuli (flash stimuli). The three subtypes of tVEPs are defined according to their stimulus and recording conditions, which provide their core clinical information (Odom, et al., 2010). The pattern-reversal tVEPs, the pattern onset/offset tVEPs, and the flash tVEPs, all have their own benefits. The pattern-reversal tVEPs are commonly utilised to diagnose visual dysfunction in MS and they are the least variable in waveform and timing. The pattern onset/offset stimulus is suited to the analysis of patients with nystagmus (involuntary eye movement) and flash tVEPs are useful when pattern stimulation is not appropriate, e.g. due to poor vision, poor cooperation, poor optics, and/or inability to maintain focus on the pattern stimulus.

The standard pattern stimulus is a high-contrast black and white checkerboard pattern. According to the International Society for Clinical Electrophysiology of Vision (ISCEV)(Odom, et al., 2010), the contrast as defined by Michelson, should be at least 80%. (Note: Michelson contrast = ([Lmax – Lmin]/Lmax + Lmin]) * 100%. Lmax = maximum luminance of white squares, Lmin = minimum luminance of black squares.) In the case of the pattern-reversal tVEP, the black and white checks of the pattern stimulus change abruptly (black squares change to white, white squares to black) at a specified number of reversals per second (ISCEV recommends two reversals per second). For the pattern onset/offset stimulus, the checkerboard pattern is intermittently presented against a diffuse grey background. The mean luminance of the diffuse grey background and the checkerboard pattern must be equal for the duration of the stimulus presentation. The checkerboard pattern should be presented for 200ms separated by 400ms of diffuse background. A flash stimulus consists of brief flashes of light presented in a dimly illuminated room at a standard rate, which for the presentation of flash stimuli is one flash per second. The major components of pattern reversal waveforms are N75 (negative peak, approximately 75ms), P100 (positive peak, approximately 100ms) and N135 (negative peak, approximately 135ms), as shown below.

Figure 2.3. The components of pattern reversal tVEP.



There is a wide range of literature supporting the conventional tVEP as a valuable analysis tool (Ziemann, et al., 2011). In particular, MS with optic neuritis (ON) show pathologically lengthened VEP P100 latency. In general, in the acute stage of optic neuritis the VEP shows an amplitude reduction that recovers while during the same period of time VEP latency increases. The recovery of the VEP amplitude can be explained by resolution of edema, whereas, reduced VEP amplitude developing in later stages of MS suggests axonal loss within the optic nerve (Ziemann, et al., 2011). Strong correlations have been found between prolonged and reduced tVEP responses in MS and the probability of having motor or visual disorders (Balnyte, et al., 2011). On the other hand, sometimes the correlations between VEP measures and visual function is weak due to VEP P100 latency prolongation usually persisting despite complete or near complete recovery of visual function (Ziemann, et al., 2011). Previous studies in MS all utilised a standard pattern reversal stimuli to generate tVEP responses (van Dijk, Jennekens-Schinkel, Caekebeke, Singh, & Zwinderman, 1992) and reported 30 MS patients to have significantly prolonged P100 latencies at occipital sites for both eyes in comparison to 19 healthy and age-matched controls. Balnyte, et al. (2011) found that P100 responses of 63 MS patients had prolonged latencies and reduced amplitudes for both eyes, when compared to the responses of 63 patients with other neurological conditions. Furthermore, a significant correlation existed between symptom duration and the prolongation of P100 in both eyes of MS patients. Moreover, delayed and reduced tVEP values indicated an increased probability of having motor and visual disorders. A retrospective longitudinal analysis (Niklas, Sebraoui, Hess, Wagner, & Then Bergh, 2009) reported that clinically, non-affected eyes of the patients with sub-clinically delayed prolonged latencies remained stable over a period of almost 5 years. In contrast, patients whose eyes were clinically affected by ON and displayed an initially delayed P100 latency, displayed a shortening of P100 latency over a 4 year period. The study

by Schlaeger, D'Souza, Schindler, Grize and Kappos, et al. (2012) showed that combined multimodal (visual, somatosensory, motor) evoked potentials can be used as markers and predictors of disability of the early phase of RRMS, as the multimodal EPs correlated well with cross-sectional clinical disability (EDSS value) over a 3-year period in 50 patients. The subsequent study (Schlaeger, D'Souza, Schindler, Grize, Dellas, et al., 2012) found that baseline EP parameters and EDSS at year 14 (and the change over 14 years) were correlated.

However, a major disadvantage of VEPs is that as they present stimuli periodically (the responses are generated approximately every 400ms), the output results must be averaged over many trials to obtain a stable VEP response; typically a minimum of 60 trials with 200-300 trials being preferable. As a result, VEP testing sessions have a lengthy duration of approximately one hour and they can often be tiresome for subjects, which leads to difficulty maintaining focus and concentration. Therefore, there is a demand for an alternative, more efficient and subject-friendly method to probe and monitor visual dysfunction in MS, such as Visual Evoked Spread Spectrum Analysis (VESPA) investigated as part of the present research project (Lalor, Kelly, Pearlmutter, Reilly, & Foxe, 2007).

2.5. Electroencephalography and P3 event-related potentials: promising methods for detecting and monitoring brain function related to cognitive impairment in multiple sclerosis

Neuropsychological tests, such as the PASAT, are generally used to measure CI in MS. Cognitive-indexing electrophysiological measures, electroencephalography (EEG) and event-related potentials (ERPs), offer information on brain functions related to cognitive dysfunction in MS. Therefore, they have the potential to provide additional information on cognitive impairment in MS. As mentioned in Chapter 1, EEG is an electrophysiological method that records the ongoing electrical voltage changes on the scalp in order to determine the underlying neural activity. EEG is caused by multiple neuronal network interactions which can be affected by white matter damage. EEG may be used as an indicator for the global status of these neuronal network interactions (Comi, Leocani, Locatelli, Medaglini, & Martinelli, 1999). ERPs are averaged responses to specified stimuli. In other words, stimulus onsets are marked in the ongoing EEG data during recording, and afterwards these trials are averaged together to form an ERP. P3 ERPs can be said to act as an index of cognitive function in MS (Leocani, et al., 2010). The early sensory processing of the brain can be explored with EEG methods such as

visual evoked potentials (VEPs). Similar, but more advanced methods such as Visual Evoked Spread Spectrum Analysis (VESPA) can provide a more ecological and in depth perspective of visual processing in MS.

Other neuroimaging methods, such as fMRI, PET and SPECT, have two main advantages over EEG analysis; better spatial resolution and an ability to detect cellular activity in structures that do not contribute to scalp EEG (i.e. in the cerebellum, subcortex). Nevertheless, EEG has several advantages over neuropsychological tests and neuroimaging methods. EEG has greater temporal resolution, and they are the summation of cortical postsynaptic potentials themselves, not just measures of cell metabolism as in other neuroimaging methods. Specifically, electrophysiological methods assess the consequences of axon demyelination and degeneration as the activity recorded on the scalp reflects the action potential conduction along central visual, auditory, somatosensory and motor pathways. The slowed action potential conduction, results from a succession of impaired cellular level processes; acute demyelination of an axon results in increased current leakage at the internodal membrane. This causes delay in producing sufficient depolarisation at the next node of Ranvier to reach the action potential threshold (Ziemann, et al., 2011). In a given fibre bundle, demyelination may variably affect different axons, resulting in disperse action potential propagation (Ziemann, et al., 2011). Furthermore, EEG technology is favourable, as it has high temporal resolution, is independent from behavioural responding, inexpensive and relatively easy to administer, portable, relatively convenient to use, tolerated better by some patients, and not adversely affected by practice effects (Barker-Collo, 2005), education, motor delay of speech or hand movement. As previously mentioned, some of the neuropsychological tasks, such as the PASAT, are very unpopular amongst patients due to its difficulty, which leads to loss of data, which is especially unfortunate in clinical trials with repeated testing (Williams, et al., 2006).

2.5.1. Electrophysiological measures

Clinical neurology continues to rely on the principle of cortical localisation of function despite recent advances in neuroscience suggesting brain to function as a distributed, parallel and recursive network. In other words, in clinical practice it is still considered that definite functions are represented in specific parts of the brain. It is thought that brain injuries disrupt these localised functions giving rise to corresponding behavioural deficits. Moreover, stimuli are processed in a serial, bottom-up fashion, in the separate hierarchically organised brain

areas performing different functions (Engel, Fries, & Singer, 2001; He, Shulman, Snyder, & Corbetta, 2007).

At the same time, it was also generally accepted that the brain is anatomically organised in widely distributed and highly parallel networks. Cortical rhythms are considered to be the main means of communication between and across these networks. Cortical rhythms can arise from synchronisation of coupled oscillators when noise is absent or small. Alternatively, they can emerge from strong feedback synaptic interactions in a predominantly asynchronous circuit, with highly stochastic spiking activity of individual cells (Wang, 2010). Efficient transfer of information within the brain has previously been theorised to depend on bottom-up mechanisms, such as modulation of firing rates or the degree of spike synchronisation (Fries, 2005). However, the most recent accounts in the research stresses the role of temporal binding through rhythmic synchronisation (Engel, et al., 2001; Fries, 2005; He, et al., 2007). Neural synchrony, i.e. coherent oscillations, is considered to be a universal feature of neural systems and to be the integration mechanism at different levels from individual neurons to local or intermediate networks up to widely distributed, long-range networks such as cortico-cortical fibres and thalamocortical reciprocal pathways (>1cm) (He, et al., 2007; Varela, et al., 2001). Synchronous cortical rhythms can emerge from the synchronisation by excitatory connections, through an interplay between excitation and inhibition, or even a by purely inhibitory neuronal network (Wang, 2010).

Neuronal synchrony is thought to play a central role in well-timed coordination and communication between neural populations simultaneously engaged in a cognitive process, such as the perception of a stimulus or the performance of a task (Fries, 2005; He, et al., 2007; Wang, 2010). These coordination mechanisms rely on the temporal patterning of neuronal activity (Fries, 2005; Uhlhaas, et al., 2009). The brain rhythms in the waking brain occur intermittently by short episodes in time, synchronisation is subtle and typically confined to restricted neural populations. It is presumed that cortical networks in wakefulness are predominantly asynchronous but at the same time weak oscillations and selective coherence are present (Wang, 2010). Synchronisation supports the encoding of information about stimuli and is also highly sensitive to context (Uhlhaas, et al., 2009). In particular, through the modulation of the strength of synchronisation (Engel, et al., 2001; Uhlhaas, et al., 2009) and the utilisation of the delays among the discharges of neurons participating in synchronised assemblies (Uhlhaas, et al., 2009). Synchronisation also results in alternating periods of excitation and inhibition, which rhythmically pace neuronal activity and therefore, the transfer of information (He, et al., 2007; Uhlhaas, et al., 2009).

The cortical rhythms are commonly measured by reporting the spectral power (absolute, relative, ratio) in specific frequency bands. Physiologically, EEG spectral power is an indirect reflection of the number of neurons that discharge synchronously, i.e. increased power implies increased synchronous firing of a group of neurons (Klimesch, 1999). Moreover, cortical rhythms recorded during cognitive tasks can be examined with relatively novel methods of event-related spectral perturbations (ERSPs; event-related mean power spectral changes) and inter-trial coherence (ITCs; event-related consistency of spectral phase). ERSPs are the mean latency-by-frequency images that show the frequencies and latencies when mean changes in log power (dB) occur from a specific mean power baseline, showing them time-locked to experimental events (Makeig, 1993). ITCs measure the trial-to-trial phase consistency at each frequency and latency relative to a set of time locking events. If ITC is close to zero at a given frequency and latency (relative to time-locking events), then the measured spectral phase is evenly distributed across trials. In contrast, if ITC is near 1, then the activity is reliably phase locked to the time-locking events (Makeig, 2002; Tallon-Baudry, Bertrand, Delpuech, & Pernier, 1996).

The cortical oscillations are divided into different EEG frequency bands. More specifically, the low frequency bands, alpha (8-13 Hz) and theta (4-8 Hz), and the high frequency bands, beta (14-30 Hz) and gamma (>30 Hz) are associated with cognitive processing and behaviour (Polich, 2007; Wang, 2010). The underlying anatomical mechanisms behind the low and high frequency bands are different. The neural synchronisation in the high frequency range (gamma and beta) has found to be mainly mediated by cortico-cortical connections, reciprocally linking cells within the same cortical area. Also, cells distributed across different areas, in turn the generation and synchronisation of oscillatory activity in the lower frequency bands (alpha, theta) seem to be dominated by subcortical structures, especially the thalamus (Uhlhaas & Singer, 2006). Furthermore, the frequency of the synchronised oscillations is associated with the distance over which synchronisation is observed. Short distance synchronisation most often manifests at gamma, and long-distance synchronisation at beta and theta (Schnitzler & Gross, 2005; Uhlhaas & Singer, 2006; Wang, 2010).

Rhythmic synchronisation plays a functional role in many cognitive functions. Transient synchronisation of neuronal discharges is thought to dynamically bind widely distributed sets of neurons into functionally coherent assemblies that represent the neural correlates of a cognitive content (Singer, 1999; Uhlhaas & Singer, 2006). Neural synchrony is also essential for the large-scale integration, as it is associated with (complex) cognitive functions that demand large-scale integration of distributed neural activity (Uhlhaas & Singer, 2006). Furthermore,

theta oscillations are related to information processing tasks, especially those of sustained attention and with high executive demands (Gevins, Smith, McEvoy, & Yu, 1997; Klimesch, 1999; Mizuhara, Wang, Kobayashi, & Yamaguchi, 2004; Mizuhara & Yamaguchi, 2007). Furthermore, theta oscillations are linked with the maintenance of working memory (Gevins, et al., 1997; Varela, et al., 2001), and the encoding and retrieval of episodic memory (Klimesch, et al., 2006; Wang, 2010).

The extent of theta synchronisation has also been related to good working memory performance (Klimesch, 1999; Wang, 2010). Theta band oscillations may play a role in top-down processing, with field theta oscillations and theta-band inter-areal synchronisation being associated with working memory and attention (Wang, 2010). In addition, reduction in alpha power has been associated with attention focused on external stimuli processed by the studied brain area (Wang, 2010). Beta oscillations have been related to movement behaviour, response inhibition and also with broader cognitive processes (Wang, 2010). Inter-areal coherence associated with selective attention, working memory, guided search, object recognition, perception, sensorimotor integration is especially conspicuous in the beta frequency range. The primary generation of beta rhythm is thought to happen in the deep layers of the cortex, which are then involved in long-distance signalling (oscillatory synchronisation) along feedback pathways arising from subcortical structures such as the hippocampus, basal ganglia and the thalamus (Wang, 2010). Gamma-band oscillations have been linked to a variety of different processes such as attentional selection of sensory information/selective attention, arousal, focused attention, perceptual readiness, response preparation, attentive listening, visual search, attention to moving stimuli, object recognition, learning, language processing and emotional evaluation (Engel, et al., 2001; Schnitzler & Gross, 2005; Uhlhaas, et al., 2009). The gamma band couples with other frequency bands during attention, which is illustrated by reports showing synchronized beta/gamma oscillations to be involved in attention dependent processes (Uhlhaas, et al., 2009), and attention to be associated with reduced alpha rhythm and increased gamma rhythm (Wang, 2010).

Recently, the importance of spatiotemporal balance of synchronisation and de-synchronisation has been recognised, and the specific synchronisation patterns have been found to be directly related to behaviour (Schnitzler & Gross, 2005). With regards to cognitive functions, superior working memory performance is related to an increase in theta power and to a decrease in alpha power after an increase in task demands, such as the presentation of less frequent target stimuli (Klimesch, 1999).

In recent years, there has been an enthusiasm to investigate if measuring the spectral content of EEG[1] can be used as a predictive biomarker for neurological conditions (Klassen, et al., 2011). The role of synchronisation of neural networks has been investigated in brain disorders, such as schizophrenia, Alzheimer's disease, and autism (Uhlhaas, et al., 2009; Uhlhaas & Singer, 2006). Most research on the electrophysiological correlates of pathological brain states have focused on the analysis of power distributions in various frequency bands of resting-state EEG activity (either eyes-open or eyes-closed) or on the irregularities in averaged ERPs. However, limited research has investigated task- or performance-related synchronisation phenomena in brain disorders (Uhlhaas & Singer, 2006).

Distinct abnormal changes in (task-related) neural synchrony most likely play an important role in not only the cognitive dysfunction, but also the overall pathophysiology of brain disorders (Uhlhaas, et al., 2009; Uhlhaas & Singer, 2006). These conditions involve the reduction of both local and long-range neural synchronisation (Uhlhaas & Singer, 2006). Therefore, it can be suggested that the cognitive dysfunction with the other overt symptoms of these disorders arise, at least in part, from dysfunction in the coordination of distributed neural activity between and within functionally specialised regions in the cerebral cortex. Reduced neural synchrony may be the consequence of a disconnection of these brain regions, but it can also be the cause of impaired coupling between brain areas. In particular, as synchronisation is required for the efficient propagation of neural responses across sparsely connected networks (Uhlhaas & Singer, 2006).

Only few studies (Cover, et al., 2006; Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Borges, et al., 2008; Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Escera, et al., 2008) have examined the task-related neural synchronisation in MS. Although, it may present as a highly valuable clinical tool as the axonal damage and demyelination (present in MS), have been shown to interfere with the temporal coordination of neuronal activity (Uhlhaas & Singer, 2006). The previous scalp-based and low-density spectral EEG studies in early-stage MS patients that related to cognitive task performance (Posner paradigm), have identified increased power of higher frequencies of beta and gamma bands in MS patients only, compared with controls over the whole scalp area after target-stimuli presentation. This was most prominent over frontal areas (Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Escera,

[1] The mathematical and statistical analysis of human EEG, such as fast Fourier transform, is sometimes referred to as quantitative EEG. The term is especially used with regards to the spectral content of the EEG and is widely used in clinical literature.

et al., 2008), and over anterior right hemisphere and bilateral posterior scalp regions (Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Borges, et al., 2008).

2.5.2. Event-related potentials: the oddball and 3-stimulus paradigms

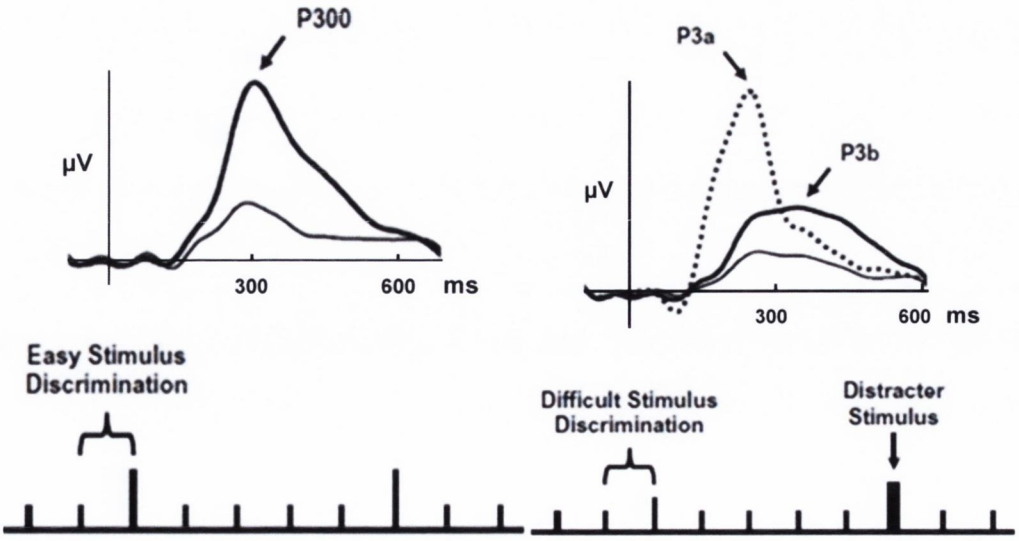
One of the fundamental skills of human beings is the ability to distinguish new events from those already experienced, which enables us to react appropriately to the stimuli in the environment. People are constantly surrounded by subtle changes in our environment, and take notice of them by comparing the new events to already existing memory traces of prior events. This stimulus discrimination process is a fundamental psychological process that determines several aspects of cognitive functioning.

Electrophysiologically, a similar process can be elicited by a two-stimulus oddball task (see Figure 2.4). First reported by Sutton, Braren, Zubin and John in 1965, the two-stimulus oddball task is one of the most popular ERP paradigms. In the oddball task an occasional target stimuli has to be detected in a train of frequent irrelevant standard stimuli. A P3b component is typically elicited approximately 300 ms after each target stimulus, and it has maximal amplitude over the parietal scalp area. The P3b is thought to reflect context updating (Polich, 2007) and the categorisation of task relevant events (Kok, 2001).

Sometimes, there are novel, non-attended events in the environment which require more attention than the small, somewhat expected changes related to P3b and context updating. In neurophysiology, this phenomenon is examined with a variant of P3b, the P3a (Figure 2.4). The P3a is considered to reflect a basic orienting response to a novel stimulus (Friedman, Cycowicz, & Gaeta, 2001), involuntary attention and response inhibition (Key, Dove, & Maguire, 2005). There are a variety of different types of P3a; the difficulty of the standard/target discrimination and the perceptual distinctiveness of the non-target stimulus alter the latency, amplitude and topography of the P3a. The most utilised P3a paradigms are the classical P3a, novelty P300 and no-go P3a. In the classical P3a paradigm, only infrequent distinct tones are presented with a train of frequent tones, which produces a P3a component best observed on the central and/or parietal scalp areas and with a relatively short latency. Novelty P300 task infrequent novel distracter stimuli are presented with more typical stimuli, which generates a novelty P300 component most visible over frontal and/or central scalp areas, and which has a relatively short latency (Polich, 2007).

The present research project focuses on no-go P3a, which is elicited by employing a three-stimulus oddball paradigm; in addition to standard and target stimuli, additional, infrequent, non-target stimuli are presented. Subjects are asked to respond only to target stimuli and refrain from responding to the additional non-target stimuli and standard stimuli. Furthermore, a difficult standard/target discrimination is employed as it results in more easily detectable P3a components. These components are higher in amplitude and shorter in latency, compared to those in an easy discrimination task. Typically, this no-go P3a component can be observed approximately 250 ms after the non-target stimuli, especially over the central scalp region, with shorter latencies in frontal areas and longer latencies in central/parietal areas (Polich, 2007). No-go P3a was the chosen P3a paradigm, as it is best suited for clinical environments where ERP components have to be reliably and objectively produced within a limited time frame. In other words, the responses to the same type of distracter stimuli in no-go P3a can be more reliably thought to reflect the same preliminary attention processes than the responses to different, novel stimuli, which may alter according to the stimulus features of each individual novel stimuli. As a result, less repetitions of the distracter stimuli are required, which reduces the overall time of the task and is, therefore, better suited to clinical populations. Moreover, the no-go P3a gives a robust response to the unattended distracter stimuli, making it a relatively easily reproduced ERP component. It also reflects a different side of the early stimulus processing, relative to traditional P3b tasks, and the multimodal employment of the two tasks enables a thorough investigation of the early, cognitive processing functions in the brain.

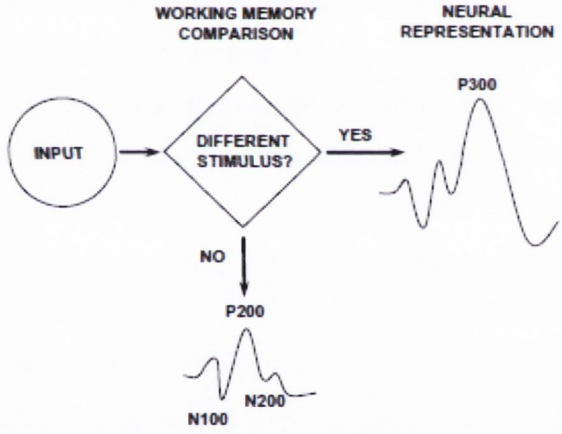
Figure 2.4. Two-stimulus oddball (P3b) and three-stimulus (no-go P3a) paradigms (adapted from Polich, 2007).



2.5.2.1. Theoretical accounts of P3 processing

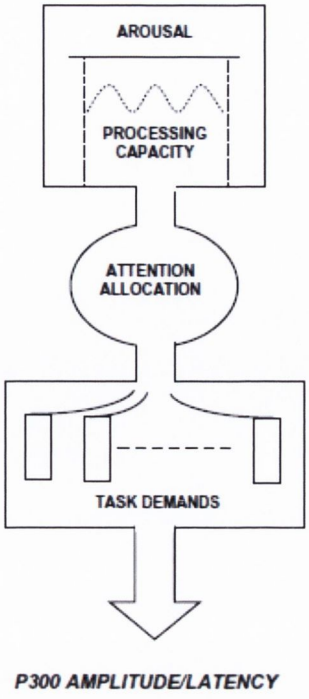
One of the main theories of the processing of the P3 regards the task as context updating; P3 component indexes brain activity arising from the comparison of incoming stimuli to the existing mental representations in short-term memory (Figure 2.5). After initial sensory processing, an attention driven comparison process evaluates the representation of the previous event in working memory. If a new stimulus is detected, the attention processes update the stimulus representation, which results in the generation of P3 peak. On the other hand, if no change is detected, the current schema of the stimulus is maintained and only evoked sensory components (e.g. N1, P2) are elicited. This process resembles orienting response, with observed P3 habituation/dishabituation. Nonetheless, the theory does not explain how and why the brain produces P3, and it does not suggest any biologically plausible hypothesis (Polich, 2007). In fact, the theory may reflect more P3a-like relatively strong initial target stimulus processing (Kok, 2001).

Figure 2.5. The context-updating theory of P3b ERP (as in Polich, 2007).



Another theory explains P3 as a result of resource allocation: the overall arousal level modulates processing systems which governs the amount of attention available for task performance (Figure 2.6). The demands of the task determine the properties of the P3 component, i.e. demanding task conditions require greater amounts of attentional resources which result in P3 amplitude to be larger and peak latency to be shorter. In contrast, when task conditions are undemanding, the P3 amplitude is smaller and peak latency longer. Furthermore, passive stimuli produce smaller P3 amplitudes than active tasks, because stimulus and non-task events engage attentional resources to reduce amplitude. The theory also assumes arousal systems to affect the availability of attention processes to modulate P3 (Polich, 2007).

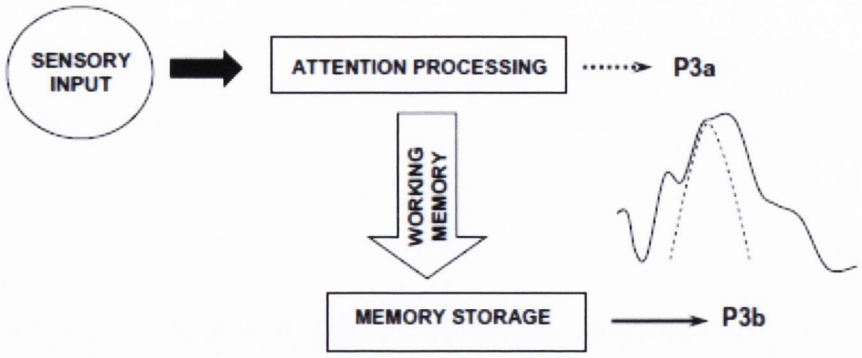
Figure 2.6. The resource allocation theory of P3b ERP (as in Polich, 2007).



After linking P3 to orienting response and attention allocation effects, the focus shifted to memory. In a series of studies P3 amplitude and/or latency were found to be associated with recall performance, memory load, memory engagement, semantic compatibility, stimulus- and response-related processing and mental function speed. In sum, stimulus encoding that promotes successful memory storage to facilitate retrieval and recognition is thought to produce increased P3-like amplitude.

These early theories did not include the P3a component, and therefore, Polich and Comerchero (2003) created a schematic neuropsychological model of P3a and P3b (Figure 2.7). The model is based on results from lesion studies, which imply a circuit pathway between frontal and temporal/parietal brain areas indicated by P3a and P3b. According to the theory, processing sensory stimuli involves attention-driven working memory changes with frontal lobe activation. This produces the P3a component if sufficient attentional focus is engaged to a rare or otherwise physically alerting stimulus. Furthermore, the subsequent attentional resource activation promotes memory updating operations in temporal/parietal areas that elicit the P3b component. Stimulus characteristics and task demands determine the distracter evaluation, and the topography and timing of P3a. The model is also consistent with the neurocognitive framework - the incoming stimuli invoke top-down attention switching, and the memory-driven bottom-up operations guide response organisation and production (Polich, 2007; Polich & Comerchero, 2003).

Figure 2.7. Schematic neuropsychological model of P3b and P3a ERPs (as in Polich, 2007).

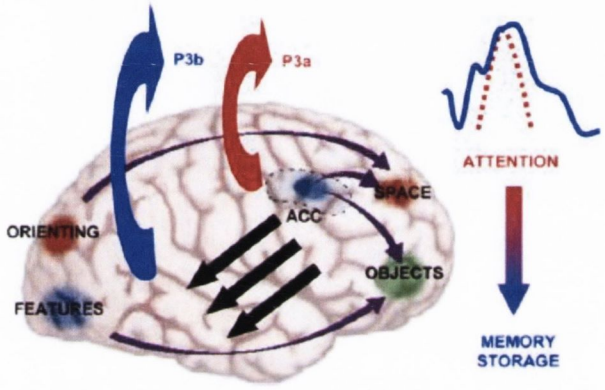


At present, the most refined theoretical model attempts, not only explain P3b and P3a generation within the cognitive and neuropsychological theoretical frame, but also includes the brain mechanisms underlying P3b and P3a generation (Figure 2.8). The model assumes there is a connection adapted by the electrical activity of the neurons between frontal and temporal/parietal structures which modulates P3b and P3a generation and activity on the scalp. According to the theory, stimulus information is maintained in the working memory of the frontal areas and monitored by anterior cingulate structures. The circuit pathway is thought to be activated when focal attention is distracted, which causes anterior cingulate cortex and related structures to generate P3a, which can be observed on frontal scalp areas. The attention-driven neural activity signal is then transmitted to temporal-parietal areas, which leads to memory-related storage operations to be engaged and the generation of P3b via temporal/parietal cortical structures. Therefore, every P3 component is presumed to be composed of both P3a and P3b subcomponents which vary with stimulus and task features (Polich, 2007). Polich (2007) links the model to a hypothesis supported by a large number of neurophysiological results. These suggest P3b components to be mainly related to inhibition processes. Thus, P3b and P3a may be caused by brain mechanisms trying to inhibit extraneous, on-going brain activation to ease the transference of stimulus/task information from frontal to temporo-parietal areas, frontal activation reflecting P3a and temporo-parietal P3b.

Minimal processing of extraneous stimuli would, therefore, facilitate the transmission of relevant incoming stimulus information from frontal to temporo-parietal areas, easing memory functions. This account is congenial with all the functions of P3. P3 is adaptive to inhibit activity unrelated to infrequent, low probability stimuli, possibly holding biologically important information subsequently eliciting large P3 amplitudes. Difficult and dual processing tasks inducing high cognitive demand limit attentional resources to resist inhibitory control

and thus, generate smaller P3 amplitudes. Arousal is suggested to modulate the level of neural inhibition engaged. The relationship between P3 peak latency and cognitive capability indexes the speed with which extraneous processes are inhibited. Declined P3 amplitude and prolonged P3 latency with aging and dementing conditions originate from breakdowns in cortical processes underlying inhibitory signals. The proposed neurotransmitter systems for P3b and P3a are consistent with the inhibition hypothesis, as the same neurochemical effects influence inhibitory signals to affect P3. In sum, a general neural inhibition approach appears to function as an underlying P3 generation mechanism, when attention-engaging stimuli are detected and engage memory operations.

Figure 2.8. Schematic representation of brain activation patterns underlying P3b and P3a generation (Polich, 2007).



Note. ACC = anterior cingulate cortex

2.5.2.2. Factors affecting P3 ERP component

P3 components are influenced by natural, induced and subject factors. Natural factors such as body temperature, heart rate, food intake and seasons with light, together with induced factors including fatigue, exercise and common drugs such as caffeine, nicotine and alcohol, all have varying effects on P3 amplitude and/or latency. P3 peaks are also affected by many subject factors including age, intelligence, handedness, gender, personality and genes (see Table 2.4. for details; (Polich & Herbst, 2000). P3 peak latency increases with normal aging (Fjell & Walhovd, 2001; Polich, 1996), and also with the increase of dementia level (Polich, 2007).

More importantly, the details involved in experimental set-up involving stimulus characteristics, subject instructions/behaviour and task and recording circumstances also have an effect on P3 components. Stimulus modality, duration, intensity and interstimulus interval

influence P3 formation, together with subject and task related factors with seating position, eyes open, active and overt response to targets, and target probability of 20% preferred. Favourably, the EEG signals should be recorded at least from the midline electrodes (Fz, Cz, Pz) with mastoid-, nosetip- or average-reference, band passed 0.01-100 Hz, and having an epoch length of 800-1200 ms and a minimum of 20 correct target trials (see Table 2.5. for details; Duncan, et al., 2009; Polich & Herbst, 2000).

Table 2.4. P3 latency and amplitude biological determinants (as in Polich, 2000).

Factor	Amplitude	Latency	Comment
Natural			
Circadian	Indirect	Indirect	Circadian body changes affect P300 measures
- Body temperature	no	yes	Increased temperature, decreased latency
- Heart rate	no	yes	Faster heart rate, decreased latency
- Food intake	yes	some	Amplitude increases, latency shorter
- Activity time	yes	some	Food interacts with activity preference time
Ultradian	some	yes	90-in latency cycles
Seasonal	yes	no	Seasons with light, increased amplitude
Induced			
- Exercise:	Indirect	Direct	Affects overall arousal level
-- Tonic	yes	yes	Increases amplitude, decreases latency
-- Chronic	no	yes	Decreased latency, variable results across studies
- Fatigue	yes	yes	Decreased amplitude, increased latency
- Drugs common	yes	yes	Specific drug, arousal level, tonic / chronic use
-- Caffeine	some	yes	Amplitude increases if fatigued, latency decreases
-- Nicotine	small	yes	Weak amplitude effects, latency decreases
-- Alcohol			
--- Acute	yes	yes	Amplitude decreases, latency increases
--- Chronic	no	no	Social drinking: no permanent long-term effects
-- Alcoholism risk	yes	no	At-risk: smaller amplitudes with visual tasks
Constitutional			
Age	yes	yes	Modality, task, response parameters important
- Children	yes	yes	Amplitude increases, latency decreases
- Adults	yes	yes	Amplitude decreases, latency increases
Intelligence	yes	yes	Amplitude from complex tasks smaller for more Intelligent, latency shorter for perceptual / speeded Classification tasks for more intelligent
Handedness	yes	yes	Amplitude: left > right for frontal / central sites Latency: left < right for frontal / central sites
Gender	small	small	Amplitude: female > male, latency: female < male
Personality	yes	no	Amplitude: introverts < extroverts
Genetic	yes	yes	Amplitude and latency genetically determined

Table 2.5. Recommended parameters for eliciting and recording P3 ERP (adapted from Polich, 2004, and Duncan, et al., 2009).

Parameter	Comment
1. Stimulus factors	Two categories of stimuli, one rare, one frequent
Auditory	Tones, environmental sounds, spoken words
- Frequencies	1000 Hz (target), 500 Hz (standard)
- Duration	50–150 ms duration, 5 ms rise/fall
- Intensity	70 dB SPL
Visual	Colours, shapes, words, pictures
- Duration	50–150 ms; stimuli should be perceptible
Interstimulus interval	1–2 s (fixed or variable), longer if categorisation task is difficult
Dimensions of change	Physical attributes of stimuli; meaning of stimuli. Stimuli must comprise two categories (high vs. low tone; female vs. male names).
Probabilities	0.10–0.20 (target), 0.80–0.90 (standard)
II. Subject and task	
Position	Seated
Eyes	Open
Sensory/health factors	Document integrity of sensory function and relevant health factors (e.g., history of central nervous system disorder or injury, medications affecting CNS).
Active or passive task	Attention to stimuli required; concurrent behavioural task with measurable performance (e.g., button press to target stimulus) is necessary.
III. Electrophysiological recording	
Electrode sites	Minimal configuration is Fz, Cz, Pz, VEOG; additional electrode sites recommended to obtain information on scalp distribution.
Reference	Nose or one earlobe used as online reference, with offline averaging with the other earlobe.
Ground	AFz
Bandpass of amplifiers	0.01–100 Hz
Digitization rate	200 Hz minimum (4 low-pass filter setting)
Epoch length	1000 ms, with a prestimulus baseline of 100–150 ms; longer epoch for difficult categorisations
Artefact reduction	EOG rejection or correction, and rejection of trials with voltages ±100 lV in any EEG channel
Minimum # trials	36 artefact-free
Digital filtering	Not recommended for ERPs prior to quantification; low-pass filtering at 12 Hz is acceptable for ERPs to be displayed.
IV. Quantification	
Average ERPs	Average ERP waveforms, excluding trials with incorrect behavioural responses, must be presented for each group and condition.
Latency	Typically peaks in the 300–400 ms range but varies according to the difficulty of categorizing the stimuli, age, and clinical status.
Amplitude	Peak amplitude in a latency window (e.g., 280–420 ms) relative to prestimulus baseline; mean amplitude in a latency window relative to prestimulus baseline also acceptable; PCA/ICA
Scalp distribution	Parieto-central in adults, becomes more equipotential in elderly.

Note. b A 'passive' task means that no response, overt or covert, is required of the participant.

2.5.3 Measuring EEG and P3 ERP components

The P3b and P3a components have traditionally been measured by assessing two aspects of the ERP; amplitude and latency (i.e. the largest positive/negative-going peak and the time from stimulus onset to largest positive/negative-going peak, respectively) (Polich, 2007). In conventional P3 ERP studies, groups are compared by examining the differences between their grand mean ERPs. The latencies and sequence of ERP waves are related to the time course of processing activity, whereas, the amplitudes of ERP waves indicate the amount of synchronised neurons and neural resources participating into specific cognitive processes (Polich & Herbst, 2000). Nevertheless, the informativeness of ERPs is restricted due to the fact that they are mostly generated by multiple, functionally distinct neural sources, and the varying source projections cause EEG potentials recorded on the scalp to overlap both temporally and spatially (Dale, et al., 2000). Furthermore, the spatiotemporal overlap makes it difficult to measure the exact sources of EEG activations (Hagoort, 2003) and impairs the ability to compare ERP phenomena related to different types of stimuli or experimental paradigms (Groppe, Makeig, & Kutas, 2008).

Event-related spectral perturbations (ERSPs) and inter-trial coherence (ITCs) are superior methods for visualizing event-related EEG dynamics since they reveal a more comprehensive overview of the underlying EEG time-frequency properties than ERPs. ERSP depicts the mean power spectral changes, time-locked to experimental events (Makeig, 1993). In addition, ITC is a measure of the consistency of the spectral phase of recorded EEG activity across single trials (Makeig, 2002; Tallon-Baudry, et al., 1996). Furthermore, a high-density EEG array (128 scalp channels) is useful in identifying differences between the different subject groups and subtle longitudinal changes within subjects, as it covers the entire scalp area. High-density EEG enables the use of more refined and advanced analysis methods, such as statistical parametric mapping (SPM), independent component analysis and clustering of independent components.

An advanced analysis tool, SPM is a more comprehensive method to investigate the differences in amplitude/power of scalp-based EEG. It includes all the activations across scalp-space and time into the analysis, without the need to average and thus possibly lose essential information regarding the activations. It is a robust statistical measure: statistical parametric maps are derived from the electrical activations recorded on the scalp and they depict activations across time and the whole scalp area. This makes it possible to discover statistically significant differences between groups in the topographical distribution of task-related P3 EEG activities over the scalp. In addition, the correlations between the brain activities and

behavioural task variables, such as neuropsychological test scores can also be examined with the method of analysis.

Independent component analysis (ICA) is an advanced method to address the problem of spatiotemporal overlap inherent in EEG. It is a relatively new linear decomposition method that attempts to separate the observed EEG signals into both physiologically and behaviourally distinct components (Delorme & Makeig, 2004). In other words, its aim is to identify temporally independent and spatially fixed ICs from high-density scalp EEG activities in which they are intermingled, yielding functionally distinct ICs which can be mapped onto 3D head space (Groppe, et al., 2008; Onton, Westerfield, Townsend, & Makeig, 2006). In contrast to other popular EEG source analysis methods such as BESA[2] (Hoechstetter, et al., 2004), ICA does not require a priori assumptions on the temporal dynamics or the spatial structure of the underlying processes. Moreover, ICA is a useful method for isolating artefactual EEG sources (such as eye blinks and muscle artefact), which are then removed, yielding a more accurate representation of neural activity. Consequently, it is possible to aggregate ICs into functionally equivalent groups (IC clustering), which provides an estimate of source EEG activities across subjects and conditions (Onton, et al., 2006).

The IC clustering method holds many advantages over the classical scalp-based ERP analysis. This approach is more informative compared to the established practice of reporting grand average ERPs between groups, which only capture the portion of the channel data that is phase-consistent at latencies relative to the time-locking events (Onton, et al., 2006). It aggregates ICs into *functionally* equivalent groups and therefore, estimates the source EEG activities across subjects and conditions. The IC activities can then be compared between the groups (and optionally conditions) 'within' the IC clusters. In contrast, the average scalp-based ERPs between groups only capture the portion of the channel data that is phase-consistent at latencies relative to the time-locking events and therefore, loses information of the activities (Delorme, Palmer, Onton, Oostenveld, & Makeig, 2012; Onton & Makeig, 2006).

This makes it possible to estimate source EEG locations more accurately, whereas the estimation with scalp-based EEG analysis is more challenging as the results are affected by the differences between the subjects' brain anatomy and therefore the underlying differences in EEG activations may be overlooked. To be more specific, the ICA approach to EEG source identification contrasts with the predominant approach of attempting to identify those EEG sources active during peaks in averaged evoked potential epochs, following abrupt onsets of

[2] BESA denotes brain electrical source analysis; a description is available online: http://www.besa.de/.

experimentally presented sensory signals. The traditional procedure assumes that these scalp maps sum the projection(s) of one or at most very few cortical source areas. However, the effects of sensory signals on the statistics of cortical field activities spread quite rapidly, concurrent with neural cross-talk and feedback between early sensory areas, beginning at about 30 ms (Hupe, et al., 2001; Stettler, Das, Bennett, & Gilbert, 2002). This makes it less likely that later peaks in average evoked potential waveforms, represent the projection of activity from a single cortical source area, making spatial source filtering by response averaging and then 'peak picking', a relatively inefficient approach to isolating and locating individual EEG source signals (Delorme, et al., 2012).

In short, modelling ICs using equivalent dipole models allows the assessment and visualisation of the spatial homogeneity of clusters of functionally similar or equivalent IC processes across subjects. The approach has been proposed by one research group (Onton & Makeig, 2006; Onton, et al., 2006), but it has not yet been adopted as a routine procedure when analysing EEG/ERP data. One reason may be that the IC clustering method requires a relatively large amount of data recorded with high-density EEG, a large number of subjects and an ERP paradigm with a clearly distinguishable, task-related peak. Otherwise the automated algorithm is less likely to reject the noise from the data and the further analysis becomes unstable and imprecise. The other limitations of the IC clustering approach include the subjective selection of the number of clusters, and the restrictions in matching IC source areas with specific anatomical regions. These limitations have been addressed by the dipole modelling of ERPs based on source locations determined from fMRI activations (see for example Wagner, & fuchs, 2001). However, fMRI measurements were not available for the present research.

2.5.4. The neural origins of P3b and P3a

The neural generators of P3b and P3a have not yet been conclusively defined. The P3b is thought to have neural origins in the temporo-parietal junction (Karch, et al., 2010; Menon, Ford, Lim, Glover, & Pfefferbaum, 1997; Verleger, Heide, Butt, & Kompf, 1994; Yamaguchi & Knight, 1991), and the involvement of different areas such as frontal lobe, anterior cingulate cortex, hippocampal formation, dorsolateral prefrontal regions, superior temporal gyrus have all been suggested (Bledowski, et al., 2004; Bokura, Yamaguchi, & Kobayashi, 2001; Brazdil, et al., 1999; Crottaz-Herbette & Menon, 2006; Daffner, Mesulam, Scinto, et al., 2000; Knight, 1996; Linden, et al., 1999; McCarthy, Luby, Gore, & Goldman-Rakic, 1997; Yamaguchi & Knight, 1991). An IC clustering study of auditory novelty P3b task in healthy, young volunteers

indicated an IC cluster reflecting P3b ERP component activities to target stimuli, to be located bilaterally around the posterior midline in the parietal region at 200-600 ms (Debener, Makeig, Delorme, & Engel, 2005a). In addition, IC clusters reflecting P3b activations showed a decrease in alpha power post-target stimulus presentation. Similarly, other studies with healthy volunteers reported IC clusters reflecting P3b activations to be located in posterior brain areas and to show a decrease in alpha power. They also specified other IC clusters related to P3b task performance, i.e. a frontal cluster reflecting decision-making, a bilateral and central 'mu' cluster related to tactile feedback, and central medial cluster linked with attentional orienting (Delorme, Sejnowski, & Makeig, 2007; Makeig, et al., 2004; Onton, et al., 2006).

Various neural substrates for P3a have also been indicated, including most importantly, frontal lobe (Bledowski, et al., 2004; Daffner, et al., 2003; Halgren, Marinkovic, & Chauvel, 1998) and in particular orbitofrontal cortex involvement (Brazdil, et al., 1999). Hippocampal formation (Daffner, Mesulam, Holcomb, et al., 2000), anterior cingulate (Brazdil, et al., 1999; Volpe, et al., 2007b), parietal areas (Halgren, et al., 1998; Volpe, et al., 2007a), and temporal areas (Strobel, et al., 2008) are also indicated.

The neurotransmitter systems underlying P3 ERP component generation are unclear. However, the findings from existing literature indicate that norepinephrine is related to P3b with temporal/parietal activity, whereas dopaminergic activity mediates P3a linked to frontal focal attention and working memory systems. The P3b neuropharmacology literature is highly varied, but a comprehensive review reported locus coeruleus-norepinephrine system to underlie parietal P3b formation (Nieuwenhuis, Aston-Jones, & Cohen, 2005). Dopaminergic involvement into especially P3a and some portion of P3b generation was supported by findings showing reduced amplitudes in Parkinson's disease and restless legs syndrome, both neurological conditions with deficits in dopaminergic circuits (Polich, 2007). The importance of dopamine for frontal P3a generation is evident in other studies utilising other neurological groups and/or pharmacological manipulations (Polich, 2007). Although, these results are consistent with the P3 topographic distributions and P3 theories of attention, arousal and memory effects, it must be noted that most of these studies have been with non-human subjects.

2.5.5. Time-frequency EEG analysis and P3 ERPs

EEG rhythms in specific frequency ranges are detectable on the background EEG during P3b and P3a ERP tasks, and they are shown to be functionally related to cognitive processing and behaviour (Polich, 2007). In previous studies theta band frequencies seem to be more engaged to novel stimuli, in comparison to non-novel stimuli. Novel visual stimuli producing P3a also created significantly more theta band activity (Demiralp, Ademoglu, Comerchero, & Polich, 2001). In addition, spectral analysis of P3b has indicated that P3b amplitude is linearly related to delta and theta band spectral power (Spencer & Polich, 1999). Alpha band spectral power covaries with P3b, which indicates ERP to be related to attention and memory processes (Polich, 1997).

Theta oscillations have also been shown to be related to information processing tasks. In particular, for those of sustained attention and with high executive demands (Gevins, et al., 1997; Klimesch, 1999; Mizuhara, et al., 2004; Mizuhara & Yamaguchi, 2007), maintenance of working memory (Gevins, et al., 1997), and the encoding and retrieval of episodic memories (Klimesch, et al., 2006), which are all essential for a favourable P3b task completion. The extent of theta synchronisation has also been related to good working memory performance (Klimesch, 1999; Wang, 2010). The successful performance of P3b tasks is dependent on these attention, memory retrieval and decision making processes. In addition, reduction in alpha power has been associated with attention focused on external stimuli processed by that brain area (Wang, 2010).

The parietal and prefrontal cortical areas are 'source areas' for top-down attentional signals to sensory systems and coherence between these areas and early visual areas is modulated by attention. The parieto-prefrontal circuit is also important for working memory, and it is suggested that it may share a common cortical circuit substrate with attention control (Wang, 2010). Slow alpha rhythm is implicated in suppressing irrelevant information in memory and attention tasks. Hence, at a given cortical site, alpha rhythm may be reduced when attention is focused on external stimuli processed by that area, but enhanced when sensory information flow to that area is behaviourally irrelevant and should be ignored (Wang, 2010).

Moreover, theta rhythm appears to be particularly prominent in the frontal midline, including anterior cingulated cortex-P3 birthplace, a subregioni of the prefrontal cortex implicated in behavioural monitoring, valuation of response outcomes and other aspects of cognitive functions (Wang, 2010). These findings can be linked to the predominant P3 ERP theory of

Polich (2007) that assumes P3b and P3a generation to be modulated by 'neuroelectric' connections between frontal and temporal/parietal structures, which are also thought to be involved in working memory and attention control through theta oscillations (Wang, 2010). Theta is prominent in the frontal midline, which encompasses anterior cingulated cortex (Wang, 2010), the same area implicated for monitoring the stimulus information, while it is maintained in the working memory of the frontal areas and thought to be involved in the generation of P3a component (Polich, 2007). Polich (2007) also suggests P3b component to be related to inhibition processes, which can be linked to alpha rhythm, thought to suppress irrelevant information during memory and attention tasks (Wang, 2010).

2.5.6. Use of EEG and P3 ERPs in diagnostics and monitoring of multiple sclerosis

EEG and ERP approaches have been utilised to examine the brain and cognitive function in different neurological and neuropsychiatrical conditions. Most P3 ERP literature concentrates on dementias, schizophrenia, mood disorders (depression, bipolar disorder), traumatic brain injury and MS (Duncan, et al., 2009). The P3 ERPs have not yet been conclusively and comprehensively utilised to investigate brain and cognitive function in MS.

2.5.6.1. Cross-sectional P3 event-related potentials and EEG as an index of cognitive function in multiple sclerosis

Several studies have investigated the relationship between CI and ERPs by employing a two-stimulus oddball (P3b ERP) task (see Table 2.6). A consistent finding is that MS patients show prolonged P3b latencies and reduced P3b amplitudes (Magnano, et al., 2006). Furthermore, the previous cross-sectional electrophysiological studies have reported MS patients to have decreased amplitudes and increased latencies in auditory P3b amplitude (Aminoff & Goodin, 2001; Ellger, et al., 2002; Gil, et al., 1993; Honig, Ramsay, & Sheremata, 1992; Piras, et al., 2003; Polich, Romine, Sipe, Aung, & Dalessio, 1992; Sailer, et al., 2001; Triantafyllou, et al., 1992) and in visual P3b amplitude compared to controls (Ellger, et al., 2002; Piras, et al., 2003). Some studies have shown latency prolongation in auditory P3b (Aminoff & Goodin, 2001; Ellger, et al., 2002; Giesser, et al., 1992; Gil, et al., 1993; Polich, et al., 1992) and in visual P3b (Ellger, et al., 2002; Piras, et al., 2003; van Dijk, et al., 1992) in MS. However, one of the studies (Sailer, et al., 2001) observed no differences between MS patients and controls in visual or auditory P3b latencies, or in visual P3b amplitudes. The P3a has also been studied in MS (Jung,

Morlet, Mercier, Confavreux, & Fischer, 2006; Sailer, et al., 2001). One study (Jung, et al., 2006) reported reduced auditory P3a amplitude while employing a mismatch negativity paradigm (also resulting in the P3a). In contrast, another study (Sailer, et al., 2001) utilising novelty P3a paradigm (i.e. the distracters were novel, instead of repeated) with an easy standard/target discrimination, did not find any differences.

There is some evidence that the ERP latency delay and amplitude reduction can be observed in varying degrees in different MS subtypes. In general, decreased P3 ERP amplitudes and delayed P3 ERP latencies can be observed more commonly in patients with secondary or primary progressive disease courses when compared to patients with a relapsing-remitting course (Ellger, et al., 2002; Gil, et al., 1993). Gil, et al. (1993) reported the progressive forms of MS to have the most delayed auditory P3b latencies. Later Ellger and colleagues (2002) found a similar result in visual modality by observing significantly reduced amplitudes for PPMS, compared to RRMS, and increased latencies for SPMS compared to both PPMS and RRMS. The rate of pathological P3 ERP latencies was also significantly higher in the SPMS subgroup, in comparison to PPMS and RRMS subgroups. In summary, these studies have found clear changes in P3 ERP activities in MS (Magnano, et al., 2006). These are most likely due to demyelination and axonal degeneration that causes the disruption of the cortical-cortical and cortical-subcortical neural connections (Leocani, et al., 2010), which is particularly evident with the progressive courses of MS.

The role of early sensory components on the P3 component in MS is disputed. The earliest components, P1, N1 and P2, are considered to only reflect sensory processing, whereas some authors regard N2 to indicate an early cognitive elaboration concerning subject's attention orientation (e.g. Piras, et al., 2003). Some studies have reported latency delays in early sensory components of N1 (Giesser, et al., 1992) and P2 (Giesser, et al., 1992; Gil, et al., 1993), and amplitude decreases in N1-P2 complex (Gil, et al., 1993; Jung, et al., 2006), besides P3 latency delay and amplitude reduction. The latency prolongation and amplitude decrease were shown to be more pronounced in progressive forms of MS (Ellger, et al., 2002; Gil, et al., 1993). The delays in early sensory components have been explained by demyelinating lesions in primary afferent pathway and thought, subsequently determining prolonged N2 and P3 components (Magnano, et al., 2006). This underlying mechanism is thought to be similar to the 'subcortical dementia' seen in Parkinson's disease and Huntington's disease (Gil, et al., 1993; Magnano, et al., 2006). Other studies have reported normal early sensory components of N1 and P2 with delayed or reduced N2 and P3 waves (Gil, et al., 1993; Honig, et al., 1992; Newton, Barrett, Callanan, & Towell, 1989; Onofrj, et al., 1991; Piras, et al., 2003; Triantafyllou, et al., 1992; van

Dijk, et al., 1992). This has been interpreted to be due to demyelinating plaques and axonal degeneration causing functional disconnection of subcortical regions from higher cortical areas (Honig, et al., 1992; Magnano, et al., 2006; Piras, et al., 2003). The disconnection causes impaired processing at cortical level, not in subcortical structures as manifested by Parkinson's disease and Huntington's disease (Honig, et al., 1992; Piras, et al., 2003). Interestingly, one of the studies (Sailer, et al., 2001) did not find any irregularities in P3a and P3b components of MS patients, but they reported visual N1 and N2 peak amplitudes to be reduced in MS patients with high lesion volume, and a correlation between visual N2 amplitude and lesion load, explaining these results to be in line with views that lesions cause partial disconnections within widespread cortical networks. Modality effects on the early components remain unclear as most of the studies only employed a unimodal paradigm (Ellger, et al., 2002; Giesser, et al., 1992; Gil, et al., 1993; Honig, et al., 1992; Jung, et al., 2006; Triantafyllou, et al., 1992), with the rest reporting deficits to be greater in visual (Piras, et al., 2003), auditory (Newton, et al., 1989), or in both modalities (Onofrj, et al., 1991). It can be concluded that the early components and their significance regarding MS require further, more thorough examination due to variability of the results and the multiple methodological limitations previously mentioned.

Nevertheless, these studies include multiple limitations, which may not allow for the full view of the changes in brain and cognitive function measured by the P3 ERPs, thus impeding the accurate interpretation of the P3 ERP activations. One of the most consistent restriction found in the research is the utilisation of low-density EEG (<30 electrodes) and typically reporting the results measured from only one electrode. This prevents statistical topographical analysis that is essential, as the pathological changes in the brains of people with MS may alter the topographical presentation of the P3 ERP. The earliest studies examining P3 ERPs in MS did not conduct any statistical analyses (Newton, et al., 1989; Onofrj, et al., 1991; Tourtellotte, Shapshak, Baumhefner, Staugaitis, & Syndulko, 1984) and only reported the rate of abnormalities in P3 ERPs. Furthermore, some studies lacked a healthy control group (Ellger, et al., 2002; Tourtellotte, et al., 1984); examined P3 ERPs in only one modality (Ellger, et al., 2002; Giesser, et al., 1992; Gil, et al., 1993; Honig, et al., 1992; Jung, et al., 2006; Tourtellotte, et al., 1984; Triantafyllou, et al., 1992); did not provide the number of patients with different disease courses (Giesser, et al., 1992; Honig, et al., 1992; Newton, et al., 1989; Sailer, et al., 2001); had a very low number (<15) of participants in each group (Giesser, et al., 1992; Onofrj, et al., 1991; Piras, et al., 2003; Ruchkin, et al., 1994; Sailer, et al., 2001); and had varied instructions

Table 2.6. A summary of the P3 ERP studies in MS.

Study	Paradigm	Modality	Patients (subgroups) / controls	Scalp electrodes N (locs, ref – result electrode)	Result				Comment
					P3b	P3a	Early comp.	topo	
Tourtellotte et al. (1984)	oddball	Aud	—	—	MS normal; CI MS abnormal	—	—	–	Only preliminary; design and procedure not described; no controls; No visual; no statistical group comparisons
Newton et al. (1989)	3-stimuli; target detection	Vis & aud	23 MS (+1 ON) / 19 C	7, mastoid - Pz	lat 57% abnormal; aud↑	—	N2 amp↓; N2 lat abnormality	—	No statistical group comparisons; MS subtype not defined
Onorf et al. (1991)	oddball	Vis & aud	10 progressive MS / 10 C	19, ? ref – Pz/Cz	lat 50% abnormal; modality—	lat 30% abnormal	N2 lat 20% lat abnormal; N2 lat →P3b lat↑	P3b 30% abnormal/absence; N2 20% abnormal	No statistical group comparisons
Honig et al. (1992)	oddball (count)	Aud	31 MS / 32 C	20, bilateral cheek – Pz/Cz	lat↑, amp↓; 65% abnormal; lat corr NP	—	—	clear 16% +mild 19% abnormal	Controls not age-matched; MS subtype not defined
Giesser et al. (1992)	double oddball with easy & hard target (RT)	Aud	6 CI & 6 non-CI MS / 7 C	14, linked mastoids – Pz	CI MS lat↑ vs. non-CI & C; lat corr NP	—	N1 & P2 lat↑ in CI MS vs. Non-CI / C	—	Low N -> weak statistical analysis; MS subtype not defined; CI MS group older; no P3 amplitude results; no MS vs. control group comparison

Triantafyllíou et al. (1992)	oddball (count)	Aud	47 RRMS (EDSS groups-cutoff 3) / 24 C	16, linked cheeks – Pz?	MS / high-EDSS lat↑ & amp↓ vs. C; high-EDSS lat↑ vs. low-EDSS	—	MS / high-EDSS lat N2↑ & amp↓vs. C; high-EDSS N2 lat↑ vs. low-EDSS	No diff	Eyes closed -> artefacts; topographic maps calculated from only 16 channels->vague results; no visual; no NP measures-no link to cognitive functions
Van Dijk et al. (1992)	Oddball (RT) with easy & hard discrimination tasks	Vis & aud	30 MS (13 RRMS, 17 progressive MS) / 19 C	3, linked ears – Fz	Easy: MS vis lat↑ vs. C; Hard: MS lat↑ vs. C;	—	Easy: MS vis N2 lat↑ vs. C;	—	Eyes closed -> artefacts;utilised one-sided t-test; only 3 electrodes; no NP measures-no link to cognitive functions; no comparison of subgroups
Gil et al. (1993)	oddball (count)	Aud	98 MS (82 RRMS, 16 progressive MS) / 71 C	1, linked mastoids – Cz	lat↑ in MS; espec. lat↑ in progressive MS; lat corr with NP	—	P2 & N2 lat↑ in MS; P2 lat↑ & N1-P2 amp↓ in progressive MS; P2 & N2 lat corr with NP	—	Only 1 electrode; no visual condition; Small group sizes for statistics
Ruchkin et al. (1994)	Phonological & visuo-spatial matching (motor response)	vis	10 MS (9 RRMS, 1 chronic progressive) / 10 C	18, linked tragus – Pz	no lat	—	—	no	Small group sizes for statistics; paradigm not established; no correlational analysis between ERP and neuropsychological variables

Boose et al. (1996)	Oddball (RT)	aud	P3: 12 MS / 12 C; other: 30 MS / 30 C	1, linked earlobes –Fz	No stat diff; 60% abnormal	—	no	—	Low N in groups included in statistical analyses; No subtype information; no neuropsychological tests included; no visual condition; only 1 electrode
Aminoff & Goodin (2001)	Oddball (count)	aud	8 RRMS / 10 C	7, linked mastoid – Cz	lat↑ & amp↓; lat & amp corr MMSE	—	N1, P2, N2 lat↑; P2 amp ↓; N2 lat, N2 amp, P2 amp corr MMSE	—	Low N; MMSE too general to measure typical cognitive profile of MS; no visual condition; controls not age- etc matched
Sailer et al. (2001)	oddball & 3-stimulus (RT)	Vis & aud	34 MS (grouped into 3 based on lesion volume) / 15 C	12, right mastoid – electrode pair P3/P4	No	no	vis N1 & N2 amp↓ in high-lesion MS; vis N2 amp corr lesion load	—	Proportions of RRMS and SPMS not specified; small groups; MS patients as one group not compared to controls; ERP and neuropsychological variables not tested for correlation
Ellger et al. (2002)	oddball (RT)	Vis	179 MS (107 RRMS, 50 SPMS, 17 PPMS) / 0 C	1, mastoid – Pz	SPMS lat↑ & amp↓ vs. RRMS; PPMS amp↓ vs. RRMS; SPMS lat↑ vs. PPMS	—	SPMS & PPMS P2, N2 lat↑ vs. RRMS	—	No control group; no neuropsychological tests; no auditory condition; only one electrode; uneven groups as PPMS group significantly smaller-effect on statistics

Piras et al. (2003)	oddball (count&RT)	Vis & aud	12 RRMS / 15 C	30, linked earlobes – Fz, FCz, Cz, CPz, Pz	vis & aud lat↑ & amp↓; vis lat & amp corr NP; vis↑	—	vis & aud N2 lat↑; vis N2 lat corr NP	no	Small group sizes; topographical differences not statistically tested; no other subtypes; no mention differences in which electrodes
Jung et al. (2006)	MMN (ignore)	aud	46 MS (19 RRMS, 21 SPMS, 6 PPMS) / 46 C	1, right mastoid – Fz	—	MS lat↓ MS vs. C; absent more in MS; no diff in CI groups	MS reduced early N1-P2 amplitude and MMN latency and area	—	reporting P3a area not amplitude; classigying to CI group based on 1 test; no correlations tested between ERP and neuropsychological variables

Note. aud = auditory, vis = visual, C = controls, MS = MS patients, RRMS = relapsing-remitting MS patients, SPMS = secondary-progressive MS patients, PPMS = primary-progressive MS patients, CI MS = MS patients with cognitive impairment, Non-CI = MS patients with no cognitive impairment, count = subjects asked to count the targets during the P3 task, ignore = subjects asked to ignore targets during the P3 task, RT = reaction time for the targets, ON = optic neuritis, diff = different, ref = reference utilised in the EEG analysis, locs = electrode(s) that used in the statistical analyses, MMN = mis-match negativity, early comp. = early ERP components, topo = topographical differences; ↑ = increase; ↓ = decrease

to the participants (i.e. overt response to target or silent counting of the number of targets; eyes open or closed; see Table 2.6.) causing methodological differences that make the reliable comparison of the results across the studies very difficult.

P3 ERPs have been found to correlate with neuropsychological test performance. Auditory (Giesser, et al., 1992; Gil, et al., 1993; Honig, et al., 1992) and visual (Piras, et al., 2003) P3b latencies increased, and auditory P3b amplitudes reduced (Honig, et al., 1992) with poor cognitive performance. Moreover, measures sensitive to impairment in learning, attention working memory were correlated with P3 waves (Giesser, et al., 1992; Gil, et al., 1993; Hohol, et al., 1997). On the other hand, some studies have reported P3b as not correlated with neuropsychological test scores (Onofrj, et al., 1991). The neuropsychological methods utilised are very limited and a more comprehensive investigation is necessary. Only one study (Giesser, et al., 1992) grouped MS patients based on their neuropsychological test scores into patients with and without cognitive decline. The comparison revealed MS patients with most cognitive impairment to have delayed auditory P3b latencies. However, the number of patients in each group was very low and the neuropsychological examination was not comprehensive enough to determine the cognitive status reliably.

P3 ERP latency delay and/or amplitude decrease are also related to lesions detected with MRI. An early study (Newton, et al., 1989) found MS patients with abnormal ERPs to have larger MRI cerebral lesions. Similarly, delayed P3b and P3a latencies were shown to correlate with the presence of extensive demyelinative lesions seen with MRI (Honig, et al., 1992). More recent investigation was more anatomically specific as it examined 15 different cerebral sites for lesions, and reported auditory P3b latency to correlate with lesions in frontal horn and brain stem (Piras, et al., 2003). Sailer and colleagues (2001) assigned 34 MS patients to low lesion volume, high lesion volume and frontal lesion volume groups based on lesion volume and distribution on T2-weighted MRI scans. Results revealed N2 amplitude to be significantly reduced in the high lesion volume and frontal lesion volume groups compared to controls. In addition, they discovered N2 amplitude to be correlated with the lesion volume. However, the P3b and P3a components did not show any differences and/or correlations with regards to lesion volume.

The few studies including sufficient numbers of electrodes to conduct topographical analyses over the scalp have reported abnormal topographies in 16-30% of MS patients (Honig, et al., 1992; Onofrj, et al., 1991). Others did not find marked differences relative to controls (Piras, et al., 2003; Ruchkin, et al., 1994; Triantafyllou, et al., 1992). Source localisation methods have

not been employed in previous literature on electrophysiological alterations in MS. This is most likely due to relatively low numbers of electrodes (<30) and because most source localisation data analysis methods have only been developed in recent years and have not been available at the time of the earliest studies. The ability to estimate EEG source location related to information processing and cognitive performance more accurately has importance in studies with neurological conditions, as it may ultimately provide insight on the locations of the brain where the changes impacting cognitive function are occurring.

2.2.7.1. Longitudinal P3 event-related potential studies examining cognitive function in multiple sclerosis

There has been some interest in studying longitudinally evoked potentials in different sensory modalities in order to better monitor the progression of disability in MS (Leocani & Comi, 2008; Pelayo, et al., 2010). P3 ERPs have been used to monitor the effects of treatment and disease course in MS. Previous studies (Flechter, et al., 2007; Gerschlager, et al., 2000a; Pliskin, et al., 1996; Pokryszko-Dragan, et al., 2009) have focused on the effects of interferon and methylprednisolone (HDMP; Filipovic, Drulovic, Stojsavljevic, & Levic, 1997) treatment on cognitive function. Flechter, et al. (2007) studied 16 MS patients using an auditory P3b paradigm prior to, and one year after, they received interferon β-1b treatment. They reported that auditory P3b latencies were shortened one year later, but they did not include a control group in the study. P3 ERPs are demonstrated to change with age (Polich, 2004) and therefore, the changes seen in the P3 components may be due to age, not disease progression or treatment. This fact makes it crucial to include a healthy control group in the longitudinal ERP studies to distinguish the changes related to MS disease progression, from normal aging related changes.

Longitudinal ERP studies that included a healthy control group failed to find any differences in visual P3b after low/high-dose interferon β -1b treatment (Pliskin, et al., 1996), in auditory P3b after interferon β -1a treatment (Pokryszko-Dragan, et al., 2009) and after interferon β -1b treatment (Gerschlager, et al., 2000a). Gerschlager, et al. (2000) also reported that three patients developed abnormal ERPs after one year. All of the studies revealed MS patients to have improved performances in neuropsychological measures of attention and memory after the treatment period (Flechter, et al., 2007; Gerschlager, et al., 2000a; Pliskin, et al., 1996; Pokryszko-Dragan, et al., 2009). These studies do not exhaustively examine whether the P3 and early ERP components remain stable or undergo progressive changes during the disease course of MS.

2.5.7. Visually Evoked Spread Spectrum Response Potential (VESPA)

By convention, visual evoked potentials (VEPs) have been employed to examine the function of the visual systems in MS patients (Polman, et al., 2011; Polman, et al., 2005). However, VEP testing sessions can be quite long (one session typically lasts approximately one hour) because the output results must be averaged over many trials to obtain a stable VEP response – typically a minimum of 60 trials, with 200 – 300 trials being preferable. Thus, VEP tests can often be tiresome for subjects which leads to difficulty in maintaining focus and concentration (already compromised in a large portion of MS patients).

Visual Evoked Spread Spectrum Analysis (VESPA) is a special case of VEP where the visual stimulus is continuously presented to the subject. The VESPA is an estimate of the linear impulse response of the visual system. The luminance of the stimulus is modulated by a stochastic waveform, with a high refresh rate (typically around 60 Hz). This method allows for the rapid acquisition of a VEP with a complete temporal profile and higher signal-to-noise ratio (SNR) than VEP, within the same timeframe. The power of the underlying waveforms used to modulate the luminance of the visual stimulus is spread over a range of frequencies and as a result are termed spread spectrum waveforms. The VESPA considers the human brain to act as a linear system. The input to the system is the stimulus brightness waveform. The resulting EEG is the output from this linear system and allowing for the addition of noise to the system, the impulse response function of this system (the VESPA) can be determined (Lalor, et al., 2006). The VESPA method generates and obtains a response from the subject for the entire duration of stimulus presentation.

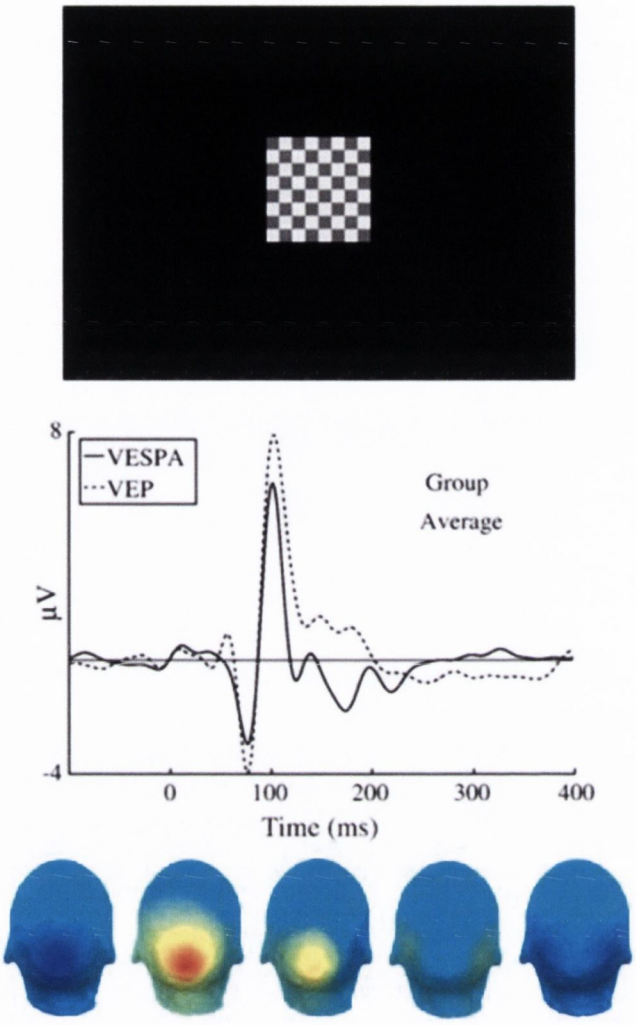
The VEPs present stimuli periodically, thus responses are only generated approximately every 400ms. The VESPA method on the other hand, allows for the presentation of simultaneous stimuli that produces separate VESPA responses (Lalor, et al., 2006). By continuously presenting a stimulus to the subject, comprehensive time-frequency data potentially lost with periodic presentation of VEPs can be acquired and more importantly, the testing time is dramatically decreased. A single VESPA run lasts for 120s and subjects usually undergo 2 runs per test sessions, which are averaged to generate a stable VESPA response. The temporal profile of VESPA responses includes a clearly defined, measurable VESPA-like P100 component, which is highly correlated with that of evoked VEPs using standard discrete stimuli (Figure 2.9). Scalp maps showing topographical evolution of VESPA indicate that this method generates highly specific scalp topographies (Figure 2.10), which may imply the involvement of different

visual neural pathways. In fact, a previous study (Lalor & Foxe, 2009) showed the full range VESPA to be biased towards parvocellular pathways.

The VESPA stimuli have been shown to target towards parvocellular pathways within the brain (Lalor & Foxe, 2009), which is one of the major pathways of the visual system with the anatomically distinct magnocellular pathways (Liu, et al., 2006). The P cells in the retina project to layers 3 - 6 in the lateral geniculate nucleus (LGN) of the thalamus. The projections of the M and P cells appear to be distinct in the LGN and remain so, from the LGN to the primary visual cortex (V1), with the M pathway terminating primarily in layer 4Cα of V1 and the P pathway terminating primarily in layers 4A and 4Cβ of V1 (Liu, et al., 2006). The parvocellular pathway shows greater sensitivity to higher spatial frequencies, lower temporal frequencies and chromatic stimuli. In addition, it is thought to be central for chromatic processing and visual acuity and to provide input to higher-order pattern processes. The parvocellular pathway can be expected to be susceptible to the pathological changes in the brains of MS patients, as the parvocellular pathway accounts for approximately 80% of optic nerve fibres. It consists of thinly myelinated ganglion cells likely to be prone to disruptions caused by widespread demyelination in MS (Cao, et al., 2011).

The group averages of VEP and VESPA responses have been found to be strongly correlated, but at an individual level the correlation was reported to be much weaker. These results indicate that while the same information regarding group averages was being extracted with both methods, the VESPA may be more sensitive to individual responses. Moreover, each of the single stimulus presentations of VEPs and VESPAs were found to be very highly reproducible (with the mean checkerboard stimulus having a higher reproducibility for both VEP and VESPA results) (Lalor, et al., 2006). Reproducibility of a method is a good indicator as to its usefulness in clinical applications. Due to the similarity of the VESPA and the VEP methods, it can be suggested that the VESPA method may hold promise to probe changes in optic nerve function, and visual function in general, in MS, likewise to the VEP (Ziemann, et al., 2011). The disadvantage of VESPA in comparison to standard VEPs is that it requires greater computational power. However, as Lalor, et al. (2006) discussed, given the power of modern computers, this should not be a factor in the choice of method, rather the speed at which results can be obtained should be prioritised.

Figure 2.9. VESPA luminance checkerboard stimuli, comparison of VESPA and VEP group averages, and a topographical illustration of VESPA signal (Adapted from Lalor et al., 2006).



2.6. Thesis objectives

Following the detailed review of the literature, the main objective of the research was to investigate if the proposed electrophysiological methods can be applied as potential tools to detect and monitor the changes associated with the dysfunctions in cognitive and brain functions in MS. Four general objectives to the research were posed.

1. To investigate cross-sectional scalp-based and source P3 EEG activations and cognitive function in MS patients. Also, to compare these between different MS subgroups (based on subtype or genetic factors). In addition, to compare the P3 EEG activations to the Paced Auditory Serial Addition Test (PASAT) performance (Chapter 4).

2. To examine change in P3 EEG activations and cognitive function in MS patients over 12 month and 24 month periods (Chapter 5).

3. To conduct a multimodal investigation of cognitive function in MS by examining associations between the findings from high-density EEG, neuropsychological examination and structural MRI (Chapter 6).

4. To examine the utility of the VESPA method to probe visual dysfunction in MS (Chapter 7).

2.7. Specific research questions

Following the detailed review of the literature, a series of specific aims and research questions remain.

1. Do the scalp-based P3 ERP activations at Month 0 differ between MS patients and healthy controls, and between MS subtypes? Does the PASAT score correlate with the scalp-based P3 ERP activations? (Chapter 4)

    1.1. Do the visual and auditory P3b and P3a ERPs differ in MS patients and in healthy controls? Or in different MS subtypes? Do the visual and auditory P3b and P3a ERPs differ in progressive MS patients versus age-matched controls? Which P3 ERP task(s) are most sensitive to detect these differences? Is there a difference between visual and auditory modalities?

    1.2. Do the PASAT scores correlate with visual and auditory latencies and/or amplitudes with the P3b and P3a ERPs? Over which brain areas and during which time period can the correlations be found?

2. Do the source P3 EEG activations at Month 0 differ between MS patients and healthy controls, and between MS patients with and without cognitive impairment? (Chapter 4)

2.1. Do the source P3 EEG activations differ in MS patients relative to controls? If so, how? Where is the source of the P3 EEG activations in MS?

2.2. Do the source P3 EEG activations differ in MS patients with impaired cognitive functions in comparison to MS patients with intact cognitive performance? If so, how? Where is the source of the P3 ERP/EEG activations in MS?

2.3. Which of the measures utilised as part of the novel independent component (IC) clustering method show the differences; ERPs, ERSPs, ITCs, spectra, or topographical maps?

2.4. Which P3 ERP paradigm (P3b vs. P3a, visual vs. auditory) detects the best of these differences?

3. Do the P3 ERP activations and the PASAT score differ between MS patients with different oligoadelynate synthetase 1 (OAS1) genotype (Chapter 4)?

3.1. Do MS patients with AA OAS1-genotype have different auditory P3 ERP latencies, amplitudes and topographies relative to MS patients with AG the OAS1-genotype?

3.2. Do MS patients with AA OAS1-genotype have different PASAT scores relative to MS patients with the AG OAS1-genotype?

4. Do the P3 ERP activations change over a 12-month period in MS? If they do, is the change greater compared to healthy controls? Does the 12-month change in the PASAT score correlate with the change in the P3 ERP activations? (Chapter 5).

4.1. Do the MS patients show greater decrease in P3 ERP amplitudes compared to controls? Do the MS patients show greater delay in P3 ERP latencies compared to controls? Do the RRMS and SPMS patients differ? Over which brain areas and what time period can this difference be observed? Which task(s) detect this difference? Is there a difference between visual and auditory modality?

4.2. Do the P3 ERP amplitudes change after 12-months in MS patients? If so, how do they change? Do the P3 ERP amplitudes change after 12-months in controls, how? Over which brain areas and during which time period can this difference be observed?

4.3. Does the PASAT score correlate with visual and auditory P3b and P3a ERPs over a 12-month period? Over which brain areas and during which time period can the correlation be found?

4.4. Does the PASAT score correlate with P3 ERP tasks at Month 0 and at Month 12? Is there a correlation when all subjects, only MS patients, or only controls are included?

5. Do the P3 ERP activations change over a 24-month period in MS? If they do, is the change greater in comparison to healthy controls? Does the change in the P3 ERP activations correlate with the change in the PASAT score? (Chapter 5).

5.1. Do the P3 ERP amplitudes change after 24-months in MS patients? If so, how do they change? Over which brain areas and during which time period can this difference be observed?

5.2. Do MS patients show greater decrease in P3 ERP amplitudes compared to controls? Do MS patients show greater delay in P3 ERP latencies compared to controls? Do RRMS and SPMS patients differ? Over which brain areas and what time period can this difference be observed? Which task(s) detect this difference? Is there a difference between visual and auditory modalities?

5.3. Does the PASAT score correlate with visual and auditory P3b and P3a ERPs over a 24-month period? Is there a correlation when all subjects, only MS patients, or only controls are included?

6. Are the findings from multimodal measures of P3 ERPs, MRI (lesion load) and neuropsychological examination associated in MS?

6.1. Do the visual and auditory P3 ERP amplitudes and latencies correlate with the neuropsychological tests of the adapted MACFIMS battery?

6.2. Do the visual and auditory P3 ERP amplitudes and latencies correlate with MRI T2 lesion loads of different brain regions? If so, which brain regions? Can the brain regions with lesions be associated to the sources of the P3 ERP activations in MS?

7. Does the VESPA method have utility to probe visual dysfunction in MS?

7.1. Do the MS patients have reduced VESPA-like P100 amplitudes and delayed VESPA-like latencies relative to the controls?

7.2. How do the VESPA-like P100 component activations differ in different MS patient subgroups; RRMS vs. SPMS, PPMS vs. SPMS, MS patients with visual dysfunction vs. MS patients with no visual deficits?

7.3. Do the VESPA-like P100 component activations change over a 24-month period? Which MS patient group(s) show the most impaired VESPA-like P100 components?

7.4. Does the VESPA-like P100 activations correlate with physical disability and/or demographic variables such as age and education?

2.7. Conclusion

This chapter reviewed thoroughly the research literature on MS disease and the cognitive deficits associated with MS. It also provided a description of the methods utilised to measure brain and cognitive function in MS, and the main findings in MS to date acquired with these methods. The shortcomings of the conventional methods and the advantages of the new methods to detect and monitor CI in MS were discussed with each method. Furthermore, the thesis objectives with the specific research questions were presented. The specified principal objective of the research was to examine if the proposed advanced electrophysiological methods can be applied as potential tools to detect and monitor the changes in the brain activations related to cognitive dysfunctions in MS.

Previous research conducted utilising the gold standards for detecting cognitive and brain function in MS, namely the neuropsychological examination and MRI, have shown cognitive deficits in MS to manifest particularly in attention, the information processing speed and working memory, and to be related to demyelinating lesions in cerebral cortex and atrophy of the brain, especially of the cortex and hippocampus. However, the cognitive-indexing electrophysiological measures present as favourable auxiliary measures to detect and monitor cognitive function in MS were due to a number of reasons. Firstly, they have higher temporal resolution, and are cost-effective, relatively easy to administer and portable, in comparison to other brain imaging measures (Polich, & Herbst, 2000). Secondly, electrophysiological measures are not confounded by practice effects (Sandman, & Patterson, 2000; Segalowitz, & Barnes, 1993; Walhovd, & Fjell, 2002), are independent from behavioural responding and thus, not dependent on physical ability (Magnano, et al., 2006), and there is no evidence to suggest that they are affected by demographic factors such as education. The previous research literature has shown MS patients to have delayed P3 ERP latencies and/or reduced P3 ERP amplitudes that index cognitive function, but the studies have numerous methodological limitations and thus, a thorough cross-sectional and longitudinal examination of the P3 ERP/EEG activations in MS utilising advanced EEG recording and analysis methods is required. The research on the differences between the MS subtypes in P3 ERP and cognitive functions is scarce and needs elucidating. Moreover, the task-related source EEG activations indexing cognitive function in MS have not been investigated earlier. The relationship between cognitive function and the P3 ERP/EEG activations, neuropsychological performance, MRI variables and genetic factors in the context of MS are largely unknown. Furthermore, the

utility of a novel method, the VESPA, in probing visual dysfunction in MS, needs to be examined.

A number of overreaching hypotheses were posed based on previous research. It was hypothesized that MS patients (especially progressive MS and those with OAS1 genotype related to increased disease activity) would have reduced and delayed scalp-based and source P3 EEG activations, and impaired PASAT performance, relative to the healthy controls. It was hypothesized that the P3 EEG activations would correlate with the PASAT scores. Moreover, the MS patients were hypothesized to show greater prolongation and decrease of the P3 EEG activations, and a reduction in the PASAT score, compared to the healthy controls over the 12- and 24-month follow-up periods. Additionally, in the multimodal study the delayed and reduced P3 EEG activations were hypothesized to be associated with poor neuropsychological test scores and increased lesion load. Lastly, the VESPA-like P100 component was hypothesized to be reduced and delayed at baseline, and show gradual delay in latency and reduction in amplitude over a 24 month period, in MS patients in comparison to healthy controls. The specific hypotheses are presented in the introduction to each study.

Chapter 3 - General Methods

In order to address the specific research questions presented at the end of the Chapter2-Literature Review, a number of advanced methods are required. This chapter provides a detailed account of each method utilised in this research.

3.1.. Study procedure

The experiments were conducted as a collaboration of the Department of Neurology in St. Vincent's University Hospital (SVUH), the Trinity Centre for Bioengineering, and University College Dublin Psychology Department. Exclusion criteria included a current use of benzodiazepines or neuroleptics with a minimum suspension period of seven days, a history of alcohol or drug misuse, head injury or stroke. The subjects were asked to participate in the study once a year over a period of three or four consecutive years.

Ethical approval was obtained from the Ethics and Medical Research Committee of the St. Vincent's Healthcare Group (Appendix 1). Informed consent was obtained from all subjects on each testing occasion. Participants were also informed that they had the right to withdraw from the study at any time for any reason and that this would not affect their future health care in SVUH. All participants were also informed that all data would be anonymous, as each data set was labelled with a code.

The research project began in 2007; 40 MS patients completed the EEG experiment with the P3 ERP and the VESPA tasks, the standard 3-s PASAT (Tombaugh, 2006) and the 21-item Hamilton rating scale for depression (HRSD) (Vieweg & Hedlund, 1979). MS patients also completed a physical examination, including Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 2008) which was administered by a neurology registrar in the Department of Neurology, SVUH.

In the second year of the research project (October 2008 – Spring 2009) structural MRI scan and neuropsychological assessment, encompassing cognitive and psychological functioning measures, were added to the study procedure. Structural MRI scans were acquired by the Department of Radiology in SVUH. Neuropsychological assessment lasted 60-90 minutes, conducted by trained psychology postgraduate students of the Department of Psychology of University College Dublin. Over half of the MS patients returned (N=24) in the second year of the project, and 8 new MS patients were recruited to the study.

The procedure comprising of the EEG experiment, structural MRI and the neuropsychological assessment was repeated in the third year (January – March 2010) and also again in the fourth year (January – February 2011). In the third year, 30 MS patients returned, although two patients from the previous year dropped out of the study. In the fourth year, 17 MS patients returned, 13 MS patients dropped out and a further 13 new MS patients were recruited, to allow a more refined cross-sectional analyses of Month 0 data.

Furthermore, in 2010 five additional PPMS patients were recruited to take part in a cross-sectional study comparing PPMS and SPMS subtypes. The patients completed the EEG experiment, the PASAT test, the HRSD-21 and the physical examination including EDSS (Kurtzke, 2008). In 2011, PPMS patients were not asked to take part in the study due to drop-outs and the small numbers in comparison to previous years.

In total, 14 MS patients completed EEG experiments over a 4-year period and 17 completed all the required measures (EEG, MRI, neuropsychological assessment) over a 3-year period. Due to the addition of the structural MRI and the complete neuropsychological battery in the second year, most of the studies include the limited data of MS patients who took part in the first year (2007 – Summer 2008). The same components applied in the following years, even though patients completed more measures, and few of the studies included the complete data with all the measures starting from the second year.

Age-matched controls, which were recruited in the second year of the study, all completed the full neuropsychological battery, in addition to the EEG experiment. Controls did not complete either physical assessment or structural MRI. The data was collected from the controls in the following second, third and fourth year.

Table 3.1. illustrates the two study procedures both with and without the full neuropsychological test battery and the structural MRI conducted in the studies. The table also includes the exact numbers of subjects at each timepoint.

Demographic details were recorded from all participants. These included age, date of birth, gender, education level, years of education and handedness. Furthermore, the MS patients were asked to provide information on their past and current drug therapies, the age they first experienced a symptom, the age when first diagnosed, history of depression and/or other psychiatric diagnoses, possible relapses and their history of visual dysfunction such as optic neuritis.

The demographic information and the behavioural task scores of subject samples are described in detail in the methods section of each study, conducted as part of the overall research project.

Table 3.1. The two study procedures with and without the full neuropsychological test battery and the structural MRI included in the studies conducted. The exact numbers of subjects are indicated at each timepoint.

	Year 1	Month 0		Year 2	Month 12		Year 3	Month 24		Year 4
time period	2007-2008	Oct 2008 - Spring 2009	Jan-Feb 2011	Oct 2008 - Spring 2009	Jan-Mar 2010	Jan-Feb 2012	Jan-Mar 2010	Jan-Feb 2011		Jan-Feb 2011
measures	EEG (+PASAT, Hamilton)	EEG, MRI, NP	EEG, MRI, NP	EEG, MRI, NP	EEG, MRI, NP	EEG, MRI, NP	EEG, MRI, NP	EEG, MRI, NP		EEG, MRI, NP
total number	40	8	13	24	8	11	22	3		14
dropouts				15		2	3	2		8
MRI notes		7 have MRI data	13 have MRI data	21 have MRI data	8 have MRI data	11 have MRI data	19 have MRI data	3 have MRI data		8 have MRI data
subtypes	RR:19	RR:1	RR: 9	RR:14	RR:1	RR: 8	RR:12	RR: 1		RR: 9
	SP:16	SP:6	SP: 4	SP:6	SP:6	SP: 4	SP:7 (8 if MS41)	SP: 2		SP: 4
	PP:2	PP:3		PP:2	PP:3		PP:2	PP: 0		PP: 1
subcodes (ERP codes)	MS01	MS39	MS49	MS01	MS39	MS49	MS01	MS42		MS02
	MS02	MS42	MS50	MS02	MS42	MS50	MS02	MS43		MS05
	MS03	MS43	MS51	MS03	MS43	MS51	MS04	MS45		MS07
	MS04	MS44	MS52	MS04	MS44	MS54	MS05			MS15
only EEG+PASAT+Hamilton:	MS05	MS45	MS53	MS05	MS45	MS55	MS07			MS17
Year 1	MS06	MS46	MS54	MS07	MS46	MS56	MS09			MS19
	MS07	MS47	MS55	MS09	MS47	MS57	MS10			MS20
Year 2	MS08	MS48	MS56	MS10	MS48	MS58	MS11			MS21
	MS09		MS57	MS11		MS59	MS12			MS22
Year 3	MS10	no EEG MS41	MS58	MS12		MS60	MS15	20 CONTROLS		MS24
	MS11		MS59	MS15		MS61	MS17			MS25
Year 4	MS12		MS60	MS16			MS18			MS28
	MS13		MS61	MS17			MS19			MS29
	MS14			MS18			MS20			MS41
MRI+EEG+NP:	MS15			MS19			MS21			
Month 0	MS16			MS20			MS22			
Month 12	MS17	43 CONTROLS		MS21	29 CONTROLS		MS24			
Month 24	MS18			MS22			MS25			
	MS19			MS24			MS26			
	MS20			MS25			MS28			
	MS21			MS26			MS29			
	MS22			MS28			MS41			
	MS23			MS29						
	MS24			MS31						
	MS25									
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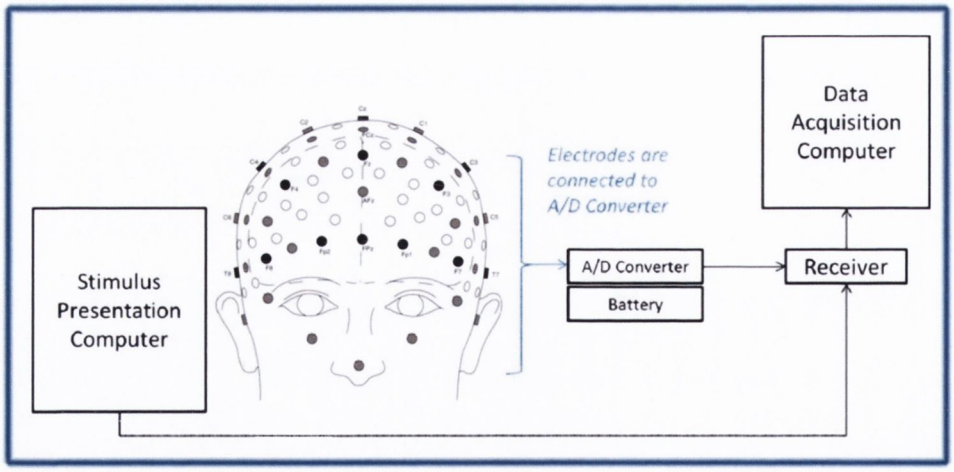
	MS37									
	MS38									
	MS40									
	MS41									

3.2. The EEG, the P3 ERP and the VESPA measures

As stated in Chapter2–Literature Review the conventional methods, such as the neuropsychological examination, MRI and VEP, have multiple limitations. The EEG, the P3 ERP and the VESPA measures are new and promising methods for investigating cognitive, brain and visual function in MS. These measures are employed in this research and are presented in detail below.

EEG was recorded with the ActiveTwo Biosemi™ electrode system from 134 electrodes, of which 128 electrodes were placed on the scalp, two electrodes on mastoids, one on the nose and two electrodes were placed bilaterally approximately 3 cm below the eye, and two on the outer canthi to record the vertical and horizontal electro-oculograms (see Figure 3.1). The scalp electrodes were organised according to the extended 10-5 system (Oostenveld & Praamstra, 2001) and digitised at 512 Hz. Subjects performed the tasks in a darkened, soundproofed room. The subjects were seated 70 cm from a cathode ray tube (CRT) computer monitor with a screen resolution of 1024 x 768 pixels, at a refresh rate of 60Hz. Data was referenced to one scalp electrode during recording and later average-referencing was conducted during the data processing as this method is considered appropriate with high-density EEG arrays.

Figure 3.1. A diagram of the EEG set-up utilised in the research project.



3.2.1. The P3 ERP paradigms

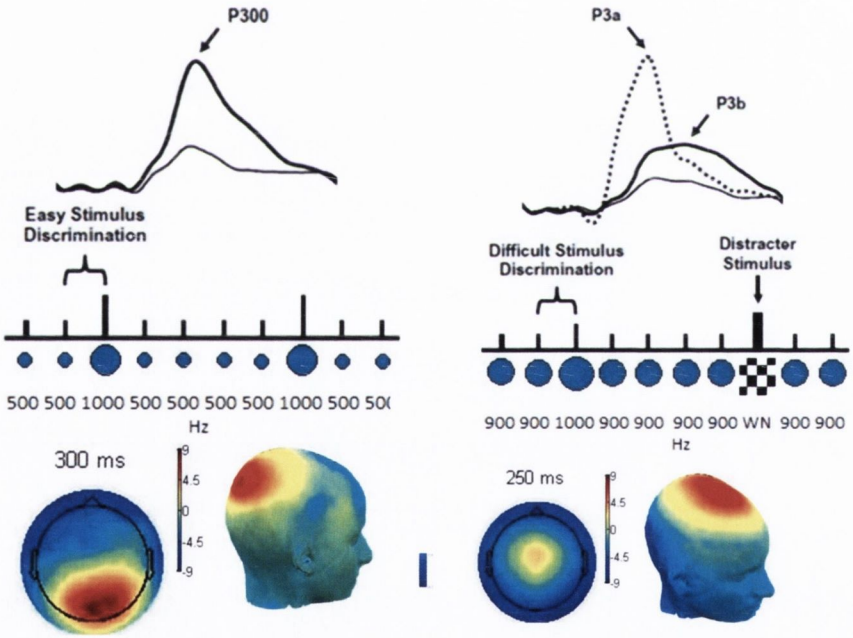
The two-stimulus oddball task is one of the most utilised ERP paradigms. It was employed in the present research, as it is one of the most robust ERP paradigms; producing a clear and easily measurable change in the EEG. In the oddball task occasional target stimuli must be detected in a train of frequent irrelevant standard stimuli. A P3b component is typically elicited approximately 300 ms after each target stimulus, and it has maximal amplitude over the parietal scalp area. In the present study, the stimuli and task parameters were based on the recommendations by Polich (2004). The visual P3b paradigm consisted of blue circles, separated by an inter-stimulus interval of 2 seconds, presented for 205 trials in a pseudorandom order. Frequent non-target (80%) and infrequent target (20%) circles were 2 cm or 4 cm in diameter, respectively. The auditory P3b paradigm consisted of tones, separated by an inter-stimulus interval of 2 seconds, presented binaurally for 205 trials in a pseudorandom order. Frequent non-target (80%) and infrequent target (20%) tones were presented at 500 Hz and 1000 Hz respectively. Subjects were instructed to press a button as quickly as possible following a target stimulus. Their reaction times were recorded and used as a part of the behavioural data analysis.

The present research project also focuses on a variant of the P3b; the no-go P3a, elicited by employing a three-stimulus oddball paradigm. In addition to standard and target stimuli, further infrequent, non-target stimuli are presented (Figure 3.2). Subjects are asked to respond only to target stimuli and refrain from responding to the additional non-target stimuli and to the standard stimuli. Furthermore, a difficult standard/target discrimination is employed because it results in more easily detectable P3a components; higher in amplitude and shorter in latency compared to those in an easy discrimination task. Typically, this no-go P3a component can be observed approximately 250 ms after the non-target stimuli. This is especially evident over the central scalp region, with shorter latencies in frontal areas and longer latencies in central/parietal areas (Polich, 2007). The visual P3a paradigm consisted of stimuli separated by an inter-stimulus interval of 2 seconds, presented for 410 trials in a pseudorandom order, across two separate runs each consisting of 205 trials. Frequent standard (80%) and infrequent target (10.24%) circles were 3.5 cm or 4 cm in diameter, respectively. The non-target stimulus was a checkerboard (9.76%) which was 5.25 cm per side. The auditory P3a paradigm consisted of stimuli separated by an inter-stimulus interval of 2 seconds, presented binaurally for 410 trials in a pseudorandom order across two separate runs of 205 trials each. Frequent standard (80%) and infrequent target (10.24%) tones were presented at 900 Hz and 1000 Hz respectively. The non-target (9.76%) stimulus was indicated

by a white-noise burst. Subjects were instructed to press a button as quickly as possible, following a target stimulus. During the EEG recording, the order of modality and task were counterbalanced across subjects. The same EEG procedure was repeated at the follow-up sessions.

The P3b and P3a components were extracted from separate paradigms. This was essential in order to compare the results from the present research to those from previous studies, most of which had only utilised the P3b paradigm. Furthermore, the use of highly established ERP paradigms, such as the oddball paradigm, is recommended with clinical groups (Duncan, et al., 2009). Also the task conditions, such as stimuli presentation frequency, influence P3 ERP waveforms (Polich, 2007). Therefore utilising two paradigms with clearly different task conditions (the well-studied P3b and the lesser-employed P3a) were deemed optimal with the MS group.

Figure 3.2. Two-stimulus oddball (P3b) and three-stimulus (no-go P3a) paradigms with examples of stimuli utilised in the research (adapted from Polich, 2007).



Note. The bottom panel illustrations visualize the topographical distribution of the electrophysiological activations recorded from 128 electrodes (based on data acquired from a healthy volunteer). WN = white-noise, ms = milliseconds.

A high-density EEG (128 scalp channels) is useful in identifying differences both between the separate subject groups and also the subtle longitudinal changes in the subjects, as it covers the entire scalp area. High-density EEG makes it possible to employ independent component analysis (ICA) and statistical parametric mapping (SPM). Advanced ICA methods allow efficient removal of noisy, artefactualy data, the depiction of EEG source activations and robust

statistical analyses. The SPM maps depict activations across time and the whole scalp area, making it possible to discover statistically significant differences in scalp EEG and P3 ERP activities.

3.2.2. The P3 ERP and EEG data analysis methods

3.2.2.1. Data analysis of P3 ERP latencies and amplitudes

The P3b and P3a components are measured conventionally by assessing the amplitude and latency of averaged P3 ERPs (see Figure 3.6). The P3 ERP amplitudes and latencies are thought to index cognitive function, and they can reflect the pathological changes occurring in the brains of MS patients. In the data processing and analysis, the EEG data is first filtered, referenced, epoched and baseline corrected. This is then followed by artefact rejection and possible interpolation of bad channels. Firstly, all the epochs of each subject for a chosen condition are averaged, which then can be grand-averaged using the averages of all subjects included in the same group. Generally, the P3b and P3a components of each subject are identified from these averaged and time-locked ERPs. Amplitude (μV) is defined as the difference between the mean pre-stimulus baseline voltage and the largest positive-going peak of the ERP waveform within an appropriate time window. Latency (ms) is defined as the time from stimulus onset to the point of maximum positive/negative amplitude, within a time window (Polich, 2007). In practice, two assessors usually identify ERP components manually, according to their polarity, amplitude, latency and topography. A higher level of objectivity can be acquired by utilising semi-automated algorithms. In particular, these enable the maximum or minimum amplitude, and its latency, to be automatically extracted from the data for all electrodes using the manually identified component as a guide to define a temporal window. Another possibility in identifying a specific component is to fit a parametric function to the ERP of a chosen channel by utilising a Gaussian profile and then determining the delay at the peak amplitude, in a pre-determined time window (Gerson, Parra, & Sajda, 2005). The components of interest can be identified in the ERPs of one channel or in the ERPs of regions of interest (ROI) which are the mean value of all the electrodes included in the ROI, typically formed by electrodes in the frontal, central and parietal areas (see Table 3.2). The specific details of pre-processing and data analysis of the EEG data are described in detail in the methods section of each study conducted as part of the overall research project.

Figure 3.6. EEG and ERP approaches with latency and amplitude measures (based on data acquired from a healthy volunteer).

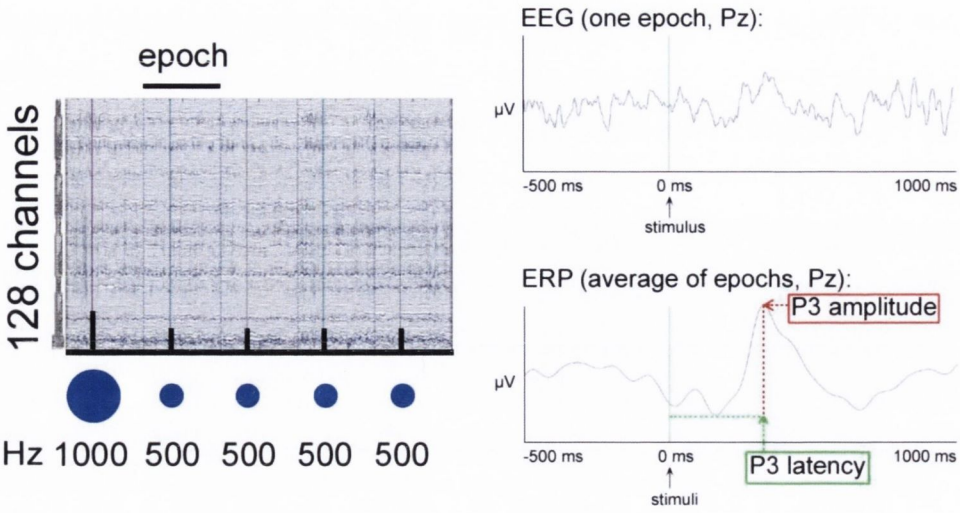


Table 3.2. Regions of interest computed from groups of electrodes and utilised in spectral power analysis.

ROI	Electrodes (10-5 system, Oostenveld 2001)
Frontal	F2', F2', AF2', AFz, AFz', Fz, Fz', F1', F1', AF1'
Central	Cz', FC2', Cz', Cz, Cz', CPz, Cz', Cz', FC1', FCz
Parietal	CPz', P1', PO1', P1', Pz, Pz', POz, PO2', P2', P2'

Note. ROI = region of interest

3.2.2.2. Statistical parametric mapping: the statistical analysis of EEG amplitude and topography

As referred to in Chapter 2-Literature Review, abnormalities in the topographical distribution of P3 ERP activations in MS are rarely reported as part previous research. However, the intervening demyelinating lesions and other pathological brain changes in MS may alter the representation of these activities on the scalp. The statistical parametric mapping (SPM)[3] is a statistical method for examining differences in brain activity recorded during EEG experiments. SPM is a more comprehensive method used to investigate the differences in amplitude of scalp-based EEG. It includes all the activations across scalp-space and time into the analysis, without the need to average and potentially lose essential information about the activations. Therefore it is a robust statistical measure to compare amplitudes and topographical

[3] The SPM tool with a detailed description is available online (http://www.fil.ion.ucl.ac.uk/spm/).

distributions between subject groups and conditions. In short, SPM involves statistical parametric maps derived from the electrical activations recorded on the scalp and they depict activations across time and the whole scalp area. This makes it possible to discover statistically significant differences in scalp EEG and P3 ERP activities between groups. In addition, SPM can enable the correlational analysis of the EEG activations and behavioural scores such as neuropsychological test scores.

EEG produces a 'map' of the scalp surface that is represented as voxels, which is the unit of measurement in the SPM method. Each voxel typically represents the activity of a particular coordinate in three-dimensional space (2 spatial dimensions + time). Statistics are used to look for the most significant difference, beyond background brain activity, to highlight the areas of activity specifically linked to the process under investigation. Prior to any statistical analysis, high-density EEG data (from all channels) needs to be preprocessed to remove noise. This data preparation process includes transforming high-density EEG data into two-dimensional sensor-space and smoothing. Smoothing involves voxels being averaged with their neighbours, typically using a Gaussian filter or by wavelet transformation, to make the data less noisy; similar to the 'blur' effect (the present studies utilised full-width half-maximum 6:6:4 a.u). Subsequently three-dimensional spatiotemporal EEG images were utilised in statistical analyses using a general linear model. In some instances, for example, with longitudinal within-subject designs, it is possible to create difference images that show the topographical difference in amplitude of one subject, across the entire scalp and time between two EEG sessions. The images can be created with the SPM8 Image Calculator tool by subtracting the subject's images for the same task. If the spatiotemporal characterisation of ERP is well known, it is beneficial to focus only on these areas by restraining the spatial characterisation of activations with spatial masks. In addition, it is more appropriate for testing specific hypotheses based on previous research as opposed to exploratory data analysis, it also has the benefit of reducing the number of statistical comparisons and the Type 1 error rate.

In relation to statistical comparison, parametric statistical models are assumed to describe the variability in the data in terms of experimental and confounding effects and residual variability at each voxel by using the general linear model. Hypotheses expressed in terms of the model parameters are assessed at each voxel with univariate statistics. Analyses may also be conducted to examine differences over a time series; using linear convolution models that measure the signal that is caused by underlying changes in neural activity. Furthermore, it is possible to examine correlations between a task variable and brain activity in a certain area. Due to the large number of statistical tests conducted, adjustments have to be made to

control for Type I errors (false positives) potentially caused by the comparison of levels of activity at a large number of voxels. In this case, a Type I error would result in falsely detecting background brain activity as activity related to the task. Based on the number of resolution elements (RESELs) in the image and the theory of continuous random fields adjustments are made, in order to set a new criterion for statistical significance that adjusts for the problem of multiple comparisons. In addition, it is possible to set a restrain for statistical results to show/retain scalp areas in which at least a certain number of voxels are significant, or to employ a stringent family-wise threshold test to further correct for multiple comparisons. Moreover, additional covariates can be added to the statistical comparisons to control for the effects of demographic variables such as age and education.

Differences in measured brain activity can be represented in a number of ways. They can be presented as a table, displaying coordinates that show the most significant differences in activity between tasks or the most significant correlations between a task variable and brain activity. However, the differences in brain activity, and correlations between a task variable and brain activity, are more often represented as pseudocolour on a scalp surface image; with the grey areas representing the location of voxels that have shown statistically significant differences between conditions. The gradient of grey is mapped to statistical values, such as t-values or r-values (the darker the area, the higher the t-value, r-value and/or statistical significance). The same areas are also represented by grey areas in the time image (time being the third dimension of the analysis). In short, these images create an intuitive means of delineating the relative statistical strength of a given area of activation in time.

3.2.2.3. Independent component analysis (ICA) and Independent component (IC) clustering

The cognitive deficits in MS are related to pathological changes occurring in the brains of MS patients, such as demyelinating lesions and atrophy. Independent component analysis (ICA) and independent component (IC) clustering can be utilised to measure the source P3 EEG activations, which reflect the brain function related to cognitive-indexing events, at the estimated brain source. Therefore, these methods offer a novel way to elucidate the relationship between cognitive and brain function in MS. The details related to ICA, modelling the IC sources and the IC clustering are depicted in the next section.

3.2.2.3.1. Independent component analysis (ICA)

The ICA was originally developed as part of 'blind source separation' problems, which attempt to separate individual source signals from multi-dimensional data in which they are mixed. Thus, when ICA is applied to high-density EEG data the aim is to distinguish separate sources, called independent components (ICs) and from scalp EEG activities in which they are intermingled. In a technical sense, ICA finds a set of fixed spatial filters, that together constitute the most distinct (i.e. temporally near-independent) signals in the data. In other words, activity of every resulting IC is a weighted sum of the signals recorded at all of the input scalp channels and every scalp channel is a weighted sum of the projected activities of all the ICs (Onton & Makeig, 2006). The general and physiologically sound assumption underlying most EEG analysis is that most of the far-field potentials measured on the scalp are generated within spatial domains of similarly oriented cortical pyramidal neurons.

EEG recordings are a time series of measured potential differences between two scalp electrodes; the potentials will sum activities from almost all active cortical EEG source domains and additionally from nearly every active artefact signal sources. As a result, EEG signals recorded on the scalp are highly variable in amplitude, frequency content and time course and have a wide band. The ICA model assumes that the far-field activities recorded at the scalp are generated in cortical EEG source domains, which project almost instantly to the scalp electrodes through volume conduction. Scalp EEG signals are therefore a mix of potentials originating from both cortical and artefact (i.e. non-neural) source domains. Accordingly scalp electrode signals can be modelled as instantaneous linear mixtures of cortical source plus non-artefact signals. In order to separate EEG source signals, ICA models make two main assumptions. Firstly, that the far-field signals produced by cortical and non-cortical EEG sources are temporally distinct and nearly temporally independent of one another. Secondly, that the cortical and artefact sources constituting together the EEG sources remain spatially fixed over time and thus project to the scalp channels with fixed weights (Onton & Makeig, 2006). These assumptions are reasonably well supported by evidence from physiological studies (Arieli, Sterkin, Grinvald, & Aertsen, 1996; Budd & Kisvarday, 2001; Freeman, 2004).

In practice, the EEG channel recordings are arranged in a matrix of n channels by t time points, which are then submitted into ICA. The ICA then performs blind separation of the data matrix (x), based on the criterion that resulting source time courses (u) are maximally independent. Subsequently the ICA finds a component 'unmixing' matrix (W) that linearly unmixes (i.e.

decomposes) the multichannel EEG data (x) into a sum of maximally temporally independent and spatially fixed components u (i.e., $u=Wx$; x and u are nxt matrices, W nxn matrix) (Onton & Makeig, 2006). The rows of the resulting matrix u are the IC activations and its columns the t time points of the input data (Jung, et al., 2001; Makeig, 2002). Columns of the inverse matrix, W^{-1}, gives the relative projection weights from each IC to each scalp electrode. It can also be expressed $x=W^{-1}u$. W^{-1} is the inverse of W, an nxn component 'mixing' matrix whose columns give the relative projection weights from each IC to each scalp electrode (Onton & Makeig, 2006; Onton, et al., 2006). Neither the IC scalp maps nor the IC activations are in original recorded units. However, despite the minor loss in subtlety these activations can be converted back to original units. Therefore the gains of the ICA method outweigh its minor disadvantages.

The quality of the ICA decomposition is highly dependent on sufficient number of time points of n-channel data, and also on the removal of 'irregular' artefact periods (i.e. large muscle movements such as clenching the jaw and yawning) prior to performing ICA on the data. ICA can be utilised in the preliminary data processing stage to identify these stereotypical artefact ICs, such as eye blinks and electromyographical (EMG) activities, and to remove them from the IC composition and also from the EEG data either manually or with automated EEGLAB[4] (Delorme & Makeig, 2004) software tools such as the Fully Automated Statistical Thresholding for EEG Artefact Rejection (FASTER[5]) (Nolan, Whelan, & Reilly, 2010). The remaining ICs can then be used in the further analyses.

The ICA method involves isolation of artefactual EEG sources (eye blinks, muscle artefacts) and removal of them from the data. This method yields a more accurate representation of neural activity compared to scalp-based ERPs with artefacts. It makes the ICA method favoured over the traditional scalp-based ERP analyses. Therefore, the independent component analysis is regularly utilised to remove artefacts from the EEG data (Groppe, et al., 2008; Nolan, et al., 2010), and results in the enhanced signal-to-noise ratio of the EEG data (Delorme, et al., 2012).

The scalp-ERP analysis procedure includes averaging, which is commonly conducted to reduce the noise in the scalp-based EEG. However, this methodology leads to the discarding of a large majority of the data and therefore has the effect of preventing the identification of the

[4] The open source EEG analysis software available from (http://sccn.ucsd.edu/eeglab/).
[5] The FASTER software tool with a detailed description is available online http://www.mee.tcd.ie/neuraleng/Research/Faster.

sources in the great majority of ongoing EEG activity that is not captured in ERP averages. The ICA approach, in contrast, allows identification of individual cortical source projections; both their scalp maps and activity time courses, and their individual contributions to the ongoing (or trial averaged) data. Therefore, it makes a wider range of source and network level analyses available. In doing so, it avoids severe confounds produced by the unavoidable summation of their signals, which are broadly volume conducted from each brain and non-brain source to most of the scalp electrodes (Delorme, et al., 2012).

3.2.2.3.2. Component source modelling

Electrode locations are usually considered only crude indicators of the locations of underlying cortical sources. However, inverse modelling of the individual IC scalp maps is a promising approach to EEG source localisation (Onton & Makeig, 2006). At its simplest, the aim is to match each observed scalp map with the best-fitting projection pattern of a single active equivalent dipole, placed in a 3D head model (Scherg & Von Cramon, 1985). Each dipole is associated with a location and a particular orientation that determines the pattern of its scalp distribution. If an IC scalp map cannot be well modelled by a single dipole it is typically not modelled. Yet if an IC scalp map appears to be bilaterally symmetrical, it can be modelled reasonably well by two dipoles symmetrically located in both hemispheres, (though possibly with differing dipole orientations). ICs accounting for most event-related dynamics are likely to be dipolar or eye artefacts, whereas non-dipolar ICs are assumed to account for relatively little in terms of the EEG signals. Therefore, they represent activity that does not fit the ICA spatial stationary assumption, or represent mixtures of small and inconsistent sources (Delorme, et al., 2012; Onton & Makeig, 2006; Onton, et al., 2006).

In practice, an equivalent current dipole model was computed for each brain activity component map. This was achieved by using a four-shell spherical head model (BESA) in the DIPFIT2[6] software tool included in the EEGLAB (Delorme & Makeig, 2004), co-registered to the Biosemi coordinates via a Talairach transformation matrix. The DIPFIT2 uses automatic single dipole source localisation algorithm for individual component maps in order to test the goodness-of-fit for modelling each IC scalp map, with a single equivalent current dipole to quantify component quality. Specifically, international 10-5 system of Biosemi utilised for EEG recording was co-registered to four-shell spherical head model (BESA), via a Talairach transformation matrix. Therefore, approximate Talairach coordinates were matched with each

[6] The DIPFIT2 software tool with a detailed description is available online (http://sccn.ucsd.edu/eeglab/plugins.html).

equivalent dipole source. The ICs were excluded from further analysis if 1) the best-fitting equivalent current dipole model had more than 15% residual variance from the spherical forward-model scalp projection (over all 128 scalp electrodes) and/or 2) the component with equivalent dipole was located outside of the model brain volume.

The component source modelling is a favoured method for estimating the location of the activation when compared to the scalp-based ERP approach. This approach assumes the recorded signals of the scalp electrodes are comparable to results achieved from equivalently placed electrodes for all the subjects. Thereby, it does not adequately take into account the physical differences of the brains of different people (e.g. differences in the orientations of cortical gyri and sulci, which cause different projections of exactly equivalent cortical sources) (Delorme, et al., 2012; Onton & Makeig, 2006). In addition, the ICA method with component source modelling more accurately reflects the spatiotemporal features of the EEG activations. The scalp-based EEG is a sum of the recorded channel activities originating from multiple, functionally distinct neural sources and non-brain sources, and therefore overlapping both temporally and spatially on the scalp (Dale, et al., 2000; Delorme, et al., 2012; Hagoort, 2003). Instead of the scalp-based EEG analysis of the locally highly-correlated source signal mixtures recorded at the two-dimensional scalp surface, the ICA decomposition is an analysis of the time courses and spatial 3-D source distributions of maximally temporally independent data sources. These separate patterns of projection via volume conduction to the scalp sensors are indicated by the decomposition (Delorme, et al., 2012). Therefore, the topographies of the ICs are not considered as informative as the 3-D locations of the equivalent dipoles. The evidence for this is shown by the minor differences across subjects in the orientation of equivalent dipoles for a set of equivalent ICs, which can produce quite different scalp maps (Onton, et al., 2006).

3.2.2.3.3. Independent component clustering

As electrode locations at the scalp are only crude indicators of the locations of underlying cortical sources, EEG recordings are generally considered to have low spatial resolution of the function of the brain. This is in contrast to functional MRI and PET which have high spatial resolution, but low temporal resolution. Typically, in order to achieve EEG source localisation, inverse modelling of EEG source locations from one or more scalp potential distributions is attempted. Nevertheless, the majority of all EEG scalp maps are the sum of the recorded activities from multiple brain and non-brain sources, and the problem has remained unsolved. Originally, by computing the average scalp distribution at specific latency/latencies following

certain task events it was hoped to minimise the number of active EEG sources. However, as more than one cortical EEG process is involved in the brain reacting to a stimulus, this approach is inadequate.

The classical scalp-channel signal analysis attempts to cluster event-related EEG phenomena across subjects. It is a relatively straight-forward procedure as each scalp electrode is assumed to be comparable with results from equivalently placed electrodes for all subjects. Nevertheless, the approach is too simplified as subject's brains have clear physical differences. In particular, the differences in the orientations of cortical gyri and sulci can cause different projections of exactly equivalent cortical sources. In addition, it is questionable whether activity at a given scalp location is equivalent in every subject. Consequently, the comparison of ICA results across subjects requires that ICs from different subjects should be similarly grouped into functionally equivalent IC clusters, despite the differences in their scalp maps.

Clustering ICs may appear imprecise at first but in comparison to the inherent disadvantages of averaging and grouping of channel activations across subjects (as achieved in the classical scalp channel signal analysis), it is a valid method in investigating the EEG source activities. In practice, clustering of ICs can be based on various features of ICs. In order to acquire comprehensive source EEG activations related to the P3b task, IC clusters can be defined by means of a joint distance measure based on these features: 3D dipole source locations and orientations, scalp maps, mean spectra, and ERP, ERSP and ITC activations. ERSPs are the mean latency-by-frequency images that show the frequencies and latencies when mean changes in log power (dB) occur from a specific mean power baseline, showing them to be time-locked to experimental events (Makeig, 1993). ITCs measure the trial-to-trial phase consistency at each frequency and latency relative to a set of time locking events. If ITC is close to 0 at a given frequency and latency (relative to time-locking events) then the measured spectral phase is evenly distributed across all trials. Whereas, if ITC is near 1, then the activity is reliably phase locked to the time-locking events (Makeig, 2002; Tallon-Baudry, et al., 1996). ITC can be considered to be very similar to cross-coherence measures as they both relate to the consistency of the phase angle from trial to trial, at each time point and frequency. However, the only difference between the two measures is that ITC represents the consistency of the phase angle for a single signal across trials, for each time point and frequency. While channel or IC cross-coherence represents the consistency of the phase angle difference between two signals across trials for each time point and frequency. The number of cycles for the time-frequency decomposition was set to [1 0.5] and the number of output frequencies to 100 for ERSP and ITC measures. The mean spectra display the log mean power spectrum for a

specified frequency range. Fast Fourier transform (FFT) was used to acquire the mean spectra (window length 256, FFT length 512 and overlap 0). The sliding windows in the frequency analysis were based on the Morlet wavelet transform (maximum 185 ms). Computation was performed on 100 frequencies from 3-256 Hz. The frequency bands of interest were theta (4-8 Hz), alpha (8-12 Hz) and beta (12-30 Hz). These cortical rhythms recorded during the P3 ERP task were examined with ERSPs and ITCs, revealing a more comprehensive overview of the underlying EEG time-frequency properties and providing a better visualisation of event-related EEG dynamics in comparison to classical ERPs. Besides ERSP and ITC, IC cluster source locations, ERP, scalp maps and mean spectra are also examined.

Principal component analysis (PCA) was utilised for pre-clustering. The open source EEG analysis toolbox EEGLAB (Delorme & Makeig, 2004) includes a clustering interface, which enables component clustering via different methods across subjects and sessions using the mentioned IC features. The IC clustering interface in EEGLAB (Delorme & Makeig, 2004) enables one to set the weights for the IC features when building a pre-clustering matrix. In the present studies, default values were used (i.e. dimensions were set to 10 for all of the measures, except for dipoles which were set to 3, and where relative weight was 1 for all of the measures, except for dipoles which was set to 10). A K-means method is the most stable algorithm that can be employed in IC clustering. It utilises Euclidean distances between the IC to find first IC cluster centroids, and then includes each IC observation into a cluster with the nearest mean. Cluster centroids include all the cluster measure centroids for each measure used to cluster the components. Dimensionality can be managed by preliminary PCA reduction. Another approach is to combine dissimilar IC activity and/or location measures in computing component 'distance' measures. Outlier components of more than two standard deviations from any of the centroid properties of the IC clusters were assigned to specific outlier IC clusters, which were then excluded from any further analyses.

The distinct IC clusters can be defined by their most prominent activities in IC features and the number of clusters set accordingly. In practice, the number of clusters can be decided based on experimentation; by increasing or decreasing the number of the clusters. Thus, the optimum number of final clusters was determined by starting with a small number of clusters, i.e. the smallest number in which activation properties were not merged across clusters. The number of clusters was then increased by one. Following the addition of an extra cluster, if IC dipole groups were divided into two, but where the other IC properties (and the differences between the groups and conditions) remained approximately the same, then the maximum

number of clusters was deemed to have been exceeded. As a result, the simplest and most informative cluster solution can be chosen.

The IC clustering interface also includes the possibility of conducting statistical analyses to compare the EEG source dynamics between the groups and/or conditions, within each IC cluster. Permutation statistics were utilised in the studies of the thesis as it corrects for multiple comparisons in EEG data (Delorme, 2006; Maris, 2004), parametric and bootstrap statistics were also available. The multiple comparisons were further controlled with Bonferroni correction and with the false discovery rate (FDR) statistical method (Benjamini & Yekutieli, 2001).

3.2.3. Visual Evoked Spread Spectrum Analysis (VESPA)

In Chapter 2–Literature Review the limitations of the conventionally utilised method to measure visual dysfunction in MS, the VEP, were discussed. Furthermore, a novel method, the VESPA, was introduced and the subtleties related to this method are detailed below.

3.2.3.1. The VESPA paradigm and experimental set-up

The VESPA is a variation of VEP where the visual stimulus is continuously presented to the subject (Lalor, et al., 2006). It is based on the assumption that the EEG response to a stimulus, whose luminance or contrast is rapidly modulated by a stochastic signal with a high refresh rate (60 Hz), consists of a convolution of that signal with an unknown impulse response plus noise, i.e.,

$y(t) = w(\tau)*x(t) + noise$[7]

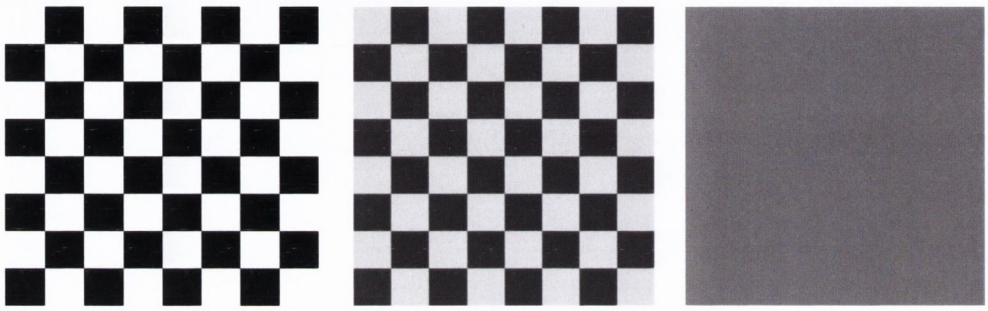
The power of the underlying waveforms used to modulate the luminance of the visual stimulus (x(t)) is spread over a range of frequencies and as a result are termed spread spectrum waveforms. The VESPA method allows for the presentation of simultaneous stimuli that produce separate VESPA responses (Lalor, et al., 2006).

VESPA stimuli presented to the subjects of this study consisted of 68 checkerboards with 64 squares, as shown in Figure 3.3., featuring a varying contrast with the luminance modulated using a stochastic signal. The mean luminance of the lighter and darker squares is

[7] $y(t)$ is the output, $x(t)$ is the known input, * indicates convolution and the noise is assumed to be Gaussian. $w(\tau)$ is the VESPA, i.e. the impulse response function to the brightness of the stimulus.

approximately equal for each of the 68 checkerboard patterns. The power of the underlying waveforms used to modulate the luminance of the visual stimulus is spread over a range of frequencies and as a result are termed spread spectrum waveforms. Each refresh of the stimulus monitor presented the subject with a new image. Each session lasted for 120 seconds and subjects underwent two sessions that were later averaged for the data analysis. Subjects were asked to maintain their focus on the presented stimulus.

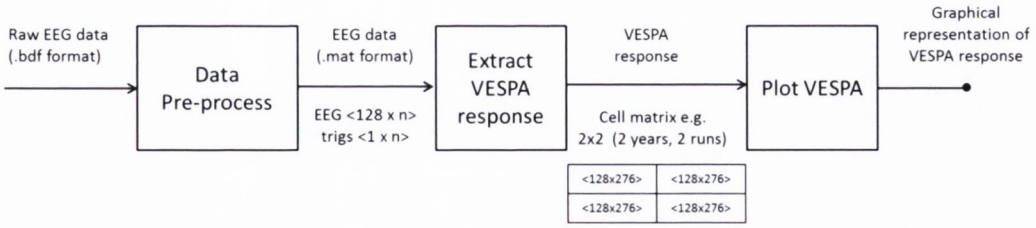
Figure 3.3. 64 square mean luminance checkerboard patterns with varying contrast.



3.2.3.2. The analysis and data processing of VESPA

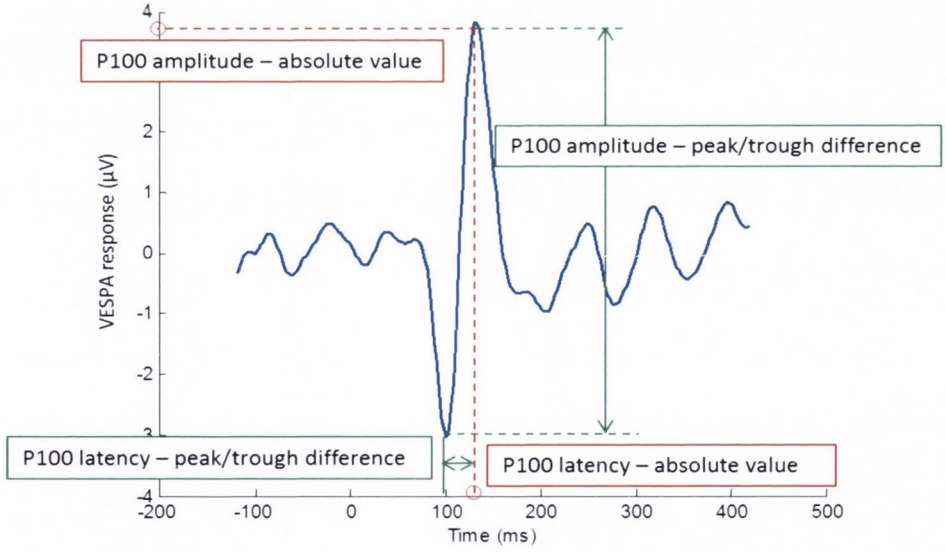
Data processing of the EEG data comprised of data pre-processing, extracting the VESPA response and plotting the VESPA responses (see Figure 3.4). Pre-processing of the raw EEG data included setting a low pass filter to cut-off frequency of 35 Hz and a high pass filter to cut off frequency of 0.5 Hz. Pre-processing also included interpolating 'bad' channels by using the 6 nearest neighbouring channels to reduce the noise in the data. EEG data was referenced to Fz, and digitised at 512 Hz. The pre-processed EEG data included EEG matrix and a vector of the triggers; the EEG matrix represented the voltages measured on the scalp for each of the 128 channels at each time point, and the triggers vector represented the input values to the system and their corresponding times. The VESPA response for each subject was extracted by deconvolving the recorded EEG with the known input signal and then was stored in a cell matrix, (each cell containing number of channels x datapoints matrix). An impulse response function was then calculated for each channel, based on the stimulation rate (60 Hz) and the number of coherencies (1). Finally, all VESPA responses for each subject were stored together in a single .mat file. In most cases, where two runs were recorded in a single session, the VESPA responses were then averaged to generate a single VESPA response for that year. The average response of the two runs was used to generate values for the statistical analysis.

Figure 3.4. Presentation of the various stages involved in VESPA data processing.



Given the known stimulus signal and the measured EEG, the VESPA impulse response can be estimated using the method of linear least squares. In the present study VESPAs were measured using a sliding window of 500 ms of data starting 100 ms pre-stimulus. VESPA responses at Oz were plotted as a function of time for each subject and for each year. Once the VESPA responses had been plotted for each of the participating subjects, the VESPA-like P100 peak amplitude, trough amplitude, VESPA-like P100 peak latency and trough latency were manually extracted. VESPA-like P100 amplitude and latency differences were calculated by comparing the peak and trough values. Therefore, the key VESPA features examined in statistical analysis were the VESPA-like P100 amplitude (absolute value), VESPA-like P100 amplitude difference, VESPA-like P100 latency (absolute value), and VESPA-like P100 latency difference (Figure 3.5).

Figure 3.5. Absolute and difference values of VESPA-like P100 amplitude and latency.



3.3. MRI measures

The pathological changes occurring in the brains of MS patients, such as demyelinating lesions, are likely to have an effect on their cognitive functioning as discussed in Chapter2–Literature

Review. Therefore, the present research incorporated MRI, to measure lesion loads in different brain regions.

All MRIs were acquired with a 1.5-T Siemens Avanto scanner. A high-resolution three-dimensional T1-weighted magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence was acquired (TR = 1160 ms; TE = 4.21 ms, TI = 600 ms, flip angle = 15°) with a sagittal orientation, a 256 × 256 matrix size and 0.9 mm isotropic voxels. A double inversion recovery DIR image (2.5 mm per slice, TR=8000 ms, TE = 95 ms, flip angle = 90 deg) was also acquired. The DIR images were thresholded and filtered in order to produce binarised lesion maps for each subject. The T1 images were segmented using SPM 8's (Wellcome Centre for Neuroimaging, London, UK) optimised segmentation, which incorporated image registration and classification into a single generative model (Ashburner & Friston, 2005). This process generated a set of normalisation parameters for converting each native brain space to standard Montreal Neurological Institute (MNI) space. The normalisation parameters from the T1 for each subject were then applied to that subject's lesion mask. Following this application, regions of interest in MNI space, were generated using anatomically defined masks (Wake Forest University PickAtlas) (Maldjian, et al., 2003). These regions of interest (ROIs) included the total, left and right volumes of the following areas: frontal, prefrontal, temporal, occipital and parietal lobes, caudate, corpus callosum, cerebellum, inferior, medial and superior frontal gyrus, globus pallidus, hippocampus, insula, lateral ventricle, limbic lobe, medulla, midbrain, nucleus accumbens, pons, precentral gyrus, precuneus, putamen, retrosplenium, substantia nigra, thalamus and Wernicke's area. Finally, the total lesion load for each ROI was calculated (i.e., the sum of the binarised lesions within that ROI).

3.4. Psychological measures

As stated in Chapter1-Introduction neuropsychological measures are widely utilised in clinical practice to measure cognitive function in MS. However, these measures can be affected by practice effects, anxiety, depression, physical disability and education (Arnett, et al., 1999; Barker-Collo, 2005; Demaree, et al., 2003; Diamond, et al., 2008; Gilchrist & Creed, 1994; Litvan, et al., 1988; Thornton & Raz, 1997). In contrast, electrophysiological measures are not confounded by practice effects (Sandman, & Patterson, 2000; Segalowitz, & Barnes, 1993; Walhovd, & Fjell, 2002) and are not dependent on physical ability and are thus, independent from behavioural responding (Magnano, et al., 2006). There is no evidence to suggest that they are affected by demographic factors such as education. One of the main objectives of this

research is to examine the associations between the cognitive-indexing electrophysiological methods and the neuropsychological tests sensitive to cognitive deficits in MS. This research makes use of the neuropsychological test battery Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) to examine the cognitive function in MS, and psychological functioning measures, to determine mood disturbances that may have an impact on the cognitive function.

3.4.1. Neuropsychological measures: Adapted version of Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) battery

An adapted version of the Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) battery was utilised in the present research to measure the cognitive function in MS (see Table 3.3). The adapted version focuses on cognitive functions found to be most adversely affected in MS (Benedict, Fischer, et al., 2002). Additional psychological functioning measures were also used in the testing process. The individual tests are described in detail in the next section.

Table 3.3. The adapted version of Minimal Assessment of Cognitive Function in Multiple Sclerosis battery (MACFIMS; (Benedict, et al., 2002) by cognitive domains and relative neuropsychological test.

Cognitive domain	Neuropsychological test
Processing Speed / Working Memory	Paced Auditory Serial Addition Test (Gronwall, 1977) Symbol Digit Modalities Test (Smith, 1982)
Learning and Memory	California Verbal Learning Test-II (Delis, Kramer, Kaplan, & Ober, 2000) Brief Visuospatial Memory Test-Revised (Benedict, 1996)
Executive Functioning	Stroop Neuropsychological Screening Test (SNST; Trenerry, Crossan, DeBoe, & Leber, 1989) The Stockings of Cambridge Test (Lowe, & Rabbitt, 1998; Rabbitt, Lowe, & Shilling, 2001)
Verbal Fluency	Controlled Oral Word Association Test (Benton & Hamsher, 1989)

3.4.1.1. Processing Speed/Working memory: Paced Auditory Serial Addition Test (PASAT) and Symbol Digit Modalities Test (SDMT)

The Paced Auditory Serial Addition Test (PASAT; (Gronwall, 1977; Rao, Leo, Bernardin, & Unverzagt, 1991) measures auditory information processing speed, working memory, divided attention and arithmetic capacity. The PASAT requires participants to add 60 pairs of randomised digits so that each is added to the digit immediately preceding it. For instance, if the first two numbers are '5' and '2', the participant will respond '7', if the next number is '3'; the next correct response is '10'. The task is presented in tape recorded form to allow equal timing (3 seconds) between each number. The subjects were asked to perform calculations silently, without writing or using fingers and a practice sequence was administered prior to the test. The subjects sat with the examiner in a quiet room and were asked to respond orally.

The dependent measure of the PASAT is the number of correct responses; scores can range from 0-60. It is a difficult task, in which normal adults typically achieve a 72% correct response rate (Fisk, & Archibald, 2001). The PASAT is demonstrated to have an internal reliability of 96% (Egan, 1988), and 93-97% test-retest reliability (McCaffrey, et al., 1995). Nevertheless, the overall validity of the PASAT has been established on numerous occasions (e.g. Drake, et al., 2010).

Symbol Digit Modalities Test (SDMT) (Smith, 1982) is a simple measure of processing speed and working memory in visual modality (Drake, Weinstock-Guttman, Morrow, Hojinacki, Munschauer, & Benedict, 2010). Participants are given a reference key that they must use to match numbers to a series of symbols. Participants have 90 seconds to complete the task. One point is allocated to each correct response (range 0-110). The present study made use of the oral form to accommodate participants with motor problems as recommended by Benedict, et al. (2002). The SDMT has yielded a reliability of 76% and it has good discrimination validity (Rathburn, & Smith, 1982).

As information processing speed, attention and working memory are the most adversely affected cognitive areas in MS (Rogers, & Panegyres, 2007), both the PASAT and SDMT are the most popular neuropsychological tests to assess CI in MS (Forn, Belenguer, Belloch, Sanjuan, Parcet, & Avila, 2011). The test scores of PASAT and SDMT have been reported to correlate with diffuse brain injury, which indicates that the performance in tests measuring these cognitive functions is associated with a diffuse disease process and thus, provides a marker of disease progression (Brochet, et al., 2008). Furthermore, the PASAT is the chosen task to

measure cognitive performance in MS functional composite (MSFC; Cutter, et al., 1999); the other two components measuring arm and leg function. The PASAT has been shown to be a sensitive measure of longitudinal changes in cognitive functions in MS (Huijbregts, et al., 2006; Rosti, et al., 2007). Above all, the PASAT has been reported to be a more reliable measure than the Symbol-Digit modalities test to detect CI in MS (Williams et al., 2006; Brochet et al., 2008). Therefore, the PASAT was chosen to be the principal neuropsychological test utilised in the present research.

3.4.1.2. Learning and Memory: California Verbal Learning Test II (CVLT-II) and Brief Visuospatial Memory Test-Revised (BVMT-R)

California Verbal Learning Test – Second Edition (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000), examines the strategies and processes used to learn and remember verbal information. The CVLT-II assesses recall and recognition of two 16-item word lists. List A is presented five times, and following each presentation, the participant is given the chance to recall as many words from the list as possible, in any order. The total free-recall score (sum of Trials 1 to 5 of List A) was utilised in the statistical analyses of the present research project. The CVLT-II demonstrates high levels of reliability (72-89%). It has been found to be a valid test to measure verbal memory in MS and to have high levels of external validity (Stegen et al. 2010).

Brief Visuospatial Memory Test – Revised (BVMT-R; Benedict, 1996), examines visuospatial learning and memory. Participants are presented with a display that consists of six geometric shapes in a 2 X 3 matrix, and are instructed that they have 10 seconds to remember as much information from the display as possible. Once the display is taken away they must try and draw the shapes as accurately as possible. Participants have an unlimited amount of time to complete this recall trial (Trial 1). This first trial is followed by two additional trials, in which the participants are asked to remember more information and to reproduce this visuospatial information during two more recall trials (Trials 2 and 3). Recall performance for Trials 1 to 3, is based on a simple scoring procedure, (a maximum score in each trial is 12). The total recall variable (Trial 1 + Trial 2 + Trial 3 raw scores) was used in the statistical analysis of the present research project. The BVMT-R has been deemed both reliable and valid (Benedict et al., 1996) with moderate to high test-retest reliability and high construct validity (Osterreith, 1944; Rey, 1941; Spreen & Strauss, 1991).

3.4.1.3. Executive functioning: Stroop Neuropsychological Screening test (SNST) and the Stockings of Cambridge Test

Stroop Neuropsychological Screening Test (SNST; version by Trenerry, Crossan, DeBoe, & Leber, 1989) is premised on the phenomenon that there is a delayed naming response for colours printed in ink of a different colour, in comparison to colour names printed in black ink, or equally, colour names printed in a corresponding ink (Lezak, et al., 2004). Recent research supports the view that slowed naming represents deficits of processing speed (Denney & Lynch, 2009; Macniven, et al., 2008). In the task, the (non-colour blind) participants are presented with the first task called the Colour Task, in which they are required to read the 112 words on a sheet as quickly and as accurately as they can in 120 seconds. Subsequently, the participants complete a second task, the Colour-Word, in which participants are asked to name aloud, the colour of the ink in which the word was printed (112 items in 120 seconds). The present study is only concerned with Colour-Word scores that were arrived at by taking the number of incorrect items from the number of correct items in the Form C-W. The SNTS yields high test-retest reliability of 90%, and it also has relatively high discriminant validity.

Cambridge Neuropsychological Test Automated Battery (CANTAB; Robbins, et al., 1994) is a computer based assessment of cognitive functioning making use of language independent tests and using touch-screen technology (Spreen & Strauss, 2006). The present study made use of the Stockings of Cambridge Test (SOC) from the CANTAB battery. The SOC is a test of executive functioning, specifically a test of spatial planning and spatial working memory abilities. Participants are shown two arrangements of coloured balls, one on the top and one on the bottom of the screen. The participants are instructed to view the balls as hanging in stockings. The main aim of the task is to make the bottom arrangement look exactly like the top arrangement, by moving the balls in a certain order, in as few moves as possible. The total of all problems solved in the least moves across the test as a whole, was utilised in the statistical analyses of the present research project. The SOC has low test-retest reliability; however, the SOC has good convergent and discriminant validity (Lowe & Rabbitt, 1998).

3.4.1.4. Verbal Fluency: Controlled Oral Word Association Test (COWAT)

Controlled Oral Word Association Test (COWAT; Benton & Hamsher, 1989; Spreen & Strauss, 1998) is a timed test that assesses language and verbal fluency. Participants are presented with three letters, F, A, and S, and are instructed to name as many words beginning with those letters as quickly as possible in three one-minute trials. The total number of correct responses

across each trial was added to form a total raw score, which was utilised in the statistical analyses. COWAT has a moderately high test-retest reliability (70-82%) (Basso, Bornstein, & Lang, 1999; Harrison, Buxton, Husain, & Wise, 2000; Lemay, Bedard, Rouleau, & Tremblay, 2004; Ross, et al., 2007; Tombaugh, Kozak, & Rees, 1999), very high inter-rater reliability (Ross, et al., 2007), high internal consistency and good construct validity (Tombaugh, et al., 1999).

3.4.1.5. Demographically corrected MACFIMS scores for the enhanced assessment of cognitive function in MS

Typically, the implementation of MACFIMS in clinical and research settings relies on manualized norms from disparate sources. These discrete norms include sets of descriptive statistics for specific age groups (Klein, Foerster, & Hartnegg, 2007). It should be noted that these age bands can be rather arbitrary causing instability in the performance rating (i.e. the corrected score could change drastically by aging a single day, when the raw scores pass from comparison with one age band/group to another). Parmenter, et al. (2010) derived regression-based norms for the MACFIMS tests to control for standard demographic variables (i.e. age, sex, education). The subjects in the large sample (395 MS patients, 100 healthy volunteers) did not differ in age, level of education, sex or race. Multiple regression analyses were conducted on the test performance of the healthy adults, and the resulting models were used to predict MS performance on the MACFIMS battery. The regression-based norms for the MACFIMS were constructed using data from healthy controls. The regression-based norms identified higher rates of impairment compared with manualised norms for many of the MACFIMS measures.

In the present research project, the raw scores of the tests included in the adapted version of MACFIMS were converted to demographically corrected scores and utilised in the statistical analyses of the two preliminary studies. SNST and the computerised version of SOC were not part of the MACFIMS battery utilised to derive regression-based norms (Parmenter, et al., 2010). Therefore, only their raw total scores were included in the statistical analyses.

The raw scores of the MACFIMS tests were converted to Z scores, based on the regression-based norms. Firstly, the raw score of one MACFIMS measure was converted into a scaled score using the raw-to-scale-score conversions originally derived from the healthy controls in the Parmenter study (2010). Then the multiple regression equations were applied to compute demographically predicted scores; taking into account age, education, sex and sex^2 for each subject. Subsequently, these predicted scores were subtracted from each subject's actual

scores and the differences were divided by the standard deviation of the original Parmenter study control group's raw residuals, to obtain a Z-score.

Z score = (scaled score – predicted score) / residual SD[8]

Predicted score = $[B_{constant} + age(B_{age}) + age^2(B_{age2}) + sex(B_{sex}) + education(B_{edu})]$[9]

The raw score-to-scaled score conversion tables, the standard deviations of the residuals of the Parmenter's healthy control group, as well as the final regression models for the MACFIMS tests are all included in the paper of Parmenter, et al. (2010).

3.4.2. Psychological functioning measures

It has been documented that depression and mood disturbance can affect performance on neuropsychological tests (Benedict, Fischer, et al., 2002). Therefore, it is widely recommended that both should be measured in conjunction with the neuropsychological battery, in order to control for confounding effects. The present study used the Hamilton Rating Scale for Depression (HRSD), the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI). Extensive details of each measure are provided below.

3.4.2.1. The Hamilton Rating Scale for Depression (HRSD)

The Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960) is a semi-structured interview used to assess depressive symptoms. It consists of 21 items, some of which are defined by increasing severity, while others are defined as equal-valued options.

The first 17 items evaluate the following features; depressed mood, guilt, suicide, insomnia (initial, middle, and delayed), work and interests, retardation, agitation, anxiety (psychological and somatic), appetite, genital symptoms, fatigue/pain/energy, hypochondriasis, insight and loss of weight. The last four items relate to diurnal variation, derealisation, paranoid symptoms and obsessive-compulsive behaviours. Some are scored on a five-point scale, while others are scored on a three-point scale, depending on the symptom in question. The HRSD was once the gold standard in the assessment of depression in both research and clinical

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[8] *Scaled scores* converted from raw scores, conversion based on the regression-based norms (Parmenter, et al., 2010, see conversion in Table 1). *Residual SD* denotes the standard deviation of the residual extracted from the sample of healthy controls for each MACFIMS task (Table 2 in Parmenter, et al., 2010)

[9] *B* = regression values extracted from Table 4 in Parmenter et al. (2010) displaying final regression models for MACFIMS measures.

settings (Williams, 2001). However, a systematic review questions its validity even though it has been found to have adequate internal reliability (Bagby, Ryder, Schuller, & Marshall, 2004).

3.4.2.2. The Beck Depression Inventory-II (BDI-II)

The Beck Depression Inventory-II (BDI-II; Beck, Steer, & Brown, 1996) is a 21-item self-report questionnaire used to assess depressive symptoms in the preceding two weeks. Each of the 21 items examines a symptom or an experience of depression, for instance; sadness, guilt, suicidal ideation, anhedonia, indecisiveness, fatigue, appetite, irritability, libido and self-esteem. Participants are instructed to select one of four statements of graded severity, for each item on the scale. Items are scored from 0 (absence of problem) to 3 (most severe form of the problem). Scores across each item are added to form a total score. The BDI-II manual reports cut-off scores for categorical purposes; whereby ≤13 is minimal depression, 14-19 is mild, 20-28 is moderate, and ≥29 is severe depression. The BDI-II has been shown to be reliable with 92-93% test-retest reliability (Beck et al., 1996) and a valid measure of depression in individuals with multiple sclerosis (Moran & Mohr, 2005).

3.4.2.3. The Beck Anxiety Inventory (BAI)

The Beck Anxiety Inventory (BAI; Beck, Epstein, Brown, & Steer, 1988) is used to assess symptoms of anxiety in the past month. This is a 21-item self-report questionnaire, whereby individuals rate items on a four-point severity scale, from 0 (not at all) to 3 (severely, it bothered me a lot). The BAI evaluates the following indications of anxiety; numbness or tingling, feeling hot, wobbliness in legs, inability to relax, fear of the worst happening, dizziness, heart pounding, unsteadiness, terror, nervous disposition, fear of choking, trembling hands, shaking, fear of losing control, difficulty in breathing, fear of dying, being scared, indigestion, fainting, flushed face and sweating (not due to heat). Scores across each item are summed to form a total score (0-63, higher scores indicating higher levels of anxiety). According to the BAI manual (Beck & Steer, 1990) 0-9 represents normal anxiety, 10-18 mild to moderate anxiety, 19-29 moderate to severe anxiety, and 30-63 severe anxiety. The BAI has high test-retest reliability (75%) and also construct validity (Spielberger, 1983).

The patients also completed the Multiple Sclerosis Impact Scale (MSIS-29), the Modified Fatigue Impact Scale (MFIS), and visual Analogue Fatigue Scale (VAS). However, these were not included in the statistical analyses of the present research project and therefore are not discussed further.

3.5. Clinical assessment measures

When examining cognitive function in MS, the level of physical disability needs to be taken into account as it may affect cognitive processes and/or performance in neuropsychological tests. This research evaluates the relationship between physical disability and cognitive function in MS.

3.5.1. Expanded Disability Status Scale (EDSS)

The Expanded Disability Status Scale (EDSS; Kurtzke, 1983) measures the extent of the participant's physical disability, as a result of having MS. Patients are evaluated on eight mutually exclusive functional systems. These include the following: pyramidal, cerebellar, brain stem, sensory, bowel and bladder, visual, cerebral or mental, and other or miscellaneous functions. These functions comprise the neurological abnormalities that can be attributed to MS lesions in both the brain and spinal cord. Each functional system is graded individually from 0-5/6, depending on the category; where 0 represents normal functioning, and scores of 5 and 6 are indicative of maximum impairment. Grades for each functional system are added to acquire a total score on a 20-point scale ranging from 0 to 10, where 0 represents a normal neurological examination and 10 represents death due to MS.

The EDSS is the most widely used scale for the measurement of disability and impairment in MS (Sharrack, Hughes, Soudain, & Dunn, 1999). It has an inter-rater reliability agreement of 96%, and an intra-rater reliability agreement of 89% (Sharrack, et al., 1999). The EDSS has also been found to have good convergent validity (Hobart, Freeman, & Thompson, 2000; Mahoney & Barthel, 1965). Very few studies have investigated the relationship between the EDSS and electrophysiological measures. The N2 and P3b component alterations in MS were associated with the EDSS scores (Triantafyllou, et al., 1992). However, the power spectral density of different EEG bands was not related to EDSS findings (Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Borges, et al., 2008). Nevertheless, there has not been an investigation into the sensitivity of EEG to patients with impairments in the EDSS functional systems. In the present research project, the EDSS was administered by a neurology registrar at each yearly test session, at St. Vincent's University Hospital.

3.6. Genetic measures

As noted in Chapter2–Literature Review, the cognitive function in MS may be affected by genetic factors. Oligoadenylate synthetase 1 (OAS1) genotyping was utilised to examine the influence of high versus low RNAseL enzyme activity to brain and cognitive function in MS patients. OAS1 is up-regulated by Type 1 interferons and a single-nucleotide polymorphism (SNP) in exon 7 of OAS1, results in differential ribonuclease L (RNAseL) enzyme activity. The G allele confers high while the A allele confers low activity. Genomic DNA was isolated from peripheral blood samples. Primers (i.e. strands of nucleic acid that serve as a starting point/platform for DNA synthesis) were designed to specifically amplify a 347-bp product surrounding the rs10774671 single-nucleotide polymorphism (SNP; a DNA sequence variation). The primer sequences were as follows: forward 5'-gggatccagatggcatgtca- 3' and reverse 5'-ctggatcaagagtcccacctg-3'. A total of 5 μg of genomic DNA was amplified by polymerase chain reaction (PCR; a method to reproduce small segments of DNA, and PCR products were digested with the ALU1 restriction enzyme (i.e. an enzyme that cuts DNA up at specific sequences). These digested products were analysed by agarose gel electrophoresis, which is used to separate DNA fragments, and genotypes were assigned.

3.7. Conclusion

This chapter presented a detailed account of the study procedure and each of the measures utilised in the present research project. Firstly, the advanced EEG and P3 ERP measures, including the SPM method, the IC clustering approach and the VESPA were presented. Subsequently, the MRI, psychological, clinical assessment and genetic measures were detailed. The sophisticated EEG and P3 ERP methods will be utilised in Chapter 4 to reveal the cross-sectional differences between MS patients and healthy volunteers and between the MS subgroups based on differing disease courses, level of cognitive function, and OAS1 genotypes. Furthermore, the relationship between the P3 ERP and neuropsychological measures is important for the studies proposed and detailed in Chapter 4. These measures will also be utilised in Chapter 5 in investigating the longitudinal changes in the cognitive function in MS. Chapter 6 will incorporate the findings acquired with both comprehensive neuropsychological test battery MACFIMS and MRI and incorporating the P3 ERP latency and amplitude variables. This is in order to examine the multimodal associations between these methods in detecting changes in cognitive function in MS. The novel VESPA method detailed in this chapter will be applied in Chapter 7, to assess its utility in detecting visual dysfunction in MS.

Chapter 4 - Cross-sectional P3 ERP studies

The present chapter examines the Research Questions 1, 2 and 3 as stated in Section2.7. Thesis objectives are included in Chapter2–Literature Review. These research questions set out to investigate the following: 1) if the scalp-based P3 ERP activations at Month 0 differ between MS patients and healthy controls, and between MS subgroups, and if the PASAT score correlates with the scalp-based P3 ERP activations, 2) if the source P3 EEG activations at Month 0 differ between MS patients and healthy controls, and between MS patients with and without cognitive impairment, and finally 3) if the P3 ERP activations and the PASAT score differ between MS patients with different oligoadelynate synthetase 1 (OAS1) genotypes.

The studies in this chapter investigated the cognitive-indexing electrophysiological activations in a relatively large sample of MS patients and healthy controls with advanced high-density electroencephalography (EEG) measures presented in Chapter3–General Methods. Based on the previous research literature, it was hypothesized that MS patients, especially with a progressive disease course, would have reduced P3 ERP amplitudes and delayed P3 ERP latencies in comparison to healthy controls. The PASAT score was hypothesized to correlate with P3 ERP amplitudes and latencies. In addition, it was hypothesized that MS patients versus controls, and cognitively impaired MS (CI-MS) patients versus non-cognitively impaired MS (non-CI-MS) patients, would show reduced theta and a smaller decrease in alpha band after P3b target stimuli over parietal brain regions 200-400 ms, which is the expected P3b peak time period. As MS patients with AA OAS1-genotype have increased disease activity, it was hypothesized that they would also have a poorer outcome in the PASAT and display reduced P3b ERP amplitudes and delayed P3 ERP latencies, compared to the MS patients with AG OAS1-genotype.

The findings of the studies in this chapter give an expanded and detailed insight into the cognitive-indexing electrophysiological activations underlying more high-level cognitive functions in MS, such as attention and working memory. These findings extend previous P3 ERP and EEG studies reported in the literature. Most of the previous studies reported in Chapter2–Literature Review utilised low-density EEG arrays, with low numbers of subjects, which were rarely compared to different MS subgroups. Previous studies also seldom attempted to link their P3 ERP findings to cognitive processes measured with neuropsychological tests.

The results of the studies in this chapter were published in the following leading peer-reviewed journals:

Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Bramham, J., O'Brien, M., Reilly, R.B., Hutchinson, M., & Tubridy, N. (2010). A high-density ERP study reveals latency, amplitude, and topographical differences in multiple sclerosis patients versus controls. *Clinical Neurophysiology, 121*, 1420-1426. (Section 4.1, Appendix 3)

Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Hutchinson, M., Tubridy, N., & Reilly, R.B. (2010). Impaired information processing speed and attention allocation in multiple sclerosis patients versus controls: A high-density EEG study. *Journal of the Neurological Sciences, 293*, 45-50. (Section 4.2, Appendix 4)

Kiiski, H., Whelan, R., Lonergan, R., Nolan, H., Kinsella, K., Hutchinson, M., Tubridy, N., Reilly, R.B. (2010). Preliminary Evidence for Correlation Between PASAT Performance and P3a and P3b amplitudes in progressive multiple sclerosis. *European Journal of Neurology, 18(5)*, 792-795. (Section 4.3, Appendix 5)

Kiiski, H., Reilly, R.B., Lonergan, R., Kelly, S., O'Brien, M., Kinsella, K., Bramham, J., Burke, E.T., O'Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N, & Whelan, R. (2012). Only Low Frequency Event-Related EEG Activity is Compromised in Multiple Sclerosis: Insights From an Independent Component Clustering Analysis. *PLoS ONE, 7(9)*: e45536. (Section 4.4, Appendix 6)

4.1. Scalp-based analysis of high-density P3b ERPs and multiple sclerosis

This section of Chapter 4 examines Research Question 1, as stated in Section 2.7. of the thesis objectives, included in Chapter2–Literature Review.

4.1.1. Introduction

The study utilised the typical oddball, P3b paradigm, in which occasional target stimuli are detected in a train of frequent irrelevant standard stimuli. The stimuli evoke a number of event-related potential (ERP) components, i.e. the early components – P1, N1, P2 – and the late components N2 and P3. The P3b timing is independent of behavioural reaction time and is therefore a suitable paradigm for subjects with potentially impaired motor ability (Polich, 2004). P3b latency and amplitude are sensitive to neural degenerative conditions and several types of dementia and psychiatric diseases (Polich, 2004). As illustrated by the comprehensive literature review in Chapter 2-Literature Review, previous electrophysiological studies of cognitive functions in MS have reported varying results in ERP differences between MS patients and controls, particularly in relation to early sensory components (P1, N1, and P2).

The variability of previous MS ERP findings may be due to several reasons. Cognitive impairment is more frequent and severe in SPMS than in RRMS (Zakzanis, 2000) and therefore the composition of the MS study group may affect results. Previous studies have employed electrode numbers ranging from 1 (Ellger, et al., 2002) to 30 (Piras, et al., 2003); although 128 electrodes are required to optimally sample the topography of the human EEG (Srinivasan, Nunez, & Silberstein, 1998). Employing low numbers of electrodes indicates that there may not be sufficient scalp coverage to detect activity from the early ERP components, which are generated across a number of subcortical regions and are observed over widespread scalp regions. Neuropsychological studies of CI in MS have provided mixed evidence of modality-specific impairments (i.e. if impairment is more pronounced in one modality than in the other, or is common across modalities (Foong, et al., 1997; Sperling, et al., 2001). It is possible that there is a modality-specific effect on ERP latency in MS because of differing neuronal pathways (King & Nelken, 2009).

The present study aims to provide a comprehensive and systematic account of the P3b ERPs in MS. A high-density EEG array was employed and a relatively large MS group was recruited,

including both RRMS and SPMS patients. The study examines the differences in latency, amplitude, and topography between MS patients and controls and also between the RRMS and SPMS patients. ERP differences with respect to modality, and the relationship between the PASAT and visual and auditory P3b amplitude and latency were also investigated. It was hypothesized that MS patients have reduced P3b ERP amplitudes and delayed P3b ERP latencies in comparison to healthy controls, as indicated by previous research (Amato, et al., 2006; Leocani, et al., 2010; Magnano, et al., 2006). The progressive MS subtypes were hypothesized to have reduced P3b ERP amplitudes and delayed P3b ERP latencies relative to RRMS patients. It was further hypothesized, that SPMS patients have reduced P3b ERP amplitudes and delayed P3b ERP latencies compared to PPMS patients, based on previous research (Ellger, et al., 2002). The PASAT score was hypothesized to correlate with P3b ERP amplitudes and latencies, as previous research has shown neuropsychological performance to be associated with P3b ERP activations in MS (Aminoff, & Goodin, 2001; Giesser, et al., 1992; Gil, et al., 1992; Honig, et al., 1992; Leocani, et al., 2010; Piras, et al., 2003). The findings of this chapter may provide additional information on the electrophysiological activities underlying the cognitive processing in MS subjects, and ultimately lead to better understanding and management of the condition.

4. 1.2. Methods

4.1.2.1. Subjects, procedure and data analysis

Seventeen right-handed RRMS patients, 16 right-handed SPMS patients and twenty-one healthy volunteers were included in the study. One RRMS patient was unable to complete the visual task, and one SPMS patient and one control subject were unable to complete the auditory task. Table 4.4.1. displays the demographic data of the subjects from whom EEG data were collected.

Table 4.4.1. Subject demographic data.

	Visual						Auditory					
	RRMS		SPMS		Control		RRMS		SPMS		Control	
N	16		16		21		17		15		20	
Male / female	5/11		10/6		9/12		6/11		10/5		9/11	
	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
Age	37.73	9.16	50.90	9.09	40.32	9.19	37.18	8.79	50.46	8.09	40.15	9.4
PASAT %	73.93	27.49	63.50	21.55	85.10	12.87	78.88	22.59	66.65	22.35	85.62	13.11
Yrs symptoms	7.76	5.85	24.0	10.67	N/A	N/A	7.95	6.07	25.06	11.99	N/A	N/A
RT (<800 ms)	376.04	65.40	396.70	60.22	383.68	54.79	372.76	70.08	356.63	60.50	370.52	72.27
	M	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*
EDSS	2.00	1.5	6.00	2.50	N/A	N/A	2.00	2.00	6.00	2.62	N/A	N/A

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = interquartile range, RRMS = relapsing-remitting MS patients, SPMS = secondary-progressive MS patients, PASAT = Paced Auditory Serial Addition Test, Yrs symptom = the number of years since the first symptom, RT = reaction time, EDSS = Expanded Disability Status Scale.

All subjects completed the standard Paced Auditory Serial Addition Test PASAT (Tombaugh, 2006) as described in Chapter 3-General Methods, approximately one hour prior to ERP recording. The details of the EEG recording procedure with visual and auditory oddball paradigms are also described in Chapter 3-General Methods.

SPSS 16 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA) was utilised for all statistical analyses, except for the topographical analysis completed using SPM 8 (http://www.fil.ion.ucl.ac.uk/spm). EEGLAB (Delorme & Makeig, 2004) was used for all the ERP analyses. The EEG data were bandpass filtered between 1 and 90 Hz, bandstopped between 48 and 52 Hz, average referenced across all scalp electrodes, epoched and baseline corrected (100 ms before baseline). Epochs with large, obvious artefacts (e.g., muscle twitch) were first rejected manually. Independent components analysis, using the infomax algorithm, was used to identify artefacts, which were subsequently removed (see Delorme, et al., 2007). Ocular artefacts were removed by identifying the components that correlated most highly with the EOG channels (minimum correlation of 0.5). Visual inspection of the EOG channel before and after removal of the component was performed in order to ensure that the ocular artefacts were removed. A 99% confidence interval was calculated across all channels for mean amplitude and variance: any channel falling outside the confidence interval was interpolated. Each ERP component was identified by two of the authors according to its polarity, latency and topography. A component was deemed to be not identifiable if the morphology of the ERP was not clear to both assessors. Next, the maximum (or minimum where appropriate) amplitude was automatically extracted from the data for all electrodes using the manually identified component as a guide to define the temporal window. If there was no peak inside a temporal window for a component at a particular electrode then data at that electrode for that component were recorded as missing. The early components were determined for P1 (visual only), N1 and P2 for trials that followed presentation of the standard stimulus. The N2 and P3 components were calculated for trials that followed presentation of the target stimulus. ERP descriptors often identify electrodes where maximum amplitudes are typically observed and differ according to modality. Electrode groups, regions of interest (ROIs), were generated and are displayed in Table 4.4.2. We used composite mean amplitude measures, i.e., the mean value of the electrodes in the ROI, computed from groups of electrodes where the peak of interest was maximal to specify its amplitude and latency.

Table 4.4.2. A list of the regions of interest (ROIs) generated using composite amplitude measures computed from groups of electrodes.

ROI	Electrodes	Modality	
		Auditory	Visual
Midline fronto-central	Cz, Cz' Fz Fz' FCz Cz' (97)	N1, P2	P2
Midline centro-parietal	Cz Cz' CPz CPz' Pz Pz' POz POz' Oz	N2	N2
Midline occipital	Pz' POz POz' Oz		P1, N1
Left inferior temporal	T7 T7' T9		N1
Right inferior temporal	T8 T8' T10		N1
Midline central	Cz Cz' CPz	P3b	P3b
Midline parietal	CPz' Pz Pz' POz	P3b	P3b

SPM 8 (http://www.fil.ion.ucl.ac.uk/spm) was used to create statistic parametric maps in order to test for topographical differences in ERP amplitude across the entire scalp and across time. Data from each subject were transformed into two-dimensional sensor-space (interpolated from 128 channels) and in peri-stimulus times from 0–250 ms for responses to the standard condition and from 230–600 ms for responses in the target condition. This transformation produced a three-dimensional spatiotemporal characterisation of the ERP, which was then compared between groups. The significance level was set at $p < .001$ and this was further restrained by only retaining scalp areas in which at least 100 contiguous voxels were significant. Furthermore, a stringent family-wise threshold was implemented separately, thus showing only significant voxels following correction for multiple comparisons.

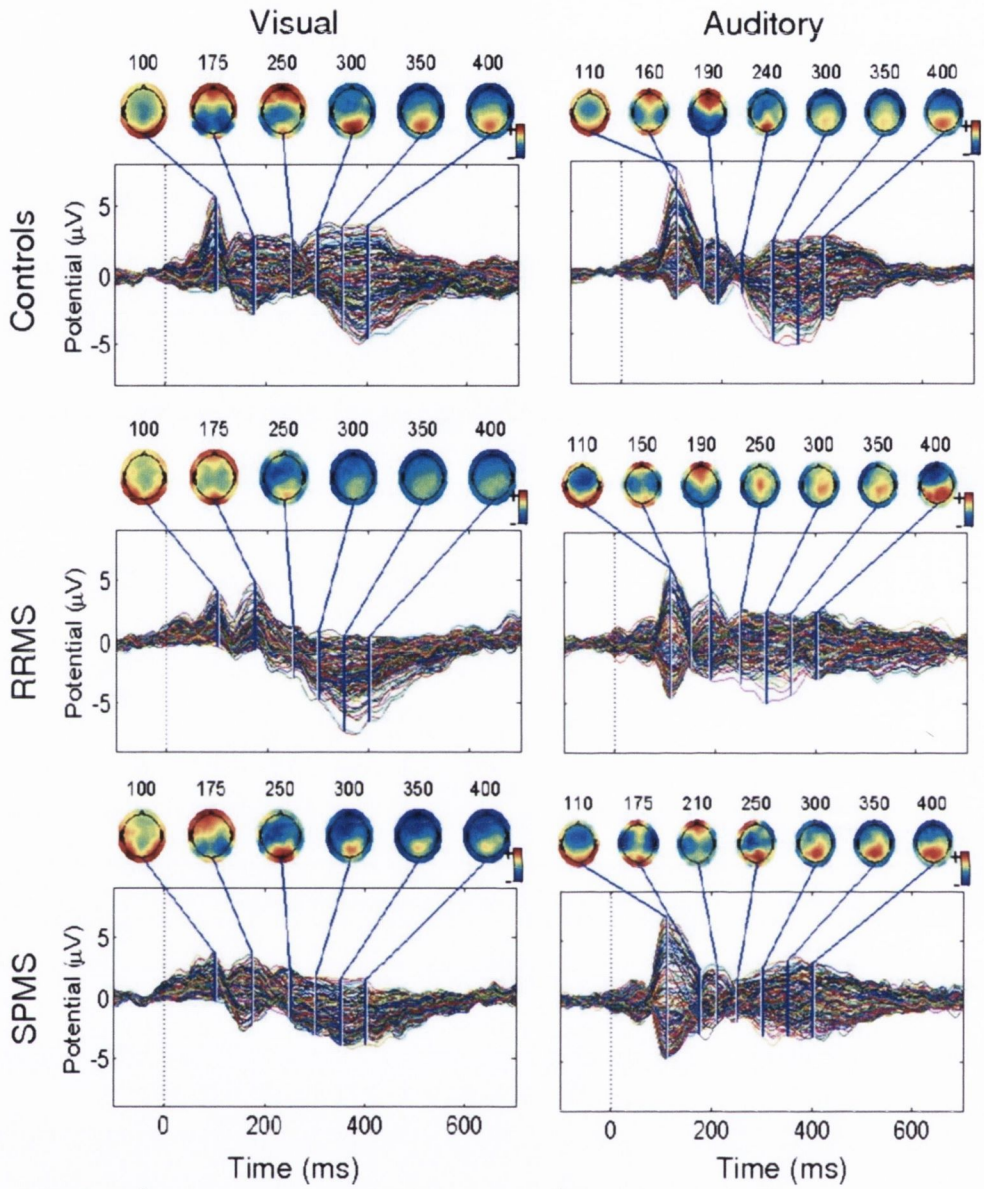
4.1.3. Results

The mean percentage of retained epochs was 86%, 84%, and 82% for the control, RRMS, and SPMS groups, respectively. Overall, 18% of independent components were identified as noise and removed. There were no significant reaction time differences between the groups for either auditory or visual modalities (Table 4.4.1.). A one-way ANOVA comparing PASAT score across groups was significant ($F(2, 46) = 3.21$, $p = .49$). A Tukey post-hoc test showed that the SPMS patients had significantly lower PASAT scores compared to the controls. Level of education was not significantly different between MS patients and controls.

4.1.3.1. ERP latency and amplitude

The descriptive and inferential results for each component are displayed in the Tables 4.1.3. and 4.1.4.. In the visual modality, P2, P3b Central and P3b Parietal amplitudes and N2 latency were significantly different across groups. In the auditory modality, P2, N2, and P3b latencies and N1 amplitude were significantly different across groups. Post-hoc tests indicated that there were no significant differences between the RRMS and SPMS patients on any ERP component. Figure 4.1.1. displays the ERP following presentation of the target stimulus from all 128 channels for both modalities and for all groups. Scalp maps of the ERP activity are also displayed. When MS patients with visual dysfunction, such as history of optic neuritis, were excluded from the analyses the results remained the same except for the comparison of auditory N1 amplitude that lost statistical significance.

Figure 4.1.1. Butterfly plots showing responses for all electrodes across all modalities and groups. Scalp maps showing the topography of the response at particular latencies are displayed above the butterfly plots.



Note. RRMS = relapsing-remitting MS, SPMS = secondary progressive MS

Table 3. Descriptive and inferential results for the visual condition.

Visual						
Component	Measure	*ANOVA*	*TUKEY*	RR MS	SP MS	Control
P1	Amp	$F(2,24) = 1.80$, $p = .181$		1.91	1.53	2.73
	Lat	$F(2,24) = .121$, $p = .887$		118.43	112.47	116.83
Mid N1	Amp	$F(2,35) = 0.23$, $p = .792$		-0.79	-0.45	-0.91
	Lat	$F(2,35) = .852$, $p = .435$		214.10	206.28	188.42
Left N1	Amp	$F(2,25) = 2.27$, $p = .125$		-1.73	-1.68	-.62
	Lat	$F(2,25) = 1.03$, $p = .371$		231.77	203.48	181.73
Right N1	Amp	$F(2,34) = 1.19$, $p = .318$		-0.57	-1.71	-1.09
	Lat	$F(2,34) = .953$, $p = .396$		197.92	208.33	182.29
P2	Amp	$F(2,36) = 3.39$, $p = .045$*	-	0.34	-1.11	0.25
	Lat	$F(2,36) = .645$, $p = .530$		271.94	268.59	247.97
N2	Amp	$F(2,32) = .46$, $p = .637$		-1.58	-1.65	-1.09
	Lat	$F(2,32) = 4.80$, $p = .015$*	RR > C	347.17	293.44	244.65
Central P3b	Amp	$F(2,49) = 4.17$, $p = .021$*	C > SP	1.18	0.84	2.35
	Lat	$F(2,49) = 0.52$, $p = .595$		409.26	397.30	379.36
Parietal P3b	Amp	$F(2,50) = 8.88$, $p = .001$*	C > RR & C > SP	2.63	2.64	4.74
	Lat	$F(2,50) = 1$ 29., $p = .283$		421.93	395.69	369.88

Note. C = control, RR= RR MS, SP = SP MS, Amp = amplitude, Lat = latency, Mid N1 = Midline fronto-central, Left N1 = Left inferior temporal, Right N1 = Right inferior temporal, *Denotes that the comparison is significant at $p < .05$.

Table 4. Descriptive and inferential results for the auditory condition.

Auditory						
Component	Measure	ANOVA	TUKEY	RR MS	SP MS	Control
N1	Amp	$F(2,26) = 6.10$, p=.007*	C > SP	-3.39	-4.22	-1.62
	Lat	$F(2,26) = 2.30$, p=.120		107.64	108.14	90.56
P2	Amp	$F(2,24) = 2.97$, p=.071		2.12	.64	1.04
	Lat	$F(2,24) = 6.78$, p=.005*	RR > C	217.32	153.21	135.00
N2	Amp	$F(2,41) = 0.69$, $p = .506$		-2.14	-1.47	-1.71
	Lat	$F(2,41) = 6.04$, $p = .005$*	RR > C	291.84	233.62	211.75
Central P3b	Amp	$F(2,47) = .212$, $p = .810$		3.18	2.31	2.63
	Lat	$F(2,47) = 2.42$, $p = .10$		366.96	416.67	356.50
Parietal P3b	Amp	$F(2,48) = .60$, $p = .551$		3.58	3.90	4.29
	Lat	$F(2,48) = 3.46$, $p = .039$*	SP > C	373.16	416.08	340.10

Note. C = control, RR= RR MS, SP = SP MS. *Denotes that the comparison is significant at $p < .05$.

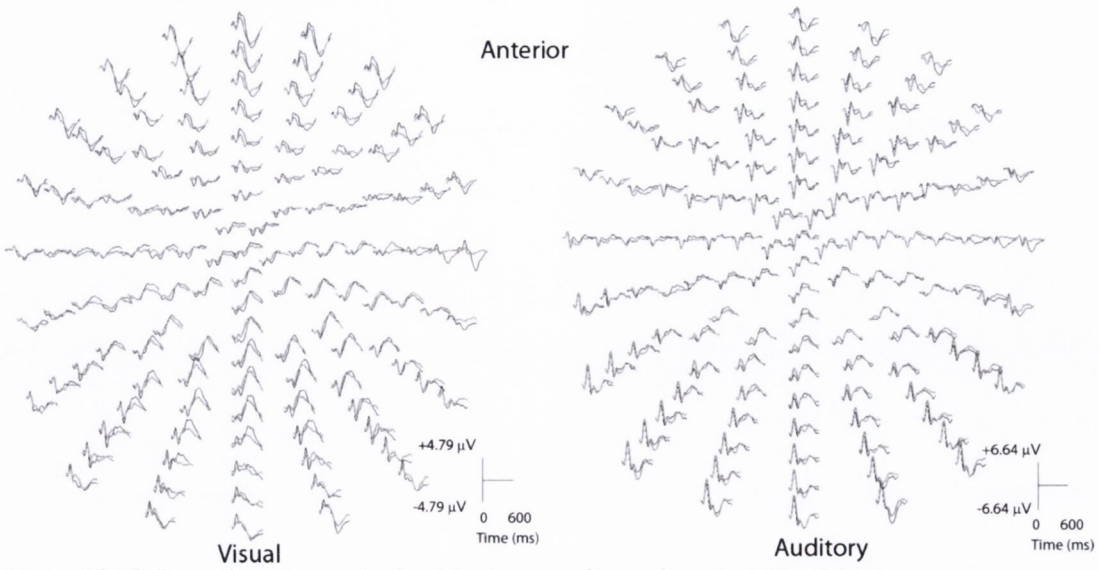
The PASAT score did not significantly correlate with education, EDSS, age or years since beginning of symptoms. The correlations remained non-significant after controlling for age ($p > .05$). Auditory P3b latency in both central and parietal regions was correlated with age in MS patients ($r = .412$, $p = .019$ and $r = .441$, $p = .011$, respectively) but not with EDSS, the level of education or the number of the years since the beginning of symptoms ($p > .05$). Visual amplitude and latency, and auditory amplitude did not correlate with age, EDSS, the years since the beginning of symptoms, or the level of education ($p > .05$). A partial correlation,

controlling for age, between auditory P3b latency and the PASAT (an auditory test of cognitive function), across all MS subjects was significant at central and parietal ROIs ($r_{partial}$ = -.483., p = .006, $r_{partial}$ = -.384, p = .033, respectively). PASAT score did not correlate significantly with latency of any early P3b component (p > .05). P3b auditory amplitude was not significant (p > .05) in either ROI. The partial correlation between the PASAT score and the visual P3b latency across all MS subjects approached significance at Central and Parietal sites ($r_{partial}$ = -.362, p = .069 and $r_{partial}$ = -.370, p = .063, respectively). The correlations across modalities were analyzed for all MS subjects for both amplitude and latency at Central and Parietal scalp locations. Visual Parietal amplitude correlated significantly auditory Parietal P3b amplitude (r_S = .507, p = .008). There were no other significant correlations for amplitude, nor for latency across modalities.

4.1.3.2. ERP topography

Figure 4.1.2 displays the ERPs of MS patients and controls following the presentation of the target stimulus from all 128 channels for both modalities. In the visual modality, there was greater activity for controls from 121 to 156 ms over the parietal area and from 218 to 277 ms over the left central area following presentation of the standard stimulus and there was greater activity for controls from 235 to 289 ms over the frontal area and from 277 to 570 ms over the central and parietal areas following presentation of the target stimulus. There was greater activity for controls over the frontal area from 82 to 148 ms and over a region of the left central area from 219 to 250 ms following presentation of the standard stimulus. There were no suprathreshold voxels following the presentation of the target stimulus in the auditory condition. Figure 4.1.3 displays the statistic parametric maps of significantly different ERP activity between all MS patients and controls. A comparison of RRMS and SPMS patients did not yield any suprathreshold voxels for early or late components in either the visual or auditory modalities (this was the case when the significance level was relaxed to p < .001). Table 5 summarizes the results of the SPM analysis.

Figure 4.1.2. ERPs to the target stimulus in the visual and auditory conditions.



Note. Black lines denote control subjects, gray lines denote MS subjects.

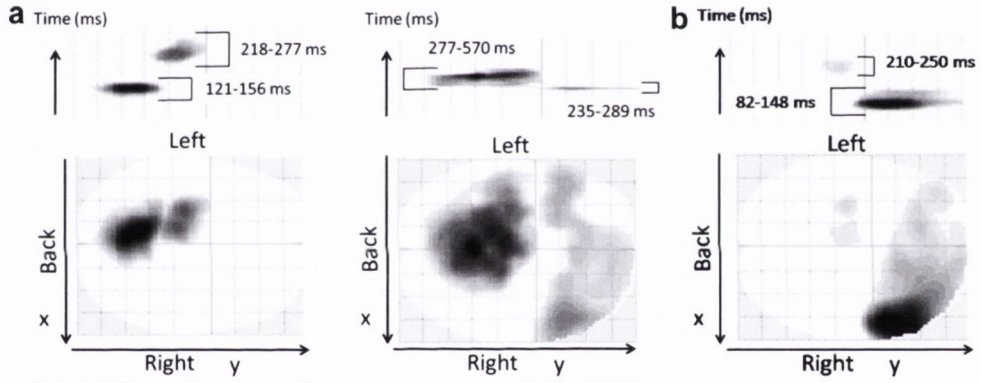


Figure 4.1.3. 3D spatiotemporal characterisation of the ERP.

Note. The left panel (a) shows responses to the standard stimulus in the visual condition. The middle panel shows responses to the target stimulus in the visual condition. The panel on the right (b) shows responses to the standard stimulus in the auditory condition.

Table 4.1.5. Results of statistical parametric mapping showing location of peak voxel.

Modality	Peak voxel				
	x	y	ms	Z	*FWE*
Visual	-11	-52	141	4.69	Yes
	-26	-19	238	4.64	No
	51	21	273	4.12	Yes
	-2	–46	422	5.37	Yes
Auditory	57	13	102	5.85	Yes
	-30	19	227	3.53	No

Note. *x* is positive-going from left to right, *y* is positive-going from posterior to anterior, and associated Z score. Family-wise error rate (*FWE*) was set at 0.05.

4.1.4. Discussion

The present study represents an advance over previous research, in that a high-density EEG array was used with a relatively large number of subjects with MS and controls (total n = 54) across both visual and auditory modalities. As hypothesized delayed latencies and reduced amplitudes were present in both sensory (N1 and P2) and cognitive components (N2 and P3b) for MS patients, in comparison to healthy control subjects. Visual P3b amplitudes were significantly reduced in MS patients when compared to control subjects. Auditory P3b latencies were significantly delayed in SPMS patients when compared to control subjects. A topographical analysis indicated that both early and late visual P3b ERP amplitudes were significantly reduced for MS patients, in comparison to controls over a large centro-parietal scalp area. The auditory P3b topography was significant only for the frontal area during the timeframe of early sensory components. The finding of delayed latencies and reduced amplitudes in the early ERP components for both modalities suggests prolonged and decreased P3b components, often observed in MS, and may at least be partly due to deficits earlier in the processing pathway.

The ROIs in the present study were identified using data from previous research with healthy subjects. However, the statistical parametric maps showed widespread regions with significantly different amplitude or latencies, between the groups, for the early sensory components. For example, in the present study, there were significant amplitude differences between MS patients and controls in over much of the centro-parietal scalp region for the visual P3b component, with greater activation over the left side. Atypical topographical distributions may be a reflection of the disease process (e.g. lesions in processing pathways) and consequently patient topographies of electrophysiological activations may not be in the same scalp areas shown by previous research with healthy populations (Polich, 2007). A high-density EEG array is therefore useful in identifying differences between the groups, as the entire scalp area can be covered. The statistical parametric approach has the benefit that multiple comparisons across scalp regions are corrected, without the need to make a priori assumptions about topographical differences between groups.

The relatively large sample size in the present study facilitated the comparison between RRMS and SPMS patients. However, contrary to the hypotheses, there were no significant differences between the groups in latency, amplitude or topography. Only two previous studies have examined the differences between RRMS and SPMS subtypes (Ellger, et al., 2002, Gil, et al., 1993), and they both reported SPMS patients to manifest delayed latencies and reduced

amplitudes. These differences have been thought to be most likely due to a higher degree of demyelination and axonal degeneration in SPMS, which causes the disruption of the cortical-cortical and cortical-subcortical neural connections (Leocani, et al., 2010). Nevertheless, the finding of no significant differences between RRMS and SPMS on any P3b ERP measure may suggest that the disease process, per se, rather than severity or duration of the disease, impacts on ERP amplitude and latency.

A correlation between PASAT score and auditory P3b latencies for all MS patients was significant for the central and parietal scalp regions, thus being consistent with the hypotheses. PASAT score did not correlate with latency of earlier auditory ERP components in MS patients. This indicates that auditory P3b latency is sensitive to the degree of CI for MS patients. Although we have shown that early sensory ERP delays may contribute to the delay of the auditory P3b latency between SPMS patients and controls, a significant correlation between PASAT and auditory P3b latency was still found within the MS group.

Some of the previous studies have not reported impaired early ERP components in MS (Ellger, et al., 2002; Gerschlager, et al., 2000a), whereas others have found latency delays in N1 and P2 (Giesser, et al., 1992; Gil, et al., 1993). Moreover, some studies have reported a trend, but not statistically significant differences, towards a reduction of visual amplitude in early components (e.g. Piras, et al., 2003). It is possible these studies did not have the statistical power or sufficient electrode coverage to detect such differences. Differences between RRMS patients and controls and between SPMS patients and controls, in the visual modality, were reflected in the reduced amplitudes of the P3b components. In addition, latency of the visual N2 was delayed for the RRMS group versus controls. In the auditory modality, latencies were prolonged for the RRMS patients versus controls for the P2 and N2 components, and for the SPMS patients versus controls for the parietal P3 latency. The widespread delayed latencies and decreased amplitudes in the early and late ERP components is in accordance with the view that demyelinating lesions, in primary afferent pathways, cause delays in early sensory components; which in turn prolongs the later, cognitive-indexing N2 and P3b (Magnano, et al., 2006). Furthermore, the N1 amplitude was significantly greater for SPMS patients in comparison to controls. An N1 amplitude increase, in conjunction with a P3b amplitude decrease, has been reported for older subjects during an auditory oddball task (Anderer, Semlitsch, & Saletu, 1996). This increase has been attributed to a higher level of general attention in older adults during the oddball task. It is plausible therefore, that a similar mechanism is involved for MS subjects with respect to the auditory N1 component.

Parietal P3b amplitude correlated across modalities. However, latency was not correlated across modalities; only the auditory P3b latency was delayed in SPMS patients relative to controls. The finding that auditory P3b ERPs were more susceptible to delay may be a consequence of the differences between visual and auditory processing pathways. More synaptic relays are involved in early sensory auditory processing (King & Nelken, 2009), in comparison to early visual processing. Consequently, there is more opportunity for demyelination to delay transmission of the early sensory auditory information. This study adds to the literature that suggests a modality-specific impairment in MS (De Sonneville, et al., 2002).

Key points:

- Both early sensory and late cognitive-indexing P3b ERP amplitude and topographical deficits in MS were more pronounced in the visual modality
- MS patients have reduced amplitudes in only early auditory P3b ERP task compared to controls
- ERP latency deficits (P2, N2, P3b) in MS were more pronounced in the auditory modality
- No differences between RRMS and SPMS patients were observed on any P3b ERP measure
- PASAT score correlated with auditory P3b latency for the MS patients
- Results show widespread deficits in sensory and cognitive processing in MS patients, especially in SPMS patients
- P3b ERP measures have potential utility in clinical research and/or practice to detect subtle cognitive dysfunction

4.2. Scalp-based analysis of high-density P3a ERPs in multiple sclerosis

This section of Chapter 4 examines Research Question 1 as stated in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. The study examined if the scalp-based P3a ERP activations at Month 0 differ between MS patients and healthy controls, and between MS subtypes. Furthermore, the correlation between the PASAT score and the scalp-based P3a ERP activations was investigated.

4.2.1. Introduction

The previous study in this chapter found that MS patients had delayed and reduced early sensory and late cognition-related P3b ERP components. This finding implied deficits in patients sensory processing and in cognitive functions of context updating and working memory. However, previous studies have reported that MS patients experience cognitive deficits, particularly in attention and often they have reduced information processing speed (Bobholz & Rao, 2003), which is typically more frequent and severe in secondary progressive MS (SPMS) than in relapsing remitting MS (RRMS) (Zakzanis, 2000). These subtle cognitive impairments have a significant impact on daily life (Rao, Leo, Ellington, et al., 1991).

Consequently, the present chapter applied another cognitive-indexing electrophysiological paradigm; the no-go P3a. The no-go P3a indexes the speed of information processing and the engagement of attention mechanisms (Polich, 2007), in order to study the cognitive deficits in MS. The no-go P3a is a variant of the P3b, and it can be produced by utilising a three-stimulus oddball paradigm. Where similar to the traditional oddball paradigm, occasional target stimuli have to be detected in a train of frequent irrelevant standard stimuli. Though in this case, infrequent additional stimuli are being presented and the subject should withhold from responding to these distracter stimuli. The non-targets are non-novel repeated stimuli in the no-go P3a paradigm. If the standard and target stimuli are difficult to discriminate, then the P3a is observed over frontal and central areas, with shorter latency in frontal areas and longer latencies in parietal areas. Auditory P3a latencies are typically shorter than visual P3a latencies (Katayama & Polich, 1999). In contrast to the P3b, which seems to be mainly affected by temporo-parietal junction disruptive lesions, P3a responses are compromised in patients with a variety of disruptive lesion sites, including the medial temporal, frontal, and parietal lobes

(Linden, et al., 1999). Therefore, the P3a may be more suitable than the P3b for detecting CI in MS, in which patients typically have widespread lesions.

The literature review revealed that only limited studies have examined the P3a component in MS, with one showing delayed and decreased P3a waveforms in MS (Jung, et al., 2006). An alternative study did not find any differences relative to healthy controls (Sailer, et al., 2001). However, no study has employed a no-go P3a oddball paradigm with MS patients. The three-stimulus no-go or P3a paradigm is utilised to examine the differences in its latency, amplitude and topography between MS patients and controls, and also between RRMS and SPMS subtypes. Likewise, similar to the previous chapter on P3b ERPs, the auditory-visual modality differences and the relationship between the PASAT and the P3a are of interest. Despite the limited research, it was hypothesized that MS patients have reduced P3a ERP amplitudes and delayed P3a ERP latencies in comparison to healthy controls. It was similarly hypothesized that the progressive MS subtypes have reduced P3a ERP amplitudes and delayed P3a ERP latencies relative to RRMS patients, as indicated by previous research on P3b ERP activations (Amato, et al., 2006; Ellger, et al., 2002; Leocani, et al., 2010; Magnano, et al., 2006). The PASAT score was further hypothesized to correlate with P3a ERP amplitudes and latencies; as previous research has shown neuropsychological performance to be associated with P3 ERP activations in MS (Aminoff, & Goodin, 2001; Giesser, et al., 1992; Gil, et al., 1992; Honig, et al., 1992; Leocani, et al., 2010; Piras, et al., 2003). The present study is anticipated to provide evidence of the utility of the no-go P3a measure to offer information on the electrophysiological functioning of MS patients, during an attention-demanding task that involves the frontal lobe.

4.2.2. Methods

4.2.2.1. Subjects, procedure and data analysis

Twenty RRMS patients and 20 SPMS patients and 34 control patients were included in the study. One RRMS patient was unable to complete the visual task, and one SPMS patient and one control subject were unable to complete the auditory task. Table 4.2.1 displays the demographic data of the subjects.

Table 4.2.1. Subject demographic data.

	RRMS		SPMS		Control	
N	20		20		34	
Male/female	7/13		14/7		19/15	
	μ	σ	μ	σ	μ	σ
Age	37.81	9.13	51.04	8.92	40.11	9.92
PASAT	74.93	28.19	64.98	21.13	85.86	12.66
Yrs symp	7.95	5.90	24.95	11.15	N/a	N/a
	M	*IQR*	*M*	*IQR*	*M*	*IQR*
EDSS	1.75	1.88	6.5	2.25	N/A	N/A
Interferon β-1a	6		4		N/A	N/A
Interferon β-1b	6		4		N/A	N/A
Natalizumab	7		0		N/A	N/A
Fingolimod	1		0		N/A	N/A
No current tx	0		12		N/A	N/A

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = interquartile range, PASAT = the Paced Auditory Serial Addition Test, EDSS = the Expanded Disability Status Scale.

The EEG recording procedure and the visual and auditory three-stimuli paradigms are described in detail in Chapter 3-General Methods. The pre-processing of the EEG data was identical to the one conducted in the previous, P3b study. Subsequently, three regions of interest (ROIs) were generated by using composite mean amplitude measures (i.e., the mean value of the electrodes in the ROI). The electrodes used in each ROI are in Table 2 (the electrode sites are labeled according to the 10–5 system).

The peak of the P3a component was found by fitting a parametric function to the ERP using a Gaussian profile to the most prominent peak of the ERP between 250–600 ms for the visual modality and between 200–600 ms for the auditory modality and determining the delay at the peak amplitude (Gerson, et al., 2005). The timeframes were set to be broad as the most prominent peak, the P3a, has been reported to be delayed in MS patients (Jung, et al., 2006; Onorf, et al., 1991), and many MS patients demonstrated with prolonged P3a latency in preliminary visual inspection. Age has been shown previously (Fjell & Walhovd, 2001) to correlate with P3a latency: therefore, latencies were compared using an analysis of covariance (*ANCOVA*), controlling for age. All statistical analyses were completed using SPSS 16 (SPSS Inc., Chicago, IL, USA). SPM 8 was used to create statistic parametric maps in order to test for topographical differences in ERP amplitude, and the correlation between the PASAT scores and the P3a amplitudes, across the entire scalp and across time. Data from each subject were transformed into two-dimensional sensor-space (interpolated from 128 channels and smoothed, full-width half-maximum 6:6:4 a.u.) and in peri-stimulus times from200–600 ms for responses to the non-target stimulus (voxel size=2.1 mm×2.7 mm×3.9 ms). This transformation

produced a three-dimensional spatiotemporal characterisation of the ERP, which was then compared between the groups. The significance level was set at $p < .001$ for the between-group comparisons, and this was further restrained by only retaining scalp areas in which at least 500 contiguous voxels were significant. Furthermore, a stringent family-wise threshold implemented separately, thus showing only significant voxels following correction for multiple comparisons. The significance level was set at $p = .005$ for the correlation between the PASAT and ERP amplitude (due to the greater sample size and the lower probability of a type-1 error), and this was further restrained by only retaining scalp areas in which at least 500 contiguous voxels were significant. The effects of age were entered as a covariate in the PASAT/ERP correlation.

Table 4.2.2. A list of locations for the Regions of Interest.

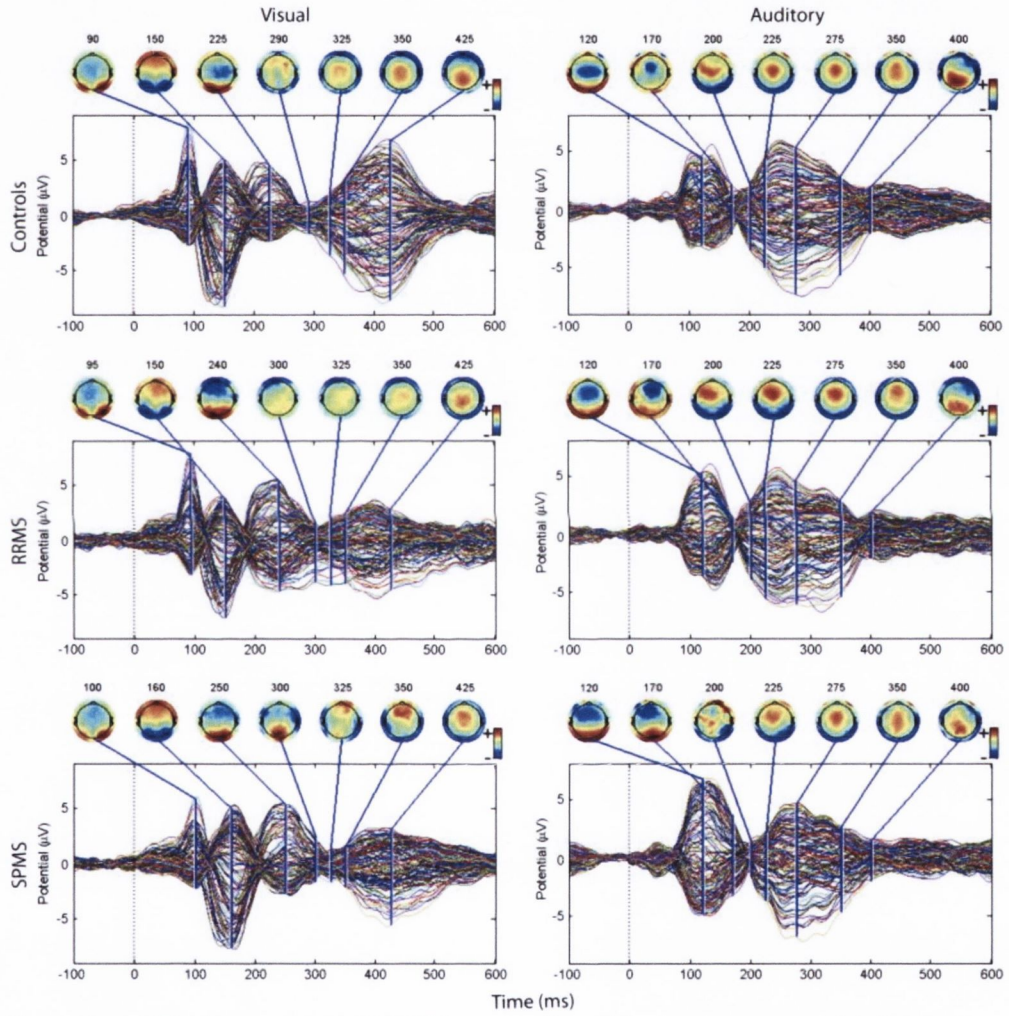
Region	Electrode locations
Frontal	F2',F2,AF2',AFz, AFz', Fz, Fz', F1,F1',AF1'
Central	Cz, Cz', CPz, CCP2h, FCC2h, FC2', FCz, FCC1h, FC1', CCP1h
Parietal	CPz', CPP1h, P1, PPO3h, PO3h, Pz, Pz', POz, CPP2h, P2, PPO4h, PO4h

4.2.3. Results

For the P3a visual task, the mean percentage of retained epochs was 87.47%, 82.00%, and 88.30% for the control, RRMS, and SPMS groups, respectively for the non-target stimulus. For the P3a auditory task, the mean percentage of retained epochs was 84.25%, 80.62%, and 84.57% for the control, RRMS, and SPMS groups, respectively for the non-target stimulus. The PASAT score did not correlate with the EDSS score ($r_S = -.20$, $p > .05$). The PASAT score was significantly correlated with age ($r_S = -.258$, $p < .05$) and consequently, all subsequent analyses involving the PASAT score were conducted with age as a covariate. A one-way *ANCOVA* comparing PASAT score across groups was significant ($F(2, 62)=4.51$, $p < .05$). A post-hoc test (least-significant difference) showed that there was a significant difference between the SPMS patients and controls on PASAT score ($p < .05$) and also between RRMS patients and the controls ($p < .05$). Figure 4.2.1 displays the ERP following presentation of the target stimulus from all 128 channels simultaneously for both modalities and for all groups. Scalp maps of the ERP activity are also displayed. Figures 4.2.2 and 4.2.3 display the latencies and significant comparisons for each group across the three locations in the visual and auditory modalities, respectively. There were significant latency differences between RRMS and SPMS patients for the visual P3a ERP component. There was a significant latency difference between controls

and MS patients over the parietal region in the auditory condition. Latencies in both auditory or visual modalities were not significantly correlated with EDSS score. Neither PASAT score nor EDSS correlated significantly with ERP latency (when the effects of age were removed). It is possible that delayed latencies in the visual condition were caused by delays in early visual processing. Therefore, the mean latencies for patients with and without optic neuritis were compared for each ROI, but no comparison was significant.

Figure 4.2.1. Butterfly plots showing responses for all electrodes across all modalities and groups.



Note. Visual P3a latency range 250-600 ms, auditory P3a latency range 200-600 ms.

Figure 4.2.2. Latencies and significant comparisons for each group across the three locations in the visual modality.

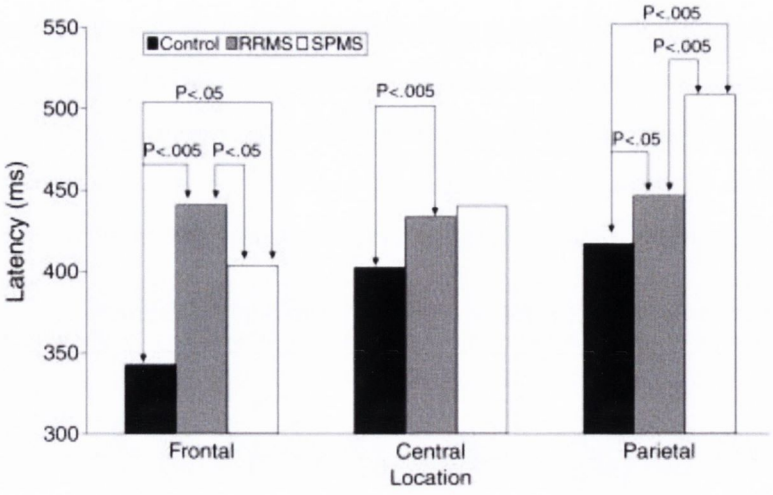
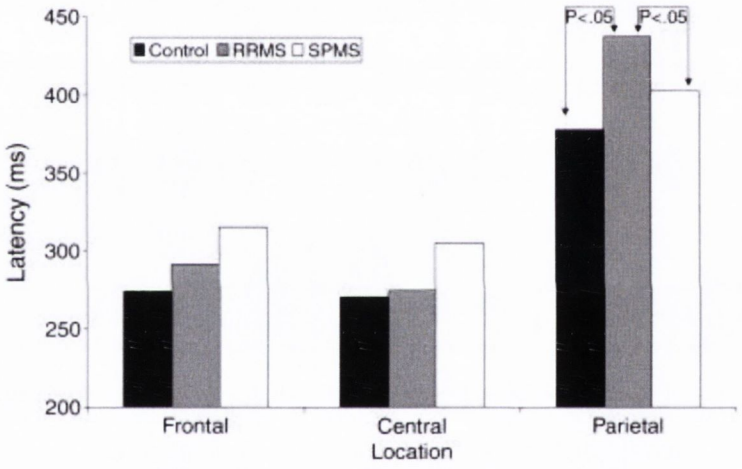
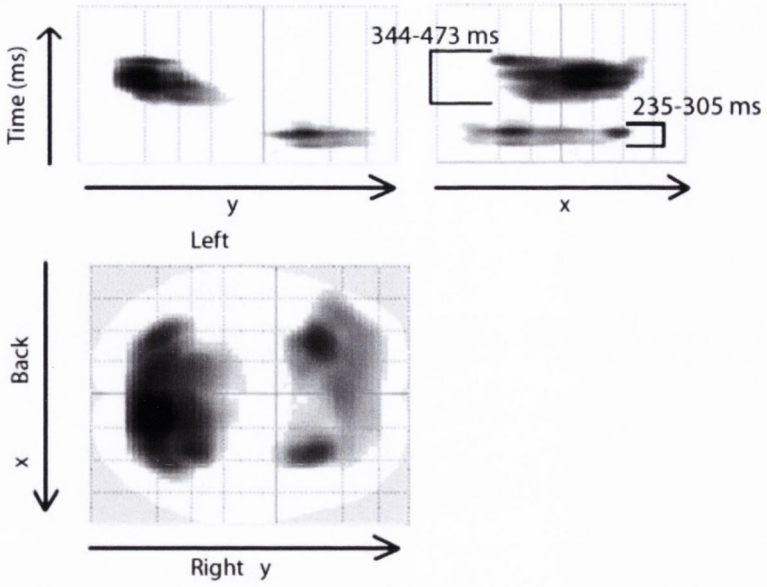


Figure 4.2.3. Latencies and significant comparisons for each group across the three locations in the auditory modality.



A SPM comparison of RRMS and SPMS patients did not yield any suprathreshold voxels in either the visual or auditory modality, and therefore the RRMS and SPMS groups were combined for subsequent analyses. Figure 4.2.4. displays the statistic parametric maps of significantly different ERP activity between all MS patients and controls in the visual modality: there was significantly greater activity for controls from 235–305 ms over the frontal area and from 344–473 ms over the parietal area following presentation of the non-target stimulus. There were no supra-threshold voxels following presentation of the non-target stimulus in the auditory condition.

Figure 4.2.4. 3D spatiotemporal characterisation of the ERP for all MS patients versus controls in the visual condition.



SPM was used to examine scalp areas and time periods in which ERP amplitude was significantly correlated with PASAT score (see Figure 4.2.5). The visual condition was significant for a positive correlation over the frontal scalp area from 223–297 ms, over the central–parietal area from 328–375 ms, and over the lateral frontal region again from 477–594 ms. The visual condition was significant for a negative correlation over the parietal/occipital area from 231–301 ms, over the frontal region from 325–395 ms and from 414–442 ms, and over the medial parietal/occipital area from 520–594 ms. It should be noted, however, that these areas are essentially the inverse of positive correlation due to the dipolar nature of the neural sources.

Figure 4.2.5. 3D spatiotemporal characterisation of the ERP for the correlation between ERP amplitude and PASAT score.

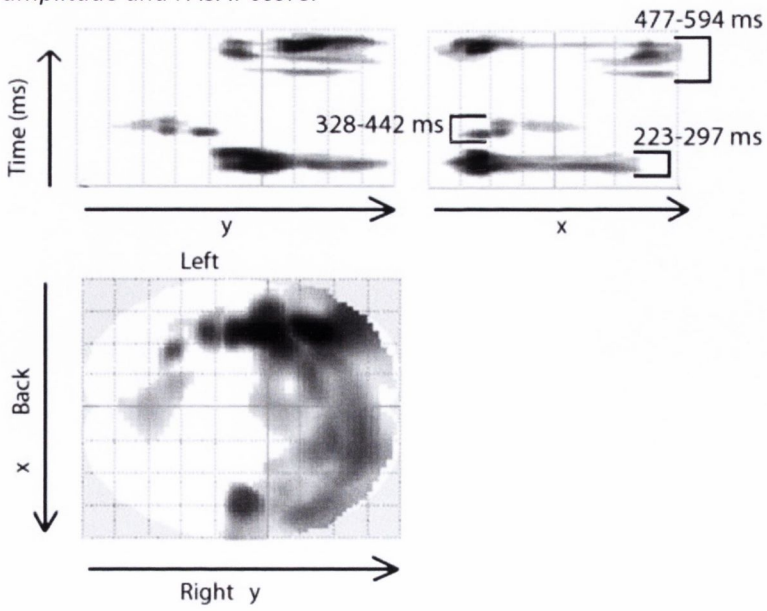
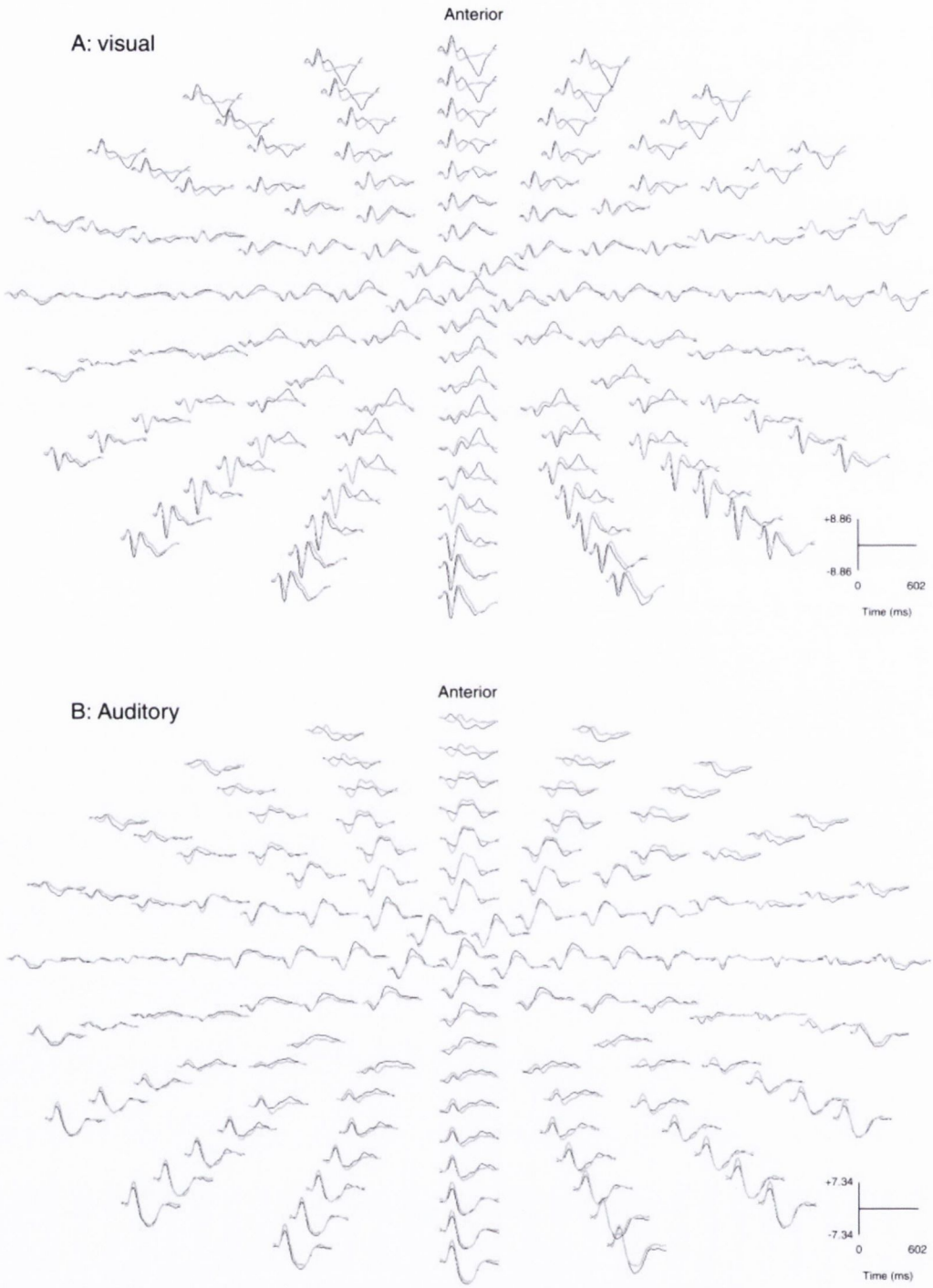


Figure 4.2.6. ERPs to the target stimulus in the visual (A) and auditory (B) conditions for all scalp channels.



Note. Black lines denote control subjects, gray lines denote MS subjects.

4.2.4. Discussion

A reduced processing speed is one of the most common cognitive deficits in MS (DeLuca, Chelune, Tulsky, Lengenfelder, & Chiaravalloti, 2004). This deficit was examined with the P3a paradigm in the present study. Consistent with the hypotheses, the results show both amplitude reduction and latency prolongation of the visual P3a ERP in MS patients, relative to healthy control subjects. Amplitude differed significantly over the frontal and parietal areas in the visual condition. The results also show significant latency differences between RRMS and SPMS patients for the visual P3a ERP component. Results further indicated a significant latency difference between the controls and the MS patients over the parietal region in the auditory condition. However, contrary to the hypotheses, there were no significant latency, amplitude or topography differences in the auditory condition between RRMS and SPMS patients. PASAT score correlated significantly with visual amplitude over a widespread area as hypothesized. The correlation was controlled for the effects of age, as it correlated with the PASAT score. The cognitive dysfunction is reported to increase with age in MS patients with early, mild cognitive deficits, whereas the cognitive performance of MS patients preserved at baseline remain stable (Kujala, et al., 1997).

The early, mild cognitive deficits were found to be predictive of future cognitive status, as the initially cognitively mildly deteriorated MS group had widespread deficits after 3 years. Yet the cognitive performance of MS patients, classified as cognitively preserved at baseline, was similar to the controls and it remained stable over 3-year period (Kujala, et al., 1997).

There was a pronounced effect of the P3a ERP modality, with more significant effects in the visual condition. Differences in stimulus characteristics may be the cause of the modality differences. It can be argued that the modality specific differences in P3 ERPs may be due to the distinctiveness of the stimuli utilised in the studies, with more similar stimuli properties increasing the difficulty of the task. It is possible that in our studies the auditory stimuli were not sufficiently similar, which would have made the auditory P3a ERP task less difficult to perform. In addition, it would have made less demands on attentional capacities and memory processes, than the visual P3a ERP tasks. This statement is supported by several previous findings. A faster ERP to auditory stimuli may indicate that it was easier to discriminate between auditory target and non-target stimuli (P3a non-target increases when targets are harder to discriminate from standards) (Polich & Comerchero, 2003). It is also possible that the white-noise burst in the auditory condition, was not as salient as the checkerboard in the visual condition. In general, previous studies have shown that the P3a to auditory stimuli is

typically faster than the P3a ERP to visual stimuli and the control data in the present study is comparable to data using similar paradigms (Katayama & Polich, 1999). This divergence in absolute magnitude of the amplitude and latency responses between visual and auditory modalities may have made statistical comparisons between groups differentially powerful. The difference in modality effects may explain why some previous studies (Sailer, et al., 2001) did not report significant differences in auditory P3a between MS patients and controls.

The PASAT makes demands on a number of cognitive domains; including speed of information processing and simultaneous allocation of attention to two tasks. A functional MRI (fMRI) study (Mainero, et al., 2006) reported that, during execution of the PASAT in RRMS patients, there was activation of the inferior and middle frontal gyrus, anterior cingulate, inferior parietal lobule, supplementary motor area, superior and middle temporal gyrus, insula, thalamus, vermis and brainstem bilaterally, as well as the right superior parietal lobule and the right lateral premotor area. The results of the ERP/PASAT correlation are consistent with a pattern of widespread frontal and parietal activation. A drawback of fMRI is that it is limited in the temporal domain (resolution is in the order of seconds).

In contrast, high-density EEG provides excellent temporal resolution (resolution in the order of milliseconds) and moderate spatial resolution (in the order of centimetres). Latency differences in the present study, in particular in the visual condition, showed that MS patients had significantly longer latencies than control subjects. This was most pronounced over frontal and parietal sites. It is notable, however, that the latencies of RRMS patients were significantly longer than those of SPMS patients at the frontal ROI in the visual condition and over the parietal ROI in the auditory condition. The results remained the same when MS patients with recent relapses (within one year) were excluded from the analyses. It is possible that initial attention allocation in RRMS patients was affected to a greater degree than the SPMS patients. This effect may be due to aggressive damage with limited opportunity for plastic repair in the RRMS groups, in comparison to the SPMS group. For example, atrophy in the neocortex (Simon, 2006) may proceed rapidly in early RRMS. The differences in location between modalities may be a reflection of the relative difficulty of the standard/target discrimination. The shorter latencies in the auditory condition show that the standard/target discrimination was less difficult than in the visual condition. It has previously been shown that P3a amplitudes are likely to be higher in amplitude over anterior areas when the discrimination is difficult (Katayama & Polich, 1999). Therefore, in the visual condition, the differences between RRMS and SPMS were observed over the frontal ROI, whereas in the auditory condition the

differences were observed over the parietal ROI (there were no significant differences among the groups at the frontal and central ROIs in the auditory condition).

Key points:

- MS patients have deficits in P3a latency, amplitude and topography, particularly in the visual condition
- MS patients have reduced amplitudes in visual P3a over frontal and parietal scalp regions
- MS patients have delayed latencies in visual P3a over frontal and parietal scalp areas, and in auditory P3a over parietal region
- RRMS and SPMS patients differed in visual P3a latency at frontal and parietal scalp regions
- Visual P3a amplitude correlated with PASAT score in all MS patients over frontal and parietal areas
- P3a ERP measures have potential utility in clinical research and/or practice to detect subtle cognitive dysfunction

4.3. Scalp-based analysis of P3b and P3a ERP amplitudes in progressive multiple sclerosis

This section of Chapter 4 examines the Research Question 1 as stated in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. The study investigated if the visual and auditory P3b and P3a ERPs differ in progressive MS patients in comparison to age-matched controls, and if the SPMS and PPMS differed in their P3 ERP activations. Also the correlation between the PASAT score and the scalp-based P3 ERP activations was of interest.

4.3.1. Introduction

The previous chapters shed light on the utility of P3 ERP measures to index cognitive function in the most common subtypes of MS patients, relapsing–remitting (RRMS) and secondary progressive (SPMS) patients. The relatively large samples consisted only from RRMS and SPMS patients, which is very common in the MS literature. However, approximately 10-15% of the MS patients experience a different type of disease progression called the primary-progressive MS (PPMS), where the disability increases steadily over time after the onset without major relapses or remission as described in Chapter 2-Literature Review (Rejdak, et al., 2010). The cognitive function of the people with the PPMS has not been studied extensively and there is a gap in the cognitive-indexing electrophysiological literature concerning the PPMS patients.

Some studies have found significant neuropsychological differences between the PPMS and SPMS (e.g. Foong, et al., 2000; Huijbregts, et al., 2004) but some have found none (e.g., Potagas, et al., 2008). Both progressive MS subtypes are regarded to have more frequent and more severe CI in comparison to RRMS patients (Huijbregts, et al., 2004; Magnano, et al., 2006). The progressive MS subtypes have a poorer outcome in the PASAT in comparison with controls (Huijbregts, et al., 2006; Huijbregts, et al., 2004). Secondary progressive patients tend to be afflicted more often and more severely. The reported results from studies comparing the CI in PPMS and SPMS patients are not conclusive and differ with respect to frequency, severity and impaired cognitive domains (Winkelmann, Engel, Apel, & Zettl, 2007). In attention-demanding tasks, SPMS patients were slower than PPMS and RRMS patients (De Sonneville, et al., 2002). SPMS patients performed worse than PPMS patients on tests requiring higher order working memory processes with the exception of tests involving sustained attention and concentration (Huijbregts, et al., 2004). A recent study showed that primary progressive MS patients revealed a poorer performance in verbal learning and verbal fluency than secondary

progressive patients (Wachowius, Talley, Silver, Heinze, & Sailer, 2005). In sum, MS patients may suffer from focused, divided and sustained attention deficits, as well as from compromised central processing stages, with SPMS patients showing the most extensive range of deficits, closely followed by PPMS patients. In contrast, RRMS patients appear to be much less affected. General slowing appears to be highest in PPMS and SPMS type patients (50% slower) versus RRMS (24% slower) (De Sonneville, et al., 2002).

Only a few EEG studies have included PPMS patients in their analyses (Arrondo, et al., 2009; Ellger, et al., 2002; S. J. Jones, Sprague, & Pato, 2002; Leocani, et al., 2000; Nagels, et al., 2007). Most of these studies (Arrondo, et al., 2009; Leocani & Comi, 2000; Nagels, et al., 2007) have not compared the MS subtypes with each other, but rather compared all the MS patients as one group to the controls. The study of Ellger, et al. (2002) is the only study so far to compare the ERPs of different MS subtypes. They reported the rate of pathological P3b latencies of PPMS patients to be significantly lower when compared to SPMS P3b latencies. Furthermore, they found visual P3b latency of PPMS to be significantly shorter compared to P3b latency of SPMS patients, and visual P3b amplitude of PPMS to be significantly reduced compared to P3b amplitudes of RRMS patients. Interestingly, the visual P3b latency correlated significantly with EDSS score in the PPMS patients. The study used only one electrode at Pz to record the P3b and focused only on visual modality. Thus, the results are not comprehensive, and there is a need for more comprehensive studies also including topographical analyses. There are no previous studies focusing solely on the task-related neurophysiology of PPMS subtype.

The same P3b and P3a ERP paradigms (Polich, 2007) that were introduced in the previous studies were utilised alongside with high-density EEG array. In the two previous studies significant differences in both auditory and visual P3b and P3a were observed between the MS patients sample comprising both RRMS and SPMS, in comparison to healthy controls. Nevertheless, the patients with RRMS and SPMS differed only in visual P3a latency over parietal scalp areas.

In the present study the P3 ERP responses of PPMS and SPMS subtype groups were compared, and these progressive MS groups were also compared to healthy age-matched controls. Patients with SPMS were employed as a patient control group because patients with RRMS are typically younger than patients with PPMS, and the P3 component has been reported to change with age (Polich, 1996). Furthermore, one of the main interests was to examine the relationship between the PASAT and the P3 ERP components. It was hypothesized that the progressive MS subtypes would have reduced P3 ERP amplitudes and delayed P3 ERP latencies

relative to healthy controls, and SPMS patients to have reduced P3 ERP amplitudes and delayed P3 ERP latencies compared to PPMS patients, based on previous research (Ellger, et al., 2002). The PASAT score was hypothesized to correlate with P3 ERP amplitudes and latencies, as previous research has shown neuropsychological performance to be associated with P3 ERP activations in MS (Aminoff, & Goodin, 2001; Giesser, et al., 1992; Gil, et al., 1992; Honig, et al., 1992; Leocani, et al., 2010; Piras, et al., 2003).

4.3.2. Methods

4.3.2.1. Subjects, procedure and data analysis

The study included ten patients with PPMS were age, sex and education matched with 10 patients with SPMS and with 10 healthy controls (Table 4.3.1). The experimental procedure including PASAT test are described in Chapter 3-General Methods. One patient with PPMS was excluded from the auditory tasks, and one patient with PPMS from visual P3b task. The ERP recording procedure, P3b and P3a paradigms, and ERP analysis method are identical to the ones described in detail in the two previous studies and in Chapter 3-General Methods. In brief, in the oddball task an occasional target stimuli have to be detected in a train of frequent irrelevant standard stimuli. A P3b component is typically elicited approximately 300 ms after each target stimulus, and it has maximal amplitude over the parietal scalp area. The no-go P3a is elicited by employing a three-stimulus oddball paradigm: in addition to standard and target stimuli, additional, infrequent, non-target stimuli are presented. Subjects are asked to respond only to target stimuli and refrain from responding to the additional non-target stimuli and to the standard stimuli. Typically, this no-go P3a component can be observed approximately 250 ms after the non-target stimuli especially over the central scalp region, with shorter latencies in frontal areas and longer latencies in central/parietal areas (Polich, 2007). SPM8 (Statistical Parametric Mapping 8, http://www.fil.ion.ucl. ac.uk/spm) was employed to test for topographical differences in ERP amplitude across the entire scalp and from 250–500 ms. The significance level was set to $P < 0.005$, which was further restrained by only retaining scalp areas in which at least 500 contiguous voxels were significant. Disease duration was entered as a nuisance variable for comparisons involving only patients with MS as SPMS patients had had their first symptoms earlier than PPMS patients.

Table 4.3.1. Demographic and behavioural data of PPMS, SPMS and controls.

	PPMS		SPMS		Control	
N	10		10		10	
Male/female	7 / 3		7 / 3		7 / 3	
	μ	σ	μ	σ	μ	σ
Age	54.52	13.35	52.96	8.75	52.33	9.58
Yrs disease	11.00	11.51	16.50	8.17	N/a	N/a
Yrs symptoms	12.70	11.15	23.90	12.13	N/a	N/a
PASAT (%)	54.50	26.46	55.50	22.81	87.50	10.66
Visual P3b RT (<800 ms)	408.40	73.95	398.64	78.16	393.07	36.01
Auditory P3b RT (<800 ms)	391.32	71.94	355.85	55.79	402.01	88.04
	M	*IQR*	*M*	*IQR*	*M*	*IQR*
EDSS	5.80	2.38	5.90	1.50	N/a	N/a
HRSD	3.5	14.00	3.50	12.00	2.00	4.00
Interferon β-1a	1		2			
Interferon β-1b			1			
Natalizumab						
No current tx	9		7			

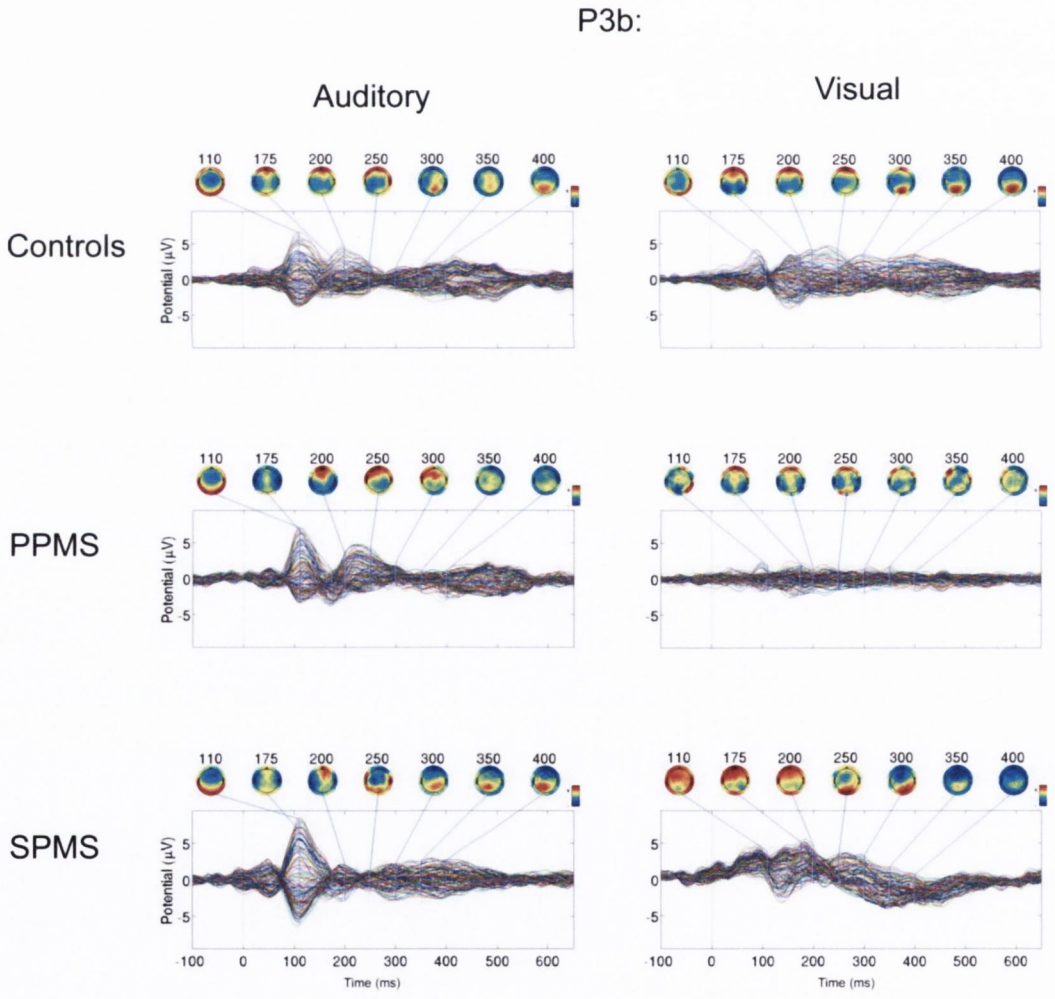
Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = inter-quartile range, PPMS = primary-progressive MS, SPMS = secondary-progressive MS, PASAT = the Paced Auditory Serial Addition Test, RT=reaction time.

4.3.3. Results

The groups did not differ in age, in educational level or in HRSD scores ($p > .05$), but controls performed significantly better in the PASAT compared to both PPMS and SPMS groups ($F(2,27) = 7.92$, $p = 0.002$; see Table 1). However, the progressive MS groups did not differ in their PASAT performance or in the EDSS score. The PASAT scores did not correlate with age, education level, EDSS, years since first symptoms, years since diagnosed, history of depression, or with HRSD score.

Activity related to P3b and P3a ERPs is depicted in Figures 4.3.1. and 4.3.2, respectively. The control group had greater amplitude than the MS groups in all the P3 ERPs (except in auditory P3b) ($p < 0.005$) (Figure 4.3.3.), when testing the contrast that controls would have greater activation than either MS group, and that MS groups would not differ from each other. Patients with PPMS had lower amplitude than patients with SPMS in the auditory P3b condition over the left parietal region from 356–438 ms, and the right parietal area from 250–305 ms (p <0.005) (Figure 4.3.4). The PASAT/P3 correlations for the PPMS and SPMS ($p < 0.005$) groups are displayed in Figure 4.3.5. This correlation was also significant when controls were included (Figure 4.3.6.)

Figure 4.3.1. Butterfly plots showing responses for all electrodes across all modalities and groups for P3b task. Scalp maps showing the topography of the response at particular latencies are displayed above the butterfly plots.



4.3.2. Butterfly plots showing responses for all electrodes across all modalities and groups for P3a task. Scalp maps showing the topography of the response at particular latencies are displayed above the butterfly plots.

P3a:

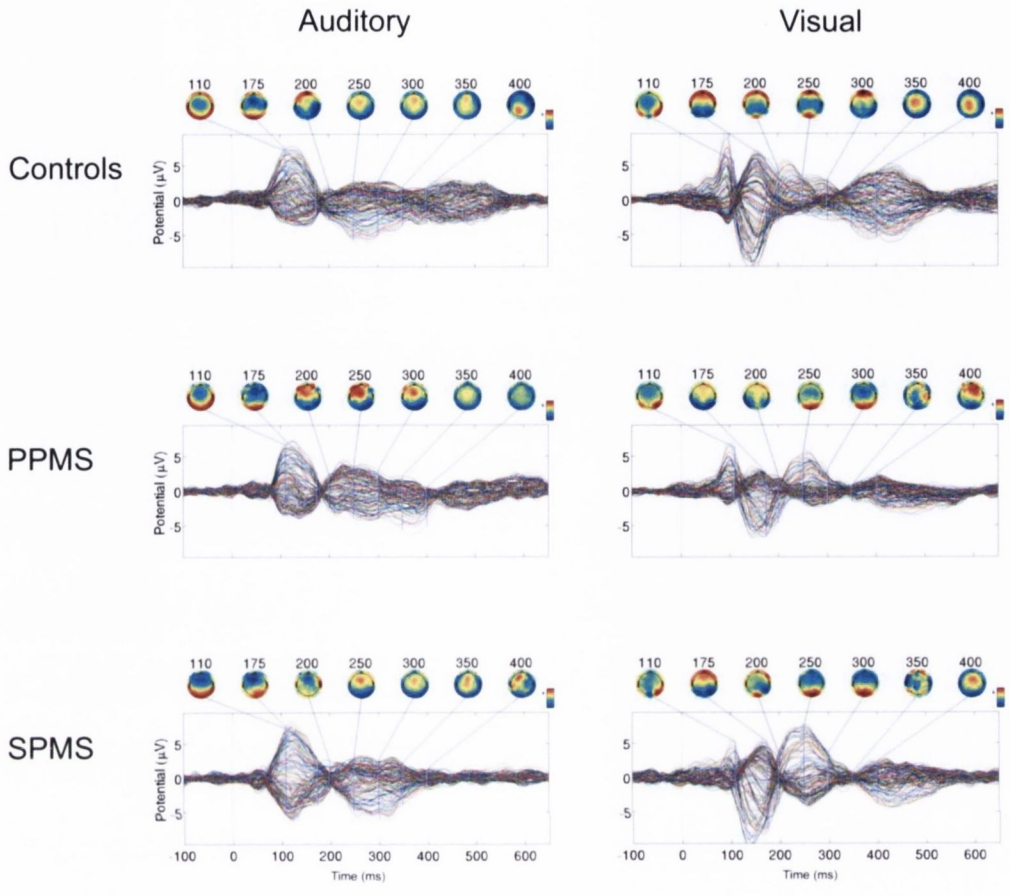


Figure 4.3.3. Progressive MS patients have reduced amplitudes in comparison to controls.

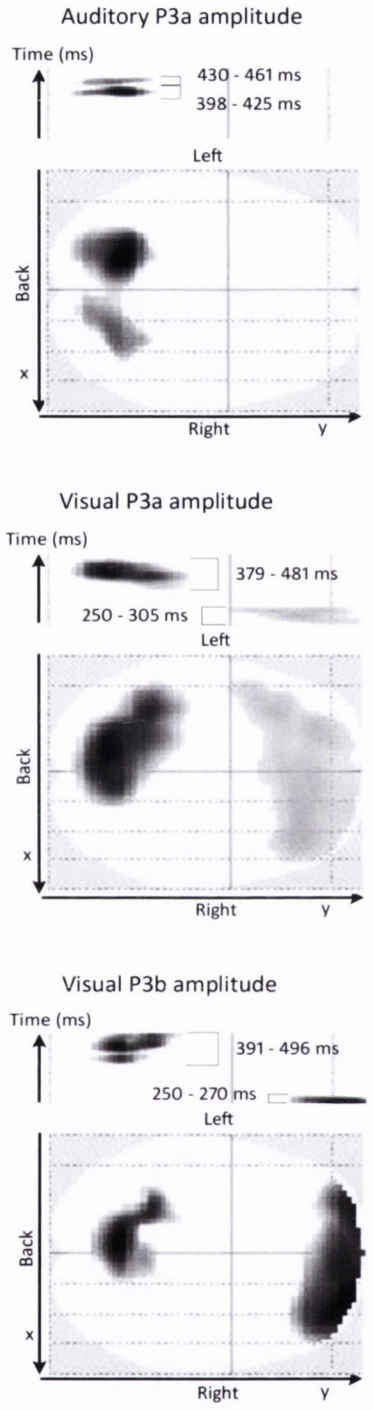


Figure 4.3.4. PPMS patients had lower amplitude compared to SPMS in auditory P3b condition.

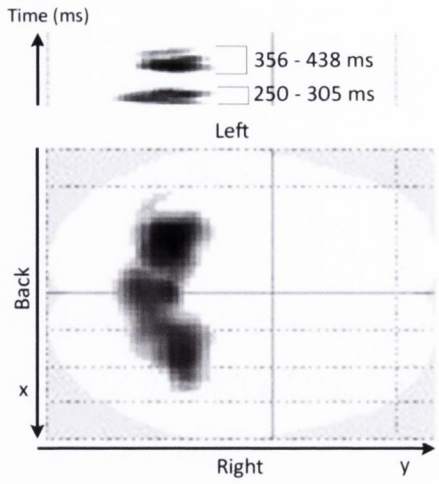
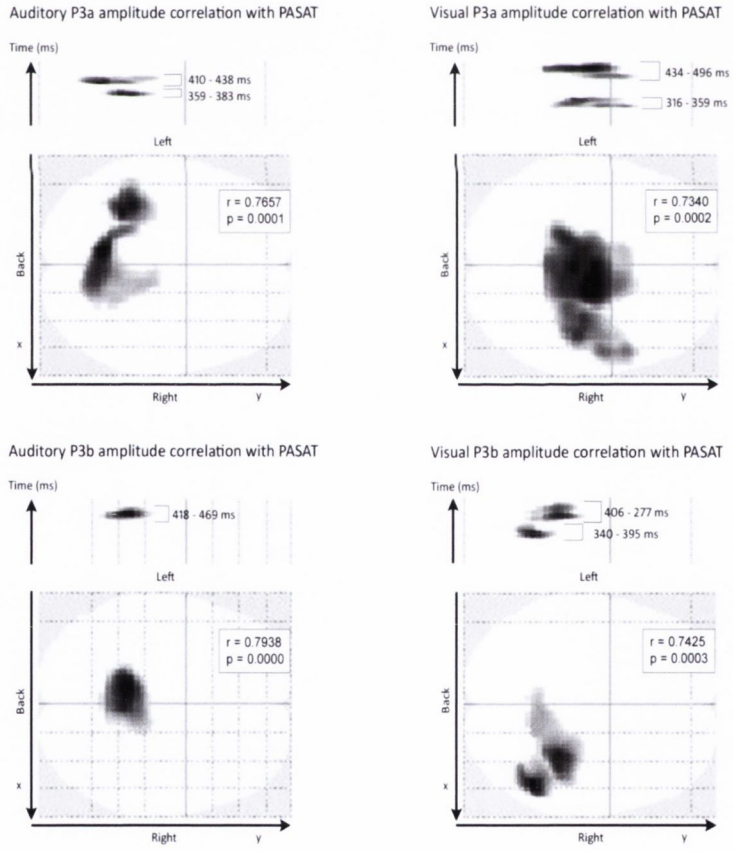
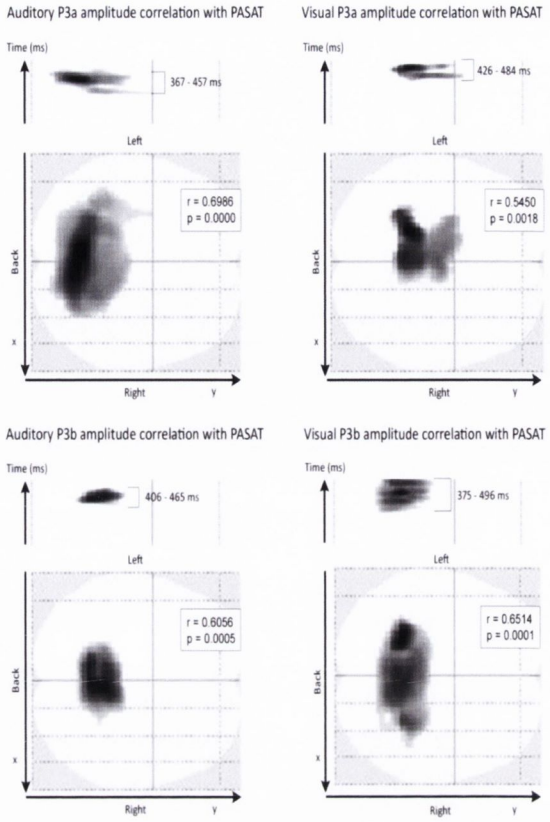


Figure 4.3.5. Significant correlation ($p < 0.005$) of the amplitudes of P3 event-related potentials and PASAT in patients with multiple sclerosis.



Note. The correlation (*Pearson's r*) between the voxel of maximum intensity and the PASAT is also displayed.

Figure 4.3.6. Significant correlation ($p < 0.005$) of the amplitudes of P3 event-related potentials and PASAT in MS patients and controls.



Note. The correlation (Pearson's r) between the voxel of maximum intensity and the PASAT is also displayed.

4.3.4. Discussion

Previous literature has not included an examination of the ERP components in PPMS using a high-density EEG array and the no-go P3a. Furthermore, both visual and auditory tasks were employed in this study. In addition, PPMS patients were compared to age-, education- and sex-matched SPMS patients and to healthy controls.

Patients with PPMS had decreased amplitude compared to SPMS patients only in the auditory P3b task, which was not consistent with the hypothesis. It is possible that these amplitude differences arise from the abnormal topographical distribution of P3 ERP activations, potentially caused by demyelinating lesions, as in the auditory P3b condition the PPMS patients have very frontal P3b-related topography, whereas the SPMS patients show activations over parietal areas (as illustrated in Figure 4.3.1). As hypothesized, the control group had greater amplitude than the progressive MS patients in all the tasks, except in the auditory P3b task. The auditory P3b task is typically less difficult than the visual P3b and the no-go P3a tasks in either modality, which is most likely due to the clearly distinctive stimuli that are easy to distinguish from another, i.e. more similar stimuli properties increasing the difficultness of the task. It is possible that in our studies the auditory stimuli were not sufficiently similar, which would have made the auditory P3b ERP task less difficult to perform, and made less demands on attentional capacities and memory processes, in comparison to the visual P3b ERP task. Therefore, a faster ERP to the auditory stimuli may indicate that it was easier to discriminate between auditory target and non-target stimuli. For example, P3a non-target latency increases when targets are harder to discriminate from standards (Polich & Comerchero, 2003). It is possible that all conditions – with the exception of the auditory P3b – were too difficult for the patients with MS, which manifested with equally prolonged and decreased P3 ERP activations.

It is possible that the differences between RRMS and PPMS reported in the study of Ellger and colleagues (2002) were partly influenced by age differences, as the PPMS patients were older (57 years). In contrast, in the present study subjects were age-matched and analyses were controlled for the effect of differences in disease duration, age, education and sex. Another factor affecting the results may be the low number of subjects in the present study (7 PPMS), which is less than in the previous study of Ellger, et al. (2002) that included 17 PPMS patients. This may have reduced the statistical power to detect potential differences between the groups. Moreover, in the present study the PPMS patients had longer disease duration (11

years), and had more severe physical disability (EDSS score 5.8) compared to the patients in the study of Ellger, et al. (2002) (45 years, disease duration 7 years, EDSS score 5.3).

The PASAT score correlated with P3 amplitude in all P3 tasks as hypothesized, suggesting that the P3 ERP may be a surrogate marker of CI in MS. Future research comparing PPMS and SPMS and using ERP tasks should be cognisant of the fact that task difficulty, age differences and methodological limitations may obscure differences between these groups. The present study provided preliminary evidence that there are subtle differences between PPMS and SPMS in electrophysiological responses during an auditory P3b task, and that the PASAT performance and P3 ERPs correlate in progressive MS subtypes.

Key points:

- PPMS patients have decreased auditory P3b amplitude relative to SPMS patients
- MS patients have reduced amplitude relative to controls in all of the tasks, except in the auditory P3b task
- PASAT score correlated with amplitudes in all of the P3 ERP tasks
- P3 ERPs may be a surrogate marker of cognitive function in MS

4.4. Independent component analysis based cluster analysis of P3 ERP source activations in multiple sclerosis

This section of Chapter 4 examines the Research Question 2 as stated in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. The principal interest of the study was to study if the source P3 EEG activations at Month 0 differ between MS patients and healthy controls, and between MS patients with and without cognitive impairment.

4.4.1. Introduction

The results of the previous studies in this chapter have provided evidence that objective and reliable cognitive-indexing electrophysiological measures have potential to complement the assessment of CI in MS by providing information on the brain functions related to cognitive dysfunction. However, these studies with the former electroencephalography (EEG) studies have concentrated on measuring *scalp* P3 event-related potentials (ERPs) and EEG time-frequency domain in MS patients, as discussed in depth in Chapter 2-Literature Review. The neural generators in healthy controls of P3b have not been comprehensively delineated, not least because they are widespread in nature, but they include the parietal, frontal, and medial temporal lobes, the cingulate cortex, and the temporo-parietal junction (Polich, 2007; Volpe, et al., 2007b).

The majority of P3b ERPs studies in MS have reported prolonged P3b latencies and/or reduced P3b amplitudes in visual and/or auditory modality (Magnano, et al., 2006), although some studies (Gerschlager, et al., 2000a; Sailer, et al., 2001) have failed to find differences between MS patients and controls. The variation in the scalp-based P3b ERP results was most likely due to the relatively small sample sizes and the use of low-density EEG arrays. In the scalp-based high-density EEG studies described in detail earlier in the present chapter a relatively large number of subjects (N=54) were shown that MS patients had reduced P3b amplitudes over frontal and centro-parietal scalp areas in the visual task, but there were no statistically significant differences in the auditory modality.

Physiologically, EEG spectral power reflects the number of neurons that discharge synchronously (i.e. increased power implies increased synchronous firing of a group of neurons)(Klimesch, 1999). *Theta* (4-8 Hz), *alpha* (8-13 Hz), and *beta* (14-30 Hz) EEG frequencies are associated with cognitive processing and behaviour (Polich, 2007; Wang,

2010). In particular, superior working memory performance is related to an increase in theta power and to a decrease in alpha power after an increase in task demands, such as the presentation of less frequent target stimuli in an oddball paradigm (Klimesch, 1999). Theta oscillations are also related to information processing tasks, particularly those of sustained attention and executive functions (Gevins, et al., 1997; Klimesch, 1999; Mizuhara, et al., 2004; Mizuhara & Yamaguchi, 2007), which are all essential for a successful oddball detection. Previous scalp-based and low-density spectral EEG studies on MS patients related to P3b ERP task performance have identified increased power of only beta and gamma bands in MS patients compared to controls over the whole scalp area, which was most prominent over frontal electrodes (Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Escera, et al., 2008), and over anterior right hemisphere and bilateral posterior scalp regions (Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Borges, et al., 2008).

4.4.1.1. Beyond scalp ERP

Previous EEG studies of cognitive functioning in MS have only examined the differences between the mean ERPs of MS patients and controls as comprehensively presented in Chapter 2-Literature Review. The informativeness of ERPs is restricted due to the fact that they are generated by multiple, functionally distinct neural sources, which cause EEG potentials recorded on the scalp to overlap both temporally and spatially (Dale, et al., 2000; Hagoort, 2003). Independent component analysis (ICA) is one of the most advanced methods to deal with the problem of spatiotemporal overlap of EEG. Its aim is to identify temporally independent and spatially fixed independent components (ICs) from high-density scalp EEG activities in which they are intermingled, yielding functionally distinct ICs which can be mapped onto 3D head space (Groppe, et al., 2008; Hoechstetter, et al., 2004; Onton, et al., 2006), as detailed in Chapter 3-General Methods. The ICs reflect the origin of the EEG activations, whereas in the traditional scalp-based ERP analysis the recorded signals from each scalp electrode are assumed to be comparable with the signals from equivalently placed electrodes for all the subjects, thus not taking into account the physical differences of the brains of different people (Hoechstetter, et al., 2004). In contrast to other EEG source analysis methods (e.g., BESA (Makeig, 1993), ICA does not require a priori assumptions on the temporal dynamics or the spatial structure of the underlying processes.

Average ERPs between groups only capture the portion of the channel data that is phase-consistent at latencies relative to the time-locking events, which may result into a potential loss of information of the EEG activities (Groppe, et al., 2008; Hoechstetter, et al., 2004). The

aggregation of ICs into functionally equivalent groups provides an estimate of source EEG activities across subjects and conditions, thus reflecting more comprehensively the EEG activities (Hoechstetter, et al., 2004). Event-related spectral perturbations (ERSPs) and inter-trial coherence (ITCs), described in Chapter 3-General Methods, are superior methods for visualizing event-related EEG dynamics because they reveal a more comprehensive overview of the underlying EEG time-frequency properties than ERPs. ERSPs are the mean latency-by-frequency images that show the frequencies and latencies when mean changes in log power (dB) occur from a specific mean power baseline, showing them time-locked to experimental events (Makeig, 1993). ITCs measure the trial-to-trial phase consistency at each frequency and latency relative to a set of time locking events.

An IC clustering study of an auditory P3b task in healthy, young volunteers showed that IC clusters reflecting P3b activations had a decrease in alpha power post-target stimulus presentation (Debener, et al., 2005a). To my knowledge, IC clustering of EEG data has not yet been utilised with a large sample (over 40 subjects), nor with a clinical group. IC clustering has potential to give a more thorough insight into how the changes in MS patients brains, due to demyelinating lesions and cortical atrophy, affect the speed of processing of the stimulus information.

Based on the previous research literature (Leocani, et al., 2010; Magnano, et al., 2006) and the earlier findings on scalp-space P3b ERP analyses in MS presented in this chapter, and on spectral EEG studies relating frequency bands to cognitive functions utilised during P3b task performance, such as sustained attention and working memory (Gevins, et al., 1997; Klimesch, 1999; Mizuhara, et al., 2004; Mizuhara & Yamaguchi, 2007), it was hypothesized that MS patients versus controls and cognitively impaired MS (CI-MS) patients versus non- cognitively impaired MS (non-CI-MS) patients would show 1) reduced theta and a smaller decrease in alpha band after target stimuli, and 2) less consistency in IC inter-trial coherences (ITCs), over the expected P3b peak time period (200-400 ms). These differences were anticipated to be more prominent in the IC clusters located in the parietal brain areas which were associated with P3b activities in previous IC clustering studies (Debener, Makeig, Delorme, & Engel, 2005b; Delorme, et al., 2007; Makeig, et al., 2004; Onton, et al., 2006), and in visual modality based on the previous studies conducted.

4.4.3. Methods

4.4.3.1. Subjects

Fifty-six MS patients (mean age = 44 years; range = 20-61 years) and 39 age-matched healthy volunteers (mean age = 42 years; range = 28-67 years) were included in the study. Table 4.4.1 displays the demographic and behavioural data of the subjects. Six patients had had a relapse within the previous year. Three MS patients were excluded from the further analysis of the PASAT performance groups due to missing information.

The effect of years of education, age and gender on PASAT scores were controlled by employing a regression-based approach Chapter 3-General Methods (described in detail in (Parmenter, et al., 2010) to obtain corrected PASAT *Z*-scores due to the differences and correlations between MS patients and controls in the years of education, age and gender, and the known correlations between PASAT raw scores and and these demographic variables (Diehr, et al., 2003; Tombaugh, 2006). The MS patients were then chosen for an extreme group analysis based on their PASAT *Z*-score. In this analysis, the top third of the MS patients (N=18) with highest PASAT *Z*-scores (mean = 0.91) denoted here as the non-cognitively impaired MS group (non-CI MS) were compared to the bottom third of MS patients (N=18) with the lowest PASAT Z-scores (mean = -1.54) denoted here as the cognitively impaired MS group (CI MS).

Table 4.4.1. Demographic and behavioural data of subjects.

	MS (N=56)		Control (N=40)		Non-CI (N=18)		CI (N=18)	
Male/female	30/26		22/18		12/6		9/9	
	μ	σ	μ	σ	μ	σ	μ	σ
Age	44.14	9.87	42.47	9.89	45.17	7.72	40.12	9.55
Mean years of disease	11.64	8.59	N/A	N/A	11.50	8.52	10.60	6.92
Mean years of symptoms	15.81	10.26	N/A	N/A	14.22	8.30	15.72	11.63
Mean years of education	14.65	3.59	17.24	2.98	12.22	2.58	17.00	3.40
PASAT (Z-score)	-0.33	1.14	0.033	1.49	0.91	0.70	-1.54	0.54
Auditory P3b RT (<800 ms)	372.81	75.12	359.82	74.64	372.52	68.36	378.01	101.43
Visual P3b RT (<800 ms)	381.92	72.90	363.45	47.27	390.53	77.53	379.49	77.18
	M	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*
EDSS	3.50	4.00	N/A	N/A	3.00	4.63	3.25	4.125
HRSD	3.00	6.50	0.50	4.50	4.50	7.00	4.00	12.00

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = inter-quartile range, PASAT = Paced Auditory Serial Addition Test, Non-CI = MS patients with the highest PASAT *Z*-score, CI = MS patients with the lowest *Z*-score, EDSS = Expanded Disability Status Scale, HRSD = the Hamilton Rating Scale for Depression, RT = reaction time.

4.4.3.2. Experimental procedure, EEG acquisition, data analysis and statistical analyses

EEG data acquisition and P3b and P3a tasks are described in detail in Chapter 3-General Methods. The experimental procedure involving the standard 3-s Paced Auditory Serial Addition Test PASAT (Gronwall, 1977; Tombaugh, 2006), 21-item Hamilton rating scale for depression (HRSD; Vieweg & Hedlund, 1979), and a measure of physical disability in MS, Kurtzke Expanded Disability Status Scale (EDSS; Kurtzke, 2008) is described in Chapter 3-General Methods.

The data analysis is depicted in detail in Chapter 3-General Methods, and only the parts of data analysis not included in the chapter are described here. Preliminary data processing employed the EEGLAB toolbox (Delorme & Makeig, 2004) in conjunction with the FASTER software tool (Fully Automated Statistical Thresholding for EEG artefact Rejection; Nolan, et al., 2010). The EEG data were bandpass filtered between 1 and 95 Hz, notch frequencies set to 50 and 60 Hz (the monitor refresh rate), average referenced across all scalp electrodes (appropriate when using a high-density EEG array), epoched from 500 ms pre-stimulus to 1000 ms post-stimulus and baseline corrected from 200 ms pre-baseline). The FASTER toolbox removed epochs with large artefacts (e.g., muscle twitch) and interpolated channels with poor signal quality.

Independent component analysis (ICA) was used to obtain independent components (ICs) from scalp EEG activity in which they were mixed (Bell & Sejnowski, 1995; Makeig, Jung, Bell,

Ghahremani, & Sejnowski, 1997) as detailed in the General methods chapter. The auditory and visual P3 data were subjected to two separate ICAs because the tasks involved different sensory processes activating different brain areas at different latencies. Based on the prior experience of our research group with ICA (e.g. developing FASTER, a fully automated method for processing high-density EEG data; (Nolan, et al., 2010) we found that combining different run data yielded suboptimal results.

FASTER (Nolan, et al., 2010) automatically identified artefactual (i.e., non-neural) ICs and removed them from the EEG data (note: the standard $z = 3$ threshold for IC rejection was lowered to $z = 2$ for the EOG channels). The remaining ICs were used in the further analyses. Next, inverse modelling of the individual IC scalp maps was performed to localize EEG sources related to P3b task performance (Onton, et al., 2006) as described in Chapter 3-General Methods.

In order to acquire comprehensive source EEG activations related to P3b task, IC clusters were defined by means of a joint distance measure based on three dimensional (3D) dipole source locations and orientations, scalp maps, mean spectra (using a Fast Fourier Transform), and ERP, ERSP and ITC activations (described in detail in Chapter 3-General Methods).

Outlier components of more than 2 standard deviations from any of the centroid properties of IC clusters were assigned to specific outlier IC clusters that were excluded from further analyses. On average, 26.8% of ICs were classified as outliers. Four distinct IC clusters were identified in the visual condition and 5 IC clusters in the auditory condition.

Permutation statistics were applied to each cluster (Delorme, 2006; Maris, 2004), in conjunction with the false discovery rate correction for multiple comparisons (Benjamini & Yekutieli, 2001). Permutation statistics were chosen as they are non-parametric and therefore do not rely on an a priori model of the data distribution (Delorme, 2006; Maris, 2004). In permutation-based approaches sets of simulated sample distributions are generated by randomly shuffling the data across trials and/or subjects. Consequently, the shuffled distributions will have all features of the original data except the effect to be tested (Delorme, 2006; Maris, 2004; Onton, et al., 2006). The false discovery rate is the expected proportion of erroneous rejections among all rejections and it can be used to control the familywise error rate (i.e. the probability of having at least one type I error among the entire set of tests conducted) (Benjamini & Yekutieli, 2001). In order to correct for the number of clusters per condition, a Bonferroni cut-off was also applied, which involves dividing the p-value threshold

by the number of comparisons performed (Delorme, 2006). Thus, the corrected significance cutoff was $p = 0.0125$ (i.e., .05/4) for the 4-cluster solution in the visual condition, and $p = 0.01$ (i.e., .05/5) for the 5-cluster solution in the auditory condition. The group statistic t-values which are below the significance cutoff are displayed in red in the ERSP and ITC subfigures showing the statistically significant differences between the groups relative to time and frequency.

In order to provide a comparison with the IC clustering method, an analysis of the scalp EEG data was also performed. The data pre-processed through FASTER were grand-averaged and statistical analysis of ERP, ERSP and ITC conducted at Pz. The false discovery rate correction for multiple comparisons (Benjamini & Yekutieli, 2001) was utilised.

4.4.3. Results

4.4.3.1. Behavioural results

MS patients and controls did not differ in age or in reaction times ($p > .05$). MS patients had significantly higher scores in the HRSD-21 than controls ($p < .001$). PASAT score did not correlate with HRSD-21 score, but it correlated with EDSS score ($p < .05$). Controls had more years of education and higher PASAT scores than MS patients ($p < 0.001$). As anticipated, the raw PASAT scores correlated with years of education in a sample of all subjects, with age and years of education in the MS patient sample, and with years of education and gender in the control sample.

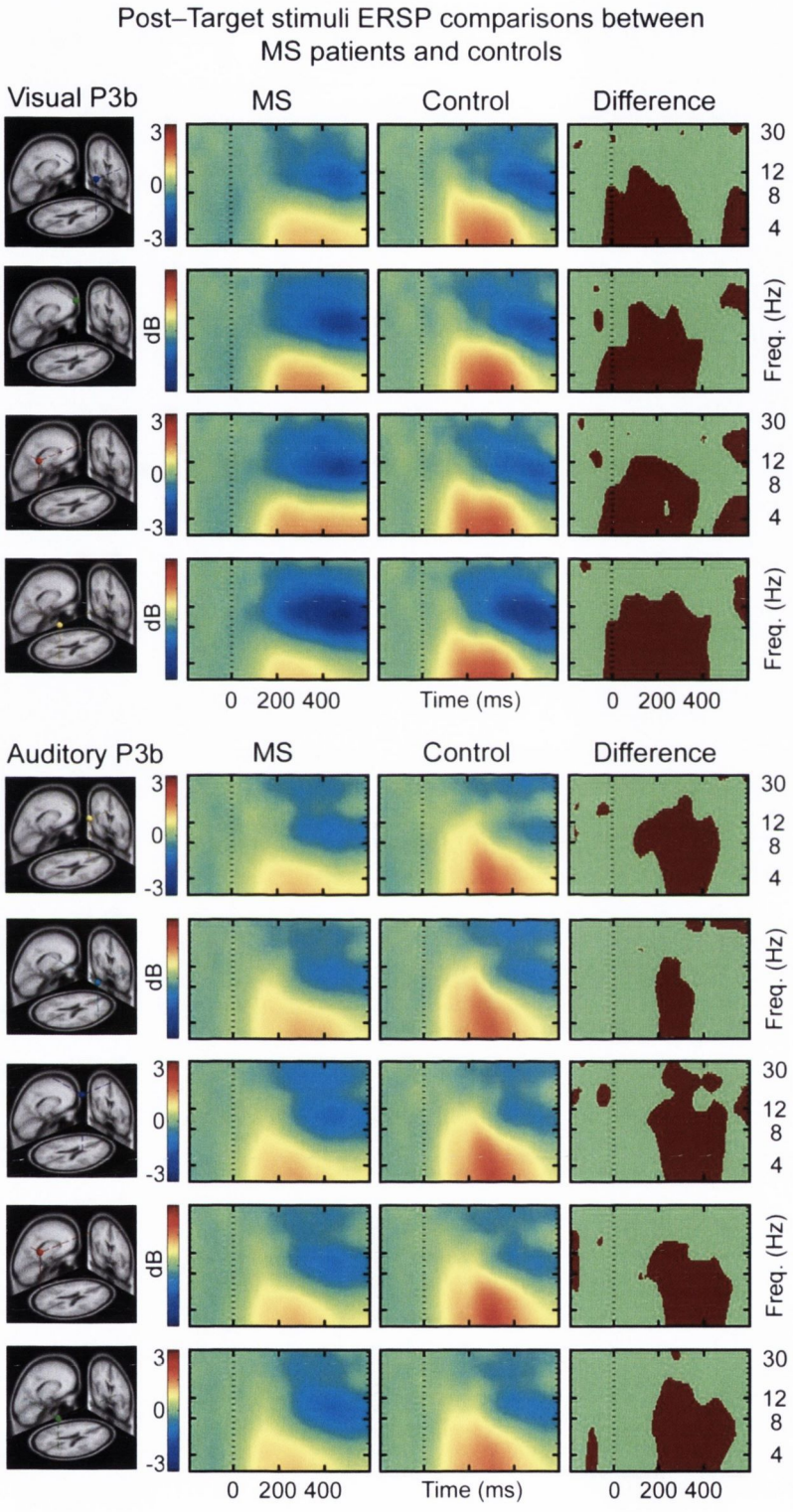
PASAT raw scores were converted to demographically corrected scores. The relatively large sample size (N=95) enabled to compare MS subgroups that were split up based on their performance in the PASAT. In specific, the MS patients were grouped into two extreme groups based on their PASAT Z-score: the MS patients with the highest PASAT Z-scores or the non-cognitively impaired MS group (non-CI MS), and the MS patients with the lowest PASAT *Z*-scores or the cognitively impaired MS group (CI MS) as detailed in Methods. The non-CI MS and CI MS groups did not differ in age, disease duration, reaction times, EDSS scores or HRSD-21 ($p > .05$). The PASAT *Z*-scores did not correlate with EDSS scores, with mean years of symptoms, or with mean years of disease but the CI MS group had more years of education than the non-CI MS group.

4.4.3.2. Comparison between MS patients and controls

The ERSPs of all of the IC clusters revealed significantly less increase in theta power and a greater decrease in alpha power at 200-400 ms post-target stimulus for the MS patients when compared to the controls in all of the IC clusters for both visual and auditory conditions. In visual condition, there was less increase in theta power and a greater decrease in alpha power 0-200 ms for MS patients relative to controls (Figure 4.4.1). The inter-trial consistency was lower for MS patients compared to controls over theta frequencies in right frontal, right parietal and left parietal IC clusters at 0-400 ms after target stimuli presentation of visual condition; and in central and left parietal IC clusters at 0-600 ms and in frontal and right parietal IC cluster at 200-600 ms of auditory condition (Figure 4.4.2). The grand mean IC cluster scalp maps are displayed in Figure 4.4.3.

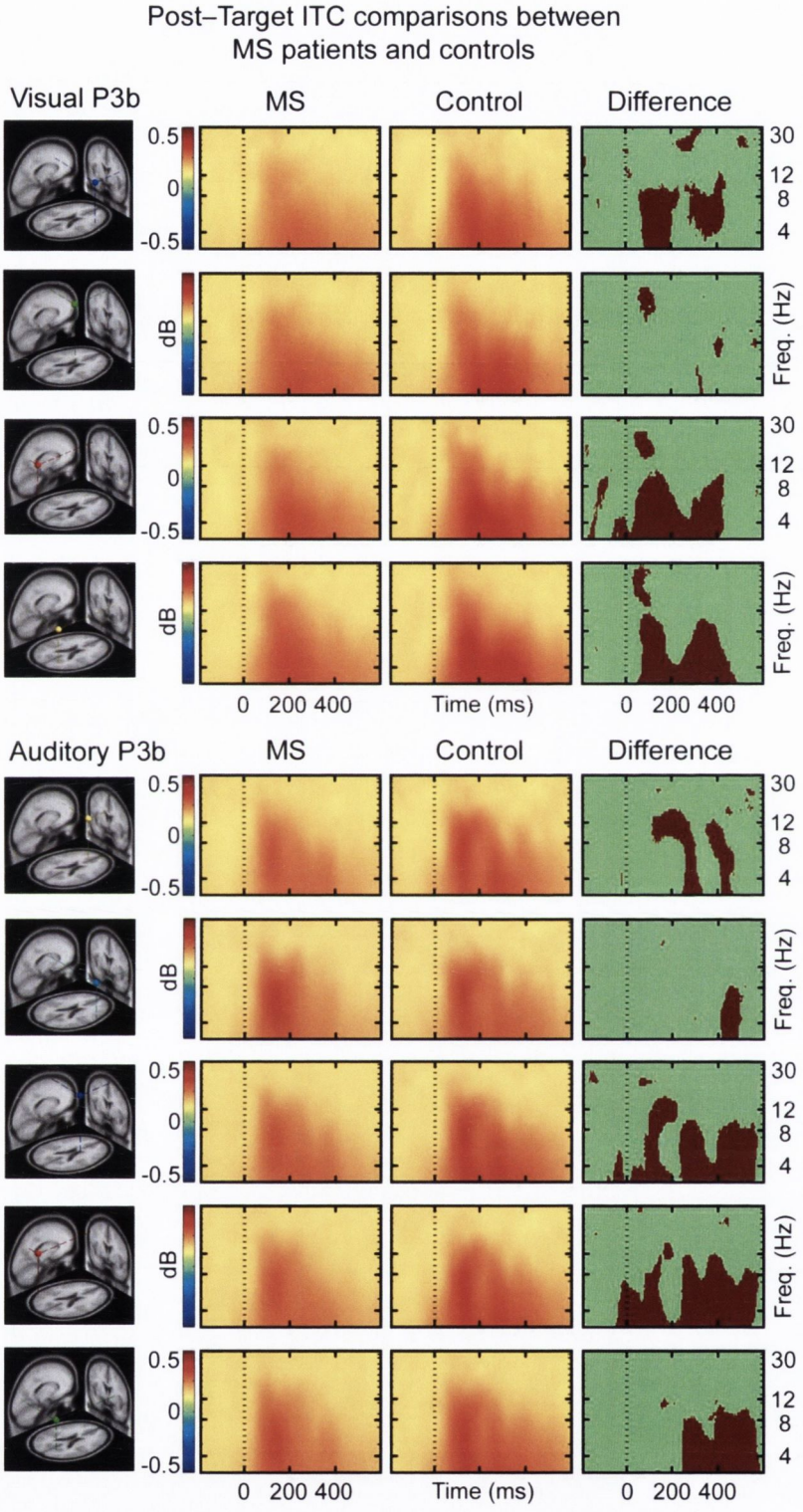
After non-target stimuli in the visual condition, the MS patients showed less increase in theta power 0-600 ms and a greater reduction in alpha power 200-600 ms compared to the controls in all ERSPs of IC clusters. In the auditory condition the MS patients showed less increase in theta, alpha and beta power around 0-200 ms and less decrease in theta, alpha and beta power 200-600 ms relative to controls in the left parietal IC cluster. The MS patients showed less increase in theta power at 100-200 ms in the frontal, right temporal and central IC clusters compared to controls (Figure 4.4.4). The inter-trial consistency was lower for MS patients relative to controls in the theta band from 0-600 ms in all of the IC clusters, and from 200-600 ms in the alpha band in left and right parietal IC clusters after visual non-target stimuli presentation; and over theta and alpha bands at 0-400 ms in frontal, central, left parietal and right parietal IC clusters, and at 200-400 ms in right temporal IC cluster of the auditory condition (Figure 4.4.5). The grand mean IC cluster scalp maps are displayed in Figure 4.4.6.
The cluster analysis of P3a showed only few differences between the groups (Figure 4.4.7). MS patients had significantly reduced P3a amplitude in central and left parietal clusters, but the groups did not differ in ERSP and ITC measures.

Figure 4.4.1. The mean ERSPs time-locked to the presentation of target stimuli in visual and auditory conditions in MS patients and controls.



Note. IC cluster mean differences shown in log spectral EEG power (dB) relative to log power in pre-stimulus EEG baseline. Red areas indicate an increase in power and blue areas a decrease in power. Statistical significance is illustrated by red/green frames beside ERSP activation frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$, controlled for multiple comparisons) differences between the MS patients and controls in time and in log spectral power.

Figure 4.4.2. The ITC showing consistency between the trials and the degree of phase-locking to the target stimuli in MS patients and controls.



Note. Highest event-related phase consistencies for each condition are plotted in red, and lowest in green (range 0 to 1, no consistency to full consistency respectively). Statistical significance is illustrated by red/green frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$ for auditory condition, controlled for multiple comparisons) differences between MS patients and controls in time and in log spectral power.

Figure 4.4.3. The grand mean topographies of MS patients and controls for each IC cluster post-target in visual and auditory conditions.

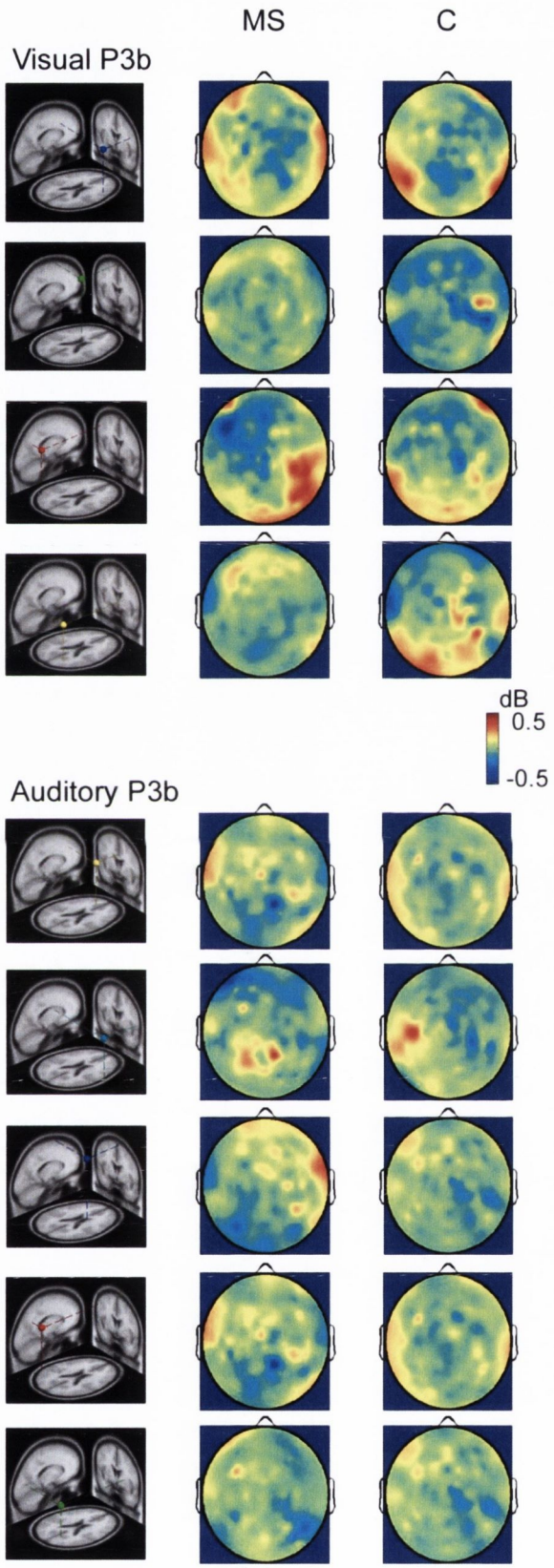
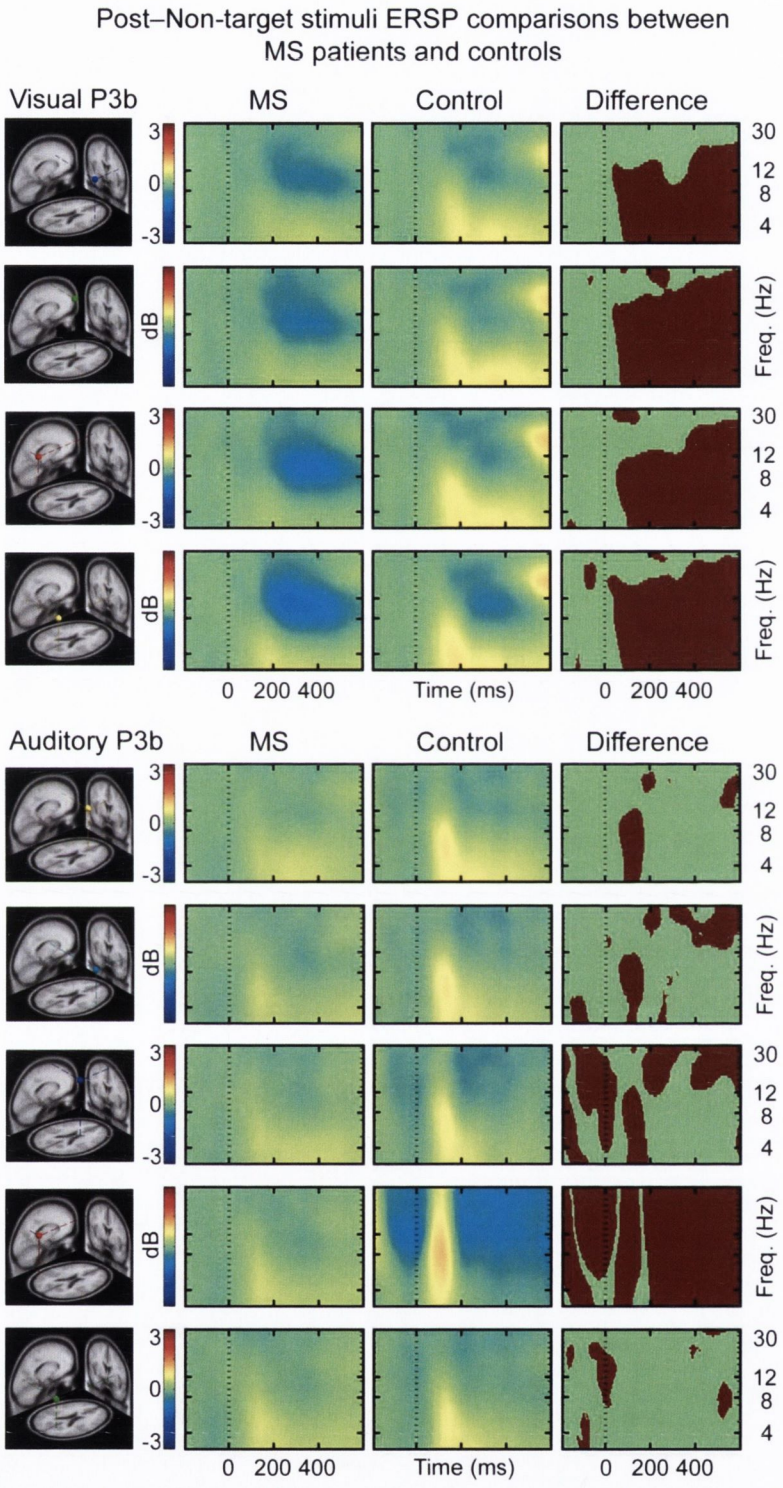
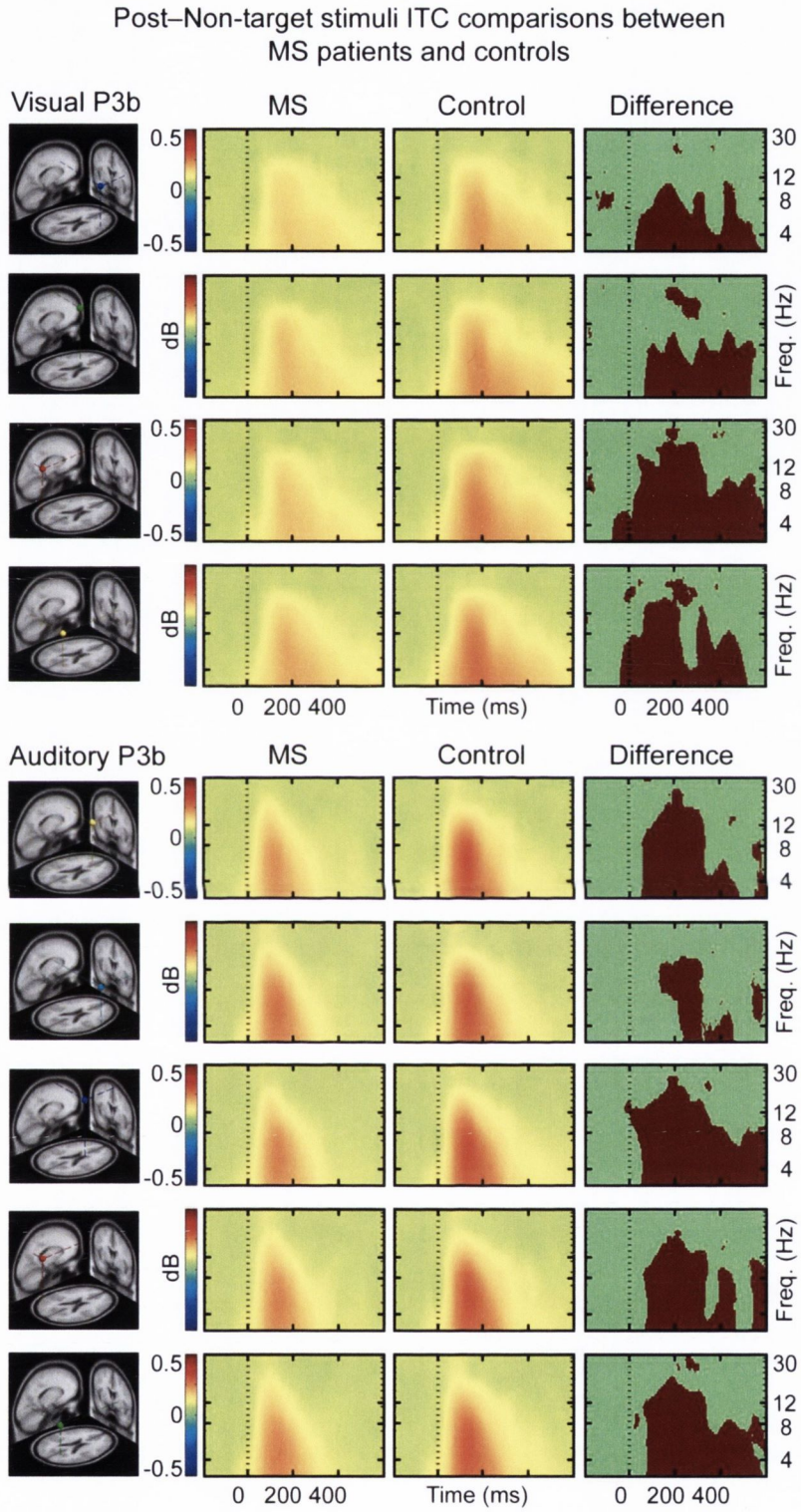


Figure 4.4.4. The mean ERSPs time-locked to the presentation of non-target stimuli in visual and auditory conditions in MS patients and controls.



Note. IC cluster mean differences shown in log spectral EEG power (dB) relative to log power in pre-stimulus EEG baseline. Red areas indicate an increase in power and blue areas a decrease in power. Statistical significance group and condition main effects, and interaction effect, are illustrated in red/green frames beside ERSP activation frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$, controlled for multiple comparisons) differences between the MS patients and controls in time and in log spectral power.

Figure 4.4.5. The ITC showing consistency between the trials and the degree of phase-locking to the non-target stimuli in MS patients and controls.



Note. Highest event-related phase consistencies for each condition and group are plotted in red, and lowest in green (range 0 to 1, no consistency to full consistency respectively). Statistical significance is illustrated by red/green frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$ for auditory condition, controlled for multiple comparisons) differences between MS patients and controls in time and in log spectral power.

Figure 4.4.6. The grand mean topographies of MS patients and controls for each IC cluster post-non-target in visual and auditory conditions.

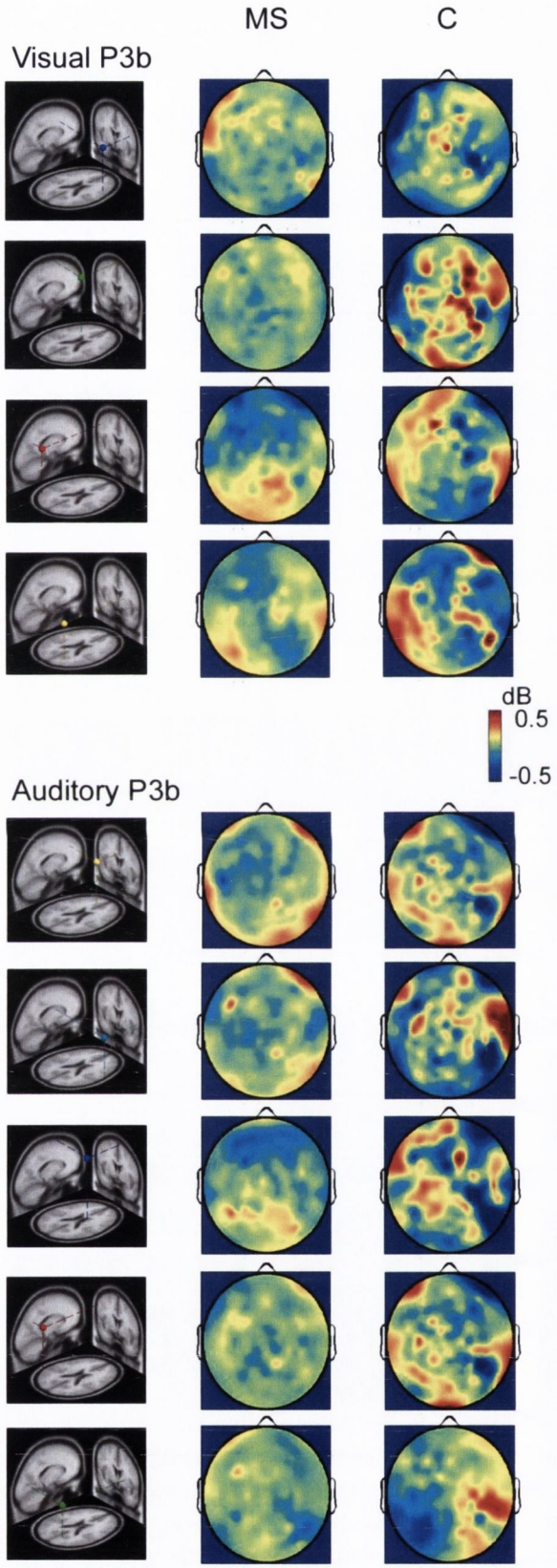
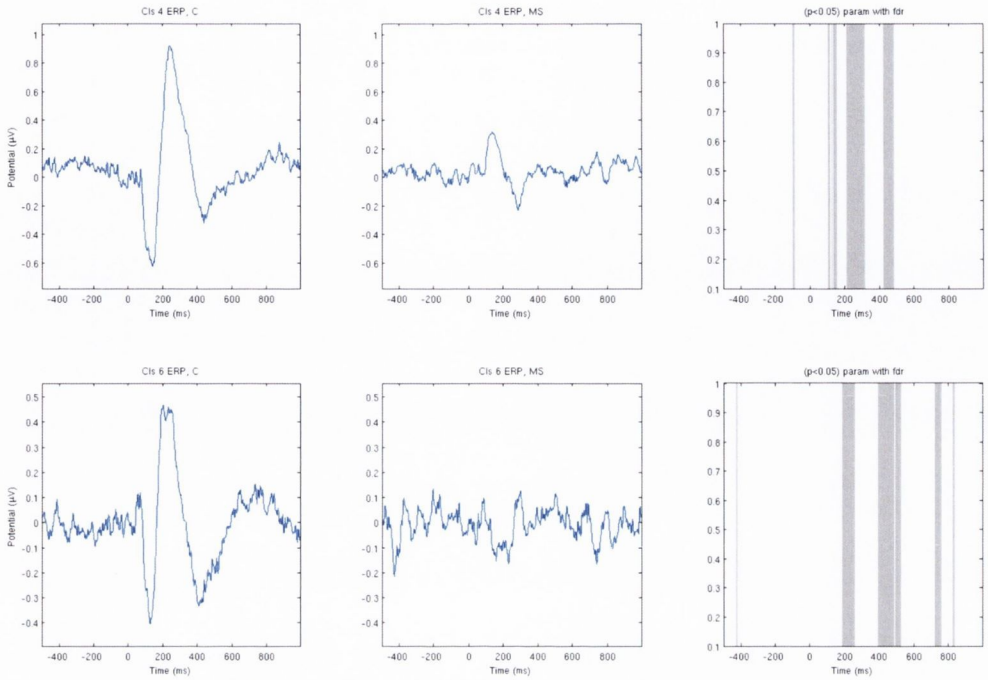


Figure 4.4.7. MS patients have significantly reduced P3a component compared to the controls in the central (above) and left parietal (below) IC clusters.



Note. P3a activation for controls on the left panels, for the MS in the central panels, and the statistically significant group differences ($p < 0.05$) with FDR correction (in grey) displayed on the right panels.

4.4.3.3. Comparison between Non-CI MS and CI MS

ERSP measures of the visual condition showed that CI MS patients had less theta, alpha and beta power at 200-600 ms post-target stimuli in the right frontal IC cluster relative to non-CI MS patients. In central and left parietal IC clusters CI MS patients showed reduced theta power at 200-600 ms post-target stimulus, relative to non-CI MS patients. The CI MS patients showed a greater decrease in alpha power in at 200-600 ms post-target stimulus compared to non-CI MS patients in right frontal, central and left parietal IC clusters (Figure 4.4.8., upper panel). The ERSP measures of the auditory condition indicate that CI MS patients had reduced theta power at 200-500 ms post-target stimuli compared to non-CI MS patients in left frontal, right frontal, and left parietal IC clusters (4.4.8., lower panel). CI MS patients had less inter-trial consistency compared to non-CI MS in the central and left parietal IC clusters in the theta band at 200-400 ms post-target stimuli (Figure 4.4.9., lower panel). There were no differences between the groups in ITC measures (Figure 4.4.9., upper panel) for the visual condition. The grand mean IC cluster scalp maps are displayed in Figure 4.4.10.

There were no clear differences in ERSP between the groups after the presentation of visual non-target stimuli, but in auditory condition there were subtle differences in theta, alpha and beta power at 0-600 ms in central and left parietal IC cluster (Figure 4.4.11). The only difference in ITC measures was in the left parietal IC cluster of auditory condition, in which CI MS patients had less inter-trial consistency compared to non-CI MS patients in theta band from 200-400 ms, and in the theta and alpha bands from 0-200 ms (Figure 4.4.12). The grand mean IC cluster scalp maps are displayed in Figure 4.4.13.

The CI MS groups did not differ significantly in ERP, ERSP or ITC measures in the IC cluster analysis of P3a.

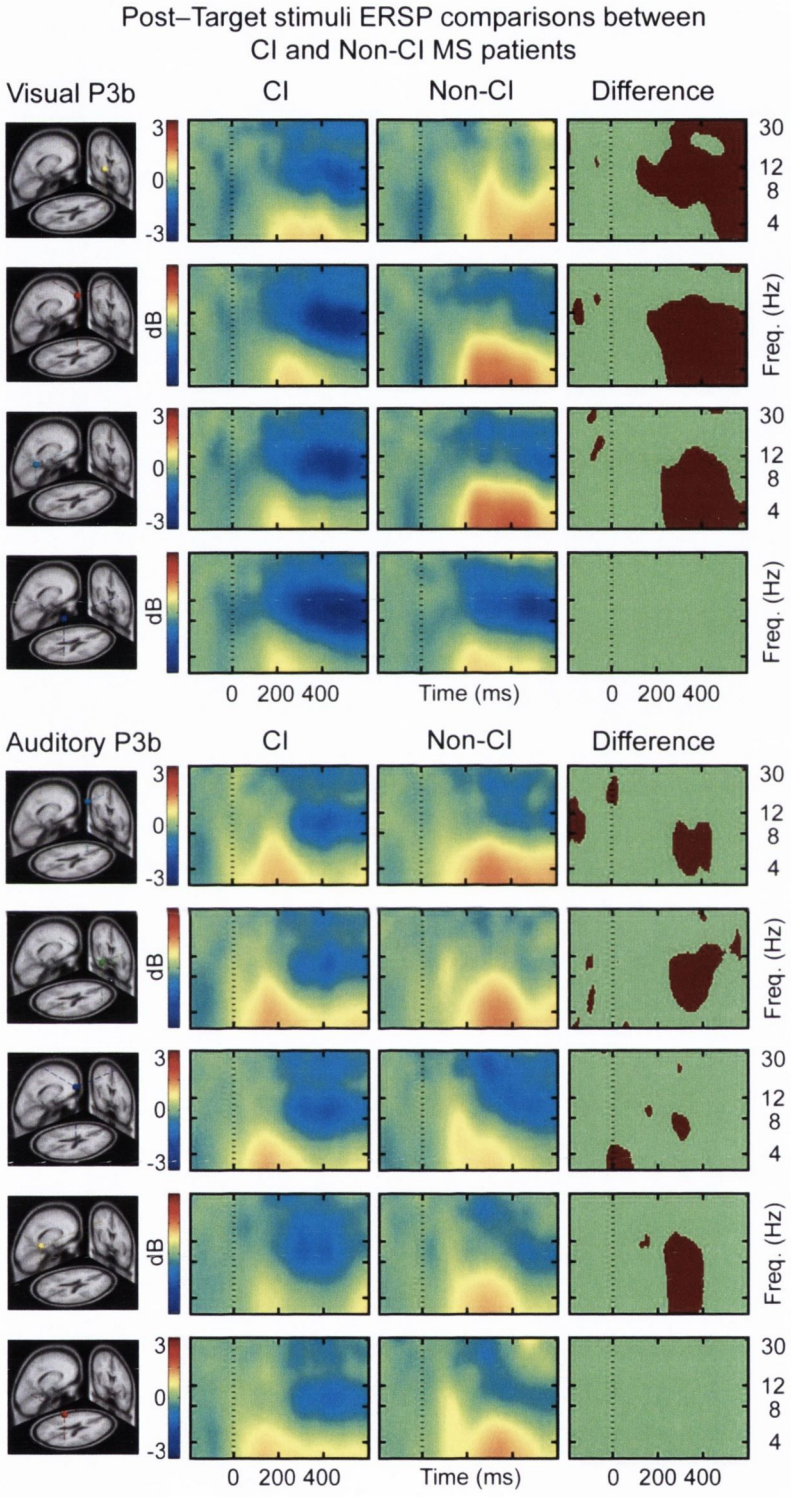
Talairach coordinates, Brodmann areas and associated brain regions of the IC cluster centroids of all of the comparisons are presented in Table 4.4.2. The numbers of independent components and subjects of each group included in clusters are reported in Table 4.4.3.

Scalp-EEG analysis results are presented in Figure4.4.14. and in Figure 4.4.15. There were no differences when the target responses were compared (Figure 4.4.14). The MS patients had a larger P2 peak after non-target stimuli relative to controls in auditory P3b (Figure 4.4.15).

The EEG-scalp analysis of recomposed ICs included in the IC clustering analysis presented in Figures 4.4.16. and 4.4.17.

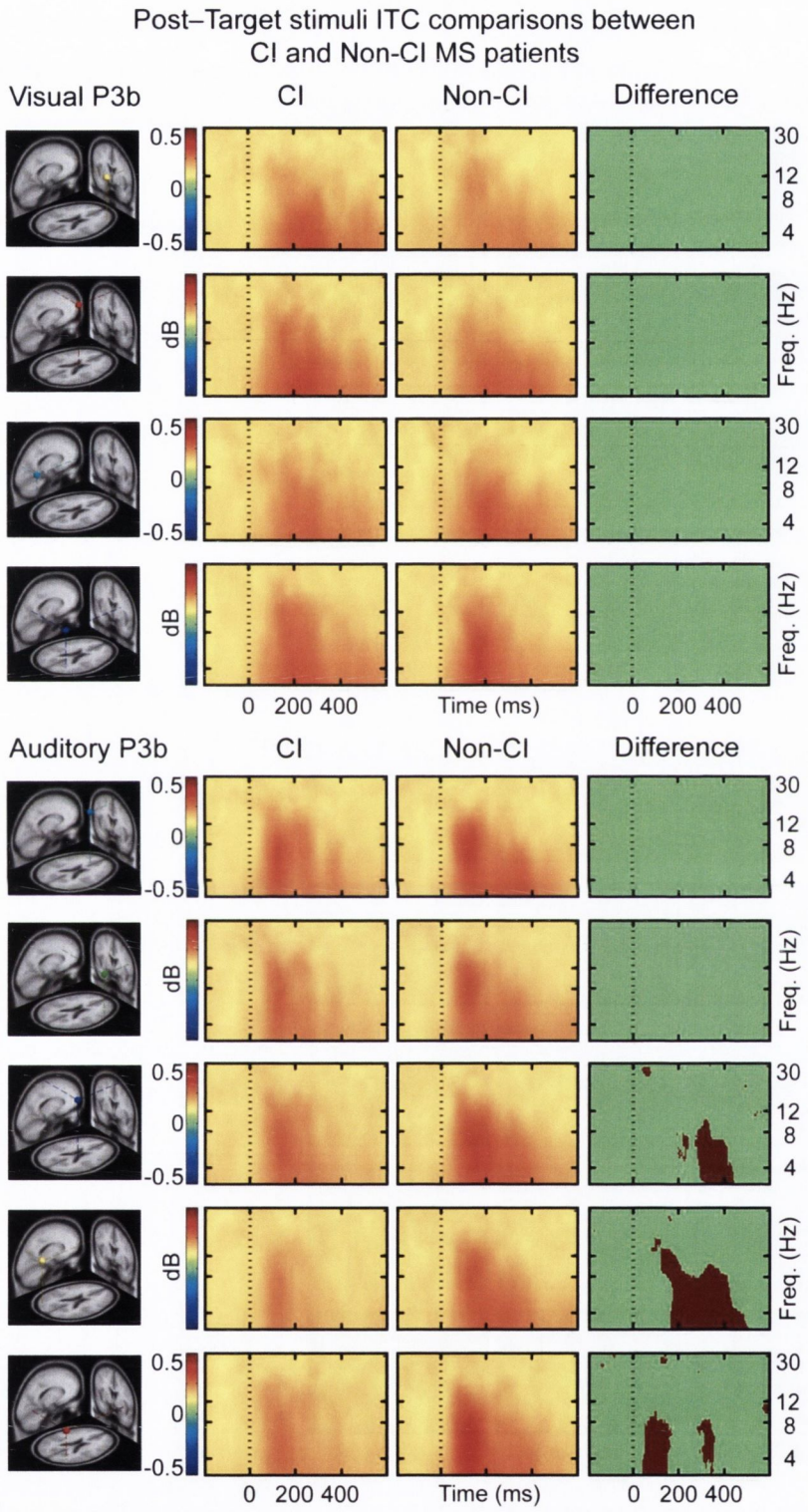
The scalp-based analysis of post-target and post-non-target activations at Pz in visual and auditory condition without FDR restriction displayed in Figure 4.4.18.

Figure 4.4.8. The mean ERSPs time-locked to the presentation of target stimuli in visual and auditory conditions in CI and non-CI MS patients.



Note. IC cluster mean differences shown in log spectral EEG power (dB) relative to log power in pre-stimulus EEG baseline. Red areas indicate an increase in power and blue areas a decrease in power. Statistical significance is illustrated by red/green frames beside ERSP activation frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$, controlled for multiple comparisons) differences between CI MS patients and the non-CI MS patients in time and in log spectral power.

Figure 4.4.9. The ITC showing consistency between the trials and the degree of phase-locking to the target stimuli in CI and non-CI MS patients.



Note. Highest event-related phase consistencies for each condition are plotted in red, and lowest in green (range 0 to 1, no consistency to full consistency respectively). Statistical significance is illustrated by red/green frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$ for auditory condition, controlled for multiple comparisons) differences between the CI MS patients and the non-CI MS patients in time and in log spectral power.

Figure 4.4.10. The grand mean topographies of CI and non-CI MS patients for each IC cluster post-target in visual and auditory conditions.

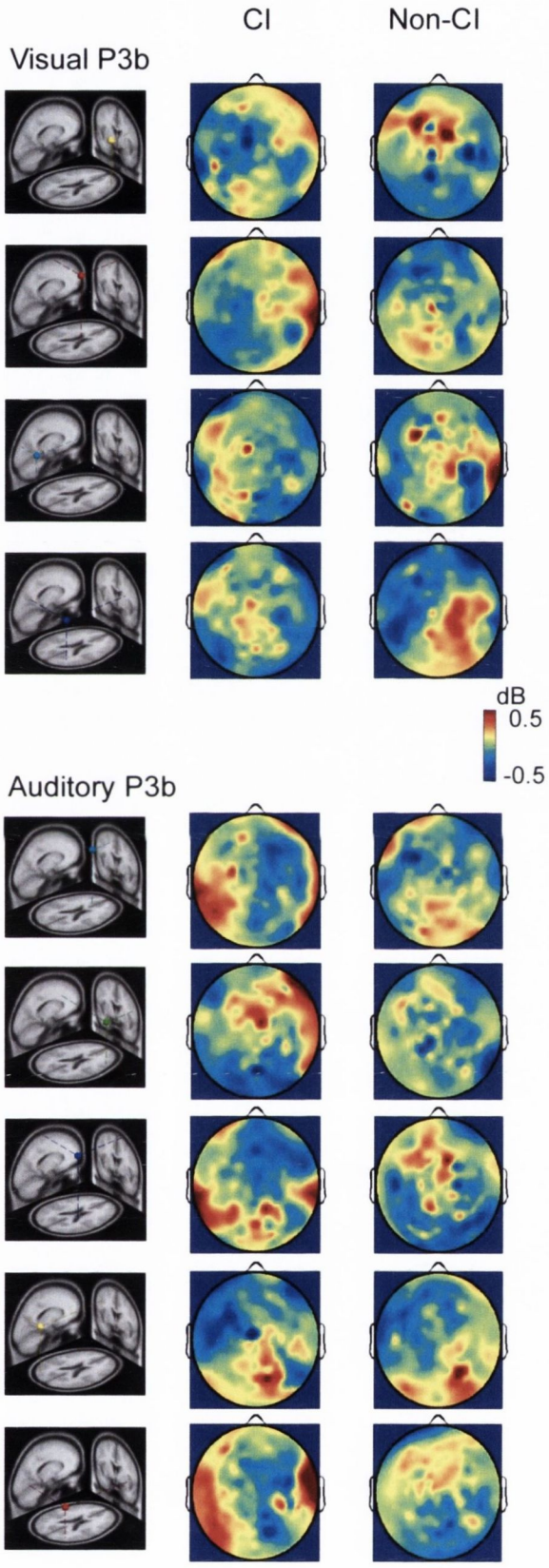
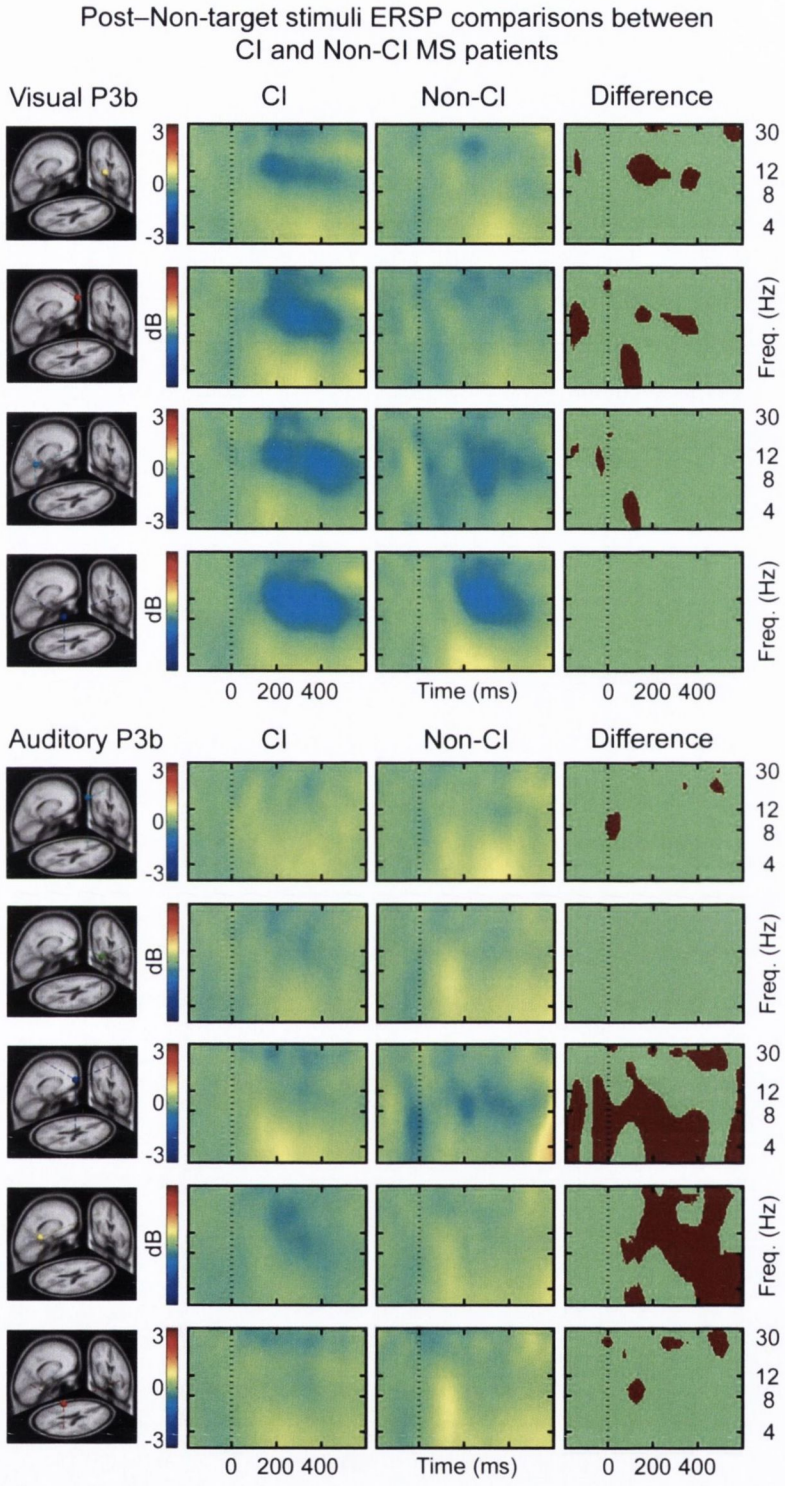
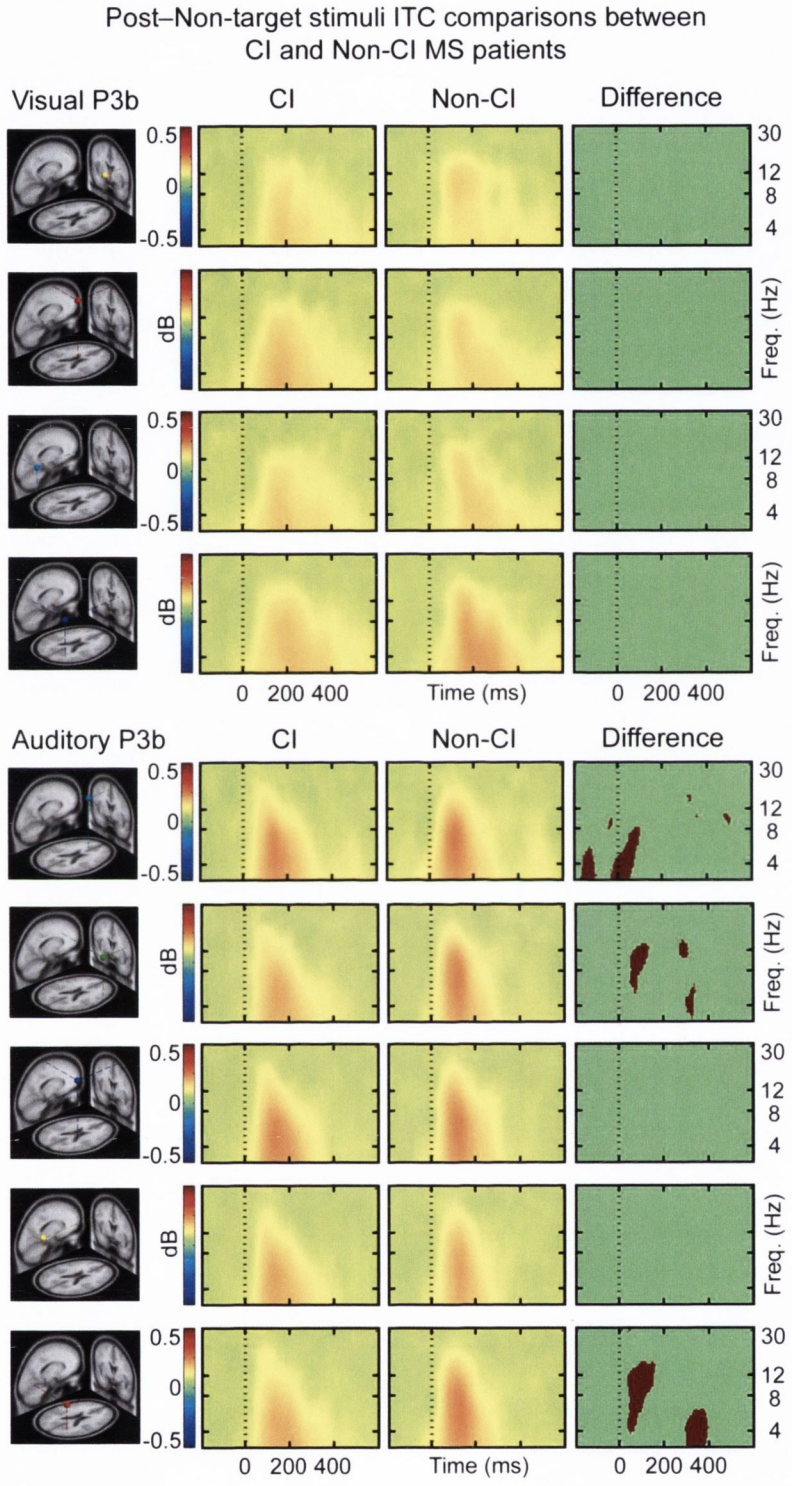


Figure 4.4.11. The mean ERSPs time-locked to the presentation of non-target stimuli in visual and auditory conditions in CI and non-CI MS patients.



Note. IC cluster mean differences shown in log spectral EEG power (dB) relative to log power in pre-stimulus EEG baseline. Red areas indicate an increase in power and blue areas a decrease in power. Statistical significance group and condition main effects, and interaction effect, are illustrated in red/green frames beside ERSP activation frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$, controlled for multiple comparisons) differences between CI MS patients and the non-CI MS patients in time and in log spectral power.

Figure 4.4.12. The ITC showing consistency between the trials and the degree of phase-locking to the non-target stimuli in CI and non-CI MS patients.



Note. Highest event-related phase consistencies for each condition and group are plotted in red, and lowest in green (range 0 to 1, no consistency to full consistency respectively). Statistical significance is illustrated by red/green frames, in which red areas signify statistically significant ($p < 0.0125$ for visual condition and $p < 0.01$ for auditory condition, controlled for multiple comparisons) differences between the CI MS patients and the non-CI MS patients in time and in log spectral power.

Figure 4.4.13. The grand mean topographies of CI and non-CI MS patients for each IC cluster post-non-target in visual and auditory conditions.

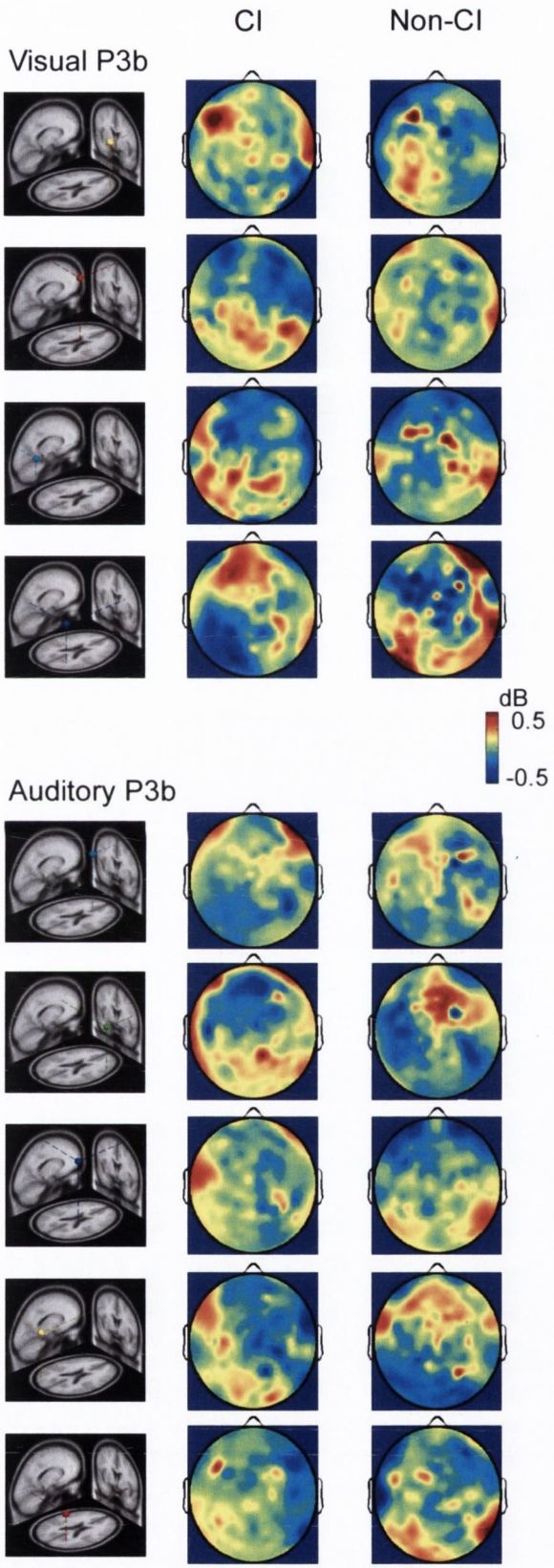
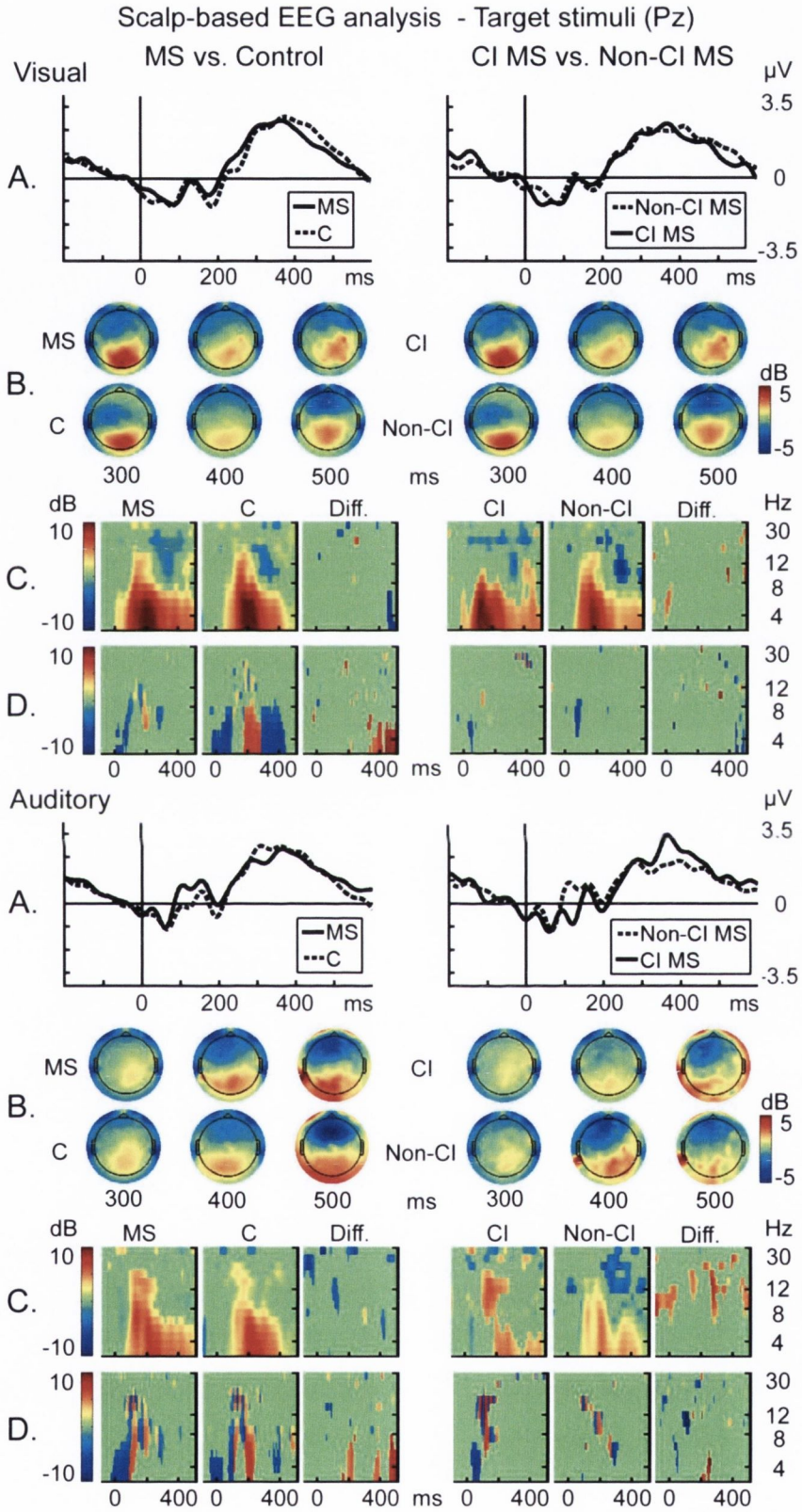
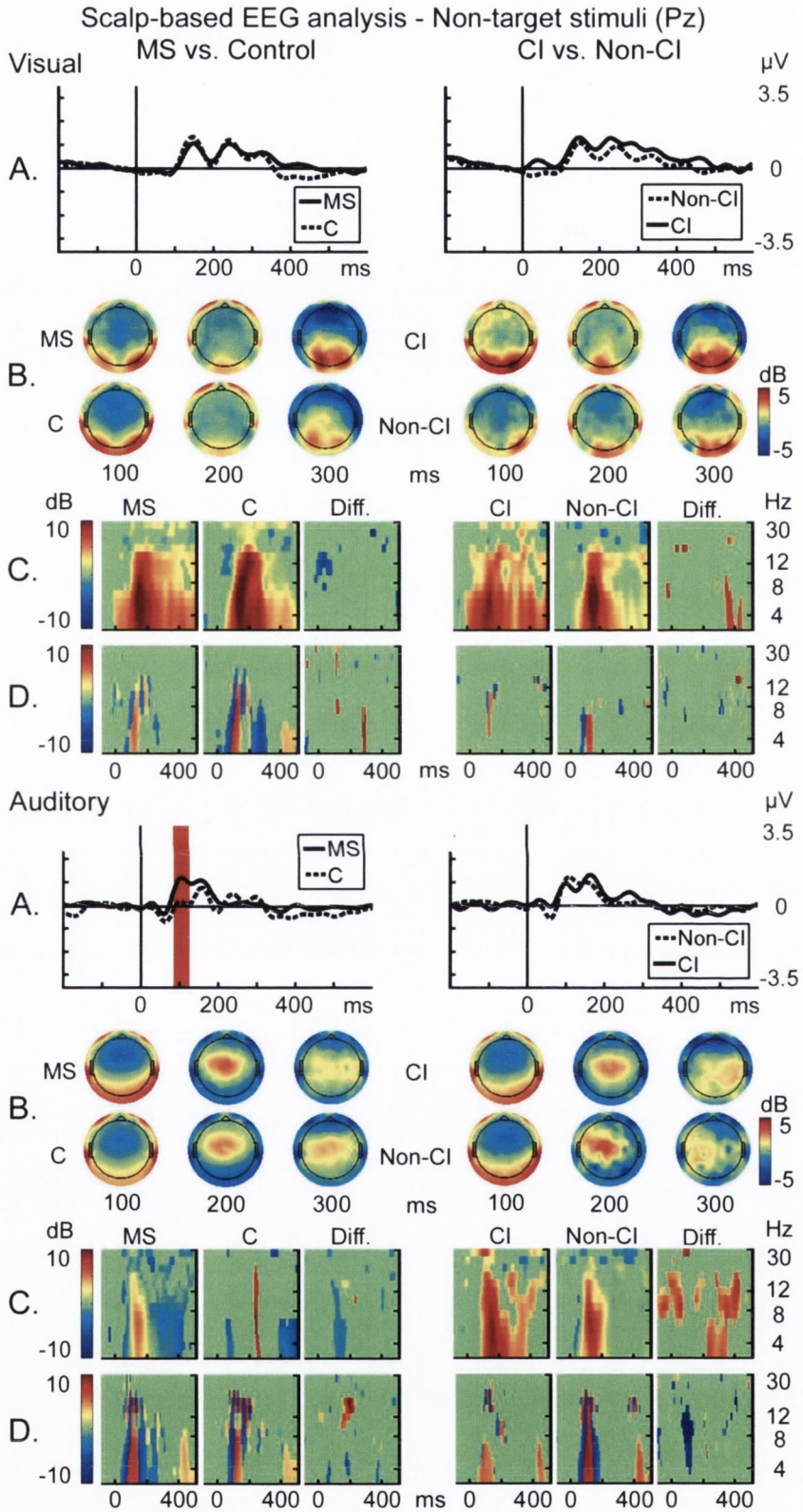


Figure 4.4.14. The scalp-based analysis of A) ERP, B) topography, C) ERSP, D) ITC of post-target activations at Pz in visual and auditory condition.



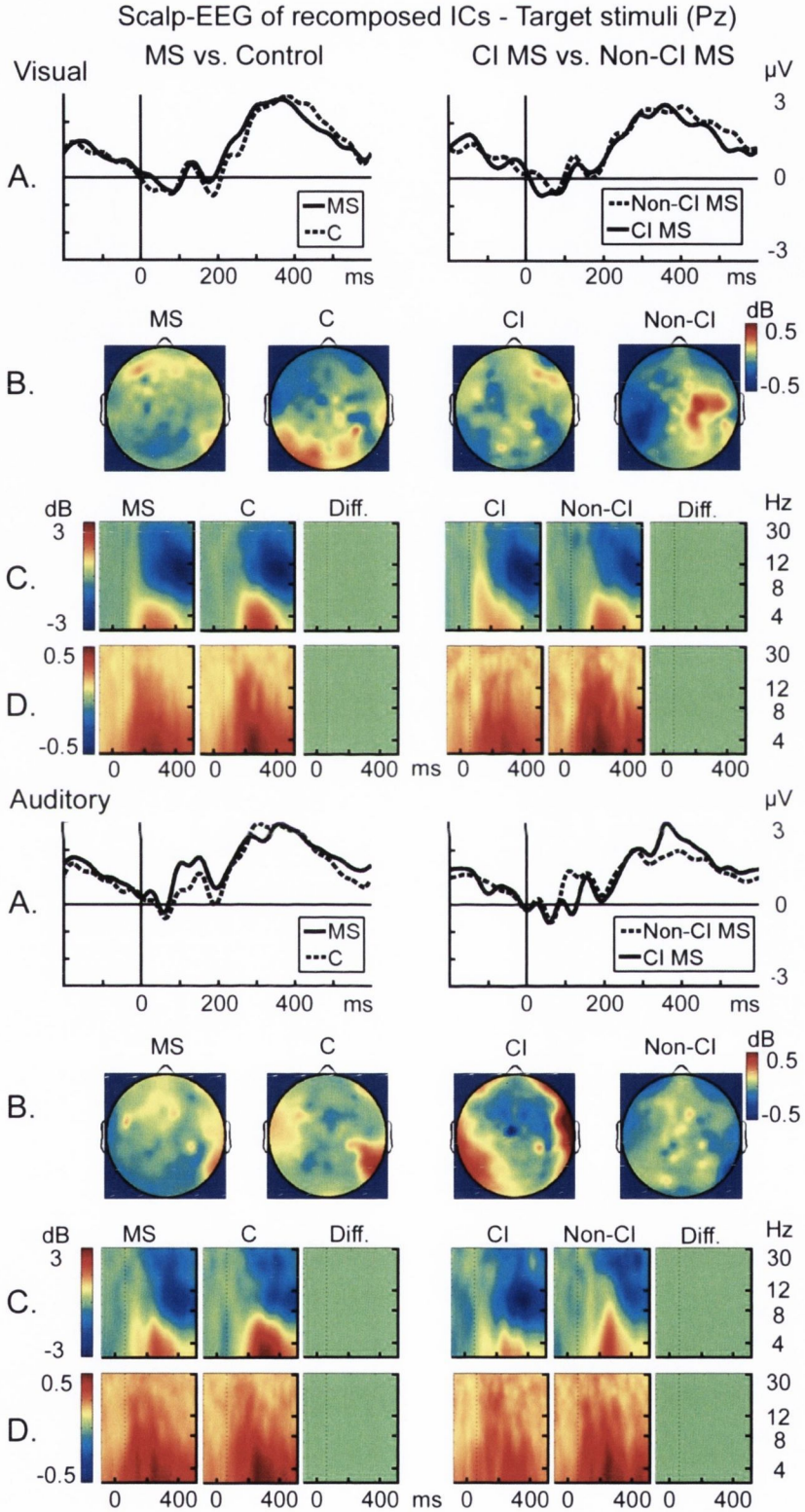
Note. The comparison of MS patients and controls on the left side, and of CI and non-CI MS patients on the right side. Statistically significant ($p < .05$) differences in ERP indicated by red, and in the ERSP and ITC difference frames by non-green areas relative to time and log spectral power.

Figure 4.4.15. The scalp-based analysis of A) ERP, B) topography, C) ERSP, C) ITC of post–non-target activations at Pz in visual and auditory condition.



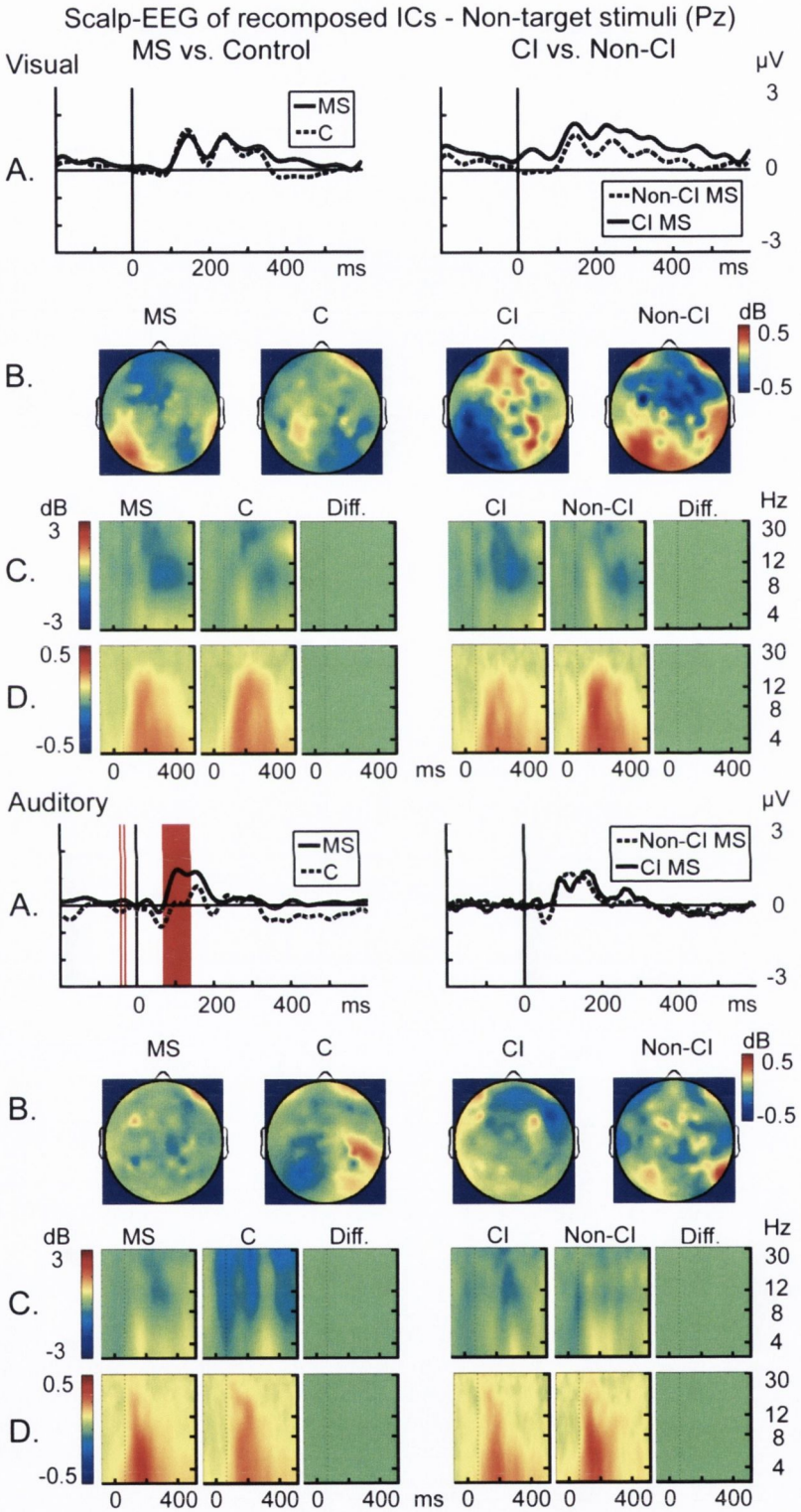
Note. The comparison of MS patients and controls on the left side, and of CI and non-CI MS patients on the right side. Statistically significant ($p < .05$) differences in ERP indicated by red, and in the ERSP and ITC difference frames by non-green areas relative to time and log spectral power.

Figure 4.4.16. The EEG-scalp analysis of recomposed ICs included in the IC clustering analysis. A) ERP, B) mean topography, C) ERSP, D) ITC of post-target activations at Pz in visual and auditory condition.



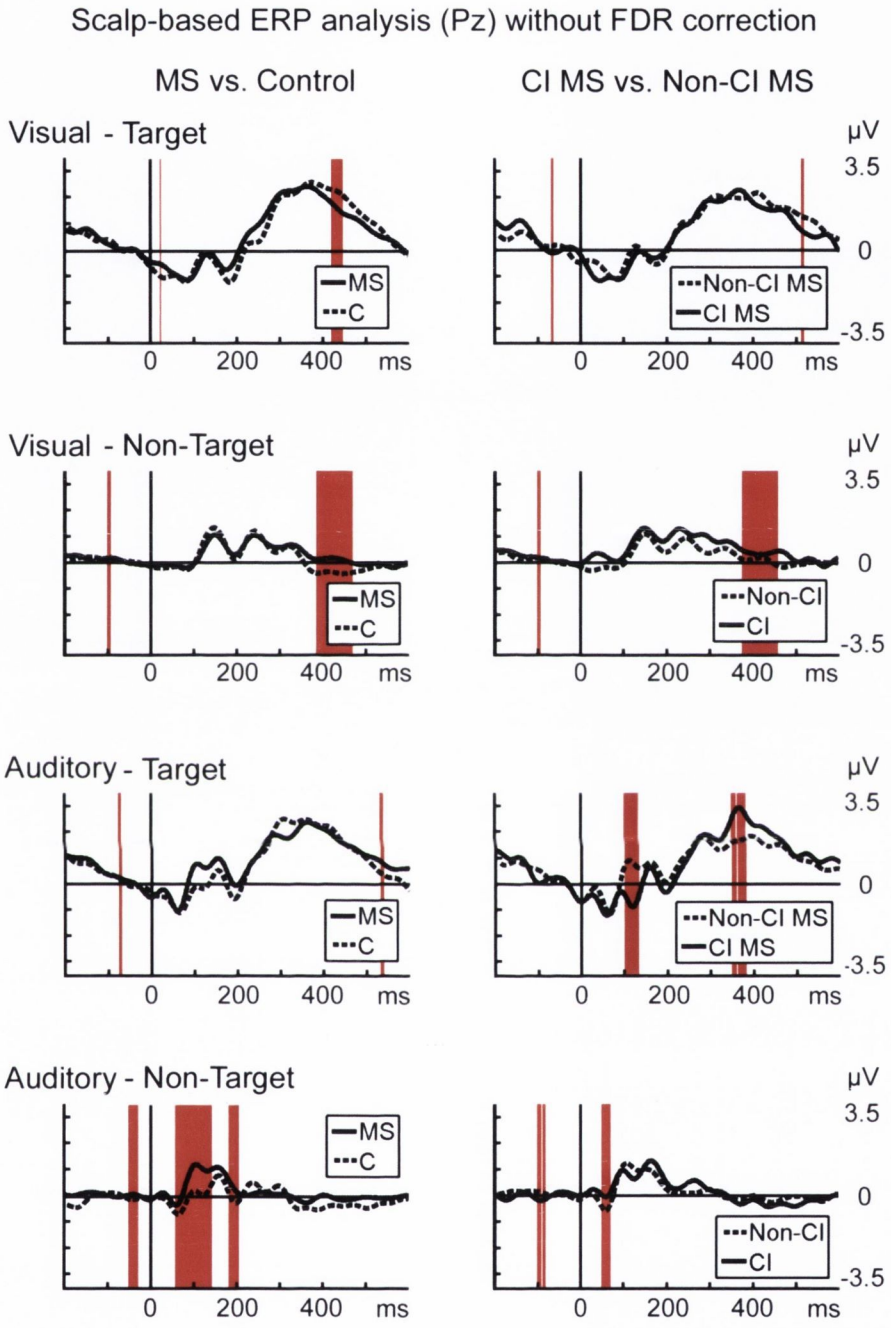
Note. The comparison of MS patients and controls on the left side, and of CI and non-CI MS patients on the right side. Statistically significant ($p < .05$, FDR corrected) differences in ERP indicated by red, and in the ERSP and ITC difference frames by non-green areas relative to time and log spectral power.

Figure 4.4.17. The EEG-scalp analysis of recomposed ICs included in the IC clustering analysis. A) ERP, B) mean topography, C) ERSP, D) ITC of post-non-target activations at Pz in visual and auditory condition.



Note. The comparison of MS patients and controls on the left side, and of CI and non-CI MS patients on the right side. Statistically significant ($p < .05$, FDR corrected) differences in ERP indicated by red, and in the ERSP and ITC difference frames by non-green areas relative to time and log spectral power.

Figure 4.4.18. The scalp-based analysis of post-target and post-non-target activations at Pz in visual and auditory condition without FDR restriction.



Note. The comparison of MS patients and controls on the left side, and CI and non-CI MS patients on the right side. Statistically significant ($p < .05$) differences in ERP indicated by red.

Table 4.4.2. Number of subjects and components in each IC cluster, and Talairach coordinates with associated Brodmann areas and brain regions of each IC cluster centroid.

	No. subjects	No. ICs	Talairach coordinates			BA	Associated brain regions
			x	y	z		
MS patients vs. controls							
Visual							
IC clusters:							
Right frontal	59	545	46	20	26	46	Frontal Lobe, Middle Frontal Gyrus
Central	59	612	-1	3	67	6	Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus
Right parietal	92	747	21	-65	-14	31	Limbic Lobe, Posterior Cingulate
Left parietal	54	475	-54	-59	31	40	Parietal Lobe, Supramarginal Gyrus
Auditory							
IC clusters:							
Frontal	66	519	-4	36	26	32	Left Cerebrum, Limbic Lobe, Anterior Cingulate
Right temporal	70	518	60	4	14	6	Frontal Lobe, Precentral Gyrus
Central	65	683	11	6	67	6	Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus
Right parietal	93	706	20	-69	9	30	Occipital Lobe, Cuneus
Left parietal	65	574	-59	-52	29	40	Parietal Lobe, Supramarginal Gyrus
CI MS vs. Non-CI MS							
Visual							
IC clusters:							
Right frontal	21	170	45	39	30	9	Frontal Lobe, Superior Frontal Gyrus
Central	22	227	-2	2	67	6	Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus
Right parietal	35	215	26	-59	21	31	Limbic Lobe, Posterior Cingulate
Left parietal	20	139	-43	-79	25	19	Temporal Lobe, Middle Temporal Gyrus
Auditory							
IC clusters:							
Left frontal	27	211	-8	36	47	8	Frontal Lobe, Superior Frontal Gyrus
Right frontal	28	219	49	25	23	46	Frontal Lobe, Middle Frontal Gyrus
Central	25	220	15	-19	68	6	Right Cerebrum, Frontal Lobe, Precentral Gyrus
Right parietal	33	217	32	-66	0	19	Occipital Lobe, Lingual Gyrus
Left parietal	28	236	-29	-80	39	19	Parietal Lobe, Precuneus

Note. BA = Brodmann area, IC = independent component, MS = MS patients, C = controls, CI MS = MS patients with CI, Non-CI MS = MS patients with no CI, No. Subjects = number of subjects, No. ICs = number of independent components

Table 4.4.3. The number of subjects and independent components from each groups in each IC cluster.

	Number of subjects		Number of components	
MS vs. C	MS	C	MS	C
Visual				
Right frontal IC cluster	38	21	305	240
Central IC cluster	35	24	389	223
Right parietal IC cluster	54	38	385	362
Left parietal IC cluster	35	19	268	207
Auditory				
Frontal IC cluster	43	23	322	197
Right temporal IC cluster	45	25	300	218
Central IC cluster	41	24	409	274
Right parietal IC cluster	54	39	397	309
Left parietal IC cluster	43	22	314	260
CI vs. Non-CI	CI	Non-CI	CI	Non-CI
Visual				
Right frontal IC cluster	11	10	62	108
Central IC cluster	11	11	91	136
Right parietal IC cluster	18	17	124	91
Left parietal IC cluster	11	9	70	69
Auditory				
Left frontal IC cluster	14	13	112	99
Right frontal IC cluster	15	13	88	131
Central IC cluster	13	12	108	112
Right parietal IC cluster	16	17	112	105
Left parietal IC cluster	16	17	139	97

Note. IC = independent component, MS = MS patients, C = controls, CI = MS patients with CI, Non-CI = MS patients with no CI

4.4.4. Discussion

4.4.4.1. The results of the present study

IC clustering of source EEG activities related to the P3b ERP is a novel approach for investigating CI in MS. To my knowledge, this is the first study to employ IC clustering method with a clinical group and it is the largest EEG IC clustering study to date with 95 subjects (56 MS patients, 39 controls). In general, consistent with hypotheses the ERSP measures showed MS patients to have reduced theta power relative to controls. A similar pattern was observed for CI MS patients vs. non-CI MS patients. Furthermore, alpha band responses were not diminished for the MS patients (and the CI-MS patients), contrary to the hypotheses.

The presentation of the target stimulus during an oddball task represents an increase in task demand, which, in cognitively intact individuals typically resulting in an increase in theta power and a decrease in alpha power post-stimulus presentation (Klimesch, 1999). Theta

oscillations are related to a number of information processing tasks, such as those requiring sustained attention and with high executive demands (Mizuhara, et al., 2004; Mizuhara & Yamaguchi, 2007). Later theta oscillations have also been suggested to reflect cortico-hippocampal feedback loops related to the evaluation of stimuli that become active after physical or context deviations and are followed by controlled processing in frontal cortex (Yordanova, Kolev, & Polich, 2001). The extent of theta synchronisation has been related to good working memory performance (Klimesch, 1999; Wang, 2010). In MS patients, a reduction in theta power may reflect the disruption of long-distance neural synchronisation (likely due to intervening lesions) which is thought to primarily occur in lower frequency bands (Wang, 2010), thus compromising early processes such as orienting and signal detection. This notion was reflected by the smaller increase of theta and alpha power in MS patients during early sensory processing (0-200 ms).

Interestingly, MS patients and CI MS patients had a greater decrease in alpha power, which was contrary to the hypotheses. The results also indicate the time range of the decrease in alpha power in the MS patients to be extended, which may be due to reduced processing speed. This may indicate that these groups were able to recruit more attentional resources later in the processing stream in order to partially compensate for the compromised earlier cognitive processes. In contrast, controls, who completed the earlier processing adequately, did not have to allocate any such additional resources. This result is concordant with the tendency of MS patients to be impaired in speed of information processing, rather than an inability to process information per se (Demaree, DeLuca, Gaudino, & Diamond, 1999). In other words, given adequate time, MS patients can complete cognitively demanding tasks.

4.4.4.2. Comparison with the previous studies

The two-stimulus oddball paradigm is one of the most popular ERP paradigms, and scalp-based EEG studies have reported P3b to be reduced in amplitude and/or delayed in latency in MS (Magnano, et al., 2006) and the findings on the previous studies in this chapter indicated P3b related activations to occur over a large parietal (and also over frontal and central) scalp regions. Most of these studies (Magnano, et al., 2006) have also observed late P3b peaks in MS patients, which in part explains the broad time range (200-600 ms) observed in the present study. Another factor influencing the timing of the P3b is age (Polich, 2004), and this study included a range of ages (MS SD = 9.87 years; control SD = 9.89), and therefore it is plausible that the age differences contributed to the range of the P3b.

The results of the present study differ from a previous study in this area (albeit one that utilized low-density, scalp-based spectral EEG), which reported increased power of higher frequencies (beta and gamma bands) in MS patients compared to controls over the whole scalp area after target-stimuli presentation. This effect was most prominent over frontal electrodes (Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Escera, et al., 2008), and around anterior right hemisphere and bilateral posterior scalp regions (Vazquez-Marrufo, Gonzalez-Rosa, Vaquero, Duque, Borges, et al., 2008), however, only MS patients in the early disease stage were included and therefore they were potentially with less CI. In contrast, the present study included patients with a wider range of CI, and also investigated the differences between patient groups divided based on their cognitive performance. Furthermore, the present results are more consistent with previous spectral studies on cognitive functions (Gevins, et al., 1997; Klimesch, 1999; Mizuhara, et al., 2004; Mizuhara & Yamaguchi, 2007; Yordanova, et al., 2001) that have reported theta power changes due to increased demands on cognitive processes. It is possible that theta power reduction is a feature of more advanced cognitive impairment.

The neural generators of (frontal-midline) theta oscillations have been reported to be located in the anterior cingulate in various EEG source analyses (see Mitchell, McNaughton, Flanagan, & Kirk, 2008), which is thought to be one of the sites for P3b ERP generation and in general to have important role in the networks contributing to cognitive function. Nevertheless, other studies indicate the generators of theta include also regions including occipital/parietal and temporal cortices (e.g. Raghavachari, et al., 2006). In the present study differences between MS patients and controls were observed in extensive areas.

The previous scalp-based P3b ERP studies in this chapter found the visual modality to be more sensitive in identifying differences between MS patients and controls. Interestingly, in the present study, IC clustering did not show the visual modality to be superior to auditory condition in detecting differences between MS patients and controls as differences in theta and alpha power in broad brain areas were visible across both modalities. On the other hand, IC clustering on the two PASAT performance groups revealed the greatest P3b-related ERSP power differences between the groups to be in visual modality.

After the presentation of non-target stimuli, controls demonstrated a greater increase in theta power compared to MS patients, and MS patients a greater decrease in alpha power relative to controls in all of the IC clusters in both modalities. These differences were apparent in the time-frame of P2 ERP component. Relatively high theta power during resting-state EEG has

been linked to effective cognitive function, particularly to processes related to sustained attention (Finnigan & Robertson, 2011; Mitchell, et al., 2008). Therefore, the apparent baseline differences between controls and MS patients may reflect the underlying differences in the background EEG due to the difficulties in maintaining sustained attention which is required for a successful P3b task performance. Moreover, it can be proposed that the MS patients have less effective modulatory processes changing the responsiveness of large neuronal ensembles in a context-sensitive manner (David, Kilner, & Friston, 2006). Furthermore, this possibly reflects the wide-spread underlying difficulties in information processing functions in the brains of MS patients which are likely to be affected by several lesions caused by the focal loss of myelin. On the other hand, there were no differences between the two PASAT performance MS groups in the visual condition, and the only difference between the groups was in left parietal IC cluster in the auditory condition. The lack of differences between the PASAT performance MS groups is most likely due to the fact that their background EEGs are relatively similar, and it can be suggested that their differences in cognitive function are not greatly related to sustained attention.

The ICA and IC clustering methods has several advantages over the scalp-based EEG analysis. Firstly, the IC clustering method aggregates ICs into functionally equivalent groups and estimates the source EEG activities across subjects and controls whereas the scalp-based ERPs between groups only capture the portion of the channel data that is phase-consistent at latencies relative to the time-locking events leading to a potential loss of information on the activities (Delorme, et al., 2012; Onton, et al., 2006). Furthermore, IC clustering reflects more accurately the spatiotemporal features of the EEG activations, as the scalp-based EEG is a sum of temporally and spatially overlapping channel activities originating from multiple, functionally distinct neural sources and non-brain sources (Dale, et al., 2000; Hagoort, 2003). In fact, the independent component analysis is regularly utilized to isolate and remove artefactual EEG sources (such as eye blinks, muscle artefacts) from the EEG data, which yields a more accurate representation of neural activity compared to scalp-based ERPs with artefacts (Nolan, et al., 2010; Onton, et al., 2006). Finally, the ICA methods estimate the origin of the activation (inverse source modelling) whereas scalp-based ERP assumes the recorded signals of the scalp electrodes to be comparable with the signals from equivalently placed electrodes for all the subjects, thus not taking into account the physical differences of the brains of different people (e.g. the differences in the orientations of cortical gyri and sulci causing different projections of exactly equivalent cortical sources (Onton, et al., 2006). For these reasons, the ERP P300 and ICA approach to EEG analysis are qualitatively different, and must be interpreted differently. It is also noteworthy that the topographies of ICs (Figures 4.4.3.,

4.4.6., 4.4.10., and 4.4.13.) are not as informative as 3-D locations of the equivalent dipoles as minor differences across subjects in the orientation of equivalent dipoles for a set of equivalent ICs can produce different scalp maps, and as the ICs are aggregated into clusters according to their functional similarity despite apparent scalp map differences Therefore, the typical centro-parietal positivity typically seen with a scalp-based ERP approach may not be seen following summation of ICs. Moreover, ICA and IC clustering methods do not require a priori assumptions (Delorme, et al., 2012; Onton, et al., 2006), which is an issue in other EEG source analysis methods such as BESA (Hoechstetter, et al., 2004). However, ICA approaches do rest on a number of assumptions, and future research (perhaps using simultaneous EEG and functional magnetic resonance imaging) should attempt to quantify the accuracy of IC spatial estimations.

On the whole, IC clustering enables a more comprehensive understanding of the dynamic EEG source activations underlying EEG recorded during cognitive performance which are likely to be missed during analysis of traditional scalp-based EEG data alone (Delorme, et al., 2012; Onton, et al., 2006). This notion is bolstered by the results of the scalp-based ERP, ERSP and ITC analysis in the present study, which did not reveal large differences among groups. The differences observed between the results from the IC earlier). Moreover, the averaging of the scalp-based event-related activations most likely does not reflect all the ongoing EEG activations as it captures only the portion of the scalp channel data that is phase-consistent at latencies relative to the time-locking events (Delorme, et al., 2012; Onton, et al., 2006). Therefore relevant information may be lost (Delorme, et al., 2012), and as a consequence there are no statistically significant differences in scalp-ERPs found between the groups. Furthermore, the IC clustering avoids (at least in part) the vagueness of spatiotemporal overlap inherent in scalp-based EEG recordings by aggregating the ICs with spatiotemporal features into functionally equivalent groups and thus estimating the source EEG activities. The group differences were not a result from a mere selection of specific ICs, as ERP activations recomposed of ICs in each IC cluster and back-projected to Pz did not show similar clear group effects to IC clustering results (see Figures 4.4.16. and 4.4.17). In addition, the EEG signals recorded on the scalp originate from multiple, functionally distinct neural and non-neural sources leading to a considerably high level of noise (besides spatiotemporal overlap) (Delorme, et al., 2012), which may mask the underlying differences between the groups. The differences in the results may also stem from the fact that physical characteristics and projection orientations from the cortex to the scalp differ across individual brains, which violates one of the main assumptions inherent in scalp-based ERP analysis that presume the position of an electrode to reflect the neural activities directly underneath it and the task-

related EEG activations in the same location to be similar across different subjects. These factors may therefore have led to the lack of statistically significant differences even though there would be a real underlying difference or a trend between the groups.

Physiologically, EEG signals originating from the brain are thought to be associated with near-synchronous field activities within a connected patch of cortical pyramidal cells sharing a common alignment near-perpendicular to the cortical surface (Nunez, 1974). The maximally temporally independent component activities with near-dipolar scalp projections may represent physiologically distinct brain sources that can be associated with field activity partially or fully synchronized across a cortical patch (or possibly across and between two anatomically well-connected patches).Thus, the individual ICs and IC clustering method hold promise to estimate more accurately EEG source locations, which in conjunction with cognitive-indexing electrophysiological experiments such as the oddball paradigm may give insight into the source and electrophysiological activities related to information processing speed and cognitive performance. The development of similar methodology is an important aim in studies with neurological conditions as it may offer better understanding of the brain location(s) where the changes impacting cognitive function are occurring. In the present study, the widespread reduction in theta and alpha power in MS patients was obvious post-target stimuli whereas the previous scalp-based P3b ERP studies have reported varied results of latency, amplitude and topography differences in MS patients relative to controls.

Objective, reliable EEG methods, such as IC clustering, may have potential to aid the detection and monitoring of cognitive impairment in MS, and therefore to complement generally utilised neuropsychological assessment by offering information on the brain functions related to cognitive dysfunction. The relationship between pathological changes in the brain white and grey matter, neurophysiological and neuropsychological cognitive function is imprecisely defined in MS (Lazeron, et al., 2005), and should be examined in future studies. Inclusion of a full neuropsychological battery may help to define better the relationships of specific cognitive domains with EEG and/or MRI findings, as may the use of more complex oddball paradigms. Moreover, future studies could explore the informativeness of more advanced EEG data analysis methods such as distributed source modelling incorporating EEG activations and structural information from individual subjects' MR images. In addition, longitudinal studies of EEG scalp and source activities spanning over several years are required to determine if EEG and ERPs have utility in predicting the changes in cognitive function in MS.

Key points:

- MS patients have significantly reduced P3b task-related ERSP theta power relative to the controls in all/most of the IC clusters
- CI MS patients have significantly reduced P3b-task related ERSP theta power in comparison to non-CI MS patients in all/most of the IC clusters
- The ITC measures were also significantly different in the theta band for most IC clusters
- MS patients have a greater decrease in P3b task-related ERSP alpha power relative to the controls
- Reduced P3b task-related theta power observed in both modalities reflects compromised connectivity, likely due to demyelination, which may have disrupted early processes essential to P3b generation, such as orientating and signal detection
- Greater decrease in alpha power observed in MS patients may be associated with enhanced cognitive function, which may reflect a compensatory mechanism in response to the compromised early cognitive processing

4.5. P3 ERP activations in multiple sclerosis as a function of oligoadenylate synthetase genotype

This section of Chapter 4 examines the Research Question 3 as stated in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. The study investigated if the P3 ERP activations and the PASAT score differ between MS patients with different oligoadelynate synthetase 1 (OAS1) genotype.

4.5.1. Introduction

The previous cross-sectional studies presented in this chapter show that auditory P3b latency and auditory P3a latency (although to a lesser degree), are sensitive to the degree of CI in MS patients, as they correlated significantly with the PASAT. The P3 ERP latency is thought to index the speed of cognitive processing (Polich, 2007), which is central for the successful performance of the PASAT.

The underlying genetic make-up of MS disease and the related cognitive deficits have not been sufficiently clarified. According to the general consensus formulated in the recent years, MS is considered to be caused by both environmental exposure and genetic susceptibility (Rejdak, et al., 2010). Genetic factors linked to MS include polymorphisms in MCH class II alleles (International Multiple Sclerosis Genetics Consortium, 2007; Dyment, et al., 2004), the interleukin-7 receptor alpha gene (IL7RA) and the interleukin-2 receptor alpha gene (IL2RA (=CD25)) (International Multiple Sclerosis Genetics Consortium, 2007), and apolipoprotein E (apoE) (Kantarci, et al., 2002).

Recently, the links between some genetic factors and cognitive function in MS have been examined. Due to its association with Alzheimer's disease, the relationship of APOE ε4 genotype and cognitive function has been investigated most extensively, although the results have been contradictory as reviewed in Chapter 2-Literature Review. Similarly, heterogenous results have been reported in studies investigating the relationship between cognitive function in MS and other genetic factors, such as human leukocyte antigen (HLA), brain-derived neurotrophic factor (BDNF), myeloperoxidase gene, and polymorphisms of interleukin 2 receptor α (IL2RA), interleukin 7 receptor (IL7R) and C-type lectin family 16 member A (CLEC16A) (Jensen, et al., 2010; Oksenberg, et al., 2008; Weinstock-Guttman, et al., 2011; Zivadinov, et al., 2007), but none of them have been linked to cognitive deficits in MS.

MS patients in the present chapter were tested for their oligoadenylate synthetase 1 (OAS1) - genotype, which is linked to type 1 interferons (IFNs). The links of OAS1, interferons, and disease/anti-inflammatory activity in MS were discussed in detail in Chapter 2-Literature Review. To my knowledge, the relationship between cognitive function in MS and OAS1 has not been investigated previously. Based on the finding that MS patients with both A alleles of OAS1 (AA MS) have decreased OAS1 disease/anti-inflammatory activity (O'Brien, et al., 2010), it was hypothesized that patients with the AA allele would have lower PASAT scores, delayed P3 ERP latencies and reduced P3 ERP amplitudes, in comparison to MS patients with both A and G alleles of OAS1 (AG MS). As the number of patients in each subgroup was very low (N=8 and 13), the statistical analyses were conducted utilizing one electrode (Pz for P3b task, and Fz for P3a task).

4.5.2. Methods

The data of 8 patient with the AA allele (mean age 42 years) and 13 patients with the AG allele (mean age 36 years) were included in the study. The MS patients had underwent OAS1 genotyping as part of another large research project conducted at St. Vincent's University Hospital, Dublin, which examined the OAS1 genotype and its relationship with MS disease activity (O'Brien, et al., 2010). The subjects also completed the neuropsychological test the PASAT (Tombaugh, 2006), and a measure of physical disability in MS, Kurtzke Expanded Disability Status Scale (EDSS; Kurtzke, 2008). Exclusion criteria included current use of benzodiazepines or neuroleptics, a history of alcohol or drug misuse, head injury, stroke, or recent relapse. Three AG MS patients did not have a clearly distinguishable P3 peaks and they were excluded from further analyses. Table 4.5.1. displays the demographic data of the subjects from whom EEG data were collected.

Table 4.5.1. Demographic and behavioural data of subjects.

	AA MS		AG MS	
N	8		10	
Male/female	6/2		6/4	
RRMS / SPMS / PPMS	4 / 1 / 3		5 / 4 / 1	
	μ	σ	μ	σ
Age	46.56	16.11	40.42	12.82
PASAT (%)	47.29	28.21	81.97	8.50
Mean years of symptoms	15.07	12.17	9.58	8.84
Aud RT (<800)	411.81	66.39	388.32	80.77
Vis RT (<800)	409.50	84.16	402.33	77.71
	M	*IQR*	*M*	*IQR*
EDSS	2.50	5.38	2.50	4.63
HRSD	3.50	4.75	3.00	6.25
Interferon β-1a	1		3	
Interferon β-1b	2		3	
Natalizumab	2		1	
Fingolimod	1		0	
No current tx	2		3	

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = inter-quartile range, AA MS = MS patients with AA OAS1-genotype, AG MS = MS patients with AG OAS1-genotype, RRMS = relapsing-remitting MS patients, SPMS = secondary-progressive MS patients, PPMS = primary-progressive MS patients, PASAT = the Paced Auditory Serial Addition Test, Aud RT = reaction times in the auditory condition, Vis RT = reaction times in the visual condition, EDSS = the Expanded Disability Status Scale, HRSD = the 21-item Hamilton depression rating scale.

The procedure of OAS1 genotyping, EEG recording and PASAT test are described exhaustively in Chapter 3-General Methods. SPSS 16 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA) was utilised for all statistical analyses, except for the topographical analysis completed using SPM 8.

EEGLAB (Delorme & Makeig, 2004) was used for all ERP analyses. The EEG data were bandpass filtered between 1 and 90 Hz, bandstopped between 48 and 52 Hz, average referenced across all scalp electrodes (appropriate when using a high-density EEG array), epoched and baseline corrected (100 ms before baseline). Epochs with large, obvious artefacts (e.g., muscle twitch) were first rejected manually. Independent components analysis, using the infomax algorithm, was used to identify artefacts, which were subsequently removed (see Delorme, et al., 2007). Ocular artefacts were removed by identifying the components that correlated most highly with the EOG channels (minimum correlation of 0.5). Visual inspection of the EOG channel before and after removal of the component was performed in order to ensure that the ocular artefacts were removed. A 99% confidence interval was calculated across all channels for

mean amplitude and variance: any channel falling outside the confidence interval was interpolated. Each ERP component was identified by two of the authors according to its polarity, latency and topography. A component was deemed to be not identifiable if the morphology of the ERP was not clear to both assessors. Next, the maximum (or minimum where appropriate) amplitude was automatically calculated for all electrodes using the manually identified component as a guide to define the temporal window. If there was no peak inside a temporal window for a component at a particular electrode then data at that electrode for that component were recorded as missing. Statistical analyses were conducted at Pz for the activations in P3b condition, and at Fz for P3a condition. Topographical maps were also plotted.

4.5.3. Results

The mean PASAT score was significantly different between groups (AA = 45%, AG= 82%; *Mann-Whitney U*, Z = -3.16, p = .002). The two OAS1 genotype MS groups did not differ in age, duration of symptoms, EDSS scores, reaction times or HRSD scores. The mean PASAT score correlated with auditory P3b latency (r_S = -.560, p = .016), but the effect disappeared when age was controlled. The EDSS score correlated with age, disease duration, auditory P3b amplitude, auditory P3a amplitude and latency. Age correlated with disease duration.

The AA OAS1 MS patients had significantly delayed mean P3b latency at Pz relative to the AG OAS1 MS patients (AA = 478 ms, AG= 366 ms; *Mann-Whitney U*, Z = -2.22, $p < .05$). Similarly, the AA OAS1 MS patient displayed prolonged mean P3a latency at Fz compared to the AG OAS1 MS patients (AA = 345 ms, AG= 273 ms; *Mann-Whitney U*, Z = -2.08, $p < .05$). Latency differences are illustrated in Figure 4.5.1. The topographical analysis confirmed these latency differences (Figure 4.5.2.) There was a trend (p = .076) indicating AA OAS1 MS patients to have reduced P3b amplitudes (AA = 2.78 uV, AG = 4.32 uV). The OAS1 genotype MS groups did not differ in latencies or amplitudes of visual P3b and P3a, which is most likely due to very low number of AA OAS1 MS patients (4 in P3b, 3 in P3a) in the visual condition as P3b and P3a peaks could not be identified.

Figure 4.5.1. AA OAS1 MS patients have delayed latencies in both auditory P3b and P3a condition.

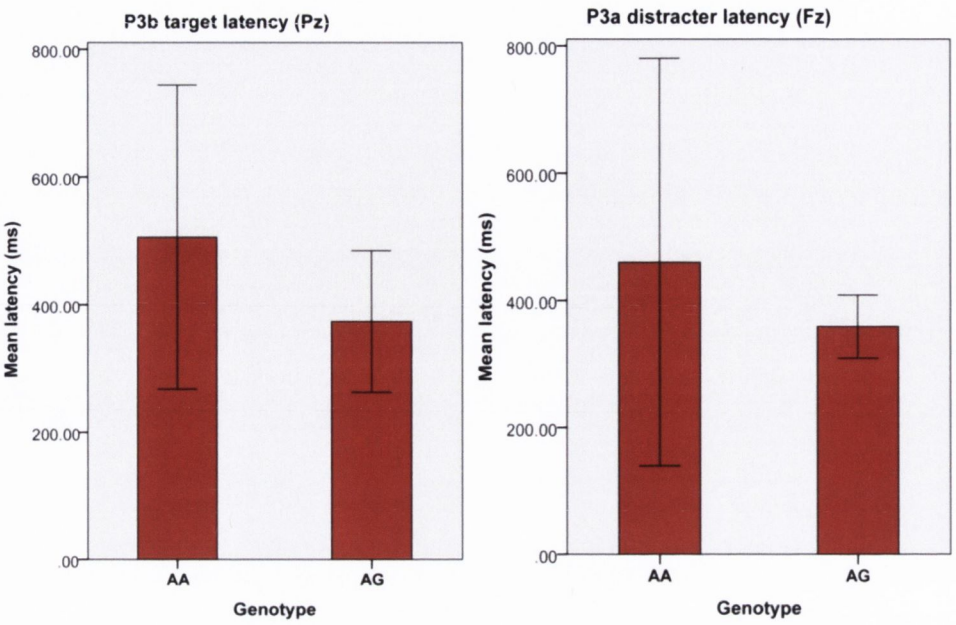
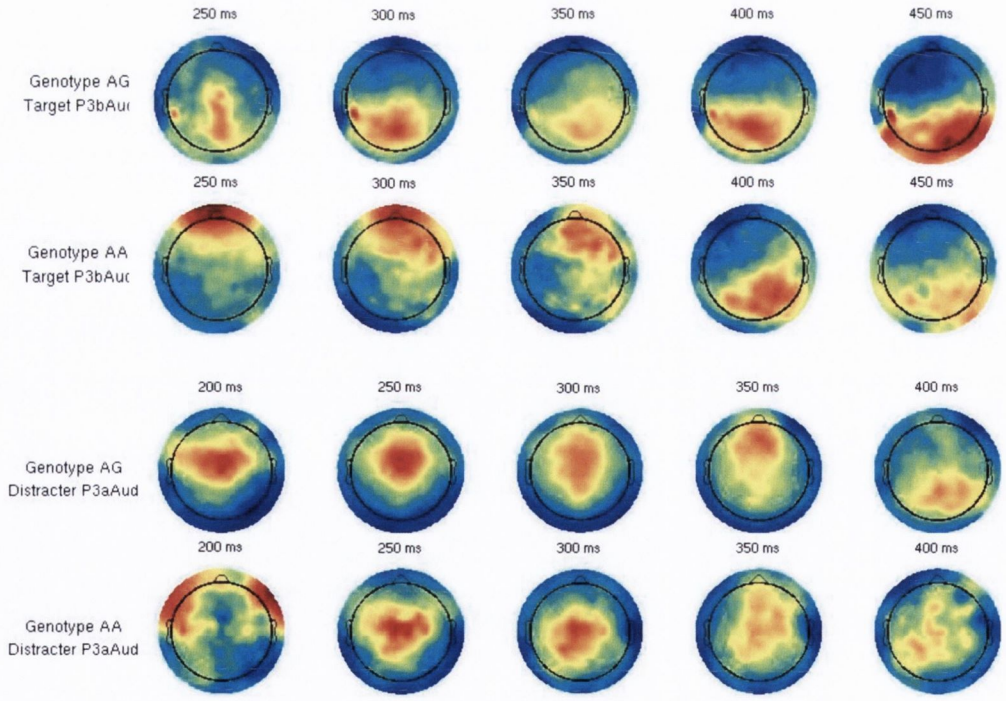


Figure 4.5.2. The AA OAS1 MS patients show delayed activations on the scalp in auditory P3b and P3a conditions.



4.5.4. Discussion

The impact of oligoadenylate synthetase 1 (OAS1) –genotype on CI in MS has not been investigated before. This study revealed a promising preliminary result of auditory P3 ERPs of MS patients to be influenced by OAS1-genotype. The previous research has shown that the patients possessing only the A allele (AA OAS1 MS) have decreased OAS1 anti-inflammatory activity in comparison to those with the G allele (AG or GG) (O'Brien, et al., 2010). In this study, MS patients with AA allele had prolonged auditory P3a and P3b latencies and performed worse in the PASAT test compared to patients with AG allele, which was consistent with the hypotheses. These findings were also evident in the topographical analyses. The topographical analyses are important for the investigation of the scalp distributions of the electrophysiological activities and offer indication if these activations are similar to healthy controls. Previous research has reported MS patients to have altered topographic presentation of the P3 ERP (Onorf, et al., 1991; Honig, et al., 1992), which is likely to be due to the pathological brain changes.

MS patients with CI are shown to have deficits in the PASAT test, which is the only cognitive test in the MS functional composite scale (MSFC; Cutter, et al., 1999). Due to the known limitations related to neuropsychological tests, cognitive neurophysiological experiments were included to complement the examination of cognitive functions in MS. Notably, AA OAS1 MS patients performed worse in the PASAT, indicating a deterioration of cognitive functions. The PASAT scores were also correlated with auditory P3b latency, which was markedly delayed in the AA OAS1 MS patients compared to the AG OAS1 MS group. In the previous chapters, the PASAT was also found to be correlated with auditory P3b and P3a in a relatively large sample of RRMS and SPMS patients, and in a group of progressive MS patients, which implies that auditory P3 latencies and amplitudes are sensitive measures of subtle cognitive decline in MS. In addition, consistent with these PASAT findings, the AA OAS1 MS group had significantly prolonged auditory P3a latencies relative to AG OAS1 MS group, providing further evidence for the assumption that AA OAS1 MS patients' cognitive functions are more adversely affected in comparison to that of AG OAS1 MS patients.

Interestingly, the level of disability measured with the EDSS scale was associated with the auditory P3 ERP components but not with the PASAT score. This implies that the deficits in the brain function are separately linked to physical disability and cognitive functioning. Also, it indicates that there is no direct association between the level of physical impairment and

cognitive performance measured with PASAT. As anticipated, the EDSS score also correlated with age and disease duration.

Previous studies have reported several linkages between cognitive performance and genes predisposing to MS, which were discussed in depth in Chapter 2-Literature Review. These include APOE ε4 genotype, human leukocyte antigen (HLA), brain-derived neurotrophic factor (BDNF), myeloperoxidase gene, polymorphisms of interleukin 2 receptor α (IL2RA), interleukin 7 receptor (IL7R) and C-type lectin family 16 member A (CLEC16A), all with contradicting findings.

It can be speculated that the mechanism underlying the poor cognitive performance of AA OAS1 MS patient group can be related to their decreased OAS1 anti-inflammatory/disease activity (O'Brien, et al., 2010). The reduced enzyme activity does not effectively cause the degradation of RNA and the suppression of virus replication (O'Brien, et al., 2010). This may lead to infection and subsequently trigger an autoimmune cascade of events (Grigoriadis & Hadjigeorgiou, 2006), possibly including a tendency for apoptosis of anti-inflammatory cells. Subsequently leading to excessive and prolonged inflammatory responses (O'Brien, et al., 2010). The exact nature of the links and mechanism behind the influence of OAS1-genotype on cognitive functions in MS require further investigation.

However, the findings may be confounded by the uneven distribution of MS subtypes in the study (1 SPMS and 3 PPMS patients in AA OAS1 MS group; 4 SPMS and 1 PPMS patients in AG OAS1 MS group). AA OAS1 MS group includes more subjects with PPMS, which may have driven the significant effects in the study. Previous literature has shown the progressive MS patients to have some subtle differences in their cognitive performance, but further investigations are required to define which cognitive areas (if any) show these differences (Foong, et al., 2000; Huijbregts, et al., 2004; Potagas, et al., 2008; Winkelmann, et al., 2007). Nevertheless, one of the studies in the present chapter found SPMS to have reduced P3 amplitude over parietal area compared to PPMS, thus indicating SPMS to have adversely affected electrophysiological activities related to cognitive processes. The various connections between subtypes, genetic factors and cognitive functions are therefore manifold; the nature of the potential connections and the directionality of these effects are not easily distinguishable.

Although the preliminary results are encouraging, the results should be interpreted with caution, as the AA OAS1 MS and AG OAS1 MS patient groups were very small, there was an

uneven number of MS subtypes included in each group, and no healthy control group was included in the study. More comprehensive future studies without these restrictions may potentially confirm this early finding of the AA OAS1 MS group to have more pronounced cognitive deficits and shed light on the intricate connections between OAS1, MS disease and cognitive performance.

Key points:

- AA OAS1 MS patients have prolonged auditory P3a and P3b latencies relative to AG OAS1 MS patients
- AA OAS1 MS patients performed worse in the PASAT compared to AG OAS1 patients
- PASAT score correlated with auditory P3b latency in the OAS1 MS sample
- Auditory modality is more sensitive to subtle deficits in cognitive-indexing P3 ERPs in a small sample of MS patients
- OAS1 genotype may have an effect on brain and cognitive functions

4.6. Conclusion

This chapter investigated the P3 ERP activations in MS and their association to the neuropsychological function measured with the PASAT at baseline. The MS patients presented with early sensory and late cognitive-indexing P3b ERP amplitude reduction and P3a latency delay and amplitude decrease, which were also apparent in the topographical statistical analysis. These P3 ERP changes were more pronounced in the visual modality, although the latencies were delayed also in the P3b and P3a in the auditory condition. The differences between the MS subtypes were almost non-existent, as the RRMS and SPMS patients differed only in the visual P3a latency, and the PPMS patients had decreased auditory P3b amplitude relative to the SPMS patients. Poor PASAT performance was associated with increased auditory P3b latency and decreased visual P3a amplitude in a relatively large sample of RRMS and SPMS patients. However, in the sample of progressive MS patients the PASAT score correlated with both visual and auditory P3b and P3a amplitudes. The source P3 EEG activations measured with a novel independent component (IC) clustering method revealed MS patients to have significantly reduced P3-task related theta power at widespread brain areas compared to the healthy controls. The MS patients with cognitive impairment displayed a similar pattern. Furthermore, the Ms patients showed a greater decrease in alpha power in the posterior sources relative to the controls. The genetic factors were also found to be related to the P3

ERP activations and cognitive functions, as the MS patients with AA OAS1 –genotype presented with prolonged auditory P3b and P3a latencies and performed worse in the PASAT in comparison to the MS patients with the AG OAS1 genotype. In summary, it can be concluded from the findings that the P3 ERPs, particularly in the visual modality, have potential utility in the clinical research and/or practice to provide information on the brain functions related to cognitive dysfunction in MS. Here the principal interest was to investigate if P3 ERPs differ in MS patients in comparison to healthy controls. Future research may also shed light on the P3 ERPs in different MS subgroups (based on subtype, genotype), and if P3 ERPs have sensitivity also to discriminate these subgroups. The following chapter will examine if and how these abnormalities change after 12-month and 24-month period.

The results of this chapter were published in the following top peer-reviewed journals:

Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Bramham, J., O'Brien, M., Reilly, R.B., Hutchinson, M., & Tubridy, N. (2010). A high-density ERP study reveals latency, amplitude, and topographical differences in multiple sclerosis patients versus controls. *Clinical Neurophysiology, 121*, 1420-1426. (Section 4.1, Appendix 3)

Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Hutchinson, M., Tubridy, N., & Reilly, R.B. (2010). Impaired information processing speed and attention allocation in multiple sclerosis patients versus controls: A high-density EEG study. *Journal of the Neurological Sciences, 293*, 45-50. (Section 4.2, Appendix 4)

Kiiski, H., Whelan, R., Lonergan, R., Nolan, H., Kinsella, K., Hutchinson, M., Tubridy, N., Reilly, R.B. (2010). Preliminary Evidence for Correlation Between PASAT Performance and P3a and P3b amplitudes in progressive multiple sclerosis. *European Journal of Neurology, 18(5)*, 792-795. (Section 4.3, Appendix 5)

Kiiski, H., Reilly, R.B., Lonergan, R., Kelly, S., O'Brien, M., Kinsella, K., Bramham, J., Burke, E.T., O'Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N, & Whelan, R. (2012). Only Low Frequency Event-Related EEG Activity is Compromised in Multiple Sclerosis: Insights From an Independent Component Clustering Analysis. *PLoS ONE, 7(9)*: e45536. (Section 4.4, Appendix 6)

Chapter 5 - Longitudinal P3 ERP studies: P3 ERPs and PASAT performance over 12-month and 24-month period in multiple sclerosis patients

The present chapter examines the Research Questions 4 and 5 as stated in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. The studies in this chapter investigated the 12-month and 24-month change in the cognitive-indexing electrophysiological activations in MS patients and healthy controls with advanced high-density electroencephalography (EEG) measures presented in Chapter 3-General Methods. Furthermore, the association between the change in the PASAT score and the P3 ERP activations was studied. Based on the previous research, it was hypothesized that MS patients would show deterioration in their cognitive and brain functions, which would manifest as a gradual decline of the PASAT performance, and as a gradual reduction of P3 ERP amplitudes and a prolongation of P3 ERP latencies over the 12 and 24 month period. It was also hypothesized that there would be no change in the P3 ERP activations, or in the PASAT score, of the healthy controls. Furthermore, the 12 and 24 month changes in P3 ERP activations were hypothesized to correlate with the 12 and 24 month changes in the PASAT score. The findings of the studies in this chapter complement and go further than the scarce research literature on the longitudinal P3 ERP activation changes in MS presented earlier in Chapter 2-Literature Review. The studies in this chapter examine longitudinally the relationship between P3 ERP activations and cognitive function (measured with the challenging neuropsychological task the PASAT), not performed previously.

The results of the first study in this chapter were published in a leading peer-reviewed Journal of the Neurological Sciences:

Kiiski, H., Reilly, R. B., Lonergan, R., Kelly, S., O'Brien, M., Kinsella, K., Bramham, J., Burke, T., O'Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N., & Whelan, R. (2011). Change in PASAT performance correlates with change in P3 ERP amplitude over a 12-month period in multiple sclerosis patients. *Journal of the Neurological Sciences, 305(1-2)*, 45-52. (Section 5.1, Appendix 7)

5.1. Longitudinal analysis of P3b and P3a ERPs in multiple sclerosis over 12-months

5.1.1. Introduction

The studies reported in the previous Chapter 4 investigated the cognitive-indexing electrophysiological activities and their link to the neuropsychological assessment cross-sectionally. As cognitive impairment is usually poorly correlated with disease duration and the level of physical disability level, and the cognitive dysfunctions typically observed in MS in speed of information processing, attention, memory and executive functions (Rogers & Panegyres, 2007) have an impact on daily life (Kalmar, Halper, Gaudino, Moore, & DeLuca, 2008; Rao, Leo, Ellington, et al., 1991), it is important to monitor its course frequently after its detection (Amato, et al., 2006). As stated earlier, the commonly utilised neuropsychological tests have a variety of shortcomings related to practice effects, education, anxiety and physical ability (Arnett, et al., 1999; Barker-Collo, 2005; Demaree, et al., 2003; Diamond, et al., 2008; Gilchrist & Creed, 1994; Litvan, et al., 1988; Thornton & Raz, 1997). However, electrophysiological measures are not confounded by practice effects (Sandman, & Patterson, 2000; Segalowitz, & Barnes, 1993; Walhovd, & Fjell, 2002), are independent from behavioural responding and thus not dependent on physical ability (Magnano, et al., 2006), and there is no evidence to suggest that they are affected by demographic factors such as education. Therefore, the cognitive-indexing electrophysiological measures may provide additional supporting information on the cognitive status of a MS patient. Thus, as stated in the Research Question 4 in the section 2.7. Thesis objectives included in Chapter 2-Literature Review, the potential of the cognitive-indexing electrophysiological measures to track brain activations related to cognitive dysfunctions is examined in the present study. In specific, this study compares the change between MS patients and controls in the visual and auditory P3 ERP tasks by employing a high-density EEG array, and examines the relationship between the change in PASAT score and the change in amplitude and topography in P3 ERPs over 12-month period.

Most previous cross-sectional literature report altered P3 ERP activations in MS patients (Magnano, et al., 2006), although some discrepancy exists regarding the relative informativeness of amplitudes and latencies, and the role of the modality of the stimuli, which may be due to the methodological weaknesses of these studies (low subject numbers, low electrode coverage of the scalp, outdated EEG analysis methods). In addition to the cross-sectional studies, there has been some interest in studying longitudinally a variety of evoked

potentials to better monitor the progression of disability in MS (Leocani & Comi, 2008; Pelayo, et al., 2010). A few studies (Flechter, et al., 2007; Gerschlager, et al., 2000a; Pliskin, et al., 1996; Pokryszko-Dragan, et al., 2009) have examined the change in P3b ERPs longitudinally in MS, but to my knowledge, the relationship between the PASAT and P3 ERPs in MS has not been studied longitudinally. These longitudinal ERP studies have focused on the effects of interferon treatment on cognitive functions, with only one study reporting auditory P3b amplitude decreased and latencies shortened one year later, but they did not include a control group in the study (Flechter, et al., 2007). Studies that included a control group failed to find any longitudinal differences in visual or auditory P3b after interferon treatment (Gerschlager, et al., 2000a; Pliskin, et al., 1996; Pokryszko-Dragan, et al., 2009). Nevertheless, these studies showed improved performance in neuropsychological measures of attention and memory after the treatment period, either indicating a practice effect or a beneficial effect of betaferon to cognitive functions in MS.

Most of the previous longitudinal studies of P3 ERPs in MS (Flechter, et al., 2007; Gerschlager, et al., 2000a; Pokryszko-Dragan, et al., 2009) employed an auditory two-stimulus oddball paradigm. However, the cross-sectional studies conducted in Chapter 4 showed that differences between patients and controls were influenced by the modality of the task and the P3 task type (i.e., either two- or three-stimulus oddball). Furthermore, the cross-sectional studies described in the previous chapters found P3b ERP latency delays (P3, N2, P3b) more pronounced in the auditory modality and early and late P3b amplitude reduction and topographical changes more pronounced in the visual modalities. Furthermore, in the P3a delayed latency, reduced amplitude and atypical topographical distribution of the activations were observed particularly in the visual modality. The association between cognitive-indexing electrophysiological measures and one neuropsychological test, the PASAT, was also established in the previous chapters as the PASAT score correlated with auditory P3b latency and with visual P3a amplitude in a sample of RRMS and SPMS patients, and with both visual and auditory P3b and P3a amplitudes in MS patients with a progressive disease course.

Moreover, all longitudinal P3 ERP studies to date have focused on investigating the effects of interferon treatment on cognitive functions in relapsing–remitting MS (RRMS) patients, and thus there is no comprehensive longitudinal information on CI and P3 ERPs in a larger MS population, which also includes secondary-progressive MS (SPMS) patients. Another issue is the lack of control group in the only study (Flechter, et al., 2007) reporting statistically significant differences in auditory P3b over one-year period. Including a control group in the longitudinal studies is important as P3 ERPs are demonstrated to change with age (Polich,

2004) and therefore the changes seen in the P3 components may be due to age, not disease progression or treatment. Furthermore, previous longitudinal studies employed low-density EEG (i.e. 3–4 electrodes).

The longitudinal investigation of P3 ERP activities of the present chapter avoided some of the methodological shortcomings of the earlier studies by utilising a high-density EEG array and including a relatively large group of both RRMS and SPMS patients, and healthy age-matched control group. The tasks encompassed both two- and three-stimulus oddball paradigms in both visual and auditory modality. High-density EEG also allowed the utilisation of an advanced method of statistical parametric mapping (SPM8) to statistically test the correlations and differences of the data. SPM has not yet been employed in longitudinal studies of P3 ERPs in MS.

Because of the limitations of the previous longitudinal P3 ERP studies (especially the lack of healthy control group and focus on examining the effects of DMTs), the hypotheses were based on cross-sectional P3 ERP studies (e.g. Magnano, et al., 2006) and previous longitudinal neuropsychological studies (Amato, et al., 2001; Denney, Lynch, & Pannenter, 2008; Huijbregts, et al., 2006). It was hypothesized that MS patients would show deterioration in their cognitive and brain functions, which would manifest as a gradual reduction of P3 ERP amplitudes and a prolongation of P3 ERP latencies over the 12-month period, and as a gradual decline of the PASAT performance. It was also hypothesized that there would be no change in the P3 ERP activations, or in the PASAT score, of the healthy controls. Furthermore, the 12-month change in P3 ERP activations were hypothesized to correlate with the 12-month change in the PASAT score.

5.1.2. Methods

5.1.2.1. Subjects, procedure and data analysis

Twenty-seven patients (mean age 43.64 years atMonth 0) and 17 age-matched healthy volunteers were included in the study. For MS patients, the mean years of symptoms was 16.26 years (SD 11.60), and the mean years of disease was 11.05 years (SD 9.64) at Month 0. One MS patient

and two controls were left-handed. Table 5.1. displays the demographic data of the subjects at Month 0 and Month 12. The median number of days between the two ERP testing sessions was 374. Two patients and two controls were unable to complete some of the P3 ERP tasks

and three controls and two patients did not complete the PASAT in either Month 0 or Month 12 (see Table 5.1). To the best of my knowledge, the MS patients did not take part in any other studies including the PASAT, or otherwise perform the PASAT, during the 12-month period. One patient had two relapses and another patient one relapse during the 12-month period.

Table 5.1. Demographic and behavioural data of subjects.

	MS (N = 27)				Control (N = 17)			
	Month 0		Month 12		Month 0		Month 12	
Male/female	12/15				5/12			
Auditory P3a & PASAT*, (RRMS, SPMS)	23 (12, 11)				13			
Visual P3a & PASAT*, (RRMS, SPMS)	23 (12, 11)				14			
Auditory P3a, (RRMS, SPMS)	25 (14, 11)				16			
Visual P3a, (RRMS, SPMS)	25 (14, 11)				17			
Auditory P3b, (RRMS, SPMS)	26 (15, 11)				16			
Visual P3b, (RRMS, SPMS)	26 (15, 11)				17			
	μ	σ	μ	σ	μ	σ	μ	σ
Age	43.64	11.21	44.61	11.11	40.30	9.53	41.75	9.29
PASAT (%)	73.59	20.39	75.13	21.88	85.00	13.67	88.10	9.36
Auditory P3b RT (<800 ms)	367.30	69.17	369.60	58.73	369.37	13.67	392.87	79.02
Visual P3b RT (<800 ms)	369.92	71.33	389.51	73.73	366.89	51.24	394.59	64.47
	M	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*
EDSS	2.50	4.00	3.00	5.00	N/A	N/A	N/A	N/A
HRSD-21	4.00	7.00	5.00	9.75	.00	5.00	2.00	3.00
Interferon β-1a	7		6		N/A	N/A	N/A	N/A
Interferon β-1b	8		7		N/A	N/A	N/A	N/A
Natalizumab	5		7		N/A	N/A	N/A	N/A
Fingolimod	1		1		N/A	N/A	N/A	N/A
No current treatment	6		6		N/A	N/A	N/A	N/A

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = inter-quartile range, PASAT = the Paced Auditory Serial Addition Test, EDSS = the Expanded Disability Status Scale, RT = reaction time, SD = standard deviation, IQR = interquartile range. *The correlation between the 12-month PASAT difference score and 12-month P3 ERP amplitude change.

The procedure was identical to the ones described in the Methods and in the previous chapters. The EEG, the PASAT and the physical examination including Kurtzke Expanded Disability Status Scale (EDSS; Kurtzke, 2008) and the 21-item Hamilton rating scale for depression (HRSD-21; Vieweg & Hedlund, 1979) were all conducted in two consecutive days for large majority of the subjects, the maximum days between the tests was 14 days. The same procedure was repeated at Month 12.

Reaction times were truncated at 800 ms in order to reduce the effects of outliers (Whelan, 2008). EEGLAB (Delorme & Makeig, 2004) was used for all ERP analyses. The pre-processing of the EEG data included the EEG data to be bandpass filtered between 1 and 90 Hz, bandstopped between 48 and 52 Hz, average referenced across all scalp electrodes (appropriate when using a high-density EEG array), epoched and baseline corrected (200 ms before baseline). Epochs with large, obvious artefacts (e.g., muscle twitch) were first rejected

manually. Independent components analysis, using the Infomax algorithm, was used to identify artefacts, which were subsequently removed (see Delorme, et al., 2007). Ocular artefacts were removed by identifying the components that correlated most highly with the EOG channels (minimum correlation of 0.5). Visual inspection of the EOG channel before and after removal of the component was performed in order to ensure that the ocular artefacts were removed. A 99% confidence interval was calculated across all channels for mean amplitude and variance: any channel falling outside the confidence interval was interpolated.

Three regions of interest (ROIs) were generated by using composite mean amplitude measures (i.e., the mean value of the electrodes in the ROI). The electrodes used in each ROI are in Table 2 (electrode sites are labelled according to the 10–5 system). The peak of the P3a was found by fitting a parametric function to the ERP in the ROI using a Gaussian profile and determining the delay at the peak amplitude (Gerson, et al., 2005) between 200–500 ms for the P3a and between 250–600 ms for the P3b task. For majority of the subjects the P3 ERP components were not distinguishable P3b peaks for frontal ROI, and no P3a peaks for parietal ROI, therefore only latencies extracted from central and parietal ROI of P3b, and from frontal and central ROI of P3a, were utilised in the statistical analyses.

Table 5.2. A list of locations for the Regions of Interest.

Region	Electrode locations
Frontal	F2',F2,AF2',AFz, AFz', Fz, Fz', F1,F1',AF1'
Central	Cz, Cz', CPz, CCP2h, FCC2h, FC2', FCz, FCC1h, FC1', CCP1h
Parietal	CPz', CPP1h, P1, PPO3h, PO3h, Pz, Pz', POz, CPP2h, P2, PPO4h, PO4h

Statistical analyses on demographic and descriptive data were completed using PASW Statistics 18.0 (Predictive Analytics SoftWare; SPSS Inc., Chicago, IL, USA, www.spss.com). SPM8 was used to test for topographical differences in ERP amplitude across the entire scalp and across time. Data from each subject were transformed into two-dimensional sensor-space (interpolated from128 channels and smoothed, full-width half-maximum 6:6:4 a.u.) and post-stimulus times were set to 230–600 ms for responses in the P3b tasks, and 200–600 ms for responses in the P3a tasks (voxel size=2.1 mm×2.7 mm×3.9 ms). This transformation produced a three-dimensional spatiotemporal characterisation of the ERP, and these images were then used in the subsequent analyses. The significance level was set to $p<0.005$ and this was further restrained by only retaining scalp areas in which at least 500 contiguous voxels were statistically significant. The 12-month difference images of each subject (showing the

topographical difference in ERP amplitude between Month 0 and Month 12 of one subject across the entire scalp and across time) were created using the SPM8 Image Calculator by subtracting Month 12 image from Month 0 image of the same P3 ERP task. These 12-month difference images were then correlated with the 12-month difference PASAT scores (computed by subtracting PASAT score (%) at Month 12 from PASAT score at Month 0). A paired t-test design was employed and each individual subject's Month 0 image was matched with the corresponding Month 12 image of the same P3 ERP tasks. The 12-month difference images were also compared between MS patients and controls, to show the difference in the 12-month change of the topography of ERP amplitude between the two groups. Based on the cross-sectional studies in the previous chapter, the spatial characterisation of activations were restrained with spatial masks: to a large parietal area for the P3b tasks (as reported in the cross-sectional P3b ERP study), and to an area covering a large area from frontal to parietal (excluding temporal areas) for the P3a tasks (as reported in the cross-sectional P3a ERP study). Employing spatial masks is more appropriate for testing specific hypotheses based on previous research as opposed to exploratory data analysis. Use of spatial masks reduces the number of statistical comparisons, and therefore the Type I error rate.

5.1.3. Results

The mean percentage of retained epochs of all the P3 ERP tasks at Month 0 was 91.45% and 84.69% for the control and MS groups, respectively. At Month 12, the mean percentage of retained epochs of all the P3 ERP tasks was 92.92% and 87.09% for the control and MS groups, respectively. The MS patients and controls did not differ in age ($p < 0.05$). EDSS score did not change significantly over 12-month period ($p < 0.05$). HRSD-21 score had not changed in either group over 12-month period but there was a group effect as MS patients had a higher score than the controls at both time points ($p > 0.05$). MS patients had lower level of education than controls ($p > 0.05$) and therefore the PASAT 12-month difference score was controlled for education level in the SPM analyses. A repeated measures ANOVA was conducted on PASAT and reaction time data. There was a group effect for the PASAT score as MS patients performed worse in both time points ($F[1,37] = 4.22$, $p = 0.047$). At a group level, there was no significant difference in the PASAT score between Month 0 and Month 12 in either MS patients or controls. RRMS and SPMS patients did not differ in the PASAT 12-month difference score, or in the PASAT performance in Month 0 or in Month 12 ($p < .05$). PASAT score did not correlate with EDSS or HRSD-21 scores at either Month 0 or Month 12 ($p < 0.05$). There were no statistically significant reaction time differences between the two groups for either visual or auditory modalities ($p<0.05$), but there was a significant time effect in the visual P3b as both

the controls and MS patients had longer reaction times at Month 12 ($F[1,41] = 6.12$, $p = 0.018$).

5.1.3.1. Longitudinal comparison of P3 ERP amplitudes

When the 12-month difference images were compared between MS patients and controls, MS patients had greater reduction in amplitude than the controls at a large centro-parietal area 242–441ms in the visual P3b task, and over a more restricted parietal area 556–598 ms in the visual P3a task (Figure 5.1). Paired t-tests of the MS patients revealed reduced amplitudes at Month 12 compared to Month 0 in visual P3b, auditory P3b and auditory P3a over the centro-parietal area (Figure 5.2). For the controls, the amplitudes were observed to be reduced in auditory P3a task at Month 12 over frontal area 243–286 ms and in visual P3a task over central area at 266–306 ms and 368–446 ms (Figure 5.3). Analyses of the MS subtypes revealed also number of longitudinal amplitude changes. Paired t-tests of the RRMS patients revealed reduced amplitudes at Month 12 compared to Month 0 in visual and auditory P3a over central region, and in visual P3b over centro-parietal area. The SPMS patients had reduced amplitudes at Month 12 relative to Month 0 in visual P3a and auditory P3b over centro-parietal areas.

Figure 5.1. MS patients have significantly ($p < 0.005$) greater decrease in amplitude over 12-month period compared to controls in visual P3b and P3a (i.e. time x group interaction).

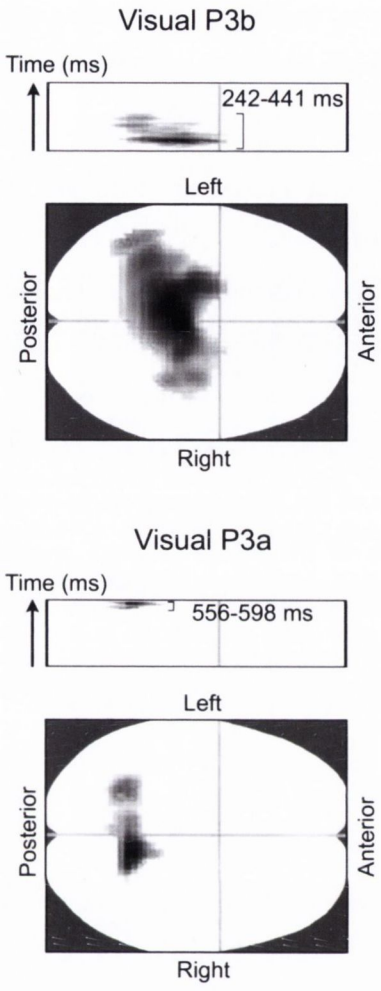


Figure 5.2. MS patients have significantly ($p < 0.005$) decreased amplitudes at Month 12 compared to Month 0 in visual P3b, auditory P3b and auditory P3a.

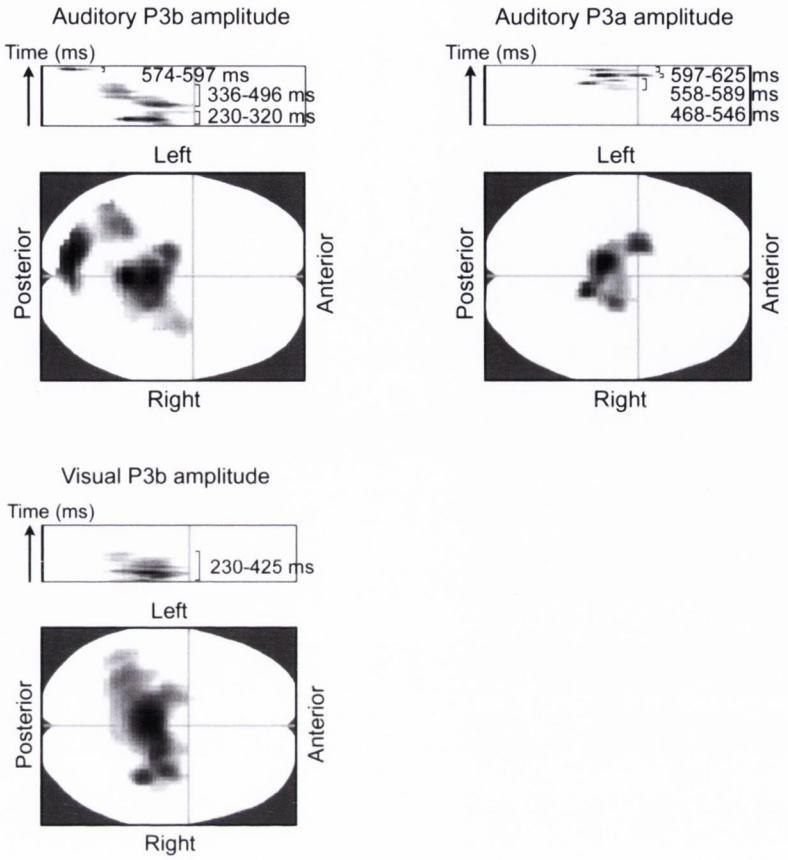
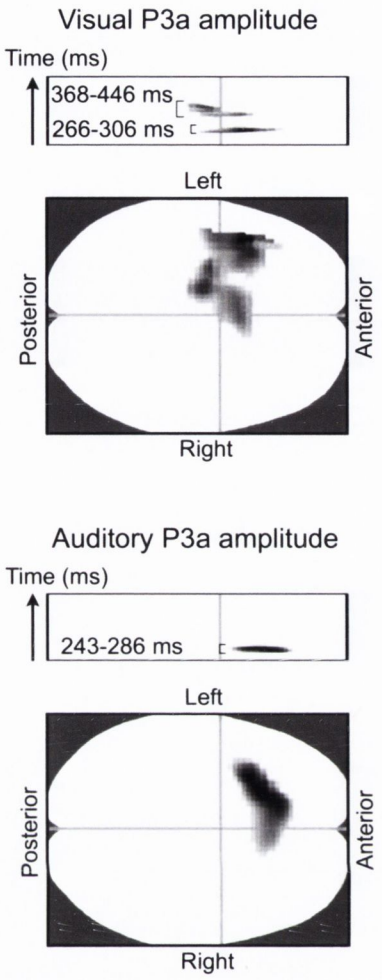


Figure 5.3. Controls have significantly ($p < 0.005$) decreased amplitudes at Month 12 compared to Month 0 in visual and auditory P3a ERPs.



5.1.3.2. Longitudinal comparison of P3 ERP latencies

MS patients showed significantly delayed latencies in auditory P3a over frontal area at Month 12 in comparison to the baseline, while the latencies of controls did not change over the 12-month period (interaction effect $F(1,38) = 6.94$, $p = .012$). At central areas the MS patients had more delayed auditory P3a latencies relative to controls at both timepoints ($F(1,39) = 4.70$, $p = .036$). In visual P3a task, the MS patients displayed more prolonged latencies over frontal region compared to the controls at both years, but the latencies did not change in one year ($F(1,39) = 6.85$, $p = .013$). The P3b tasks did not show any statistically significant time or group effects, however there was a strong trend for MS patients to have more delayed visual P3b latencies at Month 12 relative to Month 0 over central region, while the controls had no change in their latencies (interaction effect; $F(1,40) = 3.46$, $p = .070$). Paired t-tests of the MS patients showed delayed latencies at Month 12 compared to Month 0 in auditory P3a over

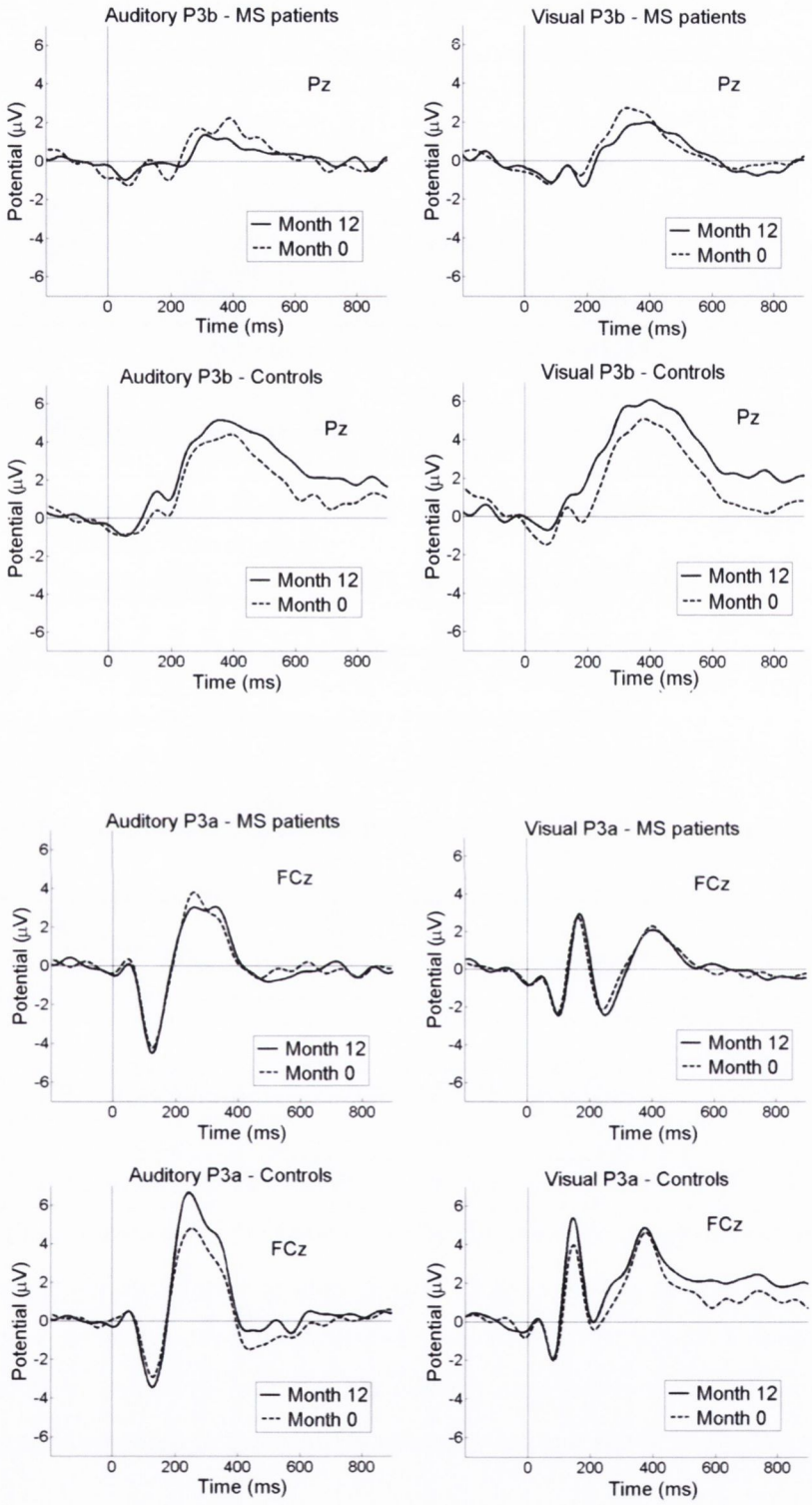
frontal ROI ($p < .05$) and there was a strong trend for latencies to be prolonged at Month12 in visual P3b condition over central sites ($p = .059$). The latencies of the controls did not differ between the two time points. Grand averaged waveforms of the responses to visual and auditory P3b (Pz) and P3a (FCz) for Month 0 versus Month 12 in MS patients and controls are displayed in Figure 5.4.

Table 5.3. The latencies (ms) at frontal, central and parietal ROIs for MS patients and controls at Month 0 and Month 12.

		MS (N = 27)				C (N = 17)			
		Month 0		Month 12		Month 0		Month 12	
	Statistical effect	μ	σ	μ	σ	μ	σ	μ	σ
Visual P3b central		401.77	135.77	449.45	99.37	425.18	74.85	408.41	78.35
Visual P3b parietal		347.35	97.13	389.88	76.10	373.28	46.38	374.17	72.86
Auditory P3b central		395.48	139.10	423.54	115.69	347.68	76.46	372.33	111.22
Auditory P3b parietal		398.74	72.95	384.70	73.34	353.25	63.15	367.34	55.48
Visual P3a frontal	MS > C	368.05	69.42	363.97	88.78	326.96	58.65	309.27	43.51
Visual P3a central		400.79	77.49	406.44	101.39	396.41	40.05	382.47	41.00
Auditory P3a frontal	MS mo12 > mo0 & C mo12 = mo0	283.34	42.11	323.31	48.06	278.09	48.20	282.08	37.04
Auditory P3a central	MS > C	285.57	40.94	289.67	42.00	266.03	43.23	262.15	24.13

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = inter-quartile range, C = controls, mo0 = Month 0, mo12 = Month 12

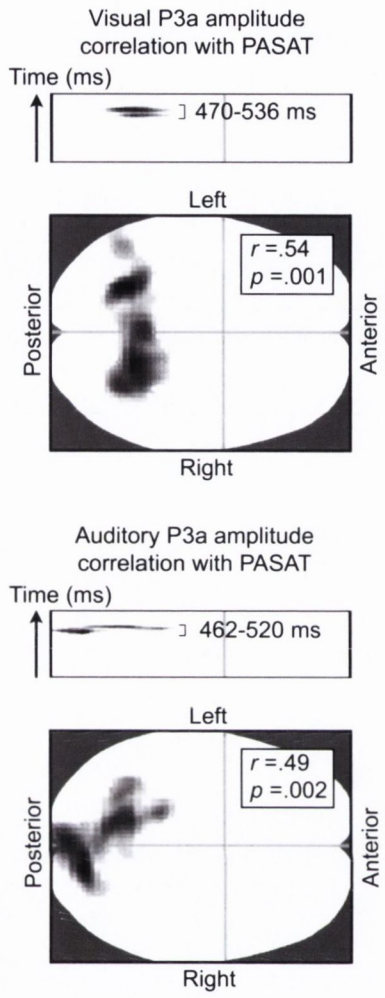
Figure 5.4. Grand averaged waveforms of the responses to visual and auditory P3b (Pz) and P3a (FCz) for Month 0 versus Month 12 in MS patients and controls.



5.1.3.3. Correlation between the 12-month change in PASAT score and the 12-month change in P3 ERP amplitudes and latencies

SPM8, a statistical method for examining differences in EEG activity, was used to examine scalp areas and time periods in which the amplitude of 12-month ERP difference was significantly correlated with the 12-month PASAT difference score. The correlations (*Pearson's r*) are reported between the voxel of maximum intensity and the 12-month PASAT difference score. For all subjects, the two difference estimates were correlated in both auditory and visual P3a tasks ($r = 0.48$, $p = 0.003$ and $r = 0.56$, $p < 0.001$, respectively), but not in auditory and visual P3b tasks (Figures 5.1. and 5.2). The correlations were apparent although at a group level the PASAT score had not significantly changed over the 12-month period. The change in the PASAT score was variable in individual subjects with some subjects' PASAT score increasing, some remaining similar and some reducing (standard deviation 9.57% in MS and 9.36% in controls). Therefore, the change in the PASAT score for individual subjects was paralleled by the individual changes in P3a ERP amplitudes. There was a significant correlation between the voxel of maximum intensity and the PASAT score at Month 0 for both auditory and visual P3a ERPs ($r = 0.56$, $p < 0.001$ and $r = 0.71$, $p < 0.001$, respectively), and at Month12 for auditory and visual P3a ERP/PASAT correlation in MS patients ($r = 0.63$, $p < 0.05$ and $r = 0.61$, $p < 0.05$, respectively). In controls, the SPM for the correlation at Month12 displayed a trend towards statistical significance ($p < .005$). The PASAT results for controls at Month12 were less variable than Month 0, and the average score was higher. Therefore, the results from the control group alone at Month 12 did not have enough variability for an accurate correlation. The latencies of 12-month ERP differences did not correlate with the 12-month PASAT difference scores. Descriptive statistics of PASAT 12-month difference scores for these two groups are presented in Supplemental Table 1.

Figure 5.5. Significant correlation ($p < 0.005$) between the 12-month amplitude changes of P3 event-related potentials and the 12-month change in PASAT with both MS patients and controls.



Note. The correlation (*Pearson's r*) between the voxel of maximum intensity and the 12-month PASAT difference score is also displayed.

Figure 5.6. Significant correlation (Pearson's r) between the voxel of maximum intensity and the 12-month PASAT difference score with both MS patients and controls.

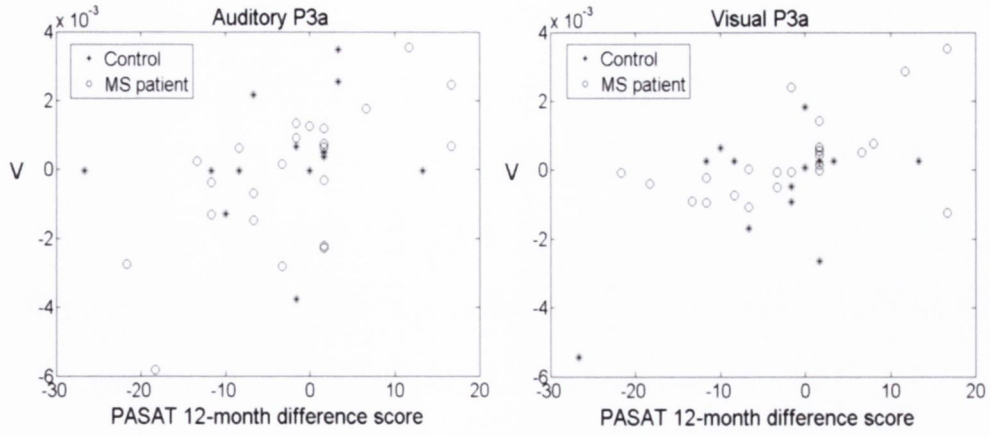


Table 5.4. The 12-month PASAT difference score in MS patients receiving disease-modifying medication and in MS patients without MS medication at Month0 and Month12.

	Medication for MS				No medication for MS			
	Month 0 (N=19)		Month 12 (N=19)		Month 0 (N=6)		Month 12 (N=5)	
	μ	σ	μ	σ	μ	σ	μ	σ
12-month PASAT difference score	-2.65	9.43	-3.11	8.41	1.94	10.63	3.67	13.51

Note. Both time points reported as one patient stopped and another patient began taking medication for MS during the 12-month period. μ = mean, σ = standard deviation.

5.1.4. Discussion

5.1.4.1. The results of the study

The present study employed a high-density EEG array across both visual and auditory modalities with MS patients and controls over 12-month period. The present study revealed the 12-month difference amplitude to be statistically significantly greater for MS patients compared to healthy age-matched controls (i.e. a significant time x group interaction), and the effect was best demonstrated with visual P3b amplitude over a large centro-parietal region (see Figure 5.1.). As hypothesized, the MS patients had also more delayed auditory P3a latency over frontal area at Month 12 relative to Month 0, while the controls did not have change in their latencies. The decrease in amplitudes over 12-month period was greater in MS patients, relative to controls, over parietal area for visual P3a amplitude. The MS patients had more delayed auditory and visual P3a latencies over fronto-central area relative to controls at both timepoints, but these latencies did not change in one year. The amplitudes of the visual P3b, auditory P3b and auditory P3a were reduced in MS patients over the centro-parietal area and the latencies of auditory P3a over frontal region were prolonged after 12 month period.

Similarly, the visual P3b latencies over central area were prolonged at Month 12 for MS patients but the comparison narrowly failed to reach significance (p = .059). When MS subtype groups were examined separately, the visual and auditory P3a, and visual P3b, amplitudes were reduced after 12-month period in RRMS. The SPMS patients showed reduced amplitudes at Month 12 in visual P3a and auditory P3b.

Visual P3a amplitudes were not significantly different in MS patients from Month 0 to Month 12. This may be due to the fact that visual P3a is a more difficult task compared to the P3b ERP tasks as reported in previous studies (Polich, 2007). Therefore, the amplitudes at Month 0 may already be at such a low level that a further decrease was difficult to measure at Month 12. More difficult standard/target discriminations are reflected in later P3 onset (Comerchero & Polich, 1998; Kok, 2001; Polich, 2007). Greater standard/target discrimination difficulty in the visual P3a task – in comparison to the visual P3b task – resulted in longer P3a onset. Therefore significant differences were later for visual P3a versus visual P3b. It is also possible that the inclusion of both RRMS and SPMS subgroups into the larger MS group may have affected the results by increasing variability of the P3a onset (thus reducing the statistical power), as when including these subgroups separately to the analyses they manifested with longitudinal amplitude changes in various P3 ERP conditions, including the visual P3a. In controls, there were no change in the latencies within the timeframe but the amplitudes of P3a ERP tasks at Month 12 were reduced compared to Month 0 over frontal and central areas, thus emphasizing the need for control groups in longitudinal studies. The visual P3a amplitude reduced over 12-month period in controls, but not in MS group, which may be due to the task difficultness affecting the P3a latency onset, and/or the smaller variability of P3a latency onset within the group, as described earlier.

In the present study the 12-month difference in the PASAT score correlated statistically significantly with the 12-month difference in amplitude of visual P3a over the parietal area, which was consistent topographically with the previous cross-sectional P3a ERP study of the previous chapter showing the visual P3a amplitude to be correlated with the PASAT scores. A similar correlation was observed with the 12-month difference in amplitude of auditory P3a. These correlations were over parietal areas, which is consistent with the centro-parietal scalp distribution of the no-go P3a reported in previous literature (Katayama, & Polich, 1998), eventhough the activations related to the no-go P3a are observed more commonly over central scalp regions (Polich, 2007). The correlations were evident even though the PASAT score – at the group level – had not significantly changed over the 12-month period. The change in the PASAT score was variable across subjects, with some subjects' PASAT score

increasing, some remaining similar and some reducing (standard deviation 9.57% in MS and 9.36% in controls). The finding that cognitive function changes over time in MS may be reflective of the disease course variability. Therefore, it is interesting that P3 ERP amplitudes reflect changes in cognitive function measured by the PASAT score.

5.1.4.2. Comparison to previous studies

It is essential to recognise CI as early as possible and to monitor its course frequently. The evolution of CI in MS has not been entirely explained so far but a general trend appears to be that cognitive impairment in MS occurs more slowly and less consistently in MS than in other degenerative conditions such as Alzheimer's disease (Amato, et al., 2006). In the present study the potential utility of cognitive-indexing electrophysiological measures to monitor the progression of cognitive dysfunction by measuring the brain functions related to CI in MS were examined.

Differences in P3 ERP amplitudes were observed over the 12-month period in the present study. Only one previous longitudinal P3 ERP study (Flechter, et al., 2007) reported change in P3b component after one year whereas the rest of the studies (Gerschlager, et al., 2000a; Pliskin, et al., 1996; Pokryszko-Dragan, et al., 2009) did not. The results of previous studies may be confounded by the variety of ways that latency and amplitude were measured. A high-density EEG array covers the entire scalp area and therefore it is possible to generate statistical parametric maps depicting activations across time and the whole scalp area. Previous studies have suggested a modality-specific amplitude reduction in MS (De Sonneville, et al., 2002). In the present study, visual modality appeared to be more sensitive to changes in the amplitudes of P3 ERPs as greater decrease was evident in MS patients, relative to controls, over 12-month period. Similar distinction between the modalities was apparent in Chapter 4 concentrating on the cross-sectional differences, which may be due to the differences between visual and auditory processing pathways. There may be more demyelination in visual processing areas and/or visual tracts, which are longer than auditory tracts. The superiority of visual condition in detecting CI-related changes in P3 ERPs can explain in part why most of the previous longitudinal P3 ERP studies (except for Pliskin, et al., 1996), which employed only auditory P3 paradigm, failed to find any changes. Nevertheless, latency differences were more prominent in the auditory condition. The latencies and sequence of ERP waves are related to the time course of processing activity, whereas the amplitudes of ERP waves indicate the amount of synchronized neurons and neural resources participating into specific cognitive processes (Polich & Herbst, 2000). Therefore, the deficits in the time course are mostly pronounced in

auditory modality in MS, and dysfunctions in the neuronal level are best reflected in visual modality as a reduction of the amplitudes.

This study is the first longitudinal P3 ERP study, to my knowledge, to focus solely on the changes in P3 ERPs related to CI in MS, and not to concentrate on the effect of disease modifying therapies (DMTs) on cognitive performance. The present study comprised of a more comprehensive patient group – including SPMS patients – who have not been part of the previously reported longitudinal P3 ERP studies (e.g. Flechter, et al., 2007). However, the results of the present study may be influenced by the different medications the patients were receiving. The DMTs alter the cerebral demyelinating process, which may in turn be associated with slower decline of cognitive functions (Bobholz & Rao, 2003). On the other hand, this link has not been established firmly, and the mix of MS patients with different treatments has the advantage of giving a more comprehensive overview of the process of CI in MS. Moreover, the utilisation of P3 ERPs in detecting and monitoring the brain functions related to cognitive impairment in MS in clinical practice would inherit the same issue, and if the differences are observable even in a study group using different types of DMTs, it supports the potential usage of P3 ERPs in clinics. In the present study it was not possible to compare the patients receiving DMTs to those without medication as the number of patients without medication for MS was too low (N=6). Increasing brain lesion load and brain atrophy are correlated with the progression of CI in MS (Amato, et al., 2006). Furthermore, the whole brain atrophy seems to be a good predictor of future cognitive and memory decline in early MS (Whitwell, 2008). The relationship between lesions, and neuropsychological and neurophysiological cognitive function is imprecisely defined in MS (Lazeron, et al., 2005), which is especially true concerning progression of the CI in MS.

One of the main objectives of the present study was to investigate the relationship between PASAT and P3 ERPs longitudinally in MS, which to my knowledge, has not been done before. The 12-month PASAT difference score was found to correlate most with both visual and auditory P3a ERP 12-month amplitude difference. A relationship between PASAT and P3b and P3a ERPs was established in the previos cross-sectional studies of Chapter 4, but they included larger number of subjects (N=54 and N=74, respectively). Nevertheless, the additional statistical power of the within-subject design in the present study made it possible to observe statistically significant PASAT/P3 ERP correlations for the within-subject change scores. There were some differences between the modalities as there was greater variability in auditory P3a amplitude. Although the visual P3a variability was less than the auditory P3a, perhaps reflecting the difficulty of the visual P3a task, there was a sufficient change between Month 0 and Month 12 for a statistically significant correlation. To be more specific, the difficulty

between the modalities is a function of perceptual distinctiveness of the standard/target discrimination, and not related to modality per se. Thus, the distinctiveness of the stimulus and the context (standard/target discrimination) contributes to P3a amplitude for the most part, and the modality of the stimuli is less influential (Polich, 1987). Therefore the visual P3a task presented was more difficult due to the stimulus properties (i.e. the standard/target discrimination was more difficult for visual compared to auditory stimuli). Nonetheless, there was a change for some subjects in visual P3a amplitude over the 12-month period (see Figure 5.6.), and this change was sufficient for a statistically significant correlation.

The encouraging findings of this study revealed the deterioration of P3 ERP activations in MS over the 12-month period, which motivated the next study in the chapter. The following study encompasses a third time point, Month 24, which was included to investigate if the longitudinal changes discovered over 12-month period reflect the gradual degradation of brain and cognitive function in MS, or alternatively if the findings merely portray fluctuations similar to the range of healthy controls.

Key points:

- MS patients have greater amplitude decrease in visual P3b and P3a tasks, and in auditory P3a condition, in 12 months relative to controls
- Visual P3b, auditory P3b and auditory P3a amplitudes had significantly decreased and auditory P3a latency prolonged in MS patients after 12-month period
- The change in visual and auditory P3a ERP activations correlated significantly with the change in the PASAT score
- Visual modality is more sensitive to changes in P3 ERP amplitudes over 12-month period
- Auditory modality is more sensitive to changes in P3 ERP latencies over 12-month period
- The P3 ERPs may have utility, in parallel with neuropsychological tests, in monitoring the course of subtle changes in cognitive function in MS

5.2. Longitudinal analysis of P3b and P3a ERPs in multiple sclerosis over 24-months

5.2.1. Introduction

The results from the first two years of P3 ERP data recorded from the MS patients were presented in the first study of this chapter. The same procedure was repeated at a third timepoint (Month 24). Due to the data collection constraints (e.g. subject recruitment and the time required to collect, pre-process and analyse P3 EEG data), the longitudinal analysis was conducted in two parts: the first study compared Month 0 and Month 12, the second study Months 0, 12, and 24. The first analysis of the 12-month period was also anticipated to give indication if aspects of study procedure had to be altered. For instance, the control group was included in the Month 24 study procedure due to the statistically significant amplitude changes over the first 12-month period in this group. Therefore, this study examines Research Question 5 as presented in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. In specific, it investigated if the P3 ERP activations change over 24-month period in MS, and if this change is greater compared to healthy controls. Also, it studied if the change in the P3 ERP activations correlate with the change in the PASAT score.

As depicted in Chapter 2-Literature Review and earlier in this chapter, none of the longitudinal P3 ERP studies with MS patients extended over a 2-year timeframe. Nevertheless, the neuropsychological longitudinal studies of cognitive functions in MS found deficits in attention and short-term spatial memory after 10 year period (Amato, et al., 1995; Amato, et al., 2001), in learning after 4 years (Jennekens-Schinkel, et al., 1990), and in the speed of information processing after 3 years (Denney, et al., 2008; Huijbregts, et al., 2006). The previous study in this chapter revealed visual P3b and P3a amplitudes and auditory P3a amplitude to show greater decrease in 12 months in MS patients relative to controls. In MS patient group, visual P3b, auditory P3b and auditory P3a amplitudes had significantly decreased and auditory P3a latency prolonged after 12-month period. Furthermore, the change in visual and auditory P3a ERP correlated significantly with the change in the PASAT score. Interestingly, visual modality appeared to be more sensitive to changes in P3 ERP amplitudes over 12-month period, and auditory modality to changes in P3 ERP latencies over 12-month period. Therefore it was hypothesized that the longitudinal P3b ERP changes found over 12-months in the earlier study of this chapter would also be found in the analysis of data spanning over 24-month period. In other words, based on the previous research and the previous 12-month study, it was

hypothesized that MS patients would show a gradual reduction of P3 ERP amplitudes and a prolongation of P3 ERP latencies over the 24-month period, and a gradual decline of the PASAT performance, which would be greater to the healthy controls. Furthermore, the 24-month change in P3 ERP activations were hypothesized to correlate with the 24-month changes in the PASAT score.

5.2.2. Methods

5.2.2.1. Subjects, procedure and data analysis

24 patients (mean age 43 years atMonth 0) and 20 age-matched healthy volunteers (mean age 43 years atMonth 0). Table 5.2.1. displays the demographic data of the subjects at Month 0, Month 12 and Month 24. To the best of my knowledge, the MS patients did not take part in any other studies including the PASAT, or otherwise perform the PASAT, during the 24-month period.

Table 5.2.1. Demographic and behavioural data of subjects.

	MS (N = 24)						RRMS (N=13)						SPMS (N=11)						Control (N = 20)					
	Month 0		Month 12		Month 24		Month 0		Month 12		Month 24		Month 0		Month 12		Month 24		Month 0		Month 12		Month 24	
Male/female	12 / 12						5 / 8						7 / 4						9 / 11					
	μ	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*	*μ*	*σ*
Age	43.24	11.54	44.46	11.64	44.92	11.99	36.19	8.77	37.22	8.75	38.27	9.11	51.57	8.54	53.87	7.4	54.9	8.37	43.23	11.1	44.36	11	39.79	30.38
PASAT %	74.64	21.02	75.99	21.68	70.8	27.48	78.75	19.59	84.17	19.89	80.51	22.08	70.15	22.53	66.17	20.41	58.17	29.7	83.98	14.32	88.8	8.44	89.75	9.6
PASAT Z	-0.21	0.99	0.09	1.26	0.06	1.27	-0.16	1.21	0.32	1.49	0.3	1.46	-0.26	0.69	-0.21	0.87	-0.24	0.98	-0.23	2.01	0.1	1.38	0.21	1.35
Auditory P3b RT (<800 ms)	364.44	70.41	474.41	231.65	368.41	57.89	368.8	79.73	424.31	120.33	358.12	43.38	359.69	62.19	362.13	63.96	380.76	72.18	354.06	64.26	397.22	71.15	362.79	51.54
Visual P3b RT (<800 ms)	368.39	74.23	460.12	199.84	414.49	62.74	345.3	60.84	428.14	115.9	404.16	67.3	395.68	81.96	384.32	87.75	425.87	58.65	355.62	33.03	388.27	55.73	383.15	59.47
	M	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*
EDSS	2.25	3.88	2	4.5	3	4.5	2	1	1.5	2	1.5	1.5	5.05	3.5	6.25	2.38	6	2.5	N/A	N/A	N/A	N/A	N/A	N/A
HRSD	3.5	6.25	5	9.25	8.5	8.75	3.25	5	5	9.5	8	13	4.5	10.75	5.5	9.25	10	4.75	2	6	3.5	3.25	3	5
Interferon β-1a	7		5		4		4		3		2		3		2		2		N/A	N/A	N/A	N/A	N/A	N/A
Interferon β-1b	7		6		6		3		2		2		4		4		4		N/A	N/A	N/A	N/A	N/A	N/A
Natalizumab	5		7		6		5		7		6		0		0		0		N/A	N/A	N/A	N/A	N/A	N/A
Fingolimod	1		1		1		1		1		1		0		0		0		N/A	N/A	N/A	N/A	N/A	N/A
No current treatment	4		5		7		0		0		2		4		5		4		N/A	N/A	N/A	N/A	N/A	N/A

Note. *μ* = mean, *σ* = standard deviation, *M* = median, *IQR* = interquartile range, RRMS = relapsing-remitting MS patients, SPMS = secondary-progressive MS patients, PASAT % = raw score of the Paced Auditory Serial Addition Test, PASAT *Z*= demographically corrected Z score of the Paced Auditory Serial Addition Test, RT = reaction time, EDSS = the Expanded Disability Status Scale, HRSD = the 21-item Hamilton depression rating scale.

The procedure was identical to the one in the previous 12-month study in the chapter and it was also described in Chapter 3-General Methods. The same procedure was repeated also at Month 24.

The data analysis procedures are described in detail in Chapter 3-General Methods, and only the parts of data analysis not included in the chapter are described here. Preliminary data processing employed the EEGLAB toolbox (Delorme & Makeig, 2004) in conjunction with the FASTER software tool (Fully Automated Statistical Thresholding for EEG artefact Rejection; Nolan, et al., 2010). The EEG data were bandpass filtered between 1 and 95 Hz, notch frequencies set to 50 and 60 Hz (the monitor refresh rate), average referenced across all scalp electrodes (appropriate when using a high-density EEG array), epoched from 500 ms pre-stimulus to 1000 ms post-stimulus and baseline corrected from 200 ms pre-baseline). The FASTER toolbox removed epochs with large artefacts (e.g., muscle twitch) and interpolated channels with poor signal quality. FASTER (Nolan, et al., 2010) also automatically identified artefactual (i.e., non-neural) ICs and removed them from the EEG data (note: the standard z = 3 threshold for IC rejection was lowered to z = 2 for the EOG channels).

The data analysis included the extraction of the latencies and amplitudes from the three regions of interest (ROIs). The ROIs were generated by using composite mean amplitude measures (i.e., the mean value of the electrodes in the ROI). The electrodes used in each ROI are in Table 2 (electrode sites are labelled according to the 10–5 system). The peak of the P3a was found by fitting a parametric function to the ERP in the ROI using a Gaussian profile and determining the delay at the peak amplitude (Gerson, et al., 2005) between 200–500 ms for the P3a and between 250–600 ms for the P3b task. For majority of the subjects the P3 ERP components were abnormal or not distinguishable P3b peaks for frontal ROI, therefore only P3b latencies and amplitudes extracted from central and parietal ROI of P3b were utilised in the statistical analyses.

The regression-based normative PASAT Z scores were calculated as described in detail in Chapter 3-General Methods.

Table 5.2.2. A list of locations for the Regions of Interest.

Region	Electrode locations
Frontal	F2',F2,AF2',AFz, AFz', Fz, Fz', F1,F1',AF1'
Central	Cz, Cz', CPz, CCP2h, FCC2h, FC2', FCz, FCC1h, FC1', CCP1h
Parietal	CPz', CPP1h, P1, PPO3h, PO3h, Pz, Pz', POz, CPP2h, P2, PPO4h, PO4h

5.2.3. Results

Age was significantly correlated only with auditory P3b latencies over central areas at Month 24. When only MS patients were included in the sample, age was only correlated with auditory P3b latencies over parietal region at Month 12. The EDSS scores of the MS patients did not change significantly over the 24-month period ($p > .05$), but the SPMS patients had higher EDSS scores and therefore more physical disability in comparison to the RRMS patients at each timepoint ($p < .05$). The MS group had significantly higher HRSD scores over the 24-month period at each session relative to the controls ($p < .01$), and the scores of the MS patients increased over time, whereas the scores of the controls remained roughly the same (time effect, $p < .05$). Closer examination revealed the SPMS patients to have high, but relatively stable HRSD scores, whereas the scores of the RRMS patients increased each session in comparison to the previous one in (time, group and interaction effects, $p < .05$).

The decrease of PASAT Z scores corrected for the effects of age, sex and gender (as described in detail in Parmenter, et al., 2010) was correlated with increase of visual P3b amplitudes over central locations at Month 12 ($r = -.43$, $p < .05$) in a sample including both MS patients and the controls. The decrease in raw, uncorrected PASAT scores was associated with increased auditory P3b amplitudes over parietal areas at Month 0 ($r = -.40$, $p < .05$) and with delayed auditory P3b latencies over parietal region at Month 12 ($r = -.47$, $p < .01$). With only MS patients included, the decreased raw PASAT scores were related to increase in the auditory P3b amplitudes over parietal areas at Month 0 ($r = -.64$, $p < .01$), with increase in the visual P3b amplitudes and delayed auditory P3b latencies over parietal areas at Month 12 ($r = -.63$, $p < .01$ and $r = -.57$, $p < .05$, respectively).

The repeated measures ANOVA showed the MS patients to have lower raw PASAT scores consistently over the 24-month period ($F(1,34) = 5.26$, $p < .05$), but the PASAT performance in either group did not change significantly over time. The demographically corrected PASAT Z

scores failed to show any significant effects. Longitudinal comparison of the raw PASAT scores of RRMS, SPMS and controls revealed an time x group interaction effect ($F(4, 66) = 3.79$, $p < .01$) and a group effect ($F(1,33) = 8.53$, $p = .001$) with SPMS patient having lower PASAT scores in all time points. However, the demographically corrected PASAT *Z* scores did not display any statistically significant effects.

The results of the repeated measures ANOVA are depicted in Tables 5.2.3 and 5.2.4. Most differences over the 24-month period occurred in the P3b latencies of visual modality. The time x group interaction effects observed showed the visual P3b latencies over parietal areas and auditory P3b latencies at central areas of MS patients to prolong over the 24-month period while the latencies of controls appeared to shorten, however, no group or time effects were present. Auditory P3b amplitudes at central scalp locations were reduced at Month 12 and Month 24 compared to Month 0 in MS patients, while the amplitudes of controls showed increase, nevertheless no statistically significant time effect was found, but there was a trend ($p = .067$) for the groups to differ significantly. The visual P3b latencies over central and parietal regions showed similar interaction effects when RRMS, SPMS and controls were compared, but no group or time effects were observed. Time and interaction effects (but no significant group differences) were observed with the auditory P3b latencies at central scalp region; the SPMS displayed increased latencies at each timepoint and RRMS patients at the two follow-up sessions in comparison to the baseline, while the latencies of the controls reduced over time at each session. Furthermore, the auditory P3b amplitudes at parietal scalp locations displayed a significant time effect, as the amplitudes reduced after baseline session in each of the subgroups.

When only the MS patients were included in the longitudinal analysis, the visual P3b latencies over parietal areas were prolonged statistically significantly each year. Also the auditory P3b latencies at central region increased after the baseline but remained stable thereafter.

Table 5.2.3. The repeated measures ANOVA results for MS patients and controls.

Component	*Repeated measures ANOVA*		MS (μ, σ)			C (μ, σ)		
			Mo0	Mo12	Mo24	Mo0	Mo12	Mo24
Latency:								
Visual – Central (16MS, 12C)	Group effect: MS > C	$F(1, 26)=4.07$, $p=.054$	411.01, 116.19	433.56, 68.35	470.44, 90.97	400.07, 53.31	394.95, 91.84	384.11, 74.86
Visual – Parietal (19MS, 17C)	Interaction	$F(2, 68)=6.94$, $p=.002$	360.47, 70.05	384.95, 81.41	427.28, 114.78	390.31, 57.32	354.51, 52.81	339.37, 52.99
Auditory – Central (13MS, 10C)	Interaction	$F(2, 42)=6.80$, $p=.003$	306.34, 82.49	376.21, 80.33	377.19, 81.43	344.86, 98.96	329.59, 52.30	307.81, 40.05
Auditory – Parietal (16MS,16C)			390.23, 65.85	390.68, 73.13	394.45, 54.76	393.54, 54.90	373.32, 53.68	365.33, 45.29
Amplitude:								
Visual – Central (16MS, 12C)			1.63, 1.33	1.14, 1.14	1.12, 0.57	1.85,1.03	1.94, 1.19	1.67, 1.12
Visual – Parietal (19MS, 17C)			3.14, 1.37	2.63, 1.41	2.18, 1.09	3.33,1.78	3.65, 2.01	3.06, 1.35
Auditory – Central (13MS, 10C)	Interaction	$F(2, 42)=3.15$, $p=.053$	2.42, 2.01	1.35, 0.84	1.45, 0.93	2.03,1.63	2.52, 2.20	2.64, 1.85
Auditory – Parietal (16MS, 16C)			2.96, 1.97	2.51, 1.38	1.92, 1.04	3.11,1.29	2.89, 1.25	2.58, 1.31

Note. Post-hoc Tukey tests utilised, C= controls, [a] indicates a trend in results, significance of $p < 0.1$, * indicates significance of $p < 0.05$, ** indicates significance of $p < 0.01$, *** indicates significance of $p < 0.001$

Table 5.2.4. The repeated measures ANOVA of visual and auditory P3b latency and amplitude for RR patients, SP patients and controls.

Component	*Repeated measures ANOVA*		RRMS (μ, σ)			SPMS (μ, σ)			C (μ, σ)		
			Mo0	Mo12	Mo24	Mo0	Mo12	Mo24	Mo0	Mo12	Mo24
Latency:											
Visual – Central	Interaction	F(4,50)=2.52, p=.053	389.80, 135.05	409.69, 51.95	499.01, 86.12	438.27, 88.85	464.25, 78.28	433.71, 89.45	400.07, 53.31	394.95, 91.84	384.11, 74.86
Visual – Parietal	Interaction	F(4, 66)=5.63, p=.001	374.41, 77.77	364.59, 64.93	447.66, 130.99	336.59, 50.77	419.84, 99.51	392.34, 76.25	390.31, 57.32	354.51, 52.81	339.37, 52.99
Auditory – Central	Time effect; Interaction	F(2,40)=3.95, p=.049; F(4,40)=3.75, p=.029	310.22, 97.19	394.81, 95.08	372.57, 88.70	301.82, 70.36	354.50, 59.90	382.57, 80.04	344.86, 98.96	329.59, 52.30	307.81, 40.05
Auditory – Parietal			376.45, 74.01	389.68, 83.69	365.53, 48.00	413.20, 46.23	392.33, 58.59	442.66, 19.77	393.54, 54.90	373.32, 53.68	365.33, 45.29
Amplitude:											
Visual – Central			1.51, 1.01	0.93, 1.15	1.20, 0.54	1.79, 1.73	1.41, 1.15	1.01, 0.64	1.85, 1.03	1.94, 1.19	1.67, 1.12
Visual – Parietal	(Group trend p=.06)		2.87, 1.40	2.42, 1.50	1.74, 0.79	3.60, 1.27	2.98, 1.26	2.94, 1.17	3.33, 1.78	3.65, 2.01	3.06,1.35
Auditory – Central			2.65, 2.27	1.56, 0.97	1.84, 1.01	2.15, 1.83	1.11, 0.65	0.99, 0.61	2.03, 1.63	2.52, 2.20	2.64, 1.85
Auditory – Parietal	Time effect	F(2, 58)=3.85, p=.027	2.39, 1.15	2.76, 1.58	1.80, 1.13	3.92, 2.74	2.09, 0.96	2.11, 0.93	3.11, 1.29	2.89, 1.25	2.58, 1.31

Note. *Post-hoc Tukey* tests utilised, RRMS = relapsing-remitting MS patients, SPMS = secondary-progressive MS patients, C = controls, Mo0 = Month 0, Mo12 = Month 12, Mo24 = Month 24, [a] indicates a trend in results, significance of $p < 0.1$, * indicates significance of $p < 0.05$, ** indicates significance of $p < 0.01$, *** indicates significance of $p < 0.001$

5.2.4. Discussion

The present preliminary study is the first to investigate the P3b ERP activations in MS over a 24-month period. The findings of the present study were largely consistent with the hypotheses. The results displayed various interaction effects; the visual P3b latencies over parietal areas and auditory P3b latencies at central areas of MS patients were shown to prolong over the 24-month period while the P3b latencies of controls shortened. Auditory P3b amplitudes at central scalp locations were reduced at Month 12 and Month 24 compared to Month 0 in MS patients, but not in controls. The visual P3b latencies over central and parietal regions showed similar interaction effects when RRMS, SPMS and controls were compared. However, no group or time main effects were present. Nonetheless, statistically significant longitudinal increase in visual P3b latencies over parietal areas and auditory P3b latencies at central areas were shown, when the analysis was performed with the MS patient group. On the other hand, the longitudinal subgroup analysis showed the auditory P3b latencies at central scalp region to change over time (time effect): the SPMS displayed increased latencies at each timepoint and RRMS patients at the two follow-up sessions in comparison to the baseline, while the P3b latencies of the controls reduced over time at each session. Furthermore, the auditory P3b amplitudes at parietal scalp locations displayed a significant time effect, as the amplitudes reduced after baseline session in each of the subgroups.

The latency results of the present study are compatible with the earlier 12-month period analysis, as both longitudinal studies showed visual and auditory P3b latencies to delay over time in MS patients. The previous study also included P3a ERPs, which showed the greatest change over time in MS patients. However, the previous 12-month study observed P3b and P3a amplitudes to change significantly in the visual modality. In contrast, the present study observed the auditory P3b amplitudes to change the most. This difference may be due to the differences in analysis methods utilised. The greater sensitivity of visual modality in detecting the subtle changes in the brain and cognitive functions in MS can be suggested to reflect the fact that the visual processing involves longer white tracts, which are more prone to demyelination and intervening lesions, effectively causing more deficits in the visual modality.

Over the 12-month period, RRMS and SPMS patients did not differ in P3 ERPs, which also was the case in the present 24-month study. However, there was a weak trend for the RRMS, SPMS and control groups to differ in auditory P3b latencies over parietal areas. Subsequent analysis comparing only the two MS groups showed the SPMS patients to have significantly delayed latencies over the 24-month period. This lack of significant results may simply be due to the

very low number of subjects in the RRMS and SPMS groups (N=8-10) reducing the statistical power. The study comparing two progressive MS subtypes, SPMS and PPMS, also found auditory P3b task to be sensitive in detecting the differences between MS groups, although the difference was present in the auditory P3b amplitude. The few studies employing neuropsychological measures and comparing the MS subtypes reported the progressive MS subtypes to show a lack of improvement in visuo-spatial processing speed and working memory (Huijbregts, et al., 2006), and SPMS patients to deteriorate more in visual information processing over a 8 year period (Bergendal, et al., 2007). These neuropsychological impairments are compatible with the P3 ERP results, as they both can be thought to measure similar information processing speed and basic working memory processes. However, the present P3b ERP results indicate differences to be more prominent in the auditory modality, whereas the neuropsychological examinations show the visual modality to be more sensitive. It is likely, that the MS groups have similar difficulties in performing the visual P3 tasks. Thereby, all MS groups perform equally poorly and have less variation in the test measures. In contrast, the auditory P3 task was seen to involve greater variation between MS patients in the cross-sectional analyses in Chapter 4, and thus be able to show statistically significant differences between the groups.

The 12-month study also found the change in visual and auditory P3a ERP correlated significantly with the change in the PASAT score. In the present 24-month study the decrease in the raw PASAT scores was related to increased auditory P3b amplitudes over parietal areas at Month 0, and to delayed auditory P3b latencies over parietal region at Month 12 in all subjects. In MS group, the decreased raw PASAT scores were related to the increase in the auditory P3b amplitudes over parietal areas at Month 0, and to the increase in the visual P3b amplitudes and delayed auditory P3b latencies over parietal areas at Month 12. With demographically corrected PASAT Z scores, only the correlation with visual P3b amplitudes over central locations at Month 12 in a sample of all the subjects was found significant.

The MS patients, driven by the poor performance of SPMS patients, had lower raw PASAT scores consistently over the 24-month period compared to the controls. No significant changes appeared in any of the groups over time. Taken together with the P3 ERP findings, it may be suggested that the P3 ERP changes in MS (particularly in SPMS) may anticipate the changes in the PASAT performance, although this proposition would require further investigation. However, the difference between the groups disappeared when the PASAT scores were age, sex and gender corrected. These results highlight the importance of demographic correction to account for the effect of age and education on neuropsychological test scores (Parmenter, et al., 2010). The correction is central as typically the SPMS patients are older relative to the

RRMS patients and controls. This was also true for this study (at Month 0 RRMS mean age = 36.19 years, SPMS = 52.96 years, controls = 44.01 years; $F(2, 39) = 8.75$, $p = .001$), and the controls often have higher level of education (MS patients mean = 17.82 years, controls mean = 14.65 years; $t(40) = -3.18$, $p < .01$).

The advantages of the present examination of P3b ERP latencies and amplitudes encompass the inclusion of 3 separate sessions with yearly intervals, allowing more comprehensive monitoring of the P3 ERP responses over time, in comparison to the previous study with only 2 sessions. This previous study showed that in some cases the P3b latencies may continue to increase, or stay approximately the same, or sometimes even decrease. Furthermore, the inclusion of a control group throughout the study avoided the misinterpretation that the P3b changes observed in MS patients would be different from the average P3 ERP changes occurring in healthy subjects over time, as there was only one statistically significant group effect (with visual P3b latencies over central areas). On the negative side, the statistical analysis may have suffered from the lack of sufficient subject numbers in each of the groups thus reducing the statistical power, particularly when the RRMS, SPMS and control subgroups were compared (N=8-10).

Key points:

- MS patients have a gradual prolongation of the visual P3b latencies over central areas over 24-month period, whereas latencies of the controls do not change
- MS patients, especially in SPMS patients, have a progressive delay of the visual P3b latencies over parietal area and auditory P3b latencies over central region over 24-months compared to the controls, whose latencies reduced during the same time period
- Auditory P3b amplitudes reduced in each group at the two follow-up sessions compared to the baseline
- The PASAT performance did not change over the 24-month period but the MS (particularly the SPMS patients) had lower raw PASAT scores at each timepoint in comparison to the controls

5.3. Conclusion

This chapter examined the longitudinal changes in the P3 ERP activations and in the PASAT performance over 12-month and 24-month period. The first study found MS patients to have a greater visual P3b and P3a, and auditory P3a, amplitude decrease in 12 months relative to the healthy controls. Furthermore, after 12 months the visual P3b, auditory P3b and auditory P3a amplitudes had significantly decreased and auditory P3a delayed. A gradual delay of the latencies was evident in MS patients over 24-month period in the visual and auditory P3b conditions. These longitudinal P3 ERP changes are apparent over the central and parietal scalp regions in both studies. No clear-cut tendencies of differences were found between the subtypes. Furthermore, the change in visual and auditory P3a ERP activities over centro-parietal areas were associated with the change in the PASAT score over 12-month period. When just the PASAT scores at each time point (not the difference scores reflecting change) were studied, the MS patients (especially the SPMS patients) performed worse in the PASAT in comparison to the healthy controls.

The results of the first study in this chapter were published in a leading peer-reviewed Journal of the Neurological Sciences:

Kiiski, H., Reilly, R. B., Lonergan, R., Kelly, S., O'Brien, M., Kinsella, K., Bramham, J., Burke, T., O'Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N., & Whelan, R. (2011). Change in PASAT performance correlates with change in P3 ERP amplitude over a 12-month period in multiple sclerosis patients. *Journal of the Neurological Sciences, 305(1-2)*, 45-52. (Section 5.1, Appendix 7)

Although the relationship between the neuropsychological test performance measured with the PASAT and the P3 ERP activations were examined in this chapter, the next chapter will include a more comprehensive neuropsychological test battery, the MACFIMS, with MRI measures of lesion loads to examine cross-sectionally the intriguing question of how the multimodal findings from P3 ERP activations, MRI lesion load variables and neuropsychological performance are associated in measuring the cognitive function in MS patients.

Chapter 6 - Multimodal approach to investigate cognitive function in multiple sclerosis: cross-sectional associations between the P3 ERPs, MRI and neuropsychological performance

The present chapter examines the Research Question 6 as stated in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. The focus of the chapter was to investigate cross-sectionally if the findings acquired with multimodal measures of P3 ERPs, MRI (lesion load) and comprehensive neuropsychological battery MACFIMS are associated when assessing cognitive and brain function in MS. These measures were described in detail in Chapter 3-General Measures. Based on the previous research, it was hypothesized that the delayed P3 ERP latencies and reduced P3 ERP amplitudes would be associated with poor performance in the neuropsychological tests measuring processing speed, attention and working memory (namely the PASAT and the SDMT), and with increased lesion loads. To my knowledge, the results of this chapter are unique and novel, as equally thorough and multimodal investigation into the cognitive function in MS has not been conducted before.

6.1. Introduction

The previous studies in this thesis reported the PASAT performance to be associated to the P3 ERP activations in a sample comprising of RRMS and SPMS patients, as auditory P3b latency, visual P3b latency (a strong trend) and visual P3a amplitude correlated with PASAT scores. Furthermore, in a sample including progressive MS patients, the PASAT was correlated with both visual and auditory P3b and P3a ERP amplitudes. The change in the PASAT scores over 12-month period was associated to the change in both visual and auditory P3a amplitudes. As reviewed in detail in Chapter 2-Literature Review, some of the P3 ERP studies conducted in MS population found the P3 ERPs to be associated with neuropsychological and MRI measures (Giesser, et al., 1992; Gil, et al., 1993; Honig, et al., 1992; Newton, et al., 1989; Piras, et al., 2003). A couple of the studies examining P3 ERPs in MS population related their findings to neuropsychological results and reported auditory (Giesser, et al., 1992; Gil, et al., 1993; Honig, et al., 1992) and visual (Piras, et al., 2003) P3b latencies to increase, and auditory P3b amplitudes to reduce (Honig, et al., 1992) with poor cognitive performance. Especially

measures sensitive to impairment in learning, attention working memory were correlated with P3 ERPs (Giesser, et al., 1992; Gil, et al., 1993). However, one study did not report an association between the P3b and neuropsychological test scores (Onofrj, et al., 1991). Only one study (Giesser, et al., 1992) grouped MS patients based on their neuropsychological test scores into patients with and without cognitive decline, and the comparison revealed MS patients with most cognitive impairment to have delayed auditory P3b latencies. However, the number of patients in each group was very low and the neuropsychological examination was not comprehensive enough to determine the cognitive status reliably. The neuropsychological methods utilised in the previous studies are very limited and a more comprehensive investigation is necessary.

P3 ERP latency delay and amplitude decrease have been associated to lesions detected with MRI in MS. Two of the earliest studies investigating P3 ERPs in MS found MS patients with abnormal ERPs to have a larger MRI cerebral lesions (Newton, et al., 1989), and delayed P3b and P3a latencies to correlate with the presence of extensive demyelinative lesions seen with MRI (Honig 1992). With the advance of brain imaging methods, the more recent studies were able to estimate the lesion loads of specific brain regions, thus reporting auditory P3b latency to correlate with lesion loads in frontal horn and brain stem (Piras, et al., 2003). Furthermore, Sailer, et al. (2001) assigned 34 MS patients to low lesion volume, high lesion volume and frontal lesion volume groups based on lesion volume and distribution on T2-weighted MRI scans. They reported N2 amplitude to be significantly reduced in the high lesion volume and frontal lesion volume groups compared to controls. In addition, they discovered N2 amplitude to be correlated with the lesion volume. However, the P3b and P3a components did not show any differences and/or correlations with to lesion volumes in various brain areas.

The aim of this study is to examine cross-sectionally how the P3 ERP activations are associated to the neuropsychological performance in the tests of the adapted MACFIMS battery, and to the MRI T2 lesion load measures of different brain areas. Based on the previous research, the P3 ERP activities were hypothesized to be correlated with the neuropsychological tests measuring processing speed, attention and working memory (namely the PASAT and the SDMT). These cognitive processes have been associated with the P3 ERP activations (Polich, 2007) and have been previously associated to the P3 ERP component activations in MS patients (Giesser, et al., 1992; Gil, et al., 1993). It was also hypothesized that brain areas with high lesion loads would be correlated with delayed P3 ERP latencies and reduced P3 ERP amplitudes.

6.2. Methods

6.2.1. Subjects, procedure and data analysis

38 patients (mean age 44 years) and 44 age-matched healthy volunteers were included in the study. Table 6.1. displays the demographic and behavioural data of the subjects.

Table 6.1. Demographic and behavioural data of subjects.

	MS (N = 38)		Control (N = 44)	
Male/female	21 / 17		24 / 20	
MRI / no MRI data	22 / 16			
	μ	σ	μ	σ
Age (EEG)	43.58	9.48	43.53	10.51
zPASAT	-0.2	1.33	0.18	1.51
zSDMT	-1.9	1.22	-0.54	1.14
zBVMT	-1.17	1.35	-0.68	1.08
zCVLT	-0.1	1.32	0.41	0.88
zFluency	-0.68	1.05	-0.29	1.31
SNST	91.05	23.16	105.74	11
SSOC	-0.55	0.88	-0.37	0.76
Auditory P3b RT (<800 ms)	376.26	80.87	359.82	74.64
Visual P3b RT (<800 ms)	383.81	66.29	363.45	47.27
	M	*IQR*	*M*	*IQR*
EDSS	3	4.5	N/A	N/A
HRSD	8.12	5.4	2.73	4.12
BDI	11.05	9.1	4.2	4.93
BAI	12.62	8.91	4.83	5.9
Interferon β-1a	7		N/A	N/A
Interferon β-1b	8		N/A	N/A
Natalizumab	8		N/A	N/A
Fingolimod	1		N/A	N/A
Glatiramer acetate	2		N/A	N/A
alemtuzumab	1		N/A	N/A
No current treatment	11		N/A	N/A

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = interquartile range, Age (EEG) = age at the EEG recording session, zPASAT = *Z* score of Paced Auditory Serial Addition Test, zSDMT = *Z* score of The Symbol Digit Modalities Test, zBVMT = *Z* score of Brief Visuospatial Memory Test-Revised, zCVLT = *Z* score of The California Verbal Learning Test II, zFluency = *Z* score of the Controlled Oral Word Association Test (FAS version), SNST = raw score of Stroop Neuropsychological Screening Test, SSOC = raw score of the Stockings of Cambridge test, EDSS = Expanded Disability Status Scale, HRSD = the Hamilton Rating Scale for Depression score, BDI = The Beck Depression Inventory-II, BAI = the Beck Anxiety Inventory, RT = reaction time.

The procedure included the EEG, the structural MRI, the MACFIMS battery and psychological functioning measures, and the physical examination including Kurtzke Expanded Disability Status Scale (EDSS; Kurtzke, 2008), which are described in detail in Chapter 3-General Methods.

The data analysis is depicted in detail in Chapter 3-General Methods, and only the parts of data analysis not included in the chapter are described here. Preliminary data processing employed the EEGLAB toolbox (Delorme & Makeig, 2004) in conjunction with the FASTER software tool (Nolan, et al., 2010). The EEG data were bandpass filtered between 1 and 95 Hz, notch frequencies set to 50 and 60 Hz (the monitor refresh rate), average referenced across all scalp electrodes (appropriate when using a high-density EEG array), epoched from 500 ms pre-stimulus to 1000 ms post-stimulus and baseline corrected from 200 ms pre-baseline). The FASTER toolbox removed epochs with large artefacts (e.g., muscle twitch) and interpolated channels with poor signal quality. FASTER (Nolan, et al., 2010) also automatically identified artefactual (i.e., non-neural) ICs and removed them from the EEG data (note: the standard Z = 3 threshold for IC rejection was lowered to Z = 2 for the EOG channels).

The data analysis included the extraction of the latencies and amplitudes from the three regions of interest (ROIs). The ROIs were generated by using composite mean amplitude measures (i.e., the mean value of the electrodes in the ROI). The electrodes used in each ROI are in Table 6.2. (electrode sites are labelled according to the 10–5 system). The peak of the P3a was found by fitting a parametric function to the ERP in the ROI using a Gaussian profile and determining the delay at the peak amplitude (Gerson, et al., 2005) between 200–500 ms for the P3a and between 250–600 ms for the P3b task. For majority of the subjects the P3 ERP components were not distinguishable P3b peaks for frontal ROI, and no P3a peaks for parietal ROI, therefore only latencies extracted from central and parietal ROI of P3b, and from frontal and central ROI of P3a, were utilised in the statistical analyses.

Table 6.2. A list of locations for the Regions of Interest.

Region	Electrode locations
Frontal	F2',F2,AF2',AFz, AFz', Fz, Fz', F1,F1',AF1'
Central	Cz, Cz', CPz, CCP2h, FCC2h, FC2', FCz, FCC1h, FC1', CCP1h
Parietal	CPz', CPP1h, P1, PPO3h, PO3h, Pz, Pz', POz, CPP2h, P2, PPO4h, PO4h

6.3. Results

The correlations between the P3b ERP variables and neuropsychological measures are displayed in Tables 6.3. and 6.4. Decrease in the demographically corrected SDMT *Z* scores was associated with increased visual P3b latencies at parietal areas (r= -.27, p < .05), with increased visual P3a latencies over both frontal (r= -.31, p < .01) and central (r= -24, p < .05) regions, and with visual P3a amplitude at central areas (r= .28, p < .05), in a sample of all subjects. In MS patient sample, SDMT *Z* scores were similarly correlated with visual P3b latencies at parietal areas (r= -.49, p < .01) and with visual P3a latencies at central regions (r= -.37, p < .05). The PASAT score was associated with visual P3b latencies at parietal scalp area (r= -.41, p < .05). Lower BVMT scores were correlated with delayed visual P3b latencies (r= -.27, p < .05) and with increased visual P3a amplitudes over central areas (r= .32, p < .01), and when the controls were excluded from the analysis they correlated also with prolonged visual P3b latencies at parietal areas (r= -.40, p < .01). These correlations were moderate in the all subjects sample, but when only the MS patients were included in the analysis the correlations turned to relatively strong (r= .35-.55). In all subjects sample the CVLT was correlated with visual P3a amplitudes over frontal area (r= -.27, p < .05) and the SNST test scores were correlated with visual P3b latencies centrally (r= -.24, p < .05), auditory P3a latencies frontally (r= -.24, p < .05) and with auditory P3a amplitudes over central area (r= .26, p < .05), but these effects disappeared when controls were excluded from the analysis. It may be argued that these correlational findings may reflect only certain aspects of cognitive function, however, the neuropsychological tests were all part of an established MACFIMS test battery directed to examine cognitive dysfunction typical in MS.

The statistically significant correlations of the P3b ERP variables and MRI measures are shown in Table 6.5. Especially increased latencies and amplitudes over frontal areas in visual P3a task were associated with increased lesion load. The remaining results showed increased lesion loads in various brain areas to be associated with prolonged latencies of the tasks. In specific, the delayed visual P3b latencies recorded from central areas were related to increased lesion loads in left insula and right precuneus, visual P3b latencies at parietal regions with right corpus callosum, auditory P3b latencies at parietal areas with occipital lobe, right inferior frontal gyrus and left hippocampus. The prolonged visual P3a latencies were associated with increased lesion load in right prefrontal lobe; and auditory P3a latencies over frontal areas with left medial frontal gyrus and putamen.

Table 6.3. Correlations between P3 ERP latencies and amplitudes and neuropsychological MACFIMS test scores in MS patients and controls.

MACFIMS Test	P3b Vis C	Vis P	Aud C	Aud P	P3a Vis F	Vis C	Aud F	Aud C
zPASAT		lat: r=-.25*						
zSDMT		lat: r=-.27*			lat: r=-.31**	lat: r=-.24* amp: r=.28*		
zBVMT	lat: r=-.27*					amp: r=.32**		
zCVLT					amp: r=-.27*			
zFluency								
SNST	lat: r=-.24*						lat: r=-.24*	amp: r=.26*
SSOC								

Note. Vis = visual, Aud = auditory, C = central scalp area, P = parietal scalp area, F = frontal scalp area, lat = latency, amp = amplitude, *indicates significance of $p < 0.05$, **indicates significance of $p < 0.01$, ***indicates significance of $p < 0.001$

Table 6.4. Correlations between P3 ERP latencies and amplitudes and neuropsychological MACFIMS test scores in only MS patients.

MACFIMS Test	P3b Vis C	Vis P	Aud C	Aud P	P3a Vis F	Vis C	Aud F	Aud C
zPASAT		lat: r=-.41*						
zSDMT		lat: r=-.49**				lat: r=-.37*		
zBVMT	lat: r=-.54**	lat: r=-.40**				amp: r=.39*		
zCVLT								
zFluency								
SNST								
SSOC								

Note. Vis = visual, Aud = auditory, C = central scalp area, P = parietal scalp area, F = frontal scalp area, lat = latency, amp = amplitude, *indicates significance of $p < 0.05$, **indicates significance of $p < 0.01$, ***indicates significance of $p < 0.001$

Table 6.5. Statistically significant correlations between P3 ERP latencies and amplitudes and the lesion loads in different brain regions in MS patients.

Brain region (lesion load)	P3b				P3a			
	Vis C	Vis P	AudC	Aud P	Vis F	Vis C	Aud F	AudC
Temporal lobe					amp: r=.48*			
-right					amp: r=.50*			
Occipital lobe				lat: r=.46*				
Parietal lobe					amp: r=.49*			
-right					amp: r=.48*			
Corpus callosum - right		lat: r=.46*						
Frontal gyrus								
-right inferior				lat: r=.52*				
-left medial							lat: r=.54*	
-superior								
-left superior								
-right superior								
Left hippocampus				lat: r=.48*	lat: r=-.52*			
insula								
-left	lat: r=.57*							
midbrain								
precuneus					amp: r=.57**			
-right	lat: r=.57*				amp: r=.50*			
Prefrontal lobe								
-right					lat: r=-.51*	lat: r=-.46*		
putamen							lat: r=.53*	
-left							lat: r=.50*	
Wernicke's area								
-left								

Note. Vis = visual, Aud = auditory, C = central scalp area, P = parietal scalp area, F = frontal scalp area, lat = latency, amp = amplitude, * indicates significance of $p < 0.05$, ** indicates significance of $p < 0.01$

6.4. Discussion

The correlational analysis of P3 ERP latencies and amplitudes with neurosychological test scores revealed several interesting associations, which for the most part supported the hypotheses. The poorer test performance in SDMT Z scores was associated with increased visual P3b latencies at parietal areas, with increased visual P3a latencies over both frontal and central regions, and with visual P3a amplitude at central areas. Lower scores in the PASAT was associated with delayed visual P3b latencies at parietal scalp area, and impaired performance in the BVMT task was correlated with delayed visual 3b latencies and with increased visual P3a amplitudes over central areas. When the controls were excluded from the analysis, the lower PASAT scores were associated with prolonged visual P3b latencies at parietal areas. The correlations were moderate in all subjects sample, but when only the MS patients were included in the analysis, the correlations became relatively strong. In all subjects sample, the CVLT and the SNST test scores were correlated with few of the P3 ERP parameters, but these effects disappeared when controls were excluded. The main limitation of the study was that it was purely correlational, and therefore included multiple number of correlational analyses, which cannot provide information on the directionality of the effects. Specifically, if increased lesion load directly affects the prolongation of the P3 ERP latencies.

A few of the P3 ERP studies conducted in MS population found the P3 ERPs to be associated with neuropsychological performance (Giesser, et al., 1992; Gil, et al., 1993; Honig, et al., 1992; Piras, et al., 2003). They reported very similar findings to the present study; the auditory (Giesser, et al., 1992; Gil, et al., 1993; Honig, et al., 1992) and visual (Piras, et al., 2003) P3b latencies were found to to increase, and auditory P3b amplitudes to reduce (Honig, et al., 1992) with poor cognitive performance. Measures sensitive to impairment in learning, attention working memory were correlated with P3 ERPs (Giesser, et al., 1992; Gil, et al., 1993). When MS patients were grouped based on their neuropsychological performance, into patients with and without cognitive impairment, the MS patients with most cognitive impairment had delayed auditory P3b latencies (Giesser, et al., 1992). Nevertheless, the study had numerous limitations, such as inadequate neuropsychological test battery and low number of patients. Moreover, the neuropsychological methods utilised in the previous studies are very limited and/or outdated, thus the tests may have not measured accurately the areas of cognition they claim to have tested, and therefore the results should be considered with caution. The present study overcome these limitations as it utilised a validated test battery MACFIMS (Benedict, et al., 2002), which has been designed to target the specific cognitive areas that are most adversely affected in MS patients.

In the present study the SDMT, the PASAT and the BVMT were the neuropsychological tests most associated with P3 ERP latencies (and amplitudes). In particular, the SDMT appeared to vary clearly with the visual P3 ERP latencies, which can be anticipated, as the task measures information processing speed and working memory in the visual modality (Drake, et al., 2010); the same cognitive processes that the visual P3 ERPs are thought to index (Polich, 2007). Similarly, the observed, yet somewhat unexpected, relationship between the BVMT scores and visual P3 ERPs can be explained by stating that they both measure similar processes, namely visuospatial learning and memory (Benedict, Schretlen, Groninger, Dobraski, & Shpritz, 1996). However, the BVMT measures these cognitive functions at a higher level. It can be suggested that the processes at a lower level of the processing stream of visual information are reflected in the visual P3 ERPs, and that they also have an effect on the higher level visuospatial learning and memory processes measured by the BVMT. The PASAT was also found to be related to the visual P3b latencies over parietal regions, which was different from the results of the previous chapters. The previous studies found the raw PASAT scores to be correlated with auditory P3b latencies, and to visual and auditory P3b and P3a amplitudes in a sample of progressive MS patients. Furthermore, age-corrected PASAT scores were associated with visual P3a amplitudes over frontal and parietal scalp areas. The change in visual and auditory P3a amplitudes correlated with the change in raw PASAT scores over 12-month period. It is possible that due to the demographic correction, some of the previously observed significant effects for auditory modality were not present in this study. Most studies agree (Benedict & Zivadinov, 2011) that the PASAT and the SDMT reflect most the level of cognitive functioning in MS patients. Therefore the close association between the visual P3 ERPs and these tests implies that the visual P3 ERPs are capable of detecting these subtle changes in the cognitive functions of MS patients.

The results also clearly indicate the visual modality in the P3 ERPs to be most correlated with the neuropsychological tasks, which has been implicated in the earlier studies of this research, but also other previous studies (De Sonneville, et al., 2002). The visual P3 ERP tasks were theorized to involve more cognitive processing as they are thought to have been more difficult. This is most likely due to the lower level of distinctiveness of the stimuli. Furthermore, the processing of visual stimuli in the brain is expected to be more affected by the demyelination and lesions, as it is more dependent on the long-range white tracts (i.e. white tracts spanning from primary visual cortices in occipital areas to frontal brain areas) in comparison to the auditory processing. This is reflected in the study by the increase in the correlation strength when only MS patients were included in the analysis. The visual P3b task

was also found to be the most sensitive to detect differences between the MS patients and controls in the previously conducted studies.

P3 ERP latency delay and amplitude reduction have also been associated to lesions detected with MRI. In the present study, increased visual P3a latencies and amplitudes over frontal areas were associated with increased lesion loads in numerous brain regions including temporal, parietal and prefrontal lobes and hippocampus. The remaining results showed increased lesion loads in various brain areas to be associated with prolonged latencies of the P3 ERP tasks. The delayed visual P3b latencies recorded from central areas were related to increased lesion loads in left insula and right precuneus, visual P3b latencies at parietal regions with right corpus callosum, auditory P3b latencies at parietal areas with occipital lobe, right inferior frontal gyrus and left hippocampus. The prolonged visual P3a latencies over frontal and central areas were associated with increased lesion load in right prefrontal lobe; and auditory P3a latencies over frontal areas with left medial frontal gyrus and putamen. Comparable to these findings, a relatively recent study reported auditory P3b latency to correlate with lesions in frontal horn and brain stem (Piras, et al., 2003). The other, less recent studies in MS found abnormal ERPs to have a larger MRI cerebral lesions (Newton, et al., 1989), and delayed P3b and P3a latencies to correlate with the presence of extensive demyelinative lesions seen with MRI (Honig, et al., 1992). Furthermore, one study reported N2 amplitude to be significantly reduced in the high lesion volume and frontal lesion volume groups compared to controls (Sailer, et al., 2001).

An explanation for the unexpected finding of increased P3 ERP amplitudes associated with poor neuropsychological test scores and increased lesion loads over wide brain regions can be drawn from the fMRI studies on cognitive function in MS (Caramia, et al., 2010). According to the theoretical account, the increased activation in various brain areas associated with poor cognitive performance reflects compensatory mechanism in which the brains of MS patients recruit additional brain regions to be able to complete cognitively taxing tasks (Mainero, et al., 2004; Penner, et al., 2003; Staffen, et al., 2002). The increased activation may be directly related to, or caused by, brain pathology that has led to reorganisation of the pathways. These new pathways may thus contribute to compensatory cognitive functioning in MS despite the pathological damage (Caramia, et al., 2010).

According to the research literature on the P3b source locations presented in Chapter 2-Literature Review (Bledowski, et al., 2004; Bokura, Yamaguchi, & Kobayashi, 2001; Brazdil, et al., 1999; Crottaz-Herbette & Menon, 2006; Daffner, et al., 2000; Knight, 1996; Yamaguchi &

Knight, 1991; McCarthy 1997; Linden 1999; Stevens 2000; Karch, et al., 2010; Verleger, Heide, Butt, & Kompf, 1994; Yamaguchi & Knight, 1991; Menon 1997), the findings of the present study are relatively consistent with the proposed neural origins of P3b, as the P3b ERP variables were found to correlate with lesion loads in the right inferior frontal gyrus and left hippocampus. However, the P3b ERP correlations with lesion loads in occipital lobe, corpus callosum, insula and precuneus, were not consistent with the P3 sources reported in previous studies. This may be due to the fact that in MS the activations related to cognitive functions are more widely distributed, which has been theorized to be caused by brain reorganisation and neuroplasticity in response to pathological damage (Caramia, et al., 2010). Interestingly, the brain areas with lesion loads associated with P3a ERP variables were more consistent with the previous research literature on the origin of P3a also discussed in Chapter 2-Literature Review (Bledowski, et al., 2004; Daffner, et al., 2003; Halgren, Marinkovic, & Chauvel, 1998; Brazdil, et al., 1999; Daffner, et al., 2000; Brazdil, et al., 1999; Volpe, et al., 2007; Halgren, et al., 1998; Volpe, et al., 2007; Strobel, et al., 2008). The P3a ERPs of the study were associated with the lesion loads in multiple, largely distributed brain areas; the temporal lobe, parietal lobe, hippocampus, precuneus, and prefrontal lobe. Moreover, these correlations suggest that the lesions in these brain areas may affect the latency delay and amplitude reduction commonly observed in the P3 ERPs in MS. However, due to the correlational nature of the study, it cannot be conclusively stipulated that these lesions directly cause these changes in the P3 ERP components. It is also possible that the originating brain areas for the P3a are not very well defined, and therefore more brain areas are indicated to be related to the P3a origin. Alternatively, the brain regions related to the P3a can be thought to be widespread in nature and thus, affected by lesions in various brain locations. On the other hand, as the origins of the P3a are better delineated, it will also be possible to relate the findings to the theoretical context of the P3a. To summarize, the correlations between lesion load locations and the P3 activations are relatively consistent with the previous literature.

Key points:

- Cognitive performances measured with SDMT, PASAT and BVMT were significantly associated with visual P3b and P3a latencies
- Visual P3a latencies and amplitudes over frontal scalp regions were correlated to lesion loads in various brain areas including temporal, parietal, prefrontal lobes and the hippocampus
- Visual P3b and P3a ERP latencies were indicated to best index the cognitive function in MS
- Visual P3a ERP latencies and amplitudes reflected the pathological changes in widespread brain areas in MS

6.4. Conclusion

This chapter investigated the associations between multimodal methods; the P3 ERP activations, the neuropsychological test performances and the MRI lesion load variables, in order to measure cognitive function in MS. The most important findings are summarized in Keypoints in the section 6.3. Discussion. It can be concluded that the prolongation and/or reduction of the visual P3 ERP components are associated with both poor neuropsychological performance and with increased lesion load, and thus they index cognitive function in MS.

In the next chapter the focus will shift from the cognitive functioning in MS to purely sensory investigation of the visual function in MS, as it examines the utility of a novel electrophysiological method, the VESPA, to probe dysfunction in the visual system of the MS patients.

Chapter 7 - Cross-sectional and longitudinal analysis of the utility of Visual Evoked Spread Spectrum Analysis (VESPA) method to probe visual dysfunction in multiple sclerosis

The present chapter examines the Research Question 7 as stated in the section 2.7. Thesis objectives included in Chapter 2-Literature Review. This chapter examines cross-sectionally and longitudinally if the novel VESPA method can probe visual dysfunction in MS. The details related to the recording and data analysis of the VESPA were presented in Chapter 3-General Methods. Based on previous research, it was hypothesized that the MS patients (particularly MS patients with a progressive disease course and/or visual dysfunction) would show delayed VESPA-like P100 latencies, and reduced VESPA-like P100 amplitudes, compared to the healthy controls. In addition, it was hypothesized that the VESPA-like P100 would show gradual delay in latency and reduction in amplitude over the 24 month period in the MS patients relative to healthy controls. To my knowledge, the findings of this chapter are novel as the utility of the VESPA method in probing visual dysfunction in the context of MS has not been investigated before.

7.1. Introduction

Visual dysfunction is a common feature of multiple sclerosis (MS). The visual system of most of the MS patients is affected during the course of the condition, and it can eventually lead to disability, along with the degradation of other central functions in motor and sensory system (The World Federation of Neurology, 2007b). Approximately 95% of MS cases (The World Federation of Neurology, 2007a) have optic neuritis (ON). Other visual dysfunctions in MS include nystagmus, internuclear ophthalmoplegia and gaze palsies (Frohman, et al., 2005). The nature of visual dysfunction depends on the site of the demyelinating lesion within cerebral white matter (Frohman, et al., 2005).

Visual Evoked Potentials (VEPs) are visually evoked electrophysiological signals extracted from the electroencephalographic activity in the visual cortex recorded from the scalp (Odom, et al., 2010). The VEP method is utilised to determine visual defects, such as ON, in clinical practice,

and VEP may be utilised as an additional test to confirm the MS diagnosis (Polman, et al., 2011; Polman, et al., 2005). According to the current McDonald diagnosis criteria for MS (Polman, et al., 2011), a patient must present with a specific combination of attacks and lesions, which are detected using magnetic resonance imaging (MRI) or VEPs. There is a wide range of literature available, which gives further support to the VEP as a valuable analysis tool. These previous studies (Balnyte, et al., 2011; Niklas, et al., 2009; Schlaeger, D'Souza, Schindler, Grize, Dellas, et al., 2012; Schlaeger, D'Souza, Schindler, Grize, Kappos, et al., 2012) examining VEPs in MS found the P100 responses of MS patients to have prolonged latencies and reduced amplitudes, and to be related to motor and visual dysfunction. Therefore, the VEP are indicated to be a highly useful diagnostic tool. These studies were discussed in detail in Chapter 2-Literature Review. Furthermore, strong correlations have been found between abnormal VEP responses and the probability of having motor or visual disorders (Balnyte, et al., 2011). In addition, the VEPs have also been reported to predict MS disability (Schlaeger, D'Souza, Schindler, Grize, Dellas, et al., 2012; Schlaeger, D'Souza, Schindler, Grize, Kappos, et al., 2012). Moreover, one study found the VEPs to be superior to MRI in the detection of lesions (Ko, 2010). Thus, the VEPs are of great utility in the diagnosis of MS.

The VESPA method has been proposed as an alternative to the VEPs (Lalor & Foxe, 2009; Lalor, et al., 2006), as it allows for the rapid acquisition of a VEP with a complete temporal profile, it has high signal to noise ratios (SNR), and it has been shown to be more sensitive to individual responses than VEPs (Lalor, et al., 2006). Most importantly, VESPA allows for similar information to be obtained in a significantly shorter period of time as a single VESPA run lasts for 2 minutes, which leads to a dramatic decrease in testing time (Lalor, Yeap, Reilly, Pearlmutter, & Foxe, 2008). It also allows for the presentation of simultaneous stimuli that produce separate VESPA responses. Moreover, as VEPs present stimuli periodically, responses are only generated about every 400 ms. In comparison, the VESPA method generates and obtains a response from the subject for the entire duration of stimulus presentation.

The utility of the VESPA method to diagnose and monitor visual dysfunction in MS is of interest of the present chapter. The VESPA is a special case of VEP where a continuous visual stimulus modulating over time is presented to the subject as detailed in Chapter 2-Literature Review and Chapter 3-General Methods. The temporal profile of VESPA responses is highly correlated with that of VEPs evoked using standard discrete stimuli (Lalor, et al., 2006). The temporal profile of the VESPA includes a clearly defined, measurable VESPA-like P100 component (Lalor, et al., 2006). Scalp maps showing topographical evolution of VESPA indicate that the method generates highly specific scalp topographies (Lalor, et al., 2006), which may indicate the

involvement of different visual neural pathways, namely the parvocellular pathways (Lalor, & Foxe, 2009). The VESPA may also provide additional, potentially different information to that of the VEPs, for instance, the VESPA has been deemed more sensitive to individual responses (Lalor, et al., 2006).

The current study aims to establish an additional use for the VESPA method in the diagnosis and monitoring of visual dysfunction in MS. If VESPA is found to be as effective at identifying visual dysfunction in patients with MS, then the benefits which VEPs have for MS patients will be directly applicable to the VESPA method. To my knowledge, this is the first time the VESPA method is examined with MS patients. Based on earlier cross-sectional VEP and VESPA studies it was hypothesized that MS patients would have prolonged VESPA-like P100 latencies and reduced VESPA-like P100 amplitudes relative to controls, as visual dysfunction has been reported to occur in most MS cases (Frohman, et al., 2005; The World Federation of Neurology, 2007a), the VEP P100 has been shown to have similar pattern of activity changes in MS patients (Ziemann, et al., 2011) and the VESPA-like P100 component has been shown to be highly correlated with that of VEP (Lalor, et al., 2006). Furthermore, the progressive MS patients and MS patients with visual dysfunction were hypothesized to have reduced VESPA-like P100 amplitudes and delayed VESPA-like P100 latencies in comparison to RRMS and MS patients with no visual defects. It was hypothesized that the VESPA-like P100 would show gradual delay in latency, and reduction in amplitude, over the 24 month period in the MS patients relative to healthy controls, as the visual dysfunction is caused by the demyelinating lesion(s) within cerebral white matter (Frohman, et al., 2005), and the lesion load is expected to increase as the disease progresses (Rejdak, et al., 2010). In addition, it was hypothesized that MS patients with visual dysfunction would show a delay of the VESPA P100, whereas the MS patients without visual dysfunction would not, as MS patients with ON have been reported to show an initially prolonged P100 latency, which displayed a shortening of P100 latency over a 4 year period, whereas the P100 latency remained stable in MS patients not affected by visual dysfunction (Niklas, et al., 2009).

7.2. Methods

7.2.1. Subjects, the VESPA procedure and EEG acquisition and analysis

56 MS patients (mean age = 42.91 years) and 53 healthy controls (mean age = 40.9 years) were included in the study. The details of the study procedure are included in Chapter 3-General

Methods. The mean time between the baseline and Month 12 sessions was 374.35 days (*SD* = 56.96), and 391.42 days (*SD* = 45.35 days) between Month 12 and Month 24. Tables 7.1. and 7.2. display the demographic and behavioural data of the subjects. 13 MS patients had had at least one relapse within 24 months from the baseline, 2 MS patients had had at least one relapse between Month 0 and Month 12, but none of the MS patients had relapses between Month 12 and Month 24.

Most subjects completed two VESPA runs per testing session, which were subsequently averaged to generate a single VESPA response for each year that the subject participated in the study although some subjects did not complete a second run due to time limitations. Subjects conducted one session per year. Number of subjects who completed a single run in a single session: Year 1 – 18 subjects, year 2 – 4 subjects, year 3 – 3 subjects. VESPA responses were plotted as a function of time for each subject and for each year.

Besides the comparison of all MS patients and controls, cross-sectional and 24-month longitudinal analyses were performed to compare RRMS, SPMS and control subgroups, and MS patients with and without visual dysfunction. Visual dysfunction was defined by the patients' clinical history of optic neuritis, nystagmus, diplopia (double vision) and other symptoms and conditions related to visual function.

Previously recorded VEP responses in clinical settings, typically part of the initial MS diagnosis process if patients have presented with visual deficits, were retrieved from St. Vincent's University Hospital records for all MS patients. The VEP results were found only for 10 of the MS patients, who were divided into two groups based on their VEP results, normal response (N=3) and abnormal response (N=7). As the number of subjects was low, and the subgroups unequal in size, further statistical analyses could not be performed. In addition, many of the VEP results obtained from hospital records were very old, some were recorded in the 1980s, thus the VEP results may no longer be valid.

A separate group of PPMS patients and age-, education- and gender-matched SPMS patients were compared. Unfortunately, following data processing, four of nine PPMS patient's responses were noisy and could not be used as part of statistical analysis. However, preliminary statistical analyses were completed to identify possible trends but no statistically significant differences could be found between the two groups in any of the VESPA-like P100 variables.

The VESPA stimuli, experimental procedure and the EEG acquisition and analysis are described in detail in Chapter 3-General Methods. All statistical analyses on demographic and behavioural data were completed using PASW Statistics 18.0 (Predictive Analytics SoftWare; SPSS Inc., Chicago, IL, USA, www.spss.com).

Results obtained exhibited VESPA-like P100 latencies which were much later than anticipated. This feature is consistent across all responses for all the sessions in different years and is most likely a result of the testing procedure or set up. As a consequence this widespread prolongation in VESPA-like P100 component did not affect the results of this study.

Table 7.1. Demographic and behavioural data of MS patients and controls.

	MS patients (N =56)						RRMS (N=23)						SPMS (N=20)						Controls (N =53)					
	Month 0		Month 12		Month 24		Month 0		Month 12		Month 24		Month 0		Month 12		Month 24		Month 0		Month 12		Month 24	
Male / female	23 / 20		12 / 12		9 / 10		10 / 13		5 /10		5 / 8		13 / 7		7 / 2		4 / 2		26 / 16		16/7		10/4	
Normal VEP / abnormal VEP	3 / 7		1 / 4		1 / 4		0 / 4		0 / 3		0 / 3		3 / 3		1 / 1		1 / 1		N/A		N/A		N/A	
Normal VF / abnormal VF	20 / 22		9 / 14		8 / 10		12 / 10		8 / 6		7 / 5		8 / 12		1 / 8		1 / 5		N/A		N/A		N/A	
ON / no ON	19 / 23		10 / 13		9 / 9		8 / 14		4 / 10		4 / 8		11 / 9		6 / 3		5 / 1		N/A		N/A		N/A	
	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
Age	42.91	10.03	41.98	10.72	42.88	11.31	37.84	8.61	37.81	9.95	38.39	10.48	48.74	8.34	48.94	8.35	52.6	5.59	40.9	9.11	43.82	11.22	45.39	11.08
Edu years	15.31	3.40	14.46	3.45	14.26	3.49	15.78	3.34	14.67	3.62	14.31	3.75	14.74	3.48	14.11	3.33	14.17	3.19	1.22	3.1	17.6	3.3	17.75	3.08
Disdur_S	15.74	9.88	15.38	11.73	15.25	12.77	8.96	6	8.07	4.99	7.86	5.25	23.59	7.32	27.5	9.31	31.27	8.44	N/A	N/A	N/A	N/A	N/A	N/A
Disdur_dx	11.30	7.73	10.28	9.39	9.72	9.61	5.96	4.94	4.34	2.73	4.16	2.85	17.48	5.39	20.17	8.01	21.77	7.65	N/A	N/A	N/A	N/A	N/A	N/A
	M	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*	*M*	*IQR*
EDSS	3.00	4.50	2.00	4.50	2.00	4.50	2.00	1.50	1.50	2.00	2.00	1.50	6.50	1.38	6.00	0.50	6.25	1.50	N/A	N/A	N/A	N/A	N/A	N/A
Relapses	9		1		0		7		1		0		2		0		0		N/A	N/A	N/A	N/A	N/A	N/A
Interferon β-1a	10		7		6		6		4		3		4		3		3		N/A	N/A	N/A	N/A	N/A	N/A
Interferon β-1b	7		7		5		5		5		4		2		2		1		N/A	N/A	N/A	N/A	N/A	N/A
Natalizumab	5		5		5		5		5		5		0		0		0		N/A	N/A	N/A	N/A	N/A	N/A
Fingolimod	1		1		1		1		1		1		0		0		0		N/A	N/A	N/A	N/A	N/A	N/A
No current treatment	10		4		2		0		0		0		10		4		2		N/A	N/A	N/A	N/A	N/A	N/A

Note. μ = mean, σ = standard deviation, *M* = median, *IQR* = interquartile range, RRMS = relapsing-remitting MS patients, SPMS = secondary-progressive MS patients, VF = visual function, ON = optic neuritis, Edu years = years of education, Disdur_S = years since the first symptom, Disdur_dx = years since the MS diagnosis, EDSS = Expanded Disability Status Scale.

Table 7.2. Demographic and behavioural data of MS patients with and without visual dysfunction.

	Visual dysfunction MS patients						No vis dysfunction MS patients					
	Month 0		Month 12		Month 24		Month 0		Month 12		Month 24	
Male / Female	13 / 9		7 / 7		5 / 5		10 / 10		5 / 4		4 / 4	
Valid (RRMS / SPMS)	10 / 12		6 / 8		5 / 5		12 / 8		8 / 1		7 / 1	
Normal VEP / abnormal VEP	3 / 4		1 / 3		1 / 3		0 / 2		- / -		- / -	
ON / no ON	12 / 10		9 / 5		8 / 2		7 / 13		1 / 8		1 / 7	
	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
Age	43.2	9.7	42.8	9.5	44.9	10.0	43.5	10.2	42.5	12.3	42.4	12.3
Edu years	15.5	3.2	14.3	3.3	14	3.1	15.1	3.8	14.6	4.1	14.4	4.3
Disdur_S	18.9	9.2	19.9	12.1	21.9	13.7	12.8	9.8	9.5	8.3	8.3	6.7
Disdur_dx	12.8	7.6	13.0	9.6	13.4	11.1	10.1	7.8	6.9	8.5	6.0	6
Relapses	2		1		0		6		0		0	

Note. μ = mean, σ = standard deviation, RRMS = relapsing-remitting MS patients, SPMS = secondary-progressive MS patients, ON = optic neuritis, Edu years = years of education, Disdur_S = years since the first symptom, Disdur_dx = years since the MS diagnosis

7.3. Results

7.3.1. Correlations between VESPA-like P100 and demographic variables

All of the correlations are conducted with all of the MS patients and controls. The EDSS scores correlated with absolute VESPA-like P100 latency at baseline (r_s = .374, p = .013) and with absolute VESPA-like P100 amplitude at Month 24 (r_s = -.507, p = .027). Age at baseline correlated with absolute VESPA-like P100 latency and amplitude at baseline (r = .367, p = .001 and r = -.24, p = .032, respectively), with latency and amplitude at Month 12 (r = .318, p = .029 and r = -.288, p = .049, respectively), and with latency at Month 24 (r = .414, p = .017). The disease duration correlated with absolute VESPA-like P100 latency at Month 0 (r = .354, p = .023). The years of education correlated with absolute VESPA-like P100 latency at baseline (r = -.256, p = .022), Month12 (r = -.365, p = .012), and Month 24 (r = -.345, p = .050), and with amplitude at Month 24 (r = .352, p = .045). Visual dysfunction correlated with both absolute VESPA-like P100 latency and amplitude at baseline (r_s = .350, p = .023 and r_s = -.354, p = .021, respectively). Age was also correlated with the VESPA-like P100 difference amplitude and latency at Month 0 (p < .05). The years of education was correlated with VESPA-like P100 amplitude difference at Month 0 and at Month 24 (p < .05). The VESPA-like P100 latency difference was found to correlate with age at Month 12 and Month 24 (p < .05). Increase in EDSS score was related to the reduction of VESPA-like P100 amplitude difference (p < .05).

Linear regression analysis did not show absolute VESPA-like P100 latency to predict later disability indicated by the EDSS score.

7.3.2. Cross-sectional differences between the MS patients and the controls in VESPA-like P100

The MS patients showed statistically significantly delayed absolute VESPA-like P100 latencies (p = .001) compared to controls (Figure 7.1. and Table 7.3.). There was a strong trend indicating MS patients to have reduced amplitudes relative to controls (p = .073). Results remained the same when MS patients with relapses within 24 months pre- and post-baseline were excluded from the analysis. When controlling for both age and the years of education with ANCOVA, the results were the same. However, when the effect of age and years of education were controlled separately, the MS did not have significantly delayed absolute or difference VESPA-like P100 latencies relative to controls, whereas the groups differed in absolute VESPA-like P100 amplitude.

Figure 7.1. The VESPA response in MS patients and controls at Month 0.

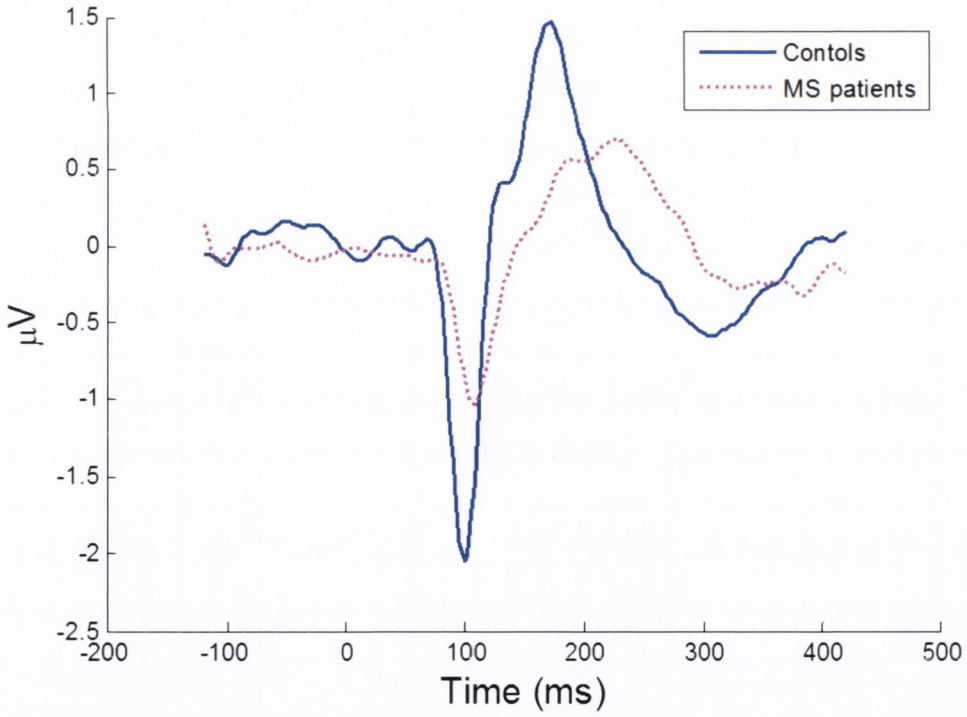


Table 7.3. Independent T-test results of VESPA-like P100 for MS patients and controls.

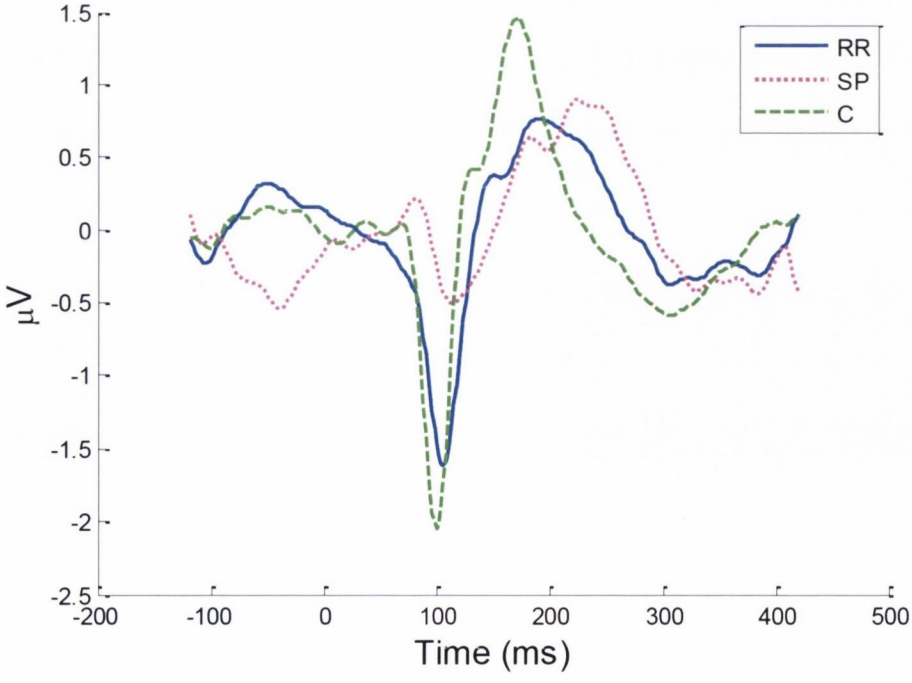
Component	T-test	MS (μ, σ)	C (μ, σ)
P100 amplitude (absolute)	$t(83)= -1.81^{a}$	1.91, 1.22	2.38, 1.19
P100 latency (absolute)	$t(72)= 3.37^{***}$	187.39, 43.19	160.94, 27.80
P100 amplitude difference	$t(83)= -1.66^{a}$	4.27, 2.54	5.12, 2.16
P100 latency difference	$t(83)= 1.68^{a}$	67.86, 31.32	57.73, 23.79

Note. C = controls, μ = mean, σ = standard deviation; [a] indicates a trend in results, significance of $p < 0.1$, * indicates significance of $p < 0.05$, ** indicates significance of $p < 0.01$, *** indicates significance of $p < 0.001$.

7.3.3. Cross-sectional differences between the RRMS patients, SPMS patients and the controls in VESPA-like P100

RRMS, SPMS and controls differed significantly in absolute VESPA-like P100 latency (p = .001) (Figure 7.2. and Table 7.4.). Tukey post-hoc tests showed that the SPMS have the most delayed absolute VESPA-like P100 latencies in comparison to the controls, but there were no other statistically significant group differences. Similar trend was also visible for the VESPA-like P100 latency difference variable. The results did not change when the effect of age and/or years of education were controlled with ANCOVA. Furthermore, when MS patients with relapses within 24 months were excluded from the analysis the results remained the same (although there was a trend of RRMS to have more delayed VESPA-like P100 latencies relative to controls).

Figure 7.2. The VESPA response in RRMS patients, SPMS patients and controls at Month 0.



Note. RRMS = relapsing-remitting MS, SPMS = secondary-progressive MS

Table 7.4. One-way ANOVA results of VESPA-like P100 for RR patients, SP patients and controls.

Component	ANOVA	Tukey	RRMS (μ, σ)	SPMS (μ, σ)	C (μ, σ)
P100 amplitude (absolute)	$F(2,77)= 1.65$		1.87, 1.09	1.95, 1.37	2.38, 1.87
P100 latency (absolute)	$F(2,77)= 36.72$**	SP > C	176.35, 41.2	200.1, 42.9	160.9, 27.80
P100 amplitude difference	$F(2,77)= 1.65$		4.52, 2.82	3.98, 2.21	5.12, 2.16
P100 latency difference	$F(2,77)= 2.96$[a]	(SP > C)	61.03, 29.5	75.71, 32.2	57.73, 23.80

Note. *Tukey post-hoc* tests utilised. RRMS = relapsing-remitting MS patients, SPMS/SP = secondary-progressive MS patients, C = controls, [a] indicates a trend in results, significance of $p < 0.1$, * indicates significance of $p < 0.05$, ** indicates significance of $p < 0.01$, *** indicates significance of $p < 0.001$.

7.3.4. Cross-sectional differences between the MS patients with and without visual dysfunction in VESPA-like P100

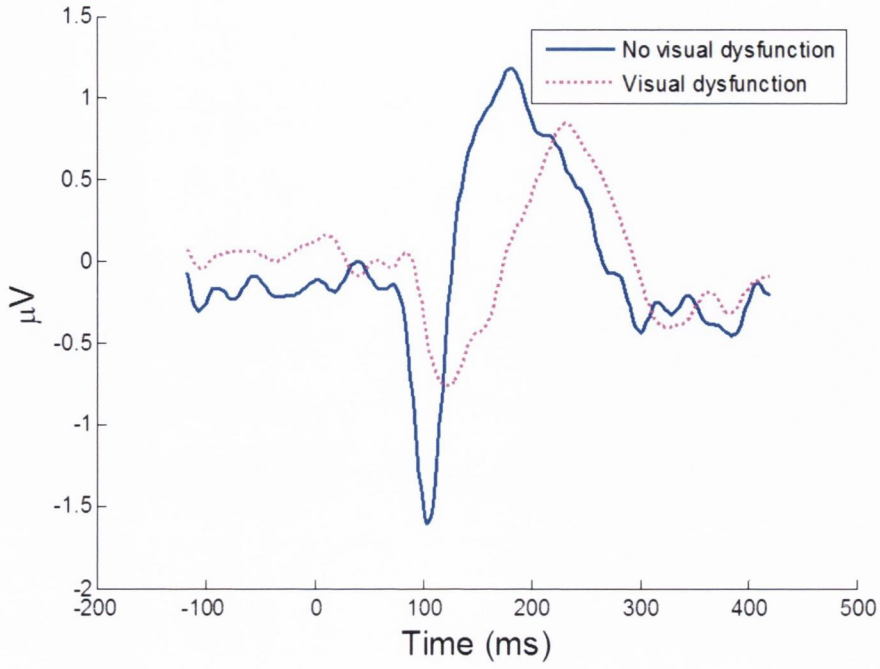
MS patients with visual dysfunction displayed significantly reduced absolute VESPA-like P100 amplitudes and prolonged absolute VESPA-like P100 latencies than patients with no history of visual dysfunction (Figure 7.3. and Table 7.5.). MS patients with no history of visual dysfunction also exhibited a trend for larger VESPA-like P100 amplitude differences. The results remained the same when the effect of age and/or years of education were controlled with ANCOVA.

Table 7.5. Independent T-test results of VESPA-like P100 for MS patients with and without visual dysfunction.

Component	T-test	Visual dysf. (μ, σ)	No visual dysf. (μ, σ)
P100 amplitude (absolute)	$t(40)= 2.40$, $p=0.021$*	1.51, 0.82	2.37, 1.45
P100 latency (absolute)	$t(40)= -2.48$, $p=0.017$*	203.40, 44.72	172.35, 35.16
P100 amplitude difference	$t(40)= 1.78$, $p=0.082$[a]	3.57, 1.77	4.94, 3.09
P100 latency difference	$t(40)= -0.95$, $p=0.346$	73.11, 30.62	63.91, 31.85

Note. Visual dysf. = MS patients with visual dysfunction, No visual dysf. = MS patients with no history of visual dysfunction, μ = mean, σ = standard deviation, *indicates a trend in results, significance of $p < 0.1$, **indicates significance of $p < 0.05$, ***indicates significance of $p < 0.001$.

Figure 7.3.The VESPA response in MS patients with and without visual dysfunction at Month 0.

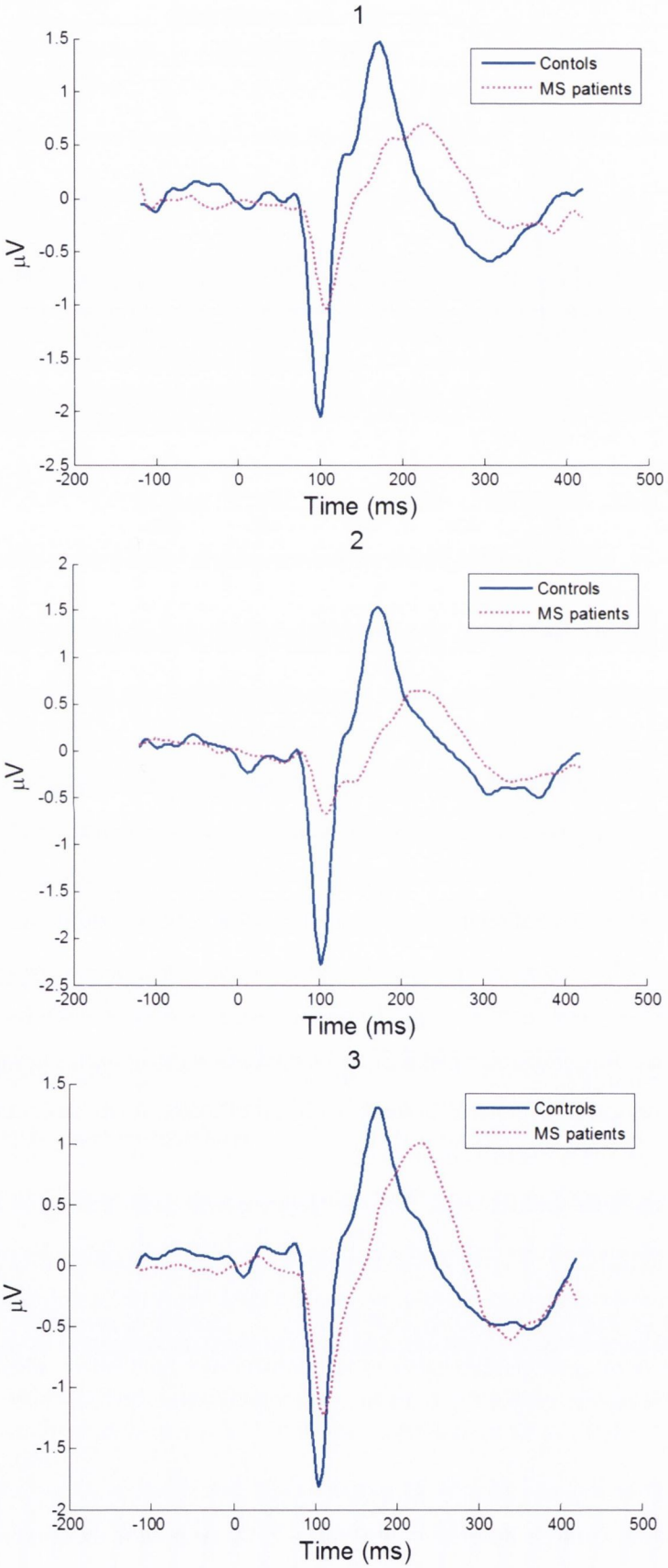


7.3.5. Longitudinal differences between the multiple sclerosis patients and the controls in VESPA-like P100

The only statistically significant finding was a group effect of absolute VESPA-like P100 latency over 24 Month period as MS patients had more prolonged latencies compared to the controls (p = .05), but otherwise there were no statistically significant time, group or time x group interaction effects neither for VESPA-like P100 latency nor for VESPA-like P100 amplitude over 24-month period (Figure 7.4. and Table 7.6.). In addition, no differences were observed when the effect of age and/or education were controlled as part of the repeated measures ANOVA analysis. However, statistically significantly prolonged absolute VESPA-like P100 latencies in MS patients, and clear trends of reduced absolute VESPA-like P100 amplitudes in MS patients, were observed when the groups were compared at each timepoint separately (i.e. time x group interaction). These effects were visible in the first two years, eventhough there was a notable trend towards MS patients to have delayed absolute VESPA-like P100 latencies at Month 24. At Month 24 MS patients showed slightly increased VESPA-like P100 amplitude, however this difference was not statistically significant and may thus reflect variability in the data.

Paired t-tests comparing the baseline and Month 24 variables did not differ in either MS patient sample or control sample. Controls differed in VESPA-like P100 amplitude differences between Month 0 and Month 12.

Figure 7.4. Averaged VESPA responses of MS patients and controls for Month 0, Month 12 and Month 24.



Note. 1 = Month 0, 2 = Month 12, 3 = Month 24.

Table 7.6. Independent T-test results for MS patients and controls.

Component	T-test	MS (μ, σ)	C (μ, σ)
P100 amplitude (absolute)			
Month 0	$t(83)= -1.81^a$	1.91, 1.22	2.38, 1.19
Month 12	$t(40)= -1.98^a$	1.80, 0.88	2.42, 1.22
Month 24	$t(31)= -0.98$	1.81, 1.26	2.22, 1.11
P100 latency (absolute)			
Month 0	$t(72)= 3.37$**	187.39, 43.19	160.94, 27.80
Month 12	$t(41)= 2.04$*	192.81, 49.87	167.45, 34.06
Month 24	$t(30)= 1.85^a$	195.98, 45.53	171.88, 29.05
P100 amplitude difference			
Month 0	$t(83)= -1.66^a$	4.27, 2.54	5.12, 2.16
Month 12	$t(45)= -2.57$*	3.90, 2.10	5.52, 2.23
Month 24	$t(31)= -0.80$	4.14, 3.15	4.97, 2.67
P100 latency difference			
Month 0	$t(83)= 1.68^a$	67.86, 31.32	57.73, 23.79
Month 12	$t(45)= 0.71$	70.11, 34.76	63.48, 29.23
Month 24	$t(31)= 0.90$	75.79, 34.82	66.59, 24.05

Note. C = controls, μ = mean, σ = standard deviation, *indicates a trend in results, significance of $p < 0.1$, **indicates significance of $p < 0.05$, ***indicates significance of $p < 0.001$

7.3.6. Longitudinal differences between the RRMS patients, SPMS patients and the controls in VESPA-like P100

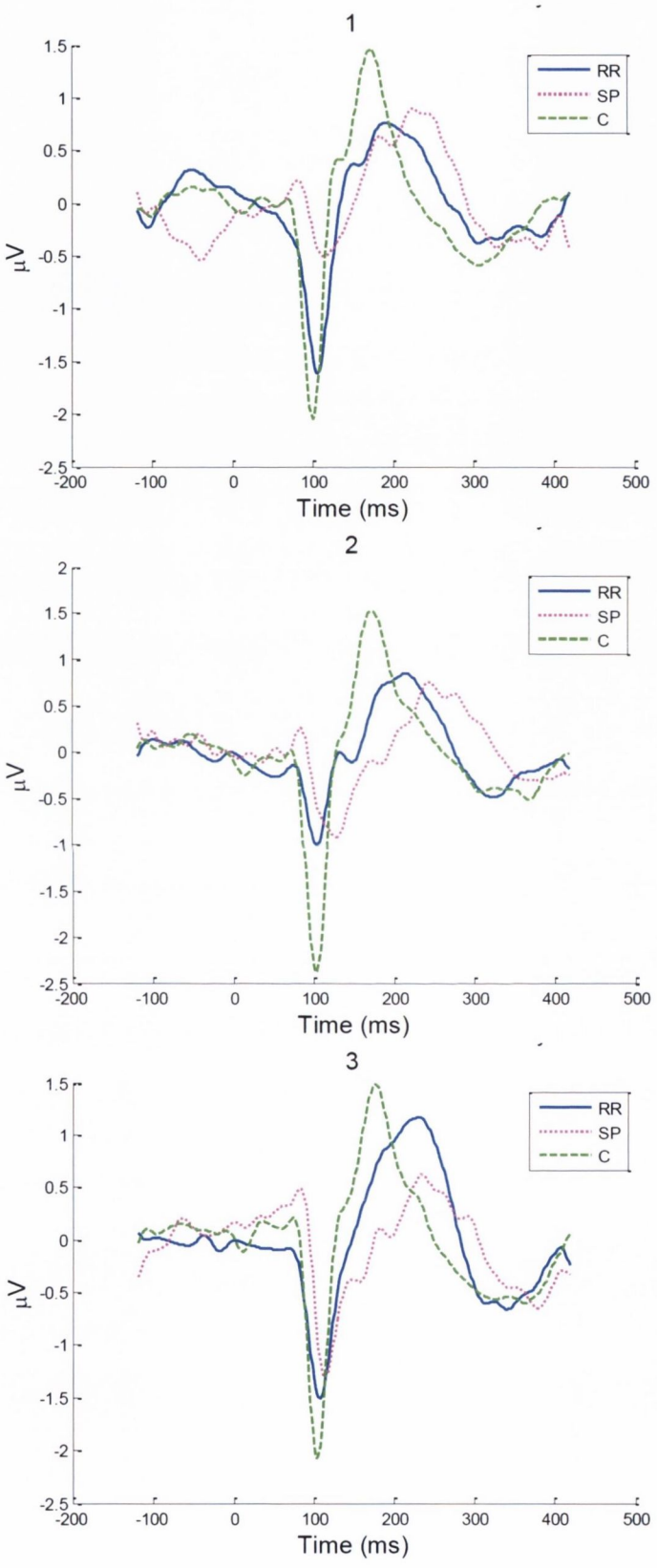
No significant group, time or interaction effects were found between the groups RRMS patients, SPMS patients and controls (repeated measures ANOVA) in any of the VESPA-like P100 variables over a 24-month period ($p > 0.05$) (Figure 7.5. and Table 7.7.). Furthermore, no significant differences were found when the 24-month repeated measures ANOVA test was controlled for age and/or number of years of education ($p > 0.05$). However, it is important to keep in mind that these statistical analyses only included 6 SPMS patients. Besides the cross-sectional differences already reported, when the groups were compared at each timepoint separately, only VESPA-like P100 amplitude difference at Month 12 showed statistically significantly controls to have larger VESPA-like P100 amplitude difference than RRMS. Paired samples t-tests revealed RRMS patients to have prolonged absolute VESPA-like P100 latency at Month 24 ($p < 0.05$). Furthermore, the controls were found to have increased VESPA-like P100 amplitude difference at Month 12 compared to Month 0.

Table 7.7. One-Way ANOVA results of VESPA-like P100 for RRMS patients, SPMS patients and controls.

Component	*ANOVA*	*Tukey*	RRMS (μ, σ)	SPMS (μ, σ)	C (μ, σ)
P100 amplitude (absolute)					
Month 0	$F(2)= 1.65$		1.87, 1.09	1.95, 1.37	2.38, 1.87
Month 12	$F(2)= 1.65^{a}$	(C > SP)	1.96, 0.99	1.53, 0.61	2.42, 1.22
Month 24	$F(2)= 1.88$		2.11, 1.29	1.16, 0.98	2.22, 1.11
P100 latency (absolute)					
Month 0	$F(2)= 36.72^{**}$	C < SP	176.35, 41.2	200.1, 42.9	160.9, 27.80
Month 12	$F(2)= 2.76^{a}$	(C < SP)	184.89, 42.3	206, 60.9	167.5, 34.06
Month 24	$F(2)=1.68$		192.00, 43.3	204.6, 53.3	171.9, 29.05
P100 amplitude difference					
Month 0	$F(2)= 1.65$		4.52, 2.82	3.98, 2.21	5.12, 2.16
Month 12	$F(2)= 3.61^{*}$	C > RR	4.18, 2.36	3.43, 1.59	5.52, 2.26
Month 24	$F(2)= 1.24$		4.75, 3.42	2.80, 2.15	4.97, 2.67
P100 latency difference					
Month 0	$F(2)= 2.96^{a}$	(C < SP)	61.03, 29.5	75.71, 32.2	57.73, 23.80
Month 12	$F(2)= 0.90$		64.36, 30.7	79.70, 40.8	63.48, 29.23
Month 24	$F(2)= 0.55$		72.76, 35.2	82.35, 36.2	66.59, 24.05

Note. *Tukey post-hoc* tests utilised. RRMS/RR = relapsing-remitting MS patients, SPMS/SP = secondary-progressive MS patients, C = controls, [a] indicates a trend in results, significance of $p < 0.1$, * indicates significance of $p < 0.05$, ** indicates significance of $p < 0.01$, *** indicates significance of $p < 0.001$

Figure 7.5. Averaged VESPA responses of RRMS patients, SPMS patients and controls for Month 0, Month 12 and Month 24.



Note. 1 = Month 0, 2 = Month 12, 3 = Month 24, RR = relapsing-remitting MS patients, SP = secondary-progressive MS patients, C = controls.

The repeated measures ANOVA test between MS patients with and without visual dysfunction over three years also yielded no significant results. Statistically significant cross-sectional differences ($p < .05$) were only present at baseline when groups were compared at each time point. No longitudinal differences were observed with paired samples t-tests.

7.4. Discussion

7.4.1. The results of the present study

The utility of the VESPA method in measuring visual dysfunction has not been investigated prior to this study. The results of the present study were consistent with the hypothesis that MS patients have significantly prolonged VESPA-like P100 latencies and reduced P100 amplitudes in comparison to responses of controls. The VESPA-like P100 latency and amplitude differences were most evident at the baseline. SPMS patients had the most delayed VESPA-like P100 latencies compared to controls. RRMS patients did not differ significantly from controls or the SPMS patients. It is likely that these differences result from a reduction in visual function due to the effects of demyelination, which is more widely spread in the brains of SPMS patients. The SPMS patients deteriorate progressively and most of them experience continuous severe disease related symptoms such as fatigue, muscular problems and visual dysfunction. These results remained the same when MS patients with relapses within 24 months pre- and post-baseline were excluded from the analysis.

MS patients with a history of visual dysfunction had significantly reduced absolute VESPA-like P100 amplitudes and prolonged absolute VESPA-like P100 latencies. Significant differences were observed for baseline data only, when the sample size was largest (N = 22). Similar differences between the two subgroups were observed for the subsequent years, although none of these differences reached statistical significance. It can be suggested that the visual functioning remains roughly at the same level over a 3-year period in MS patients. The deficits in visual function are likely to be caused by adverse disease processes, which affect longer white tracts involved in visual processing that are more prone to demyelination and intervening lesions. It is also possible the small sample size reduced the statistical power of the longitudinal analyses. Although the differences observed between the two subgroups contradicted the findings of Balnyte et al. (2011), they were in line with the hypothesis that as VESPA is a measure of visual functionality, patients with reduced visual functions such as ON, diplopia (double vision), blurred vision, and nystagmus, should have slower response times and smaller amplitudes. This may be because Balnyte et al. (2011) looked at ON history only,

whereas this study encompassed a wider range of visual dysfunctions including nystagmus and diplopia.

Contrary to the hypotheses, there was not any clear, consistent pattern in the longitudinal analysis, showing the prolongation of VESPA-like P100 latencies and the reduction of VESPA-like P100 amplitudes in MS patients relative to controls over the 24-month period. However, a few significant differences and notable trends were found cross-sectionally when the groups were compared within each year. There was also a lack of longitudinal changes between the MS patients with and without visual dysfunction. The most noteworthy finding was that the absolute VESPA-like P100 latency was prolonged at every timepoint relative to the controls. The lack of clear hypothesized longitudinal statistical differences is most likely due to the reduced statistical power as the sample size was much lower at Months 12 and 24. On the other hand, it may imply that the visual functions remained largely at the same level in the MS patients investigated during the 24-month period. The results of repeated measures ANOVA controlled for age and the number of years of education did not yield further significant results. This may be due to the reasonably weak associations found between number of years of education, age and VESPA variables, which implies the lack of influence of age and the years of education to the change in VESPA-like P100 responses. These analyses further supported the conclusion that the MS do not present with longitudinal changes in their visual function over a 24-month period.

The clear differences between the groups at Month 0 and at each follow-up session in absolute VESPA-like P100 latency, and the non-existent longitudinal differences, highlight the reproducibility of the VESPA-like P100 response, also confirmed by the findings of Lalor and colleagues (2006). Reproducibility is an important feature when choosing a reliable diagnostic tool as it allows for meaningful comparisons to be made between separate responses.

One methodological constraint of the study was that the results obtained exhibited VESPA-like P100 latencies, which were much later than those anticipated and typically presented in the literature (Lalor, et al., 2006). However, this feature is consistent across all responses for all years and is most likely a result of the testing procedure or set up. As a consequence, this widespread prolongation in VESPA-like P100 component did not affect the results of this study.

The absolute VESPA-like P100 latency (and amplitude) were found to be correlated with MS physical disability status measured with the EDSS score, which may indicate deteriorating brain changes affecting visual and physical functions at a comparable rate. The absolute VESPA-like

P100 latency and amplitude were shown to correlate modestly with age and the years of education. The VESPA-like P100 latency can be expected to prolong with age, similarly to VEP P100 latency (Kuba, et al., 2012). The finding of absolute VESPA-like P100 latency to increase with less years of education suggests the early visual processing mechanisms to be related to the general cognitive performance of a subject. However, the mechanisms and the nature of these associations are unknown and may be mediated by other factors, such as overall brain function and intelligence of a subject.

7.4.2. Comparison with previous studies

The results of the present VESPA study are similar to the VEP findings of (Balnyte, et al., 2011) as in their study MS patients also displayed greater VEP P100 latency differences and reduced VEP P100 amplitude differences compared to controls. Although the results of the previous VEP studies are not directly applicable to the current VESPA study, they highlight the usefulness of VEPs in relation to the study of MS and its treatments. The more important VEPs are for the diagnosis and treatment of MS, the greater the potential for the VESPA method in a similar role. Moreover, the present results are promising, as the VEPs has been shown to be a more sensitive measure than MRI at detecting abnormalities, including clinically silent lesions (Ko, 2010). This implies a potential clinical utility of the VESPA method to complement the conventional MRI examination. The VEPs have been shown to depend on the functionality of the patient's central vision at any level of the visual pathway (the eye, retina, optic nerve, optic radiations and occipital cortex) (Odom, et al., 2010). The VEP P100 waves are likely to reflect 'brain resetting' activity occurring between the presentations of each stimulus, an event which does not occur during the presentation of VESPA stimuli (Lalor, et al., 2006).

As mentioned in Chapter 2-Literature Review, the VESPA stimuli were hypothesized to target mainly parvocellular pathways within the brain, based on a finding from a study by Lalor and Foxe (2009). The parvocellular and magnocellular pathways are one of the major pathways of the visual system and anatomically distinct from eachother (Liu, et al., 2006). The parvocellular pathway is thought to be central for chromatic processing and visual acuity, to provide input to higher-order pattern processes, to be sensitive to higher spatial frequencies, lower temporal frequencies and chromatic stimuli (Cao, et al., 2011). The view is consistent with the results of the present chapter, as the pathological changes in the brains of MS patients can be expected to affect most the parvocellular pathway. The parvocellular pathway accounts for approximately 80% of optic nerve fibers, and it consists of thinly myelinated ganglion cells likely to be prone to disruptions caused by widespread demyelination in MS (Cao, et al., 2011).

Parvocellular pathways have a lower luminance contrast gain, but do not have a tendency to saturate. On the other hand, magnocellular pathways have a high luminance contrast, which tends to saturate at contrasts greater than about 32% (Kaplan & Shapley, 1986). Lalor and Foxe (2009) adjusted the contrast levels of stimuli to target the pathways, and they reported the full range VESPA to be biased towards parvocellular pathways. Furthermore, the lack of longitudinal differences is most likely due to no significant changes in the pathological changes affecting the visual system, and/or the low number of subjects in the second and third year reducing statistical power of the analyses. The role of remyelination has been previously suggested to be one of the reasons of no visible prolongation effects of VEP P100 latencies over time, or even the shortening of P100 latencies in the VEP follow-up sessions (Niklas, et al., 2009), which may explain the lack of VESPA-like P100 changes over time in MS.

The present study avoided many shortcomings of the previous studies in the area, such as the inclusion of neurological patients as controls (Balnyte, et al., 2011; Niklas, et al., 2009). In addition, the study groups were observed unequal amount of time, i.e. patients diagnosed with MS were observed over a longer period than those who were not affected (Niklas, et al., 2009). Furthermore, in the study of Balnyte et al. (2011), about one third of the MS patients examined in the study had a history of ON. This may have been a contributing factor to the high level of VEP abnormalities observed within the patient group. Nevertheless, the present study encompassed a few limitations. The VEP was not part of the experimental procedure in the present study, and thus the clinical utility of the VESPA method in detecting visual dysfunction will need to be further confirmed with a more comprehensive study comparing the two methods. The longitudinal analysis could also be enhanced through large sample sizes in the follow-up years. The further investigation of VESPA as a supporting or preferred diagnostic method to VEPs is endorsed.

The VESPA method has multiple advantages over the traditional VEP method as it allows for the rapid acquisition of a VEP with a complete temporal profile, it has high signal to noise ratios (SNR), and it has been shown to be more sensitive to individual responses than VEPs (Lalor, et al., 2006). Most importantly, VESPA allows for similar information to be obtained in a significantly shorter period of time as a single VESPA run lasts for 2 minutes, which leads to a dramatic decrease in testing time (Lalor, et al., 2008). It also allows for the presentation of simultaneous stimuli that produce separate VESPA responses. Moreover, the VESPA method generates and obtains a response from the subject for the entire duration of stimulus presentation in comparison to periodically generated VEP responses. The disadvantage of VESPA, in comparison to standard VEPs, is that it requires greater computational power.

However, Lalor et al. (2006) state that given the power of modern computers, this should not be a factor in choice of method and rather the speed at which results can be obtained should be prioritised. Moreover, it has not been yet conclusively delineated if the VESPA-like P100 reflects the same underlying visual processes as the VEP P100, although the P100 responses acquired with both methods have been reported to be linked (Lalor, et al., 2006). It is possible that the VESPA method offers increased subtlety to the assessment of visual function, and may reveal additional information not present in the VEP P100 response. Likewise, the VESPA-like P100 response may reflect differently the level of demyelination occurring in the white matter tracks part of the visual system in comparison to the conventionally measured VEP P100. In sum, the VESPA method has potential utility in detecting visual dysfunction in MS.

Key points:

- Absolute VESPA-like P100 latency and amplitude were correlated with MS physical disability status, age and the years of education
- Absolute VESPA-like P100 latencies were delayed in MS compared to controls at each yearly session over 24-month period
- SPMS patients have most pronounced delay in absolute VESPA-like P100 latency relative to RRMS patients and controls
- MS patients with visual dysfunction have reduced absolute VESPA-like P100 amplitudes and delayed absolute VESPA-like P100 latencies compared to MS patients with no clinical history of visual dysfunction
- No consistent longitudinal VESPA-like P100 peak differences were observed between MS patients and controls, or between any of the other subgroups, indicating the VESPA to be a reproducible tool to probe visual dysfunction in MS
- VESPA method, especially the absolute VESPA-like P100 latency, has potential clinical utility

7.5. Conclusion

This chapter set out to investigate the utility of the novel VESPA method to probe the functioning of the visual system in MS. The central findings are summarized in Key points in the section 7.4. Discussion. The VESPA method was deemed a reproducible electrophysiological method as no consistent longitudinal VESPA-like P100 peak changes were observed between MS patients and healthy controls over 24-month period, or between any of the other subgroups. Reproducibility of a method is a good indicator as to its usefulness in clinical applications, and therefore the VESPA method is proposed to have potential clinical or diagnostic utility in MS. The next chapter reviews comprehensively the findings acquired in the

studies of this thesis, and it considers how they are associated to one another and to the previous research literature. Subsequently, the next chapter discusses in-depth how these exciting new cognitive-indexing electrophysiological findings are related various topics surrounding the the research of brain and cognitive functions in MS and other neurological conditions, and what kind of impact these findings may have on the clinical management of CI in MS, and on the clinical research and practice in general.

Chapter 8 - Discussion

The overall objective of this thesis was to determine if brain and cognitive dysfunction in MS, can be successfully detected and monitored with electrophysiological measures. Detailed research questions were posed and presented in the section 2.7. Thesis objectives were included in Chapter 2-Literature Review. The first research question asked if the P3 ERPs recorded at baseline differed between MS patients and healthy controls, and between MS subtypes RRMS, SPMS and PPMS. It also investigated the relationship between neuropsychological performance measured with the PASAT and the P3 ERP activations. The second objective of the thesis was to employ a novel independent component (IC) clustering method, to examine the source P3 EEG activations at baseline. In addition, this study also aimed to determine if the source EEG activations differed between MS patients and healthy controls and between MS patients, with and without cognitive impairment. The third aim was to examine the effect of genetic factors on CI in MS. In particular whether MS patients possessing AA OAS1-genotype and with increased disease activity would show delayed P3 ERP latencies and reduced P3 ERP amplitudes, and perform more poorly in the PASAT, relative to the other AG OAS1-genotype MS patients. The fourth and fifth research questions set out to examine change, if any, in the P3 ERP activations and the PASAT, over a 12-month and 24-month period. A primary objective was to investigate if the MS patients show signs of deterioration in the electrophysiologically measured brain and cognitive functions over time, and if these changes can be linked to cognitive performance measured with the PASAT. The sixth aim of the research was to investigate if the P3 ERP activities were associated with the neuropsychological performance, as measured with tests in the adapted MACFIMS battery. This part of the research also examined if the P3 ERP activities were related to MRI T2 lesion load in different areas of the brain. The final objective of the thesis was to examine the benefits of the VESPA method in probing visual dysfunction in MS. The findings from these studies are discussed in depth in this chapter.

8.1. Main findings of the thesis

The studies conducted as part of this thesis demonstrated electrophysiological measures to detect, and in some cases to monitor, brain function related to subtle cognitive processing in MS. The primary focus was on the electrical activities generated by the oddball (P3b) and three-stimulus (P3a) paradigms, which were examined cross-sectionally and longitudinally, by utilising advanced signal processing analysis methods. In MS patients and in subgroups, the

high-density EEG recordings enabled the localisation of the amplitude and latency deficits to present more accurately on the scalp space and over a timescale. It also allowed the utilisation of source EEG analysis with IC clustering. Similarly, advanced methodology has not been utilised to the same extent in previous electrophysiological studies of MS (Magnano, et al., 2006). Neither has a high-density EEG been associated as comprehensively with other measures of cognitive and brain functioning; either neuropsychological examinations or brain MRI.

The first study in Chapter 4 investigated the hypothesis that MS patients would show reduced amplitudes and delayed latencies in one of the most established ERP components; the P3b. As anticipated, widespread deficits were observed in MS patients in electrophysiological activations, measuring early sensory processing and those indexing subtle cognitive functions. These dysfunctions were most evident for amplitudes and latencies, during the visual tasks, over the large frontal and centro-parietal areas. The observations in the auditory condition included delayed latencies over the parietal region and reduced amplitudes of the early sensory components. In accordance with these findings, visual modality was more sensitive in detecting prolonged P3a latencies and decreased P3a amplitudes in MS patients, particularly those observed over the frontal and parietal scalp regions. The only P3a differences in the auditory task were observed in the latencies recorded from the parietal region of interest. Progressive MS patients were found to have reduced visual P3b and P3a amplitudes and decreased auditory P3a amplitudes, in comparison to controls.

P3 ERP source EEG activities and their informative time-frequency properties were also included in Chapter 4, as they were investigated with a novel IC clustering analysis method. The results revealed reduced low-frequency theta and alpha activations after P3b target stimuli were presented, in widespread brain source sites in MS patients. MS patients also showed a greater reduction in alpha power in the posterior sources. These findings were similar in both modalities. The reduced P3b task-related theta power, observed in MS patients, was thought to reflect compromised connectivity, which is most likely due to demyelination, which may have disrupted early processes essential to P3b generation. These processes include orienting response and signal detection. The greater decrease in alpha power, on the other hand, may be associated with enhanced cognitive function in MS patients, possibly reflecting a compensatory mechanism in response to the compromised early cognitive processing.

In the longitudinal analysis described in Chapter 5, the MS patients were hypothesized to show greater prolongation and decrease of the P3 EEG activations, and a reduction in the PASAT score, compared to the healthy controls over the 12- and 24-month follow-up periods. MS patients had greater visual P3b, visual P3a and auditory P3a amplitude decreases over 12 months compared to the controls. The MS patients also displayed significant decreases of visual P3b, auditory P3b and auditory P3a amplitudes and delayed auditory P3a latencies at 12-month follow-up. The 12-month longitudinal analysis showed visual amplitudes and auditory latencies to be most sensitive to change over the relatively short one-year period. The 24-month investigation of the P3b latencies and amplitudes confirmed the earlier longitudinal findings, as visual modality was deemed predominant in detecting the changes. However, over the 24-month period, the changes in MS occurred in the visual P3b latencies, over the parietal areas, but not so in the amplitudes. In addition, auditory P3b latencies showed prolongation over central areas, particularly in SPMS patients. At the same time, the latencies of controls reduced over the 24-month timeframe. It is plausible that the inconsistencies between the findings may be masked by an effect that was not controlled for in the present studies, but has been reported in other previous studies. It may be that MS patients present with two courses of cognitive functioning. One group of early stage MS patients displayed mild cognitive deterioration, which accumulates over the years, while the cognitive performance of the initially preserved MS patients, remains more or less stable over several years (Bergendal, et al., 2007; Camp, et al., 1999; Kujala, et al., 1997).

In the context of the present research project, a similar, purely neuropsychological cross-sectional and longitudinal analysis would have been possible. However, as the focus of the present research was on electrophysiology and its associations with neuropsychological examination and MRI, the comprehensive longitudinal neuropsychological analysis was conducted by the collaborating researchers in the UCD School of Psychology. The differences between 12 month and 24 month studies may also be due to a methodological constraint. For instance, the peak picking method fitting a Gaussian curve on a selected ERP component and/or time window (Gerson, et al., 2005), may not have measured the peak amplitudes sufficiently accurately. Thus, to get a more accurate picture of their change over time, the P3b amplitudes should be further examined with more advanced analysis methods, such as SPM utilised in the 12-month study.

In the studies in Chapters 4 and 5, another major aim was to find associations between the P3 ERP measures and cognitive functions. This task was implemented by correlating the extracted P3 ERP variables to PASAT performance scores, and by dividing the MS patients into groups

based on their PASAT performance and then finally comparing their cognitive-indexing electrophysiological activities. At baseline, in a sample of RRMs and SPMS patients, the PASAT scores correlated with auditory P3b latencies, and with visual P3a amplitudes, over a large frontal scalp region. When only progressive MS patients were included, the PASAT score correlated with all the P3 ERP conditions, i.e. visual and auditory, P3b and P3a tasks over centro-parietal areas. In a small sample of MS subjects tested for their OAS1 genotype, the PASAT score was associated with auditory P3b latency. Source analysis conducted with IC clustering methods showed MS patients with the lowest PASAT scores to have significantly reduced theta power, relative to the MS patients with the best PASAT performance. This effect was visible in widespread brain source regions. Longitudinal analyses also provided support for the link between P3 ERPs and cognitive functions when measured with the PASAT. The 12-month change in both visual and auditory P3a was associated with the change in the PASAT score.

8.2. Differences in cognitive-indexing electrophysiological activations between multiple sclerosis patient subgroups

With MS-related deterioration, there is no single uniform course of cognitive function, and many of the studies report individual patients to perform below their estimated premorbid levels at follow-up, yet this is not always seen at a group level (Jennekens-Schinkel, et al., 1990). Interestingly, some of the studies (e.g. Kujala, et al., 1997), have suggested that the portion of MS patients manifesting early signs of cognitive problems, typically develop the most cognitive deterioration over the disease course. On the other hand, the patients with no cognitive concerns early in their disease course, usually remain somewhat stable in their cognitive performances. Some researchers have linked this phenomenon to the concept of cognitive reserve; MS patients with larger cognitive reserve withhold the signs of cognitive deficits, more so, than those who possess limited cognitive reserve, at the start of the disease progression (Arnett, 2010).The notion of cognitive reserve in MS is also suggested as a reason why only one third to one half of the variance is explained in the strong correlations between the brain MRI measures and the cognitive performance of the patients. The MS patients with a larger cognitive reserve would thus have behavioural adaptation or compensation, which enables improved cognitive performance in the setting of cerebral disease (Benedict & Zivadinov, 2011). This improvement was demonstrated by recent research, where the correlation between atrophy and cognitive performance, was shown to be insignificant with high-level cognitive reserve MS patients, whereas it was robust in low intelligence MS patients

(Sumowski, et al., 2009). Reports of increased cerebral activity in response to cognitive tasks, (which is correlated with improved cognitive performance), are in favour of a cognitive reserve hypothesis (Chiaravalloti, et al., 2005). Furthermore, a longitudinal study has reported a substantial decline in patients with low cognitive reserve, but no remarkable change of cognitive functions in patients with high cognitive reserve. This is particularly apparent in the speed of information processing (Benedict, et al., 2010). In conclusion, high premorbid levels of cognitive functioning appear to protect against the progression of cognitive impairment in MS.

The relevance of the conventional subtype classification to the cognitive status of the patient is unclear. The longitudinal neuropsychological studies agree that the patients with a progressive disease course, generally exhibit more cognitive decline compared to patients in the relapsing-remitting phase (Benedict & Zivadinov, 2011). As shown in the studies in Chapters 4 and 5, the P3 ERP measures did not reveal differences at baseline or over a 24-month period, between RRMS and SPMS patients, on either early or later cognitive-indexing P3b amplitudes or latencies. Nevertheless, SPMS patients displayed prolonged visual P3a latencies over the parietal scalp areas, when compared to RRMS patients at baseline. The PPMS patients displayed decreased auditory P3b amplitudes in comparison to other progressive MS subtype SPMS patients. These observed differences between the MS subtypes, could be related to the level of difficulty in the P3 ERP task performed.

The visual P3a task was considered to have a difficult stimulus discrimination task; which typically causes a subtle delay in the latencies of the healthy controls. The visual P3a task may have been too challenging for the information processing functions of the SPMS, as they have more brain abnormalities affecting these functions compared to the RRMS patients. Similarly, it was suggested that the observed difference between PPMS and SPMS patients in auditory P3b amplitudes was caused by the low level of difficulty in the task, which increased the variation of the amplitudes, making a difference between the groups observable. Whereas, the other tasks presented more demands on information processing, and produced a similar level of dysfunction in both progressive MS groups.

The lack of clear, consistent differences between the MS subtypes on P3 ERP measures, suggests that the disease process itself (e.g. demyelinating lesions in processing pathways), impacts on P3 ERP amplitude and latency, rather than the severity or duration of the disease. It is also possible that the subtype classification is somewhat outdated and that other means of grouping MS patients into subtypes, should be considered as more reflective of their

symptoms and disease course. For example, it may be suggested that PPMS is a distinct neurological condition due to its clearly defined disease progression and clinical features. The recommendation of an update in the MS subgroup classifications may also be supported by the recent debate around two theoretical accounts of its origin. MS can be seen to be more of an autoimmune disorder or in contrast, more a result of neurodegenerative processes. The two conflicting theories and evidence of the cause of MS, suggest that a more complex classification system should be designed, to account for the large variety in clinical presentations of MS. This heterogeneity is also apparent in the cognitive functions in MS. Benedict & Zivadinov (2011) suggest two distinct aspects related to the cognitive disorder in MS. Firstly, it can be an inflammatory and demyelinating process found in all patients, or secondly, a neurodegenerative process that is strongly related to neuropsychological compromise, and is seen in many, but not all MS patients.

One potential and a more objective criterion, could be to apply the genetic information of the MS patients, such as OAS1 genotype (described in Chapter 2-Literature Review), to define specific groups of MS patients that have similar disease activity, severity, course and pathological changes. As part of the current research, in Chapter 4, the MS patients were tested for their OAS1 genotype, which was then utilised to divide them into AA and AG subtypes. Previous studies revealed the AA MS patients to have more anti-inflammatory disease activity than the other AG and GG MS patients. It was also revealed that the AA genotype occurs at higher frequencies in MS patients, compared to the large healthy control sample (O'Brien, et al., 2010). Accordingly, the AA MS patients of the present research displayed signs of an impaired cognitive profile. The patients performed significantly worse in the PASAT and had prolonged auditory P3 ERP latencies in comparison to the AG MS group. Notably, auditory P3b latency proved to be a good indicator of cognitive function, when it was associated with the PASAT score.

8.3. The relationship between P3 ERP task properties and the P3 ERP findings in multiple sclerosis: the role of modality

Previous research literature (De Sonneville, et al., 2002; King & Nelken, 2009), has also suggested, that with differing neuronal pathways, there is a modality-specific effect on P3 ERPs in MS. In the majority of studies in this research project, visual modality proved to be more sensitive in detecting latency delays and amplitude reductions in the P3 ERPs, than auditory modality. Remarkably, MS patients also presented with similar source EEG activities

across the modalities and across widespread brain areas. In short, the Chapter 4 studies showed reduced visual P3b and P3a amplitudes and delayed visual P3b and P3a latencies cross-sectionally, in a relatively large group of RRMS and SPMS patients, as well as in a sample of MS patients at progressive MS stage.

In the longitudinal analysis presented in Chapter 5, visual P3 ERP amplitudes were the tasks most sensitive to change. It was also observed, that the auditory P3 ERPs deteriorated in one year and were most visible with auditory P3a latency delay. Auditory P3 ERPs did not show similarly consistent effects, as only auditory P3a latency showed delay in the RRMS and SPMS group, and auditory P3a amplitude in the progressive MS sample. Nevertheless, the auditory P3 ERPs showed promising results in detecting subtle differences between MS subgroups. The PPMS patients displayed more reduced auditory P3b amplitude; relative to age-matched SPMS patients. Likewise, the patients with AA genotype of OAS1, had prolonged auditory P3 ERP latencies in comparison to patients with the AG genotype, which is known to cause a decrease in disease activity (O'Brien, et al., 2010).

Consequently, one can propose that the visual modality is more sensitive at differentiating between MS patients and controls. This may be due to the inclusion of less adversely affected RRMS patients, who had presented with very few P3 ERP latency delays or amplitude reductions. However, the subtle effect of MS disease progression on cognitive functions is visible in the visual modality, which may be attributed to the widely spread lesions that are already affecting the fibrous part of the visual processing system in the brain. These white-matter tracts are longer than those in auditory processing, and are therefore more readily affected by widespread lesions typical in MS. In turn, it can be inferred, that most of the white-matter fibres involved in auditory processing remain largely intact at the early stage of the disease. As a consequence, it is also plausible to suggest that the RRMS patients do not show as much deterioration in the speed of information processing as the SPMS patients. This became apparent in the present research and was revealed by a large delay in the visual P3a latency of the SPMS patients.

Accordingly, once the disease has progressed and the adverse brain changes (including demyelination and axonal degeneration), affect more auditory processing areas, the progressive MS patients have similar levels of dysfunction in the visual processing. Nevertheless, the auditory P3b task is able to show, with easily distinguishable stimuli, that PPMS patients have a larger decrease in auditory P3b amplitudes, relative to the SPMS patients. One can suggest, that the differences seen in the auditory P3 ERPs may be due to the

synaptic relays involved in early auditory processing (King & Nelken, 2009). They are therefore more affected by demyelination in PPMS patients and in MS patients with AA genotype of OAS1, which in turn delays the transmission of the later auditory information related to cognitive processes.

Similar predominance was shown in the visual over auditory modality, in a previous P3b ERP study by van Dijk, et al. (1992). They found MS patients to have considerably delayed P3b latencies relative to healthy controls in the visual modality, whereas the same groups had more or less similar P3b ERPs in the auditory task. Accordingly, some of the neuropsychological studies have confirmed this modality discrepancy, by reporting MS patients as displaying a poorer performance in neuropsychological tasks, which require mostly visual processing (De Sonneville, et al., 2002). The findings of the neuropsychological studies do not generally compare the modality effects, but instead, they stress the deficits some MS patients show in speed of processing, attention, executive functions and (working) memory (Rogers & Panegyres, 2007). For instance, as part of the present studies presented in Chapter 4, the performance in the auditory presented PASAT task was demonstrated to correlate repeatedly with visual P3a amplitudes across the studies. The auditory P3 ERPs presented with a more variable cognitive pattern; as auditory P3b latency correlated with the PASAT scores, in the relatively large group of MS patients and healthy controls, and with a small group of OAS1 genotyped MS patients. In the progressive MS group, the PASAT score was related to both auditory P3b and P3a amplitude. The one year change in the PASAT score was associated with the change in auditory P3a amplitude in a sample of MS and healthy controls.

However, the proposed auditory modality effect is in contrast to the 24-month longitudinal study. That study showed the demographically corrected PASAT scores only correlated with visual P3b amplitudes at Month 12. Even though the uncorrected raw PASAT scores were found to be in line with the prior cross-sectional result, as they were found to be related to auditory P3b amplitudes at Month 0 and auditory P3b latencies at Month 12. Thus, it may be possible that the earlier results of significant correlations between auditory P3 ERP parameters and uncorrected PASAT scores were affected by intervening factors, such as the age and education level of the subjects. Emphasising this impact of demographic variables on the early correlational analyses, the preliminary results reported in Chapter 6, revealed almost exclusively that only the visual P3 ERPs are associated with the neuropsychological performance of the subjects. The tests are generally regarded as the most sensitive and specific to the cognitive deficits in MS, the SDMT and the PASAT (Forn, et al., 2011). They are most strongly associated with the prolonged visual P3b latencies over parietal areas and visual

P3a latencies over central scalp regions. This key finding indicates that the visual P3 ERP latencies, not the auditory P3 ERPs, to be the most sensitive in indexing cognitive functions in MS.

It can be argued that the modality specific differences in P3 ERPs may be due to the distinctiveness of the stimuli utilised in the studies, with more similar stimuli properties increasing the difficulty of the task. It is possible that in our studies the auditory stimuli were not sufficiently similar, which would have made the auditory P3b and especially the auditory P3a ERP task less difficult to perform. It would have also placed fewer demands on attentional capacities and memory processes, than the visual P3 ERP tasks. A faster ERP to auditory stimuli may indicate that it was easier to discriminate between auditory target and non-target stimuli, as P3a non-target latency has been shown to increase when targets are harder to discriminate from standards) (Polich & Comerchero, 2003). It is also possible that the white-noise burst, used as an infrequent non-target in the auditory P3a condition, was not as salient as the checkerboard in the visual condition. In general, previous studies have shown that the P3a to auditory stimuli is typically faster than the P3a ERP to visual stimuli, and the control data in the present research are comparable to data using similar paradigms (Katayama & Polich, 1999). On the other hand, van Dijk, et al. (1992) modulated the distinctiveness of the stimuli in both visual and auditory P3b tasks, but could only find significant differences in the visual modality; not in either the easy or the hard auditory condition. Their finding supports the proposition that deficits in subtle information processing and cognitive functions indexed by P3 ERPs, are in fact more apparent in visual modality and are not just due to stimuli distinctiveness and/or task difficulty. Therefore, it is difficult to exhaustively determine if the modality differences were affected by the fact that the auditory P3 ERPs were less sensitive to subtle cognitive-indexing changes, or if they were due to the differences of the stimuli properties inherent in the paradigms. Future studies employing P3 ERP paradigms with clinical populations, should take the stimuli properties into account when designing new study procedures. Moreover future research should include a sufficient number of pilot studies, in order to determine the most suitable task features for the patient population in question.

The longitudinal 12-month study in Chapter 5, was in accordance with the prior findings, as the visual P3a was implied to have a higher level of difficulty, compared to the P3b ERP tasks, as reported in previous studies (Polich, 2007). Therefore, the amplitudes at Month 0 may already be at such a low level, that a further decrease was difficult to measure at Month 12. More difficult standard/target discriminations are reflected in later P3 onset (Comerchero & Polich, 1998; Kok, 2001; Polich, 2007). Greater standard/target discrimination difficulty in the

visual P3a task, in comparison to the visual P3b task, resulted in longer P3a onset. Therefore, significant differences were later for visual P3a in contrast to visual P3b. The subsequent longitudinal study, including an additional session at Month 24 in Chapter 5, found the modalities to be more related to either latency delays or amplitude reductions of the P3b ERPs. Visual was highlighted to decrease in amplitudes and auditory and to increase in latencies. One explanation of these findings, is that the adverse changes in the processing of the auditory information are reflected in the P3b ERP time course, whereas, the changes in the visual processing are manifested in the reduction of neural resources allocated to a cognitive process. These inferences are based on the previous theoretical distinction, stating that the latencies and sequence of ERP waves are related to the time course of processing activity. Whereas in contrast, the amplitudes of ERP waves indicate the amount of synchronised neurons and neural resources participating into specific cognitive processes (Polich & Herbst, 2000).

No consistent differences could be detected between the two cognitive-indexing electrophysiological paradigms; the P3b and the P3a, although it is clear that they measure slightly different brain and cognitive processes. Possibly the clearest difference between the P3 ERP tasks were their association with cognitive performance measured with the PASAT. The visual P3a amplitude correlated consistently with the PASAT scores in cross-sectional and longitudinal samples of MS patients and controls and with a sample of progressive MS patients.

8.4. The associations between P3 ERP findings and pathological brain changes in multiple sclerosis

The various changes found in the P3b and P3a ERP components in MS are most likely due to demyelination and axonal degeneration, which causes the disruption of the cortico-cortical and cortical-subcortical neural connections (Leocani, et al., 2010). In the multimodal investigation, the delayed and reduced P3 EEG activations were hypothesized to be associated with increased lesion load. The baseline investigation of the correlations between the P3 ERPs and lesion loads in various brain regions in Chapter 6, found the increase in the visual P3a latencies and amplitudes to be most related to the increase in lesion load in multiple and extensive brain areas, including the temporal, parietal, prefrontal lobes and the right hippocampus. This finding implies that in contrast to the P3b, which seems to be mainly affected by temporo-parietal junction disruptive lesions, the P3a responses are compromised

in patients with a variety of disruptive lesion sites, including ones in the medial temporal, frontal, and parietal lobes (Linden, et al., 1999). Therefore, the P3a may be more suitable than the P3b in detecting CI-related brain dysfunctions in MS, in which patients typically have widespread lesions.

On the other hand, there were also some correlations between visual P3b and auditory P3 ERPs and lesion loads in different brain regions. The increase in the visual P3b latencies was associated with an increase in the lesion loads of more specific brain areas such as the right corpus callosum, left insula and the right precuneus. Also, the auditory P3b and P3a latencies were correlated with a number of brain regions, namely occipital lobe, frontal gyrus, left hippocampus and putamen. Nevertheless, due to the correlational nature of the analysis, it cannot be stated that these increases in the specified brain regions, directly caused the observation in the P3 ERP latency prolongations and/or amplitude reductions. Although the preliminary correlational results may be thought to implicate the involvement of these brain areas in the changes encountered in the P3 ERPs in MS, these findings require further, more comprehensive examination.

The findings of the cross-sectional P3b study included in Chapter 4 are also consistent with the predominant view that the latency prolongation and amplitude reduction are most apparent in the MS patients with a progressive disease course (Leocani, et al., 2010). The findings of this study also go further and extend the understanding of brain and cognitive function in MS. In the first cross-sectional study examining the preceding ERP components of the P3b, the early sensory components were clearly delayed and reduced in MS patients, which was particularly evident in the N2 latency and the auditory condition. The latency delays and amplitude reductions in early sensory components are in accordance with the theoretical accounts. These accounts attribute their cause to be demyelinating lesions in the primary afferent pathway, leading to prolonged cognitive-indexing N2 and P3 components (Magnano, et al., 2006). This underlying mechanism is thought to be similar to the 'subcortical dementia' seen in Parkinson's disease and Huntington's disease (Gil, et al., 1993; Magnano, et al., 2006). Furthermore, the N1 amplitude was significantly greater for SPMS patients than for controls. An N1 amplitude increase, in conjunction with a P3b amplitude decrease, has been reported in older subjects during an auditory oddball task (Anderer, et al., 1996). This feature has been attributed to a higher level of general attention in older adults during the oddball task. In reference to the auditory N1 component, it is plausible that a similar compensatory mechanism is implied in MS subjects.

Other studies reporting deficits only in the later N2 and P3 waves (Gil, et al., 1993; Honig, et al., 1992; Newton, et al., 1989; Onofrj, et al., 1991; Piras, et al., 2003; Triantafyllou, et al., 1992; van Dijk, et al., 1992), are in favour of the view that the cognitive dysfunction is due to abnormal processing at the cortical level, as the higher cortical areas are thought be disconnected from subcortical regions, due to demyelinating plaques and axonal degeneration (Honig, et al., 1992; Magnano, et al., 2006; Piras, et al., 2003). Sailer, et al. (2001) reported visual N1 and N2 peak amplitudes to be reduced in MS patients with high lesion volume. This study also found a significant correlation between visual N2 amplitude and lesion load. These results agreed with the views that lesions cause partial disconnections within widespread cortical networks. In a wider context, it has been suggested that the prolongation and reduction of the early sensory components are intrinsically part of the electrophysiological profile of neurological patients with cognitive deficit (Giesser, et al., 1992).

The findings from the source EEG analysis with IC clustering method, included in Chapter 4, offer more support for this view that the cognitive impairment in MS is due to disruption of cortical-subcortical, in addition to cortico-cortical neural connections caused by demyelination and axonal degeneration. The abnormalities were observed in the oscillatory activity of the low-frequency bands theta and alpha, of which, generation and synchronisation is thought to be dominated by subcortical structures, in particular the thalamus. Whereas neural synchronisation in the high frequency range (gamma and beta) seem to be largely mediated by cortico-cortical connections. These connections reciprocally link cells within the same cortical area and are distributed across different areas, even hemispheres (Uhlhaas & Singer, 2006).

Cognitive impairment can occur in the absence of physical disability and vice versa, but the physical disability can have an effect on the cognitive function in some MS cases (e.g. Thornton & Raz, 1997). Some of the researchers have even implied that the cognitive impairment may have a use in predicting disease progression (Portaccio, et al., 2009). The relationship between physical disability and P3 ERP measures remains unclear. The studies in Chapters 4 and 5 did not find associations between the physical disability indicated by EDSS scores and P3 ERPs, with a large sample of RRMS and SPMS patients. However, a link was found between the disability level of progressive MS patients and auditory P3 ERP measures. Furthermore, the change in EDSS score, was associated with the change in P3 ERPs, and markedly so in the P3a component and in visual modality.

8.5. The utility of novel VESPA method to probe and monitor visual dysfunction in multiple sclerosis

A further aim of the present research project, (as per Chapter 7), was to investigate if a novel method to probe visual dysfunction, the VESPA (Lalor, et al., 2006), would indicate visual deficits in MS patients. In addition, it was examined if the VESPA can also monitor changes in visual functioning over a 3-year period. In general clinical practice, pattern-reversal visual evoked potentials (VEPs) are recorded from the suspected MS cases, as one of the most common first presenting symptoms of MS is optic neuritis. The VEPs are also included as an additional confirmatory test for the diagnosis of MS in the widely accepted revised 2010 McDonald diagnostic criteria (Polman, 2011). Some studies have even found it to be a more sensitive diagnostic tool than the gold standard MRI scans (Ko, 2010). The VEPs, however, are a rather inefficient way to collect data concerning the function of the visual system in MS, as they present the stimuli periodically to the subject, and the brain responses are later averaged during the data processing stage. Thus causing potential loss of informative data. Due to this intermittent presentation, acquiring sufficient amount of data requires hundreds of repetitions, which increases the testing time to up to as much as an hour. It can also become a tiresome and inconvenient experience for the neurological patients being tested. Particularly if they already have compromised attention processes. In contrast, the VESPA takes only a few minutes to conduct and it may provide a more expanded and detailed view of the visual processing, as the visual stimulus is continuously modulating over time.

The VESPA-like P100 component was hypothesized to be reduced and delayed at baseline, and show over a 24 month timeframe, a gradual delay in latency and reduction in amplitude in MS patients in comparison to healthy controls. The present research found evidence to support the clinical value of the VESPA as a reliable and robust measure to probe visual function in MS. The VESPA-like P100 latencies were found to be delayed in MS patients in comparison to the controls, both at baseline and over the 24-month timeframe. The effect was most pronounced in the SPMS patients. Further confirmation of the advantage of the VESPA method, was provided by a finding that the MS patients with a clinically recorded history of visual dysfunction presented with reduced VESPA-like P100 amplitudes and delayed VESPA-like P100 latencies, compared to MS patients with no history of visual dysfunction. The reproducibility of the test was confirmed by the lack of significant changes over a 3-year period. The VESPA-like P100 component latency and amplitude were also associated with the physical disability of the MS patients. The clinical potential of the VESPA method needs to be further validated with

a study comparing the responses extracted from the standard, clinically tested VEP paradigm and the VESPA in a large, representative MS population. In addition, the similarities and differences between the P100 responses acquired with these different methods, demand further research as discussed in the Discussion in Chapter 7. Furthermore, in the future, the clinical merit of the VESPA method may be enhanced with the inclusion of a simple, low-density electrode array. The findings from the comprehensive high-density EEG studies of the VESPA-like P100 response and its topographical distribution in MS, will indicate the required number and optimal locations of the electrodes.

8.6. The advantages of sophisticated EEG data analysis methods, statistical parametric mapping and independent component analysis methods, over the classical scalp-based EEG analysis

A great majority of the previous electrophysiological studies, investigating the cognitive and brain function in MS, have utilised a classical scalp-based, averaged ERP approach (Magnano, et al., 2006). A small number of these studies (e.g. Honig, et al., 1992; Onofrj, et al., 1991) have reported topographical results. However, these results have been subjective evaluations of the abnormality of the individual and/or averaged scalp-topographies of MS patients. As described in detail in Chapter 3-General Methods, the present research project included a relatively new method, i.e. statistical parametric mapping, which allowed statistical analysis of the topographical distributions of amplitudes between the groups. As MS patients commonly have lesions in various brain locations, their electrophysiological activities on the scalp may be affected. For example, instead of the typical (centro-)parietal distribution of P3b, patients may show an altered distribution of the activations on the scalp. Thus, the conventionally utilised ERP methods recording and comparing activations only from one, or a restricted number of electrodes, do not take into account this fact. These conventional methods can potentially lose information, due to inappropriate electrode placement, so leading to false positive or false negative results.

Furthermore, the averaged ERPs do not reflect the full range of time-frequency properties underlying electrophysiological activities. The average scalp-based ERPs between groups, only capture that portion of the channel data that is phase-consistent at latencies, relative to the time-locking events, and therefore, loses information of the activities (Delorme, et al., 2012; Onton, et al., 2006). Whereas, the IC clustering method (presented in detail in Chapter 3-General Methods), aggregates ICs into functionally equivalent groups, and therefore estimates

the source of EEG activities across subjects and conditions. In addition, the ICA and IC clustering methods reflect more accurately the spatiotemporal features of the EEG activations. One of the main assumptions underlying scalp-based ERP analysis, is that the position of an electrode reflects the neural activities directly underneath it, and that the task-related EEG activations in the same location, would be similar across different subjects (Onton, & Makeig, 2006). However, the physical characteristics and projection orientations from the cortex to the scalp, differ across individual brains, which may contravene the assumptions inherent in an ERP analysis. Furthermore, the scalp-based EEG is a sum of the recorded channel activities originating from multiple, functionally distinct, neural sources and non-brain sources, and therefore overlapping both temporally and spatially on the scalp (Dale, et al., 2000; Delorme, et al., 2012; Hagoort, 2003). Instead of the scalp-based EEG analysis of the locally highly-correlated source signal mixtures, recorded at the two-dimensional scalp surface, the ICA decomposition is an analysis of the time courses and spatial 3-D source distributions of maximally temporally independent data sources whose separate patterns of projection, via volume conduction to the scalp sensors, are given by the ICA decomposition (Delorme, et al., 2012). Averaging is commonly conducted to reduce the noise in the scalp-based EEG data as part of the scalp-ERP analysis procedure. But averaging also leads to discarding data, and it is a relatively inefficient way to isolate and locate individual EEG source signals (Delorme, et al., 2012). Moreover, the ICA and IC clustering methods enable isolating EEG artefact sources, i.e. eye blinks, and muscle artefacts, and removing them. This then yields a more accurate representation of neural activity, compared to scalp-based ERPs with artefacts. The independent component analysis is regularly employed to remove artefacts from the EEG data (Groppe, et al., 2008; Nolan, et al., 2010), which results in the enhanced signal-to-noise ratio of the EEG data (Delorme, et al., 2012). This advantageous ICA approach was used in the studies to pre-process the EEG data (i.e. to remove artefacts), and was also applied in the investigation of the source P3 task-related EEG activities in Chapter 4.

IC clustering has the potential to offer a more thorough insight into how the changes in MS patients' brains (due to demyelinating lesions and cortical atrophy) affect the processing of the stimulus information obtained from the outside world, and so therefore the cognitive functions in general. These changes affecting the cognitive functions, such as sustained attention and working memory, may be mediated through compromised neural synchronisation. The ICA and IC clustering methods estimate the location of the activation (inverse source modelling). Whereas, scalp-based ERP assumes the recorded signals of the scalp electrodes to be comparable with results from equivalently placed electrodes for all subjects. Therefore, it does not sufficiently take into account the physical differences of the

brains of different people. For example, the differences in the orientations of cortical gyri and sulci can particularly cause diverse projections of exactly equivalent cortical sources (Delorme, et al., 2012; Onton, et al., 2006).

8.7. The benefits of the proposed cognitive-indexing electrophysiological measures relative to conventional methods, to detect and monitor brain and cognitive function in multiple sclerosis

A conventional clinical approach to cognitive dysfunction is to conduct a comprehensive neuropsychological examination, which can often be unavailable, be costly, or have interfering factors affecting the subjects' performance on these tasks, such as depression. Therefore, there is a demand for ancillary measures to complement the examination of the cognitive performance and related brain functions. Approaches such as cognitive-indexing electrophysiological measures, may provide additional information on the brain processes involved in the cognitive processes in MS. While at the same time avoiding some of the disadvantages of neuropsychological examination, such as practice effects (Barker-Collo, 2005). It may also avoid the impact of demographic factors such as education, required minimum interval times between two test sessions with same subject, the effect of hand, speech and/or motor dysfunction on the ability to perform the tasks (Magnano, et al., 2006). In addition, many patients dislike performing some of the most challenging neuropsychological tests such as the PASAT (Tombaugh, 2006), and should be considered especially important in the context of clinical trials in which repeated testing may increase the drop-out rate (Williams, et al., 2006).

The research conducted during the course of the present thesis, and the findings from the studies of Chapters 4, 5 and 6, found the PASAT performance to be associated to the P3 ERPs. The first cross-sectional study found the poor performance in the PASAT, as measured with the raw, uncorrected scores, to be associated with an increase in the auditory P3b latency at central and parietal sites. The lower PASAT scores, which were controlled for the effect of age, showed a correlation with a decrease of the visual P3a amplitudes over frontal and parietal areas in MS patients. The subsequent study with progressive MS patients, observed a correlation between the raw PASAT scores to and both visual and auditory P3b and P3a amplitudes over centro-parietal scalp regions. Similarly, the source EEG study found the MS

group with the poorest PASAT performance, to have reduced P3b task-related theta power in widespread brain regions, in comparison to MS patients with the highest raw PASAT scores.

The longitudinal studies, however, revealed somewhat discrepant findings. The 12-month study found a change in the raw PASAT score, to correlate with the change in visual and auditory P3a amplitudes over parietal scalp-areas. Whereas the preliminary results from the 24-month study found the demographically corrected PASAT scores and the P3 ERPs to correlate only at Month 12, while the raw PASAT scores and P3 ERPs were correlated at Month 0 and 12 but not at Month 24. A more comprehensive investigation of the 24-month change of both the PASAT and the P3 ERPs, including a greater number of subjects and comprising more advanced EEG analysis methods such as SPM, is warranted. Furthermore, the 24-month study found the MS patients to have a poorer outcome in the PASAT each year in comparison to the controls. Yet when the scores were corrected for age, education and sex this difference no longer existed. These somewhat inconsistent results stress the importance of demographic correction as part of the neuropsychological testing procedures, in order to improve the evaluation of the cognitive status of the MS patient. Moreover, also it is necessary to exclude the potential effects that demographic factors (particularly age) have on the neuropsychological performance. The observed correlations in the cross-sectional P3b ERP study are therefore, not entirely comparable with the subsequent studies, as the significant correlations of raw, uncorrected PASAT scores may well represent the effect that age, education and gender may have on the P3 ERP parameters.

The longitudinal 12-month study in Chapter 5 included a seemingly contradictory result. In particular, the groups did not show changes in their PASAT scores after 12 Months, but these changes still correlated with the P3a ERP amplitudes over centro-parietal areas. It was revealed that although the visual P3a variability was less than the auditory P3a, perhaps reflecting the difficulty of the visual P3a task, there was a sufficient change between Month 0 and Month 12 for a statistically significant correlation. To be more specific, the difficulty between the modalities is a function of perceptual distinctiveness of the standard/target discrimination, and not related to modality per se. Thus, the distinctiveness of the stimulus and the context (standard/target discrimination) largely contributes to P3a amplitude and the modality of the stimuli is less influential (Polich, 1987). Therefore, the visual P3a task presented was more difficult due to the stimulus properties (i.e. the standard/target discrimination was more difficult for visual compared to auditory stimuli). Nonetheless, there was a change for some subjects in visual P3a amplitude, over the 12-month period (see Figure 5.6.) and this change was sufficient for a statistically significant correlation.

The multimodal investigation presented in Chapter 6 examined the hypotheses that delayed and reduced P3 EEG activations would be associated with poor neuropsychological test scores. Results revealed that the visual P3 ERP latencies best index cognitive function in MS. The visual P3 ERPs were found to relatively strongly correlate with the neuropsychological tasks; commonly considered to be the most sensitive and specific for the cognitive functions in MS, while they also correlated with widespread brain regions. A functional MRI (fMRI) study (Mainero, et al., 2006) reported that, during execution of the PASAT in RRMS patients, there was bilateral activation of the inferior and middle frontal gyrus, anterior cingulate, inferior parietal lobule, supplementary motor area, superior and middle temporal gyrus, insula, thalamus, vermis and brainstem, as well as unilateral activation of the right superior parietal lobule and right lateral premotor area. These correlations of P3 ERPs with low neuropsychological test scores and lesion loads over widespread brain areas, may reflect the fact that the P3 ERPs, especially the P3a, involve large widespread brain regions, which when affected by lesions may have a deteriorating effect on the P3 ERPs and also the higher-order cognitive functions measured by the PASAT. As such it can be proposed that the compromised speed of information processing, due to demyelinating lesions, is reflected in the latency delay and amplitude reduction of the visual P3 ERPs, which eventually emerge on the higher processing level as cognitive difficulties. These results, which have yet to be further investigated and confirmed, indicate that the electrophysiological measures, especially in the visual modality, have utility in the detection of cognitive disturbances in MS.

The present research also proposed novel signal processing methods, detailed in Chapter 3-General Methods, offering a more comprehensive and informative view on the neurophysiological activities in MS. One of the main aims was to examine the source EEG activities during P3 ERP tasks, in addition to the traditional scalp-based and response averaged ERP waveforms. The IC clustering approach identified abnormalities especially in the low-frequencies in MS; showing less increase in theta band power and more reduced alpha band power 200-400 ms after the presented target stimuli, observed across a wide range of brain areas. In previous studies, spectral analysis of P3b has indicated that P3b amplitude is linearly related to delta and theta band spectral power (Spencer & Polich, 1999). Alpha band spectral power covaries with P3b, which indicates ERP to be related to attention and memory (Polich, 1997). Theta oscillations are also found to be related to information processing tasks which improve P3b task completion; especially those of sustained attention, high executive demands (Gevins, et al., 1997; Klimesch, 1999; Mizuhara, et al., 2004; Mizuhara & Yamaguchi, 2007), maintenance of working memory (Gevins, et al., 1997) and the encoding and retrieval of episodic memories (Klimesch, et al., 2006).

The extent of theta synchronisation has also been related to good working memory performance (Klimesch, 1999; Wang, 2010). The successful performance of the P3b task is dependent on these attention, memory retrieval and decision making processes. It has also been proposed that the parieto-prefrontal circuit is important for working memory, and that working memory and attention control may share a common cortical circuit substrate (Wang, 2010). This is in line with Polich's (2007) theoretical account of the neural processes underlining P3 ERPs. In addition, hippocampal theta rhythm is believed to play a role in the formation and retrieval of episodic and spatial memory. Theta rhythm in the neocortex was found to be enhanced in various neocortical sites during working memory. It appears to be particularly prominent in the frontal midline, including anterior cingulate cortex, a sub region of the prefrontal cortex implicated in behavioural monitoring, valuation of response outcomes and other aspects of cognitive functions (Wang, 2010). Moreover theta rhythm has also been suggested to be the brain area where P3 response originates (Polich, 2007).

Furthermore, slow alpha rhythm is implicated in suppressing irrelevant information in memory and attention tasks. Hence this links alpha rhythm to the P3 ERP processes, in accordance to Polich's (2007) inhibition hypothesis discussed in Chapter 2-Literature Review. Hence, at a given cortical site, alpha rhythm may be reduced when attention is focused on external stimuli processed by that area, but enhanced when the flow of sensory information to that area is behaviourally irrelevant and should be ignored (Wang, 2010). Differences in the beta rhythm may reflect differences in the motor system between MS patients and controls, as beta rhythm has been associated with the motor functions, especially with the preparation and inhibitory control in the motor system, and with sensorimotor integration and top-down signalling (Wang, 2010).

These findings support recent advances in neuroscience, which suggest that the brain functions as a distributed, parallel and recursive network, challenging the generally accepted principle of cortical localisation of function as a core concept of clinical neurology. Usually in clinical practice it is considered that specific functions are represented in certain parts of the brain and that brain injuries disrupt these localised functions, giving rise to corresponding behavioural deficits. Moreover it is anticipated that stimuli are processed in a serial fashion in separate brain areas performing different functions (He, et al., 2007). It can also be suggested that the damaged brain should be viewed as a whole new system, not simply as the old system minus the lesioned parts (He, et al., 2007). It is also important to remember that the neurological deficits do not simply reflect the primary effect of a lesion, but also the secondary effects of the lesion on other structures. This approach fits well with the study investigating

the source P3 ERP activations, as the MS patients exhibited reduced theta and alpha ERSP power over widespread brain regions. Thus damage in the brain was not observed in any specific source location, but more of a general reduction of the brain activations in a widespread brain network was revealed.

In addition, an interesting approach for future investigation would be to examine the MS patients with different 'new brain systems' of which some are more adaptive than others (for example due to higher cognitive reserve). The degree of how adaptive the new brain system is could be examined with similar methods as utilised in the present research. First the neuropsychological assessment could be utilised to determine the premorbid cognitive status and the present level of cognitive functioning. These findings could then be mirrored to the MRI findings, such as lesion load, to examine the level of adaptation of the brain system. For instance, the new brain system would be considered adaptive if there was evidence of widespread increase in lesion loads, while the neuropsychological performance remained stable and/or at similar level to the healthy age-matched controls. The advanced high-density EEG methods could then be utilised to investigate differences in the spatiotemporal patterns of the brain activations related to cognitive functions, between the MS patients with and without adaptive brain systems.

Nevertheless, this approach is incongruous to some extent with the correlational baseline study conducted in Chapter 6, but in fact may be more revealing with regards to the mechanism depicting how the electrophysiological changes, affect cognitive performance. Thus, the network view of the brain should be considered more often when conducting the correlational studies. Yet the observed correlations between visual P3 ERPs and lesion loads were widespread, which implicates theoretical accounts regarding the brain as one system. In particular, in which the processing of the incoming information is conducted by synchronisation of various brain areas, not just merely by serial, non-simultaneous processing, occurring in separate brain areas.

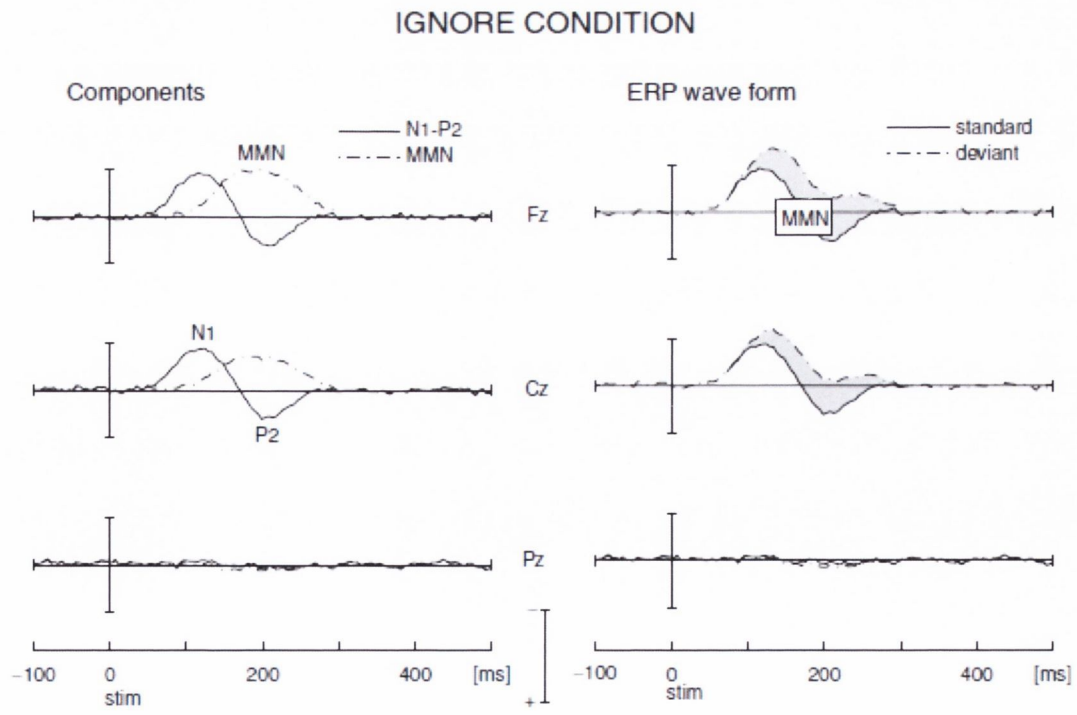
8.8. The potential utility of electrophysiological methods to assess brain and cognitive function in other neurological conditions

The present research project investigated the utility of EEG and ERP methods to detect and monitor cognitive and brain function in MS, however, similar EEG and ERP methods can also be applied to other neurological conditions. For example, similar reduction of alpha- and beta-band synchronisation compared to healthy controls, during maintenance of information in working memory was reported in Alzheimer's disease patients (Pijnenburg, et al., 2004). Similarly, findings from multiple studies imply that P3 latency differentiates between subcortical and cortical dementia, dementia and depression and early Alzheimer's disease and normal aging. Reduced auditory and/or visual P3b amplitudes have been reported in schizophrenia, alcoholism and traumatic brain injury. Reduced P3b amplitude and delayed P3b latency results have been found in people with bipolar disorder, yet the results have not shown consistent deviations for depression relative to healthy controls (Duncan, et al., 2009). Furthermore, changes in event-related potentials are shown in many childhood disorders such as attention-deficit/hyperactivity disorder, autism, dyslexia, auditory processing, language impairments and psychiatric conditions (Duncan, et al., 2009). The intra-subject test–retest correlation coefficients for the P3b compares favourably with clinical assays; 0.50 to 0.80 for amplitude and 0.40 to 0.70 for peak latency (Segalowitz & Barnes, 1993).

In addition to the P3 paradigm, other ERP paradigms, such as mismatch-negativity (MMN), have been utilised to investigate cognitive and brain function in neurological conditions (Duncan, et al., 2009). The (auditory) MMN is a negative event-related potential (ERP) with the highest activity showing over fronto-central scalp area at around 100-250 ms (Garrido, Kilner, Stephan, & Friston, 2009; Näätänen, Kujala, & Winkler, 2011). In a typical MMN task, subjects listen to auditory stimuli of which they are asked not to attend (See Figure 8.1). Frequent standard stimuli elicit a negative peak N1 around 100 ms, reflecting early auditory processing at the primary auditory cortex. However, when the repetitive stimulation is interrupted by a stimulus audibly different from the preceding ones, an enhanced negative potential around 100-200 ms is visible. MMN is typically reported as a difference wave as it becomes clearly visible only through a subtraction procedure (Näätänen, et al., 2011). MMN process is thought to reflect subtle attentional processes (i.e. the automatic switching of the organism's attention to auditory change) (Näätänen, et al., 2011).

Based on the findings with various neurological populations, it was investigated whether the MMN can be utilised as a neurophysiological marker, to aid the early differential diagnostics between two common neurological conditions sharing similar symptoms, namely the early Parkinson's disease (PD) and essential tremor (ET). Parkinson's disease (PD) is one of the most common neurodegenerative diseases and its diagnosis is usually based on clinical features, which include tremor (for a comprehensive review see Lees, Hardy, & Revesz, 2009). The differential diagnosis of PD and essential tremor (ET) can be challenging, and ET is commonly misdiagnosed as PD, especially when the tremor is of large amplitude, starts in old age and continues into the resting state (Lees, et al., 2009). The fact that some patients with PD have dementia with deficits in attention and that ET is characterised with physical symptoms (excluding cognitive impairment), offers a novel possibility for differential diagnostics. The MMN is an index of subtle attentional effects, which has shown to be impaired in PD (Lees, et al., 2009). The MMN is typically largest over frontal areas (Näätänen, et al., 2011), which opens up the possibility of early identification of PD patients at risk of dementia. Since there is growing evidence that subtle frontal lobe deficits may hold prognostic value in identifying PD patients at risk of dementia (Woods & Troster, 2003).

Figure 8.1. The mismatch negativity (MMN) and associated event-related potential components in non-attended task (Näätänen, Kujala, & Winkler, 2011).



A preliminary study was conducted with 9 Parkinson's disease (PD) and 11 essential tremor (ET) patients, in collaboration with the Department of Neurology in St. Vincent's University Hospital. Based on the literature showing PD patients to experience dysfunction in attention (Lees, et al., 2009) and the MMN to measure subtle attentional changes (Näätänen, et al., 2011), it was hypothesized that the PD patients would show a reduced scalp-space and source MMN activations, in comparison to the ET patients. Neither of these approaches revealed statistically significant differences in MMN profiles of the two groups. The lack of differences may be due to the small group sizes, which reduce the statistical power of the analysis, but it is also likely that the MMN paradigm utilised was not a sufficient method to find differences, as no clear trends were present in the data. The MMN paradigm or the selected stimulus features may not have been optimal in order to detect differences between the two samples of neurological patients. It is also possible that the subtle cognitive difficulties, present in the early PD disease course, may not have been sufficiently targeted with the MMN. From a signal-processing point of view, it can also be noted that electrophysiological measuring is technically highly challenging, with patient populations presenting with substantial motor impairments, such as tremor, which introduces large motion artefacts to the data. In fact, large motion artefacts were observed in the data for the majority of the PD and ET patients. Therefore, the data pre-processing methods utilised (both manual procedure and FASTER method described in Chapter 3-General Methods) may have not removed a satisfactory amount of the artefacts, which has subsequently lead to a reduced signal-to-noise ratio. The MMN is often regarded as a not easily detectable ERP component, and may have thus had small amplitude, therefore not revealing differences between the PD and ET patients. The sample sizes of the groups were also small, which may have reduced the statistical power of the analysis and added to the lack of observed differences. However, more studies are required to determine if the MMN may have the potential to be utilised in clinical research, in addition to neuropsychological tests and especially in trials seeking new drug therapies for cognitive dysfunction in PD.

8.9. The advantages and future potential of electrophysiological measures in the study of neurological conditions

The advent of MRI and other neuroimaging methods has significantly reduced the clinical utilisation of EEG in MS (Polman, et al., 2011; Polman, et al., 2005). However this is hard to comprehend, as the information provided by EEG is quite different from that provided by MRI. EEG measures action potential conduction, along central, visual, auditory, somatosensory and

motor pathways, and therefore, assesses the consequences of axon demyelination and degeneration in MS (Ziemann, et al., 2011). Despite the recent surge of highly advanced brain imaging technologies, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) offering high spatial resolution and ability to detect cellular activity in subcortical structures of the brain, EEG measures still have various major advantages. Most importantly, with the higher time resolution, the EEG (and MEG) can detect changes in brain activity faster than the newest brain imaging technologies. EEG is a direct reflection of the summation of cortical postsynaptic potentials, which is a distant reflection of the brain's inner workings (da Silva & Spekreijse, 1991), whereas recent brain imaging methods merely measure the cell metabolism of neurons (blood flow, blood volume, oxygenation) (da Silva & Spekreijse, 1991).

In addition to these benefits, EEG technology also has in its favour psychological and physical convenience, low cost, portability and a long history of investigation spanning over eighty years (Millett, 2001). Measuring EEG involves wearing a simple cap or string of electrodes and it is commonly acquired from young children and neurological and psychiatric populations, which are likely to find noisy fMRI and PET machines frightening and intimidating. Furthermore, the future of EEG looks promising as advances in telemetry and dry electrodes, have the potential to eliminate the inconveniences related to measuring EEG. When considering the expense, the EEG is much more affordable in comparison to the newest technologies (MRI, fMRI, PET, MEG). In recent years simultaneous EEG and fMRI (co-registration) has gained in popularity as it can build on and complement the strengths of both approaches. However, at present it still requires further development before it can become a viable method for clinical practice and clinical research.

Importantly, the role of synchronisation of neural networks should be more comprehensively investigated in various brain disorders. Most of the research on electrophysiological correlates of pathological brain states, have focused on the analysis of power distributions in various frequency bands, of resting-state EEG activity (either eyes-open or eyes-closed) or on the changes seen in averaged ERP components. Nevertheless limited research has investigated task- or performance-related synchronisation phenomena in brain disorders (Uhlhaas & Singer, 2006). Distinct abnormal changes in (task-related) neural synchrony, most likely play an important role in not only the cognitive dysfunction, but also the overall pathophysiology of brain disorders, such as schizophrenia, Alzheimer's disease and autism (Uhlhaas, et al., 2009; Uhlhaas & Singer, 2006). These conditions involve the reduction of both local and long-range neural synchronisation. Therefore, the cognitive dysfunction with the other overt symptoms of

these disorders arises from dysfunction in the coordination of distributed neural activity, between and within functionally specialised regions in the cerebral cortex. Reduced neural synchrony can be the consequence of a disconnection of these brain regions, but it can also be the cause of impaired coupling between brain areas. In particular, as synchronisation is required, for the efficient propagation of neural responses across sparsely connected networks (Uhlhaas & Singer, 2006).

The EEG methodology enables the flexible utilisation of a wide range of different task paradigms, of which the P3 paradigm is one of the most investigated. Future research could employ other similar task paradigms; targeting the subtle cognitive functions while measuring the brain activations. The information of the brain functions, related to cognitive dysfunction, acquired with the P3 and the alternative ERP task paradigms could be compared to find the most suitable and most effective ERP task design. This would challenge damaged brain networks in MS and/or other patients affected by a neurological condition.

As technology develops these advantageously, developed cognitive-neurophysiological measures may in the future be an auxiliary part of the standard diagnosis procedure for MS or other neurological conditions. The measures such as EEG have several advantages; namely non-invasiveness and objectiveness. These measures have increasing potential to act as valuable diagnostic tools. Specifically as the detection and monitoring of disease progression and the efficiency of therapeutic interventions has advanced, novel methods for time series analysis are being refined and developed (Uhlhaas & Singer, 2006). Moreover they can act as additional measures, complementing the in-person, comprehensive neuropsychological examination and brain imaging results. If the recording procedure becomes less cumbersome and time consuming, as the technology advances, these cognitive-neurophysiological measures can act also as initial screening methods for cognitive deficits. Future technological advances would enhance the applicability of EEG measures in clinical practice. These technological advances may include the dry electrodes, high-density EEG and the widely available and more effective signal-processing methods with easily approachable user-interfaces. One appealing opportunity would be to utilise similar technologies as biofeedback therapies, to test if the cognitive functions can positively affect an improvement in quality of life of the neurological patients suffering from these subtle but high-impact cognitive deficits (Uhlhaas & Singer, 2006).

8.10. The impact of the present research on the clinical research and clinical practice

The advanced electrophysiological methods examined, were shown to have the potential to be adapted as part of the clinical procedure. That is, to detect, and in some cases to follow the progression of, brain functions related to subtle cognitive processes of information processing speed, attention and working memory, and the existence of visual dysfunction in MS. The findings of the present research enhance the existing knowledge on brain and cognitive function in MS, as it includes a comprehensive, 3-year multimodal examination of a relatively large MS patient sample, with age-matched healthy controls. Furthermore, advanced signal processing analysis methods with high-density EEG, were utilised for the first time in the context of MS research.

EEG technology is already commonly employed as part of diagnosis in various neurological conditions; in MS to detect visual dysfunction, in epilepsy to detect the nature of the epileptic seizures and identify their cause and in sleep disorders to track the function of the brain to give indication of the underlying difficulties (Duncan, et al., 2009), to name a few. The cognitive-indexing electrophysiological measures of P3 and MMN ERPs have particularly favourable utility in clinical research of neurological conditions (Duncan, et al., 2009). They are well suited in the assessment of brain function related to cognitive performance, during the initial investigation of new emerging treatment options for different neurological conditions like MS or PD, to complement the information from neuropsychological batteries and from MRI or other brain imaging measures. The electrophysiological information can provide information on the brain functions related to cognitive dysfunction, and thus, complement the findings acquired with conventional neuropsychological examination and structural MRI scans. After clinical research has determined the scalp distribution, pattern and origin of cognitive-indexing ERP activations in patients with a neurological condition, the number of electrodes could be reduced and in clinical practice only a few electrodes would be required to be placed on the scalp.

The findings from the present research support the notion that P3 electrophysiological measures have utility in assessing and monitoring brain functions related to cognitive dysfunction in MS. They have desirable value especially in clinical research, and can be applied in their present form to study brain and cognitive function. Furthermore, the P3 electrophysiological measures have potential utility in clinical practice to complement

neuropsychological and MRI findings. The present research supports the development of these measures to be an auxiliary part of the clinical management of MS.

8.11. Electrophysiological measures and the cognition-specific drug therapies in multiple sclerosis

Although neuropsychological rehabilitation can improve attention, information processing and executive functions in MS patients with low disability levels (Mattioli, Stampatori, Zanotti, Parrinello, & Capra, 2010), there is a drive to find drug therapies that have positive effects on cognitive function in MS patients. The disease-modifying medications alter the cerebral demyelinating process, which may in turn be associated with slower decline of cognitive functions (Bobholz & Rao, 2003). On the other hand, this link has not been firmly established and the mix of MS patients with different treatments would have the advantage of giving a more comprehensive overview of the process of CI in MS. In the present research project, there was not adequate numbers of subjects in each treatment group, to be able to satisfactorily conduct statistically powerful analyses. Furthermore, studies examining the influence of drug therapies on the cognitive performance of MS patients should monitor their effect systematically; measuring the cognitive functions prior to treatment trial, during and after treatment with large age-, education- and gender-matched MS samples of equal size.

As drug therapies typically target specific neurotransmitter systems, which are involved in neural synchrony, the cognitive-indexing electrophysiological methods reflecting the function of neurotransmitters may have the potential to be part of the design process of these novel therapies. Although some of the studies have included the P3 ERPs, so far only one has reported changes in P3 ERP activations after treated with disease-modifying therapies (Flechter, et al., 2007). The VESPA method could also be utilised to measure the effect of drug therapies on visual function. Indeed one study employed this approach and found the VEP to be improved by a marked percentage of MS patients, after natalizumab treatment compared to the same patients measured during a run-in phase prior to treatment (Meuth, Bittner, Seiler, Gobel, & Wiendl, 2011). For instance, intrinsic theta or gamma oscillations in individual neurons appear to critically depend on voltage-gated sodium and potassium channels (Wang, 2010). Various neurotransmitter mechanisms have been implicated to underlie P3b generation; however these systems still remain unclear (Polich, 2007). Most of the studies indicate that, dopaminergic activity mediates the P3a link to frontal focal attention and working memory systems, whereas norepinephrine is related to P3b with temporal/parietal

activity. However, more human studies investigating the neurotransmitter systems underlying P3 ERP components are required, in order to improve the understanding of their neurophysiological mechanisms and yield important clinical applications (Polich, 2007).

There is growing evidence for abnormal cortical information processing in schizophrenia, to be associated with dysfunctions in cholinergic, dopaminergic and GABAergic neurons and neurotransmitter systems (Uhlhaas & Singer, 2006). It is possible that the irregularities in neural synchrony and also in the generation of neural synchrony, related to cognitive functions found in MS, can be linked to neurotransmitter systems. This may have invaluable implications for the future development of cognition-sensitive drugs for MS. EEG methodology has the potential to act as an indispensable metric during the development of more precise pharmacological interventions for neurological disorders such as MS.

8.12. Limitations of the present research

The present research had various limitations, which have to be taken into account when interpreting the results of these studies. Most importantly, some of the studies in Chapters 4, 5, 6 and 7 included a low number of subjects (especially the ones comparing different MS subgroups and/or including the data acquired at Month 24 into the analysis). In particular, the failure to recruit sufficient numbers of PPMS over the 24-month period was unfortunate, as the rare MS disease course requires a more in-depth examination. It can be proposed that with the inclusion of more MS patients with different disease courses in future research, it would be possible to reach a more comprehensive understanding. In particular in relation to the abnormalities and changes occurring over time in the cognitive functions present in MS patients, and to discover how these findings relate to the differing disease courses and physical disability. Accordingly, with the inclusion of a wide variety of patients in different disease courses it is important to investigate if the cognitive-indexing electrophysiological measures show reproducible pattern not only on the group level but also for each subject. The future studies could, thus, ascertain the utility of cognitive-indexing electrophysiological measures also at an individual level.

Furthermore, while some MS patients were treated with different disease-modifying therapies (DMT) potentially affecting their cognitive performance, others (particularly in the progressive disease course) did not receive any treatment for MS. As discussed earlier in this chapter, the effect the DMTs have on cognitive function in MS is still largely unknown and the issue needs

to be addressed in future research. Principally it would be intriguing to utilise the cognitive neurophysiological measures, in addition to the neuropsychological and brain imaging methods, to determine if they can provide supplemental insight into the topic.

The findings of the 24-month longitudinal study presented in Chapter 5 are considered preliminary. Future studies could utilise advanced EEG analysis methods, including SPM and source EEG analysis measures such as IC clustering and sLORETA. This would more accurately represent the group and time differences between MS patients and healthy controls. Moreover, a large number of MS patients dropped out from the study during the 24-month period, and it is plausible that the MS patients with most disability and/or cognitive impairment may have been the first ones to leave the study. Thereby, potentially distorting the results through insufficiently reflecting the whole MS patient population. It is vital to address these issues in future longitudinal studies on the cognitive function in MS, by for instance, making the participation to the study more accessible to MS patients with home visits or similar arrangements. Moreover, the change in P3 ERP activations in MS over a longer periods of time (such as three years) has not been studied before, although there are a few longitudinal studies employing neuropsychological and/or MRI measures up to a time period of ten years (Amato, et al., 2001). Thus, future studies focusing on the cognitive function in MS and spanning over three years should include cognitive-indexing electrophysiological measures into the study procedure, in order to gain a full view of the cognitive and brain changes occurring.

The effect of genetic factors on the cognitive function in MS was implied by a study in Chapter 4 showing MS patients with the AA OAS1-genotype (indexing increased disease activity) to have more prolonged and reduced P3 ERP components and poorer performance in the PASAT test, in comparison to the other genotype, AG OAS1. It must be acknowledged that it is not sufficient to examine the influence that only one potential genetic factor has on cognitive function in MS, one at a time, as done in this study and in the previous research literature (Cerasa, et al., 2010; Enzinger, et al., 2004; Ghaffar, et al., 2010; Jensen, et al., 2010; Manna, et al., 2006; Oksenberg, et al., 2008; Oliveri, et al., 1999; Parmenter, et al., 2007; Portaccio, et al., 2009; Savettieri, et al., 2004; Shi, et al., 2008; van der Walt, et al., 2009; Weinstock-Guttman, et al., 2011; Zivadinov, et al., 2007). Thus, the matter requires a thorough investigation, with a large number of MS patients tested on various different genetic factors. The cognitive-indexing electrophysiological measures provide a new, auxiliary method to examine the effect that genes have on the brain and cognitive function in MS, in addition to the neuropsychological and MRI measures.

The preliminary results from the cross-sectional multimodal study of Chapter 6, that found associations between P3 ERP, MRI and neuropsychological measures are promising, but need to be further examined with more advanced statistical and signal processing methods, such as SPM. This would enable a more accurate representation of P3 ERP amplitudes and topography in relation to neuropsychological scores and MRI variables. Similarly, the initial results from the 3-year P3 ERP study have to be confirmed with SPM to reveal the topographical distributions and differences between the subjects. In addition, advanced source analysis methods such as IC clustering and LORETA, may provide more significant information of the underlying electrophysiological activities and the brain locations where the activations of MS patients differ from those of healthy controls. One interesting approach would also be to apply a connectivity analysis of the data with measures such as diffusion-tensor imaging (DTI).

8.13. Future perspectives

To summarise, the main research objectives for the thesis, presented the thesis objectives in section 2.7. included in Chapter 2-Literature Review, were achieved and the electrophysiological measures such as P3 ERPs and the VESPA have potential for utility in clinical research and/or practice associated with the diagnosis and treatment of MS. The research highlighted that the VESPA has potential diagnostic use, but its value as a diagnostic tool will need to be confirmed with a study comparing the VEP and the VESPA responses. The P3 ERPs, especially in the visual modality, offer an auxiliary tool for clinical research to detect and monitor cognitive functions and the related brain processes. The advantages of cognitive-indexing electrophysiological methods are far-reaching and include: reliable and easier short-term replication, high temporal resolution, cost-effectiveness, high level of objectivity and less interference from subject-related factors such as mood disorders, physical disability and education. Due to these beneficial properties the inclusion of the cognitive-indexing electrophysiological measures into clinical practice is encouraged. It can be stated that these measures may hold utility in clinical practice, although this may be dependent on further development of the EEG methodology. At present, the sophisticated EEG data analysis and statistical procedures utilised in the studies of this thesis are not incorporated in clinical practice. Nevertheless, they hold promise as they could be utilised as part of clinical practice, if the data and statistical analysis processes could be fully automated and the results presented in a user-friendly manner to clinical professionals. For instance, it is plausible to create a clear and simple graphical user interface where the acquired EEG data can be entered for automatisation and advanced data and statistical analysis. The output from the EEG

analysis would be presented in the form of an easily approachable result summary with self-evident visualisations and text delineating the main differences (in comparison to the data of the healthy controls). The clinical professionals could then make use of the acquired information as part of their treatment plans.

Moreover, recently there has been an intense interest into the EEG frequencies during resting-state paradigms. However, these do not measure the activities associated with cognitive processes. EEG paradigms, similar to the P3 ERPs, which target specific cognitive processes, should be further developed by altering the paradigms, in order that they would be more sensitive to the specific cognitive deficits in different neurological conditions, such as MS. This type of EEG paradigms would be beneficial in clinical research on cognitive and brain functions in neurological and/or neuropsychiatric conditions. Novel, advanced methods have vast potential for similar research investigating brain functioning related to cognitive function in different neurological conditions. Relatively new methods have been developed to enable spatiotemporal analysis of multichannel EEG, i.e. the analysis of the spatial properties of the field topographies (Brunet, Murray, & Michel, 2010). Moreover, the ICA clustering method has since been made more adaptable by adding other K-means clustering algorithms such as the Affinity Product Method[10], and introducing the measure projection method. It is a novel probabilistic multi-subject inference method that searches voxel by voxel for brain regions that have event-related IC process dynamics, which exhibit statistically significant consistency across subjects and/or sessions, as quantified by the values of various EEG measures.

By abandoning the notion of distinct IC clusters, the Measure Projection Analysis (Bigdely-Shamlo, Mullen, Kreutz-Delgado, & Makeig, 2013)[11] involves lower levels of subjectivity. In particular as the user is not required to set the clustering parameters, such as the number of clusters to be extracted and the relative weights of different EEG measures, it overcomes the difficulty in proper statistical evaluation of cluster memberships and the discontinuous nature of IC clustering. With regards to multimodal investigations, a novel neuroelectromagnetic forward head modelling toolbox (NFT; Delorme, et al., 2011) enables the building of realistic electrical-head models from available magnetic resonance (MR) images and/or from measured EEG sensor coordinates. Also studies utilising fMRI to examine brain activations related to cognitive functions, provide insight on the spatial locations of these activations. Combined

[10] Description of the Affinity Product Method available online: http://sccn.ucsd.edu/wiki/Chapter_05:_Component_Clustering_Tools#Preparing_to_cluster_.28Pre-clustering.29_with_Affinity_Product_Method

[11] Description and further details on the Measure Projection Analysis available online: http://sccn.ucsd.edu/wiki/MPT

with electrophysiological methods, it offers a unique and comprehensive approach to study the cognitive function-related brain activations. In the future, similar highly sophisticated methods may prove beneficial when examining how brain abnormalities affect the function of the brain in rest or while performing cognitively demanding tasks. The functional connectivity analysis measures, such as source information flow toolbox (SIFT; Delorme, et al., 2011), electrophysiological connectome (eConnectome; He, et al., 2011) and diffusion tensor imaging (DTI; Sundgren, et al., 2004), have recently gained in popularity and may be remarkably interesting in the context of MS, which involves adversely affected connections in the brain. Furthermore, the studies utilising brain imaging methods with higher specificity, have found correlations between cognitive dysfunction and low magnetisation transfer imaging ratios, N-acetylaspartate:creatine ratio using magnetic resonance, diffusion MRI and diffusion MRI metrics (Benedict & Zivadinov, 2011).

The information acquired with these novel neurophysiological and brain imaging methods could then serve to determine the particular features characteristic of the brain and cognitive (dys)function in MS. The profound understanding of the abnormalities could then be used to create and develop innovative ways to measure the brain and cognitive function in MS. This could include advanced and user-friendly adjustments of the research methods that would be easily applicable to clinical practice; such as self-explanatory graphical user interfaces, dry electrodes, simultaneous neuropsychological and electrophysiological testing.

Future ERP studies could also incorporate a full neuropsychological battery to longitudinal ERP and MRI studies, which may clarify how the changes in specific cognitive domains are related to longitudinal neurophysiological and/or MRI findings. The association between the lesions and neuropsychological and neurophysiological cognitive function is not clearly defined in MS, which is particularly true in relation to the progression of CI in MS. A comprehensive multimodal and longitudinal study would enable the examination of abnormalities in normal-appearing white and grey matter, which may be related to the severity and progression of CI, but are commonly undetectable by conventional imaging (Bagert, Camplair, & Bourdette, 2002). Previous studies have reported increasing brain lesion load and brain atrophy to be correlated with the progression of CI in MS (Amato, et al., 2006). Furthermore, the whole brain atrophy seems to be a good predictor of future cognitive and memory decline in early MS (Whitwell, 2008). These multimodal, comprehensive investigations are a necessity in the future research of CI in MS.

The proficient functioning of cognitive processes is central to human beings, especially in the demanding work and social environments of modern life. Typically the smooth cognitive performance goes unnoticed in most MS patients. Indeed before the adverse-disease changes affecting these functions become apparent and cause difficulties, for instance, in the ability to concentrate and remember everyday details. The subtle cognitive deficits in attention, information processing speed and working memory may manifest as difficulties in common everyday life situations, for example, in maintaining concentration while driving and in difficulties completing more complex tasks at work. Therefore, it is crucial to catch these adverse changes in time, in order to assess their impact on daily tasks such as work and driving and to make adaptations to work and social environments and conditions. As many MS patients may try to hide these deficits from their family and employers, causing themselves considerable stress, loneliness and frustration, it is essential that clinical professionals communicate effectively that cognitive deficits can be accommodated into everyday life in the form of part-time work, additional breaks, restructuring of work tasks, driving in the mornings, among others. Moreover, the family of the patient can be seen as a resource, as they can report recent changes in the patient's behaviour, enable necessary alterations in the daily life and provide support to the patient.

The importance of having an outlook on the cognitive functioning of an individual MS patient becomes even more vital in the context of controversial topics, such as the management and treatment of MS symptoms. The cognitive disturbances may affect a patient's ability to make informed decisions and fully understand all of the risks and legal implications involved in the alternative treatment options. Some MS patients may disagree with their doctor about the best treatment option for them, which may cause substantial stress to both parties and the possible cognitive impairment of the patient may play a role in these disputes. For instance, there are numerous alternative treatments available, which may include travel to the other side of the world to a country with less stringent medical legislation, with costly (and possibly risky) procedures, some even involving surgery[12]. Similarly, using cannabinoids (e.g. marijuana) may ease muscle spasticity but at the same time cause dizziness, fatigue and slow mental functions, not to mention the uncertainty about the possible long-term effects and the potential legal consequences to the patient[13]. Most controversial debate of all, euthanasia[14],

[12] A recent controversy surrounds alternative treatment named chronic cerebro-spinal venous insufficiency (CCSVI) (http://www.cbc.ca/news/health/story/2012/05/10/multiple-sclerosis-ccsvi-fda-warning.html).

[13] The issue is discussed in a news article: http://www.reuters.com/article/2012/05/14/us-marijuana-sclerosis-idUSBRE84D0RS20120514.

[14] A recent case reported by the press: http://www.nzherald.co.nz/politics/news/article.cfm?c_id=280&objectid=10834168

highlights the importance of determining the patient's level of cognitive function in its core: if and when can a patient be judged to be cognitively fit enough to make a decision to end his/her own life?

In light of all these ethical questions it is essential to be able to accurately detect any cognitive difficulties as early as possible, and to monitor their potential progression over time. This allows MS patients to plan their life and accommodate to the future challenges. One of the difficulties at present, is the accurate diagnosis of cognitive impairment in MS, as the deficits apparent in neuropsychological performance can be masked by fatigue, depression and other mood disorders such as anxiety (Arnett, et al., 1999; Demaree, et al., 2003; Diamond, et al., 2008; Gilchrist & Creed, 1994; Litvan, et al., 1988; Thornton & Raz, 1997), which may lead to inappropriate treatment. Therefore, the interdisciplinary care and the utilisation of multimodal methods to detect and monitor CI in MS are essential, in order to gain a full understanding of the cognitive functioning in each patient. As it happens, the cognitive-indexing electrophysiological measures may prove to be a central part of the solution.

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Appendices.

Appendix 1. Participant information and consent form



St. Vincent's Healthcare GROUP LIMITED



PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE: Cognitive Functioning in Multiple Sclerosis: A Longitudinal ERP Analysis

NAME OF PRINCIPAL INVESTIGATOR: Professor Niall Tubridy, Consultant Neurologist, St. Vincent's University Hospital, Dublin

You are being invited to participate in a research study. Thank you for taking time to read this.

WHAT IS THE PURPOSE OF THIS STUDY?

The aim of the study is to see how people with a diagnosis of MS complete a range of mental tasks, when compared to healthy volunteers. It also aims to examine whether or not any difficulties with mental tasks are related to abnormalities seen on traditional and newer types of MRI brain scan.
As part of this study, we also record the electrical activity of the brain from the scalp surface. The method of recording electrical brain activity is called Electroencephalography (EEG), and it is a safe, non-invasive method. During an EEG recording the subject listens to, and/or watches some stimuli. These stimuli are called events, and the electrical activity associated with these events are called Event Related Potentials (ERPs).

We aim to observe changes in these measures over time; they will be performed at three points in time (at the start of the study, 1 year later and 2 years after the study begins).

WHY HAVE I BEEN CHOSEN?

You are being asked to participate in this study because you have a diagnosis of Multiple Sclerosis (MS).

WHAT WILL HAPPEN IF I VOLUNTEER?

Your participation is entirely voluntary. If you initially decide to take part you can subsequently change your mind without difficulty. This will not affect your future treatment in any way. Furthermore your doctor may decide to withdraw you from this study if he/she feels it is in your best interest.

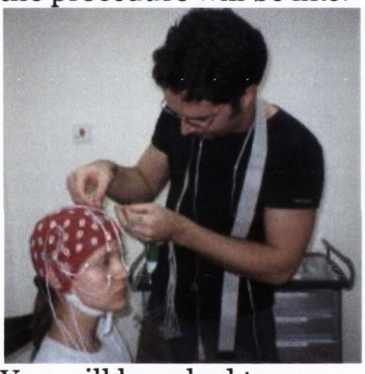
This study involves tests in the neurology clinic, the ERP laboratory and MRI department. Therefore, you will be asked to attend St. Vincent's Hospital twice in one week during the first, second and third years. The following outlines what will happen during your visits to St Vincent's Hospital:

DAY ONE

On the first day, before the ERP test, you (the study subject) will be asked to complete some mental tasks, for example addition of a series of numbers called out on CD.

You will also be asked some questions regarding your mood and other aspects of mental health, and how you think MS impacts on your life. Some participants may find these questions disturbing or intrusive. If you experience any anxiety you will be offered a referral to counselling services provided by the Dept of Psychiatry at SVUH and UCD. The contact number is 01 2774228.

The ERP test involves placing a cap with 128 electrodes on your head. Four electrodes will also be placed under and to the side of your eyes using sticky paper. Each electrode makes contact with the scalp via conductive water-based gel, which will have to be washed out afterwards. The picture below shows what the procedure will be like.



You will be asked to:

(1) Listen to a series of tones (beeps) through a pair of earphones, and press a button when you hear a high-pitched tone.

(2) Watch to a series of letters on a computer screen, and press a button when you see the letter X.

(3) Watch a checkerboard pattern (no response needed)

(4) Listen to random noises (no response needed)

(5) Remember a series of numbers

The ERP study will take approximately 2.5 hours.

DAY TWO

The brain scan (MRI) will be performed in St. Vincent's Hospital on the day of your second visit. It will take about 30 minutes. The scan causes no discomfort. After the brain scan, a psychologist will meet you and bring you to the Neurology Department, St Vincent's Hospital. You will be asked to participate in a neuropsychological assessment. This assessment will require you to complete a number of tests which will assess different aspects of your cognitive functioning. The neuropsychological assessment will take approximately one hour.

ARE THERE ANY BENEFITS FROM MY PARTICIPATION THIS STUDY?

You will not benefit directly from taking part in this study but the information we will obtain will provide further knowledge of this condition.

ARE THERE ANY RISKS INVOLVED IN PARTICIPATING IN THIS STUDY?

ERP TEST

We are using a very safe way of measuring brain activity, with no known adverse effects. Very occasionally, participants feel uncomfortable wearing the hat.

MRI SCANNING

MRI scanning is a very safe procedure, which uses a strong magnetic field to study the brain. Prior to having the MRI, you will be asked to go through a checklist of questions to make sure that it is safe for you to have the scan. If you are a woman the scan will not be done if there is any possibility that you may be pregnant, even though MRI scanning has been shown to be safe even in early pregnancy. People with cardiac pacemakers, defibrillators or other non-MRI compatible implanted devices cannot have an MRI. MRI is also unsafe if there are metallic foreign bodies in the eyes/orbits, tattoos or permanent eye make-up or recently inserted ferromagnetic surgical implants. As no MRI contrast agents (dyes) will be used for this study, patients with previous allergies, asthma or diabetes will be identified but not excluded from participation.

The MRI scan will require you to lie still in a relatively enclosed space, which some people may find uncomfortable. For this reason the scan will not be done if you have a history of claustrophobia.

Rarely, the scan might show an unexpected change in the brain. All the scans will be checked for any abnormalities by a consultant radiologist. One of the neurology doctors will speak to you about the scan after it is performed.

WHAT HAPPENS IF I DO NOT AGREE TO PARTICIPATE?
If you decide not to participate in this study your treatment will not be affected in any way.

CONFIDENTIALITY

Every effort will be made to protect the names of the participants in this study. Specifically, each participant is given a unique study number that is used to identify their data in place of their name. Test data are stored with this number code only. Names of participants and their study numbers are kept in a locked filing cabinet in a locked office, as well as in a database that only the researchers can access. This information is stored separately from all test data.

For the purpose of this research, your name will never be used in any publication that may result from this study. However, there is no absolute guarantee that the information you provide will not be obtained by legal process or court order. Research data may be shared, as required by law, with St. Vincent's University Hospital authorities. Furthermore, if you give us information about the abuse or neglect of a minor or incapacitated adult, or make threat of harm to yourself or others, we are obliged to report this information to the relevant statutory authorities.

The data collected in this study will be used for the purpose described in this information leaflet. Unless otherwise specified, test data gathered from this study will be maintained indefinitely.

Anonymous data may also be shared with other researchers to conduct additional related studies. This information will not identify you. Information that could identify you will not be released beyond that required for the purposes of conducting this research study, or as may be required by law (see above).

If you choose to withdraw from the study, you are entitled to take back your approval for the use of your data. To do this, you should contact the researcher *in writing*. Data that has already been collected will then be destroyed (unless we are obliged under law to disclose it to the relevant statutory authorities, or unless you agree to allow us use the data already collected).

These tests are being conducted for research purposes only. Therefore, it is not possible for participants to obtain any of the data collected. Participants may obtain information about their test results, if required.

COMPENSATION

Your doctors are adequately insured by virtue of their participation in the clinical indemnity scheme.

WHO IS ORGANISING AND FUNDING THIS RESEARCH?

This study is organised by the Neurology research programme at St. Vincent's University Hospital and University College Dublin, Ireland. There will be collaboration with the School of Psychology in University College Dublin and the Schools of Engineering and Medicine in Trinity College Dublin. This study is partly funded by an Enterprise Ireland project grant titled *eHealthCare based on Biomedical Signal Processing and ICT for Integrated Diagnosis and Treatment of Disease and IRCSET.*

You will not receive any financial reward for taking part in this study but we will cover expenses you incur as a result of your participation.

IS THIS STUDY SAFE AND BENEFICIAL?

The St. Vincent's Healthcare Group, Ethics and Medical Research Committee have reviewed and approved this study.

CONTACT DETAILS

Any further information can be obtained by contacting Dr.Siobhan Kelly

Phone: 01 2213674

Address:
Department of Neurology,
Carew House,
St. Vincent's University Hospital,
Elm Park,
Dublin 4.

PLEASE TICK YOUR RESPONSE IN THE APPROPRIATE BOX

I have read and understood the Participant Information YES ☐ NO ☐

I have had the opportunity to ask questions and discuss the study YES ☐ NO ☐

I have received satisfactory answers to all my questions YES ☐ NO ☐

I have received enough information about this study YES ☐ NO ☐

I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care YES ☐ NO ☐

I agree to take part in the study YES ☐ NO ☐

Participant's Signature: _____ Date: _____

Participant's Name in print: _____

Witness Signature: _____ Date: _____

Witness Name in print: _____

Investigator's Signature: _____ Date: _____

Investigator's Name in print: _____



St. Vincent's Healthcare GROUP LIMITED



INFORMATION FOR HEALTHY VOLUNTEERS ABOUT PARTICIPATING IN RESEARCH—MEASURING BRAIN ACTIVITY

Study Title: Cognitive Functioning in Multiple Sclerosis: A Longitudinal ERP Analysis

NAME OF PRINCIPAL INVESTIGATOR: Professor Niall Tubridy, Consultant Neurologist, St. Vincent's University Hospital, Dublin.

We are asking you to participate in a research study. Your participation is entirely voluntary. You are being asked to participate in this study because you are a healthy volunteer (a 'control').

WHAT IS THE PURPOSE OF THIS STUDY?

The aim of the study is to examine brain activity and see how people with a diagnosis of Multiple Sclerosis (MS) complete a range of mental tasks, when compared to healthy control volunteers.

HOW LONG DOES THE STUDY TAKE?

Your involvement in this study will take approximately 3.5 hours for the full assessment. However, because this is a follow-up study of people with MS, we are also asking control volunteers to return for a second assessment approximately one year after their initial assessment.

What does your involvement in the study entail?

For the assessment, you will be asked to attend St. Vincent's Hospital on one occasion for 3.5 hours or twice in one week for shorter periods of time, if that suits you better.

Your involvement in the study involves the following four components:

(1) When you attend St Vincents Hospital, you will be asked to complete some mental tasks, for example addition of a series of numbers called out on CD.

(2) We will also record the electrical activity of your brain from the scalp surface. The method of recording electrical brain activity is called Electroencephalography (EEG), and it is a safe, non-invasive method.

During a recording of brain activity, the subject listens to, and/or watches, some stimuli. These stimuli are called events, and the electrical activity associated with these events are called Event Related Potentials (ERPs).

The ERP test involves placing a cap with 128 electrodes on your head. Four electrodes will also be placed under and to the side of your eyes using sticky paper. Each electrode makes contact with the scalp via conductive water-based gel, which will have to be washed out afterwards. The picture below shows what the procedure will be like.



You will be asked to:

(a) Listen to a series of tones (beeps) through a pair of earphones, and press a button when you hear a high-pitched tone.

(b) Watch a series of letters on a computer screen, and press a button when you see the letter X.

(c) Watch a checkerboard pattern (no response needed)

(d) Listen to random noises (no response needed)

(e) Remember a series of numbers

(3) When you attend St Vincent's Hospital, you will also be asked some questions about your mood and other aspects of mental health.

Some participants may find these questions somewhat disturbing or intrusive. If you experience any anxiety you will be offered a referral to counselling services provided by the Dept of Psychiatry at SVUH and UCD. The contact number is 01 2774228.

(4) Additionally, when you attend St Vincent's Hospital you will be asked to undertake a neuropsychological assessment. This assessment will require you to complete a number of tests that will assess different aspects of your cognitive functioning (e.g. attention, concentration, memory).

DATA COLLECTION

The data collected as a result of your involvement in this study includes:

Demographic information such as age, gender, and date of birth

Information about your mood and mental health

Neuropsychological tests data

The data collected from the brain activity measure (ERP) described above

Medical history data such as current medications and current and past medical conditions.

ARE THERE ANY BENEFITS FROM MY PARTICIPATION?

You will not benefit directly from taking part in this study. The current study is part of a body of research that aims to examine the effects of MS on cognitive functioning. In time, it is hoped that the results of the current study, and of the studies that follow it, will contribute to the development of interventions that impact on MS. If you take part in the study, you will contribute to this body of research. Without people like you, the research would not be possible.

ARE THERE ANY RISKS INVOLVED IN PARTICIPATING?

We are using a very safe, non invasive way of measuring brain activity, with no known adverse effects. Very occasionally, participants feel uncomfortable wearing the hat.

You should report any problems to the director of this study: Siobhan Kelly (01-2213674)

WHO IS ORGANISING AND FUNDING THIS RESEARCH?

This study is organised by the Neurology research programme at St. Vincent's University Hospital and University College Dublin, Ireland. There will be collaboration with colleagues at the School of Psychology in University College Dublin and the Schools of Engineering and Medicine in Trinity College Dublin. This study is partly funded by an Enterprise Ireland project grant titled *eHealthCare based on Biomedical Signal Processing and ICT for Integrated Diagnosis and Treatment of Disease.*

CONFIDENTIALITY

Every effort will be made to protect the names of the participants in this study. Specifically, each participant is given a unique study number that is used to identify their data in place of their name. Test data are stored with this number code only. Names of participants and their study numbers are kept in a locked filing cabinet in a locked office, as well as in a database that only the researchers can access. This information is stored separately from all test data.

For the purpose of this research, your name will never be used in any publication that may result from this study. However, there is no absolute guarantee that the information you provide will not be obtained by legal process or court order. Research data may be shared, as required by law, with St. Vincent's University Hospital authorities. Furthermore, if you give us information about the abuse or neglect of a minor or incapacitated adult, or make threat of harm to yourself or others, we are obliged to report this information to the relevant statutory authorities.

The data collected in this study will be used for the purpose described in this information leaflet. Unless otherwise specified, test data gathered from this study will be maintained indefinitely.

Anonymous data may also be shared with other researchers to conduct additional related studies. This information will not identify you. Information that could identify you will not be released beyond that required for the purposes of conducting this research study, or as may be required by law (see above).

If you choose to withdraw from the study, you are entitled to take back your approval for the use of your data. To do this, you should contact the researcher *in writing*. Data that has already been collected will then be destroyed (unless we are obliged under law to disclose it to the relevant statutory authorities, or unless you agree to allow us use the data already collected).

These tests are being conducted for research purposes only. Therefore, it is not possible for participants to obtain any of the data collected.

WHO SHOULD YOU CALL WITH QUESTIONS ABOUT THIS STUDY?

Questions about this study may be directed to the researchers in charge of this study during normal business hours:

Katie Kinsella, Neurology Research Nurse, St. Vincent's University Hospital, Elm Park Dublin 4 (01-2214821)

or

Sean O Donnachadha (Psychologist in Clinical Training, HSE/UCD 087 2713429)

WHAT ABOUT THE COSTS OF THE STUDY?

There will not be a charge to you for any aspect of this study.

Will you be paid to participate in this study? No.

WHAT HAPPENS IF YOU GET SICK OR HURT FROM PARTICIPATING IN THIS STUDY?

All appropriate emergency care will be provided in this instance.

Appendix 3.Journal paper examining baseline P3b ERPs in multiple sclerosis.

Clinical Neurophysiology 121 (2010) 1420–1426

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

A high-density ERP study reveals latency, amplitude, and topographical differences in multiple sclerosis patients versus controls ☆

R. Whelan [a,b,*], R. Lonergan [a], H. Kiiski [b], H. Nolan [b], K. Kinsella [a], J. Bramham [c], M. O'Brien [c], R.B. Reilly [b], M. Hutchinson [a], N. Tubridy [a]

[a] Department of Neurology, St. Vincent's University Hospital/University College Dublin, Ireland
[b] Trinity Center for Bioengineering, Trinity College Dublin, Ireland
[c] School of Psychology, University College Dublin, Ireland

ARTICLE INFO

Article history:
Accepted 17 March 2010
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Keywords:
Multiple sclerosis
Evoked potentials
Auditory
Visual
Neuropsychology
Cognition

ABSTRACT

Objective: To quantify latency, amplitude and topographical differences in event-related potential (ERP) components between multiple sclerosis (MS) patients and controls and to compare ERP findings with results from the paced auditory serial addition test (PASAT).
Methods: Fifty-four subjects (17 relapsing remitting (RRMS) patients, 16 secondary progressive (SPMS) patients, and 21 controls) completed visual and auditory oddball tasks while data were recorded from 134 EEG channels. Latency and amplitude differences, calculated using composite mean amplitude measures, were tested using an ANOVA. Topographical differences were tested using statistical parametric mapping (SPM).
Results: In the visual modality, P2, P3 amplitudes and N2 latency were significantly different across groups. In the auditory modality, P2, N2, and P3 latencies and N1 amplitude were significantly different across groups. There were no significant differences between RRMS and SPMS patients on any ERP component. There were topographical differences between MS patients and controls for both early and late components for the visual modality, but only in the early components for the auditory modality. PASAT score correlated significantly with auditory P3 latency for MS patients.
Conclusions: There were significant ERP differences between MS patients and controls.
Significance: The present study indicated that both early sensory and later cognitive ERP components are impaired in MS patients relative to controls.
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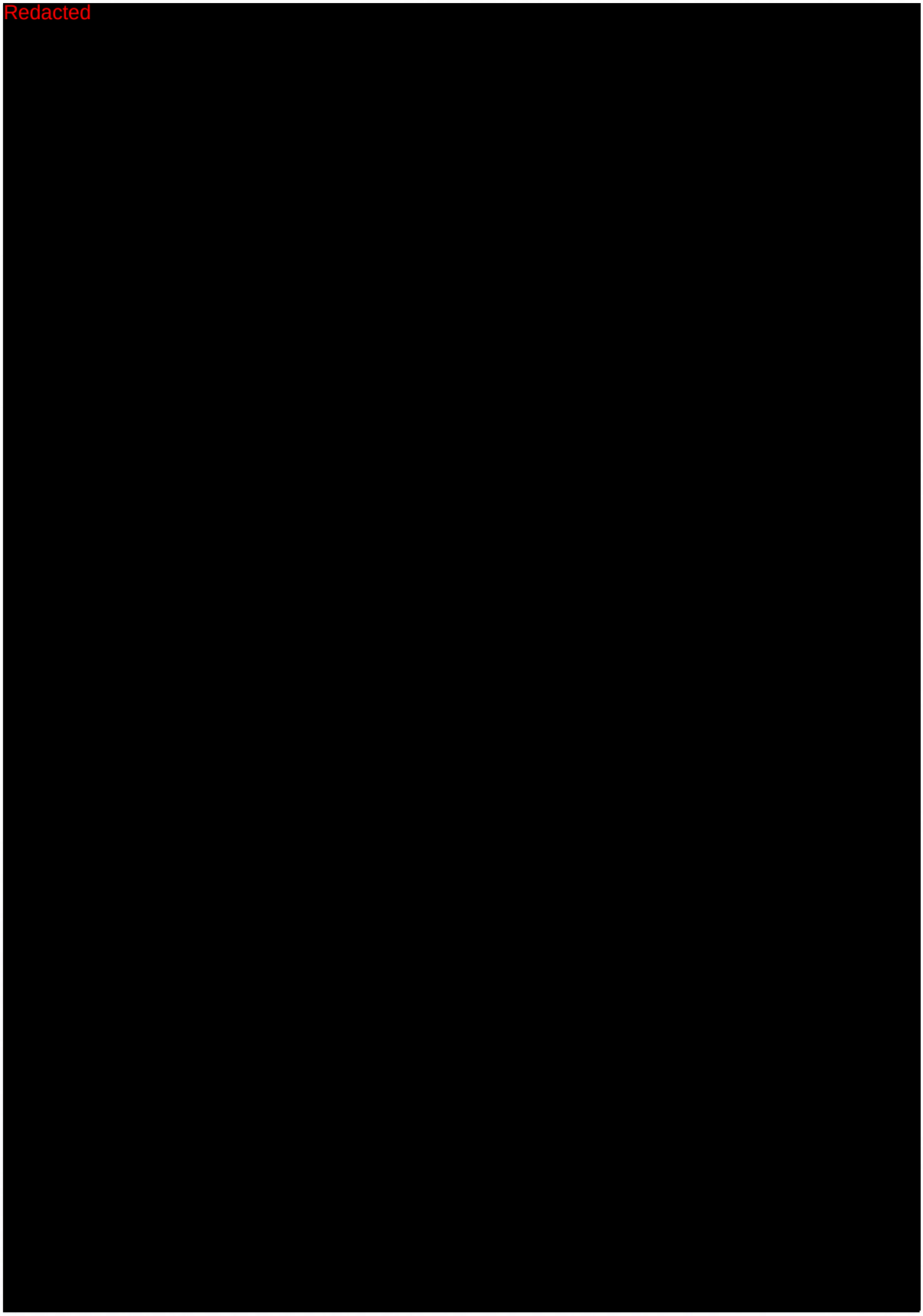
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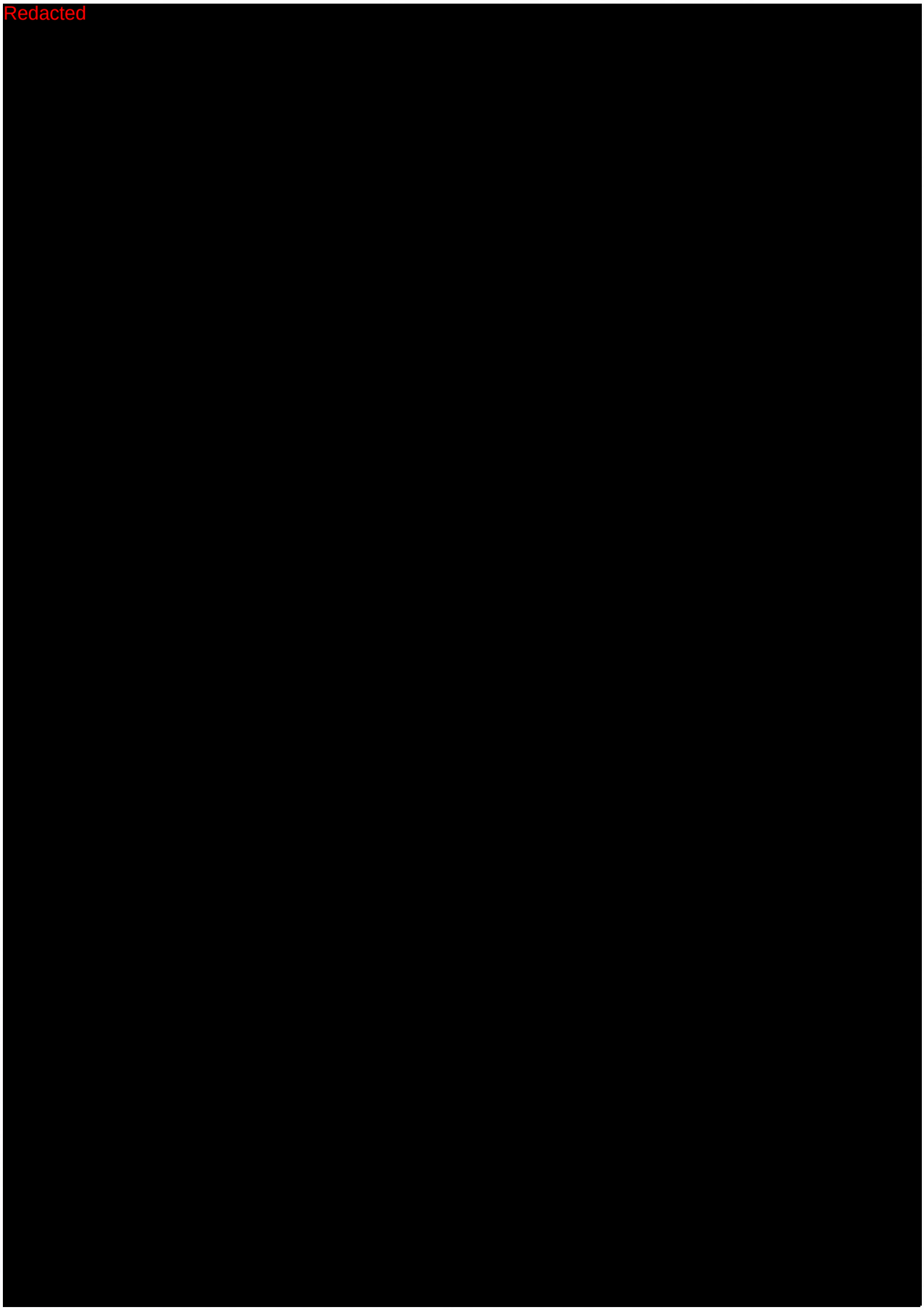

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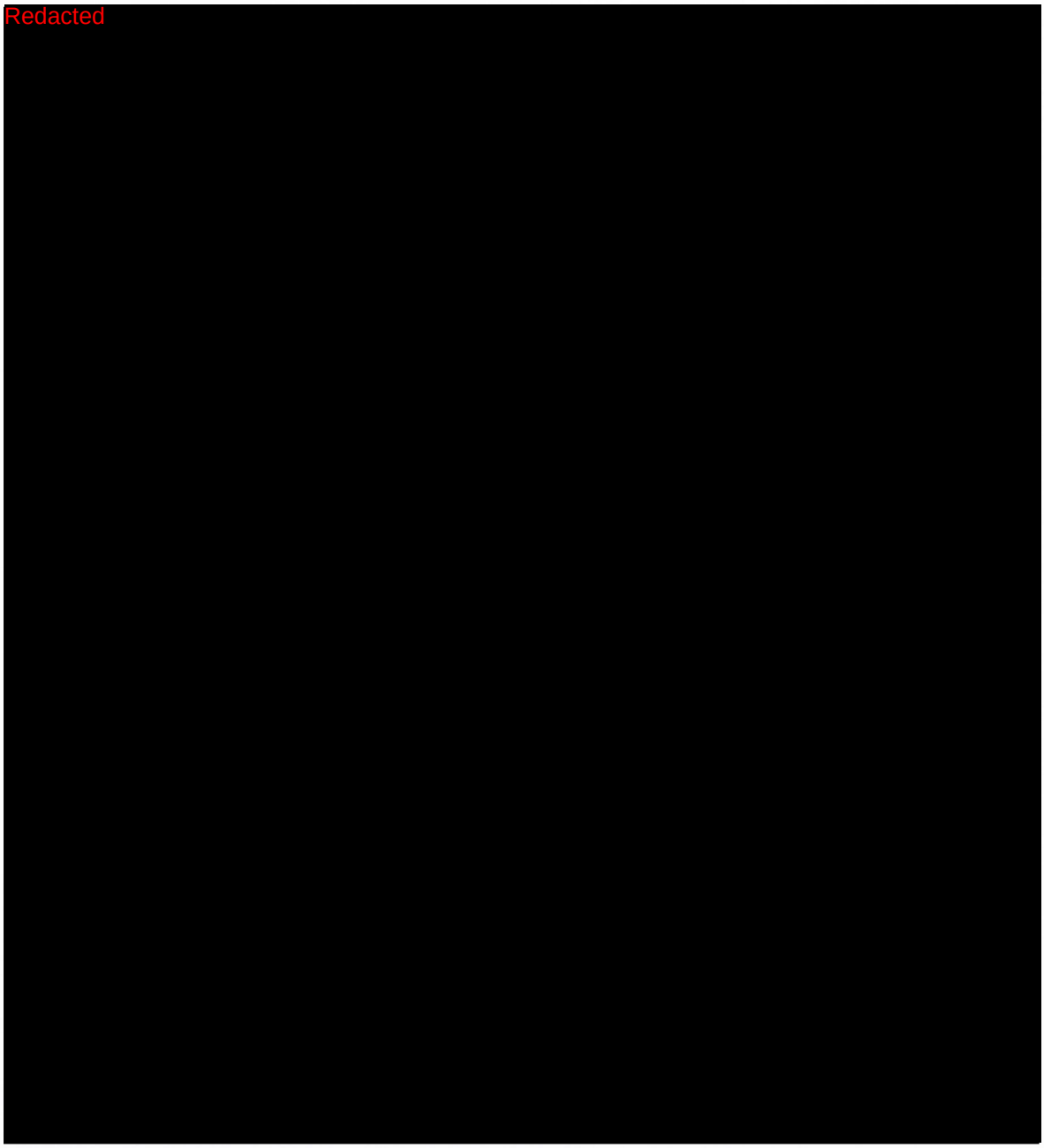
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Appendix 4. Journal paper examining baseline P3a ERPs in multiple sclerosis.

Journal of the Neurological Sciences 293 (2010) 45–50

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Impaired information processing speed and attention allocation in multiple sclerosis patients versus controls: A high-density EEG study

R. Whelan [a,b,*], R. Lonergan [a], H. Kiiski [b], H. Nolan [b], K. Kinsella [a], M. Hutchinson [a], N. Tubridy [a], R.B. Reilly [b]

[a] Department of Neurology, St. Vincent's University Hospital/University College Dublin, Ireland
[b] Trinity Center for Bioengineering, Trinity College Dublin, Ireland

ARTICLE INFO

Article history:
Received 18 December 2009
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Accepted 11 March 2010

Keywords:
Multiple sclerosis
Event-related potentials, auditory
Event-related potentials, visual
Neuropsychology
Cognition
Attention

ABSTRACT

Background: The no-go P3a is a variant of the P300 event-related potential (ERP) that indexes speed of information processing and attention allocation. The aim of this study was to compare ERP findings with results from the paced auditory serial addition test (PASAT) and to quantify latency, amplitude and topographical differences in P3a ERP components between multiple sclerosis (MS) patients and controls.
Patients and methods: Seventy-four subjects (20 relapsing remitting (RRMS) patients, 20 secondary progressive (SPMS) patients and 34 controls) completed a three-stimulus oddball paradigm (target, standard, and non-target). Subjects participated in separate visual and auditory tasks while data were recorded from 134 EEG channels. Latency differences were tested using an ANCOVA. Topographical differences were tested using statistical parametric mapping.
Results: Visual P3a amplitude correlated with PASAT score in all MS patients over frontal and parietal areas. There were significant differences in latency, amplitude, and topography between MS patients and controls in the visual condition. RRMS and SPMS patients differed in visual P3a latency and amplitude at frontal and parietal scalp regions. In the auditory condition, there were latency differences between MS patients and controls only over the parietal region.
Conclusion: The present results demonstrate that information processing speed and attention allocation are impaired in MS.

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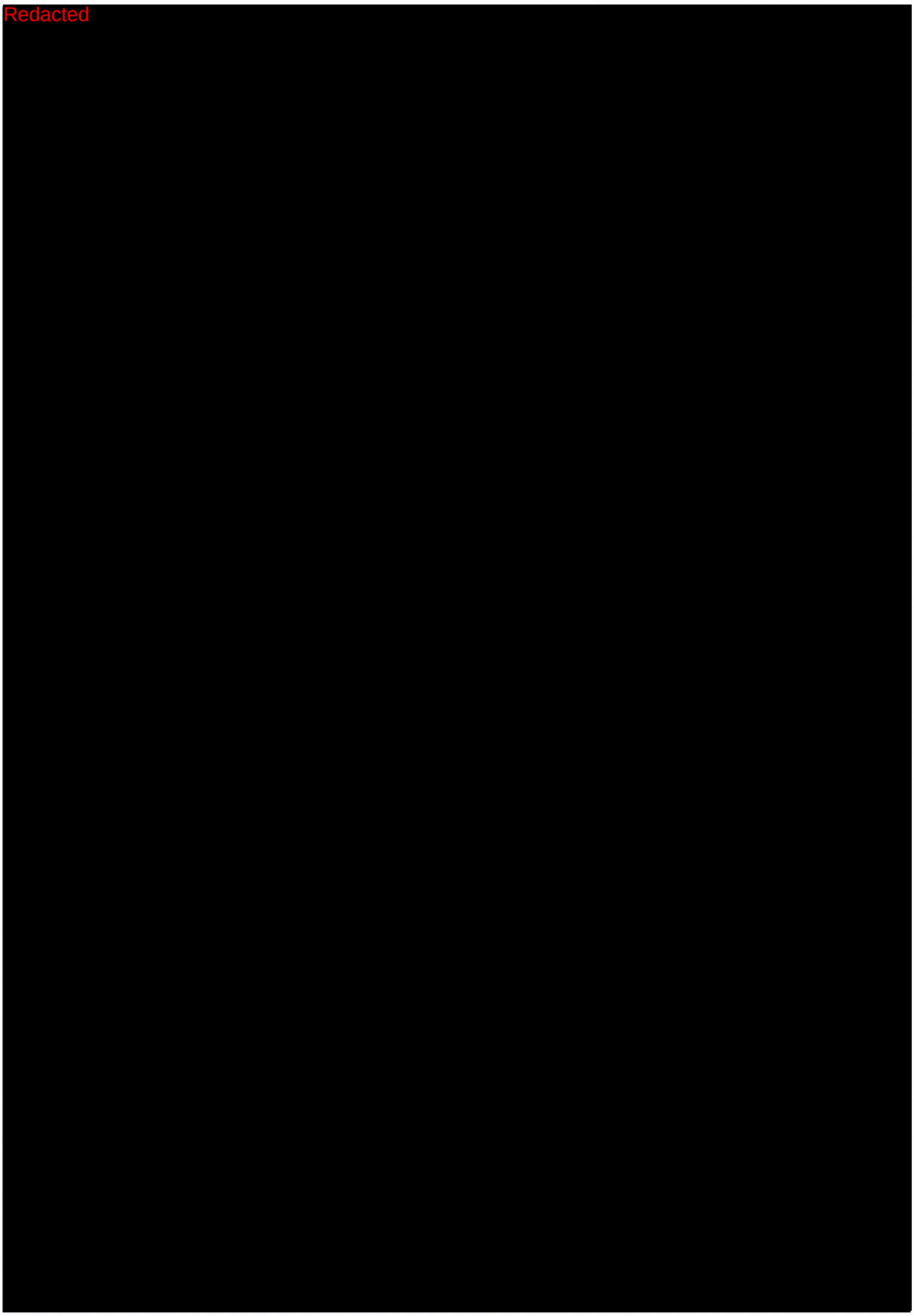
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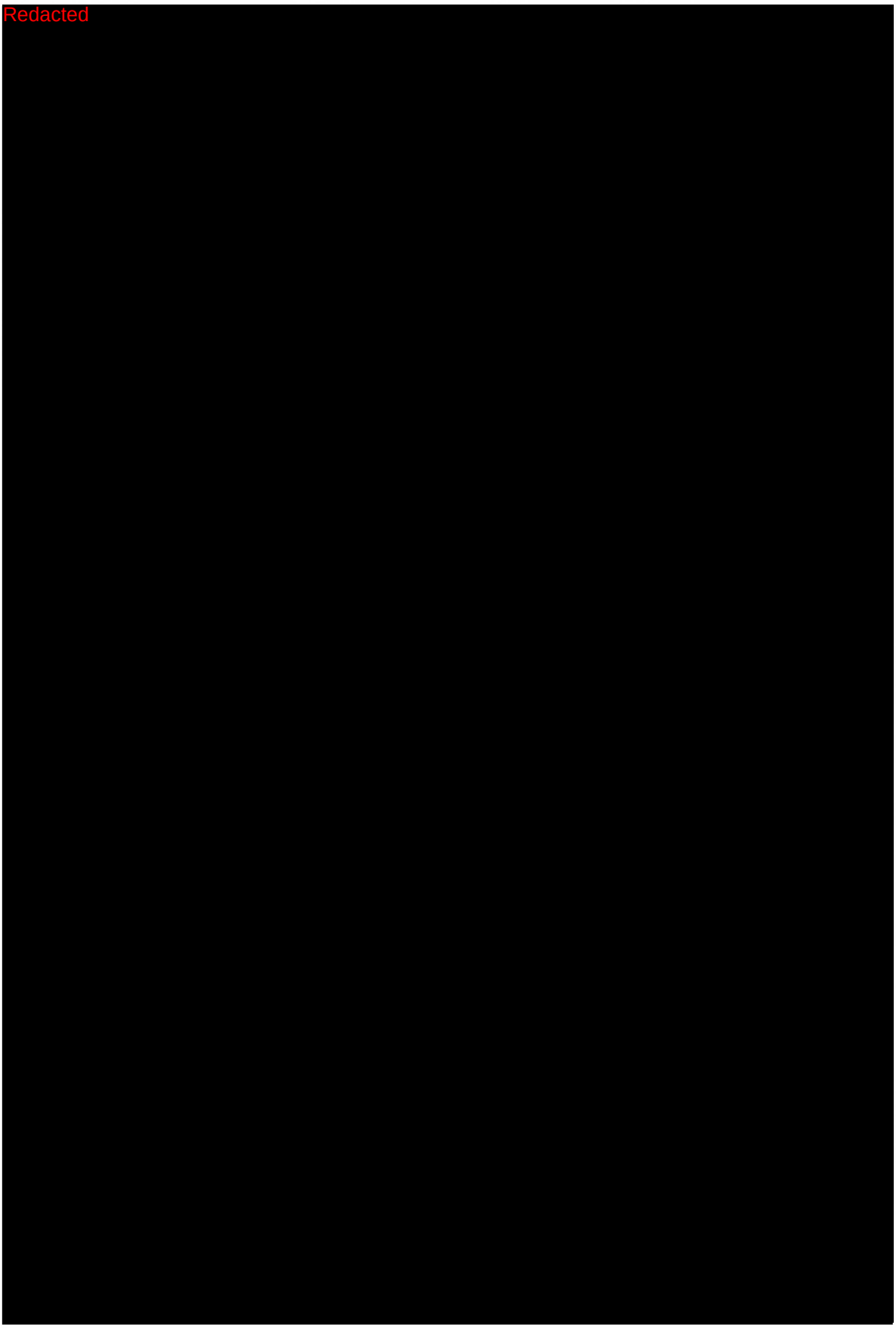
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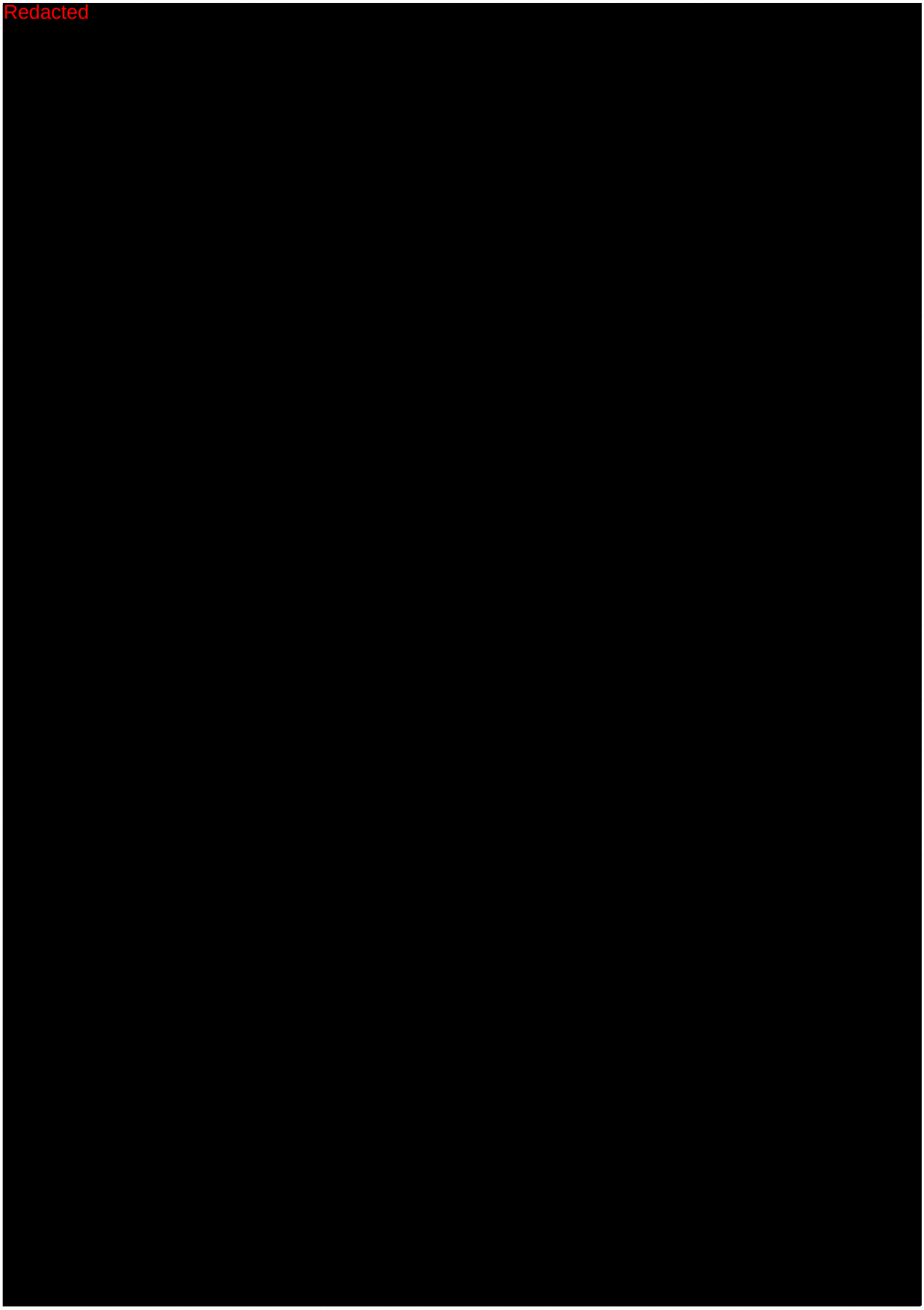
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Appendix 5. Journal paper examining baseline P3 ERPs in progressive multiple sclerosis patients.

European Journal of Neurology 2010 doi:10.1111/j.1468-1331.2010.03172.x

SHORT COMMUNICATION

Preliminary evidence for correlation between PASAT performance and P3a and P3b amplitudes in progressive multiple sclerosis

H. Kiiski[a], R. Whelan[a], R. Lonergan[b], H. Nolan[a], K. Kinsella[b], M. Hutchinson[b], N. Tubridy[b] and R. B. Reilly[a]

[a]*Trinity Centre for Bioengineering, Trinity College Dublin, College Green, Dublin, Ireland; and* [b]*Department of Neurology, St. Vincent's University Hospital, Elm Park, Dublin, Ireland*

Keywords:
attention, auditory event-related potentials, cognition, multiple sclerosis, neuropsychology, visual event-related potentials

Received 30 March 2010
Accepted 21 June 2010

Background: The no-go P3a event-related potential (ERP) is a measure of attentional engagement and the P3b is a measure of context updating. The aim of this study was to compare ERP topographies: (i) to Paced Auditory Serial Addition Test (PASAT) results, (ii) of visual and auditory P3a and P3b of patients with primary progressive multiple sclerosis (PPMS) versus patients with secondary-progressive multiple sclerosis (SPMS) and (iii) of both progressive subtypes to healthy controls.

Methods: Thirty subjects (10 PPMS, 10 SPMS and 10 age-matched controls) completed visual and auditory no-go P3a and P3b tasks whilst data were recorded from a 128-scalp channel electroencephalography (EEG) array. Data from scalp channels were converted into continuous interpolated images (incorporating the entire scalp and time). Topographical differences and correlations were then tested using statistical parametric mapping.

Results: For the patients with multiple sclerosis (MS), PASAT score correlated significantly with parietal regions in the auditory P3b, auditory P3a and visual P3b conditions, and with central regions in the visual P3a condition. Patients with PPMS had significantly lower amplitude than patients with SPMS in the auditory P3b condition over the parietal area. The control group had greater amplitude than the patients with MS in all the P3 tasks, with the exception of the auditory P3b.

Conclusions: These data suggest that PASAT performance and P3 ERPs correlate for MS progressive subtypes and that PPMS and SPMS differ in electrophysiological responses during auditory P3b tasks.

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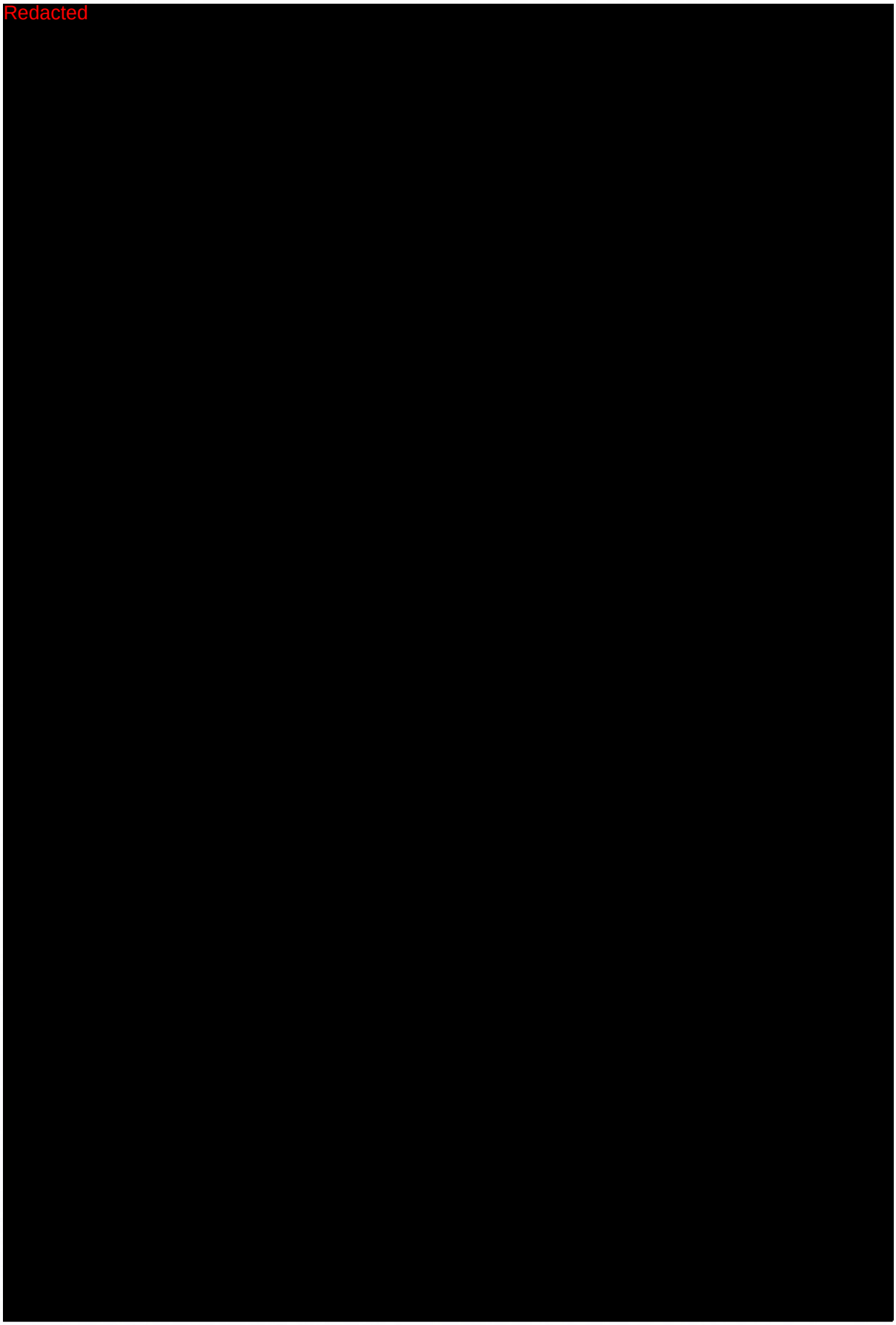
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Appendix 6. Journal paper examining P3b task-related source EEG activations in multiple sclerosis.

OPEN ACCESS Freely available online

PLOS ONE

Only Low Frequency Event-Related EEG Activity Is Compromised in Multiple Sclerosis: Insights from an Independent Component Clustering Analysis

Hanni Kiiski[1], Richard B. Reilly[1], Róisín Lonergan[2], Siobhán Kelly[2], Marie Claire O'Brien[3], Katie Kinsella[2], Jessica Bramham[3], Teresa Burke[3], Seán Ó Donnchadha[3], Hugh Nolan[1], Michael Hutchinson[2], Niall Tubridy[2], Robert Whelan[1,4]*

1 Trinity Centre for Bioengineering, Trinity College Dublin, Ireland, **2** Department of Neurology, St. Vincent's University Hospital, Elm Park, Dublin, Ireland, **3** School of Psychology, University College Dublin, Belfield, Dublin, Ireland, **4** Department of Psychiatry, University of Vermont, Burlington, Vermont, United States of America

Abstract

Cognitive impairment (CI), often examined with neuropsychological tests such as the Paced Auditory Serial Addition Test (PASAT), affects approximately 65% of multiple sclerosis (MS) patients. The P3b event-related potential (ERP), evoked when an infrequent target stimulus is presented, indexes cognitive function and is typically compared across subjects' scalp electroencephalography (EEG) data. However, the clustering of independent components (ICs) is superior to scalp-based EEG methods because it can accommodate the spatiotemporal overlap inherent in scalp EEG data. Event-related spectral perturbations (ERSPs; event-related mean power spectral changes) and inter-trial coherence (ITCs; event-related consistency of spectral phase) reveal a more comprehensive overview of EEG activity. Ninety-five subjects (56 MS patients, 39 controls) completed visual and auditory two-stimulus P3b event-related potential tasks and the PASAT. MS patients were also divided into CI and non-CI groups (n = 18 in each) based on PASAT scores. Data were recorded from 128-scalp EEG channels and 4 IC clusters in the visual, and 5 IC clusters in the auditory, modality were identified. In general, MS patients had significantly reduced ERSP theta power versus controls, and a similar pattern was observed for CI vs. non-CI MS patients. The ITC measures were also significantly different in the theta band for some clusters. The finding that MS patients had reduced P3b task-related theta power in both modalities is a reflection of compromised connectivity, likely due to demyelination, that may have disrupted early processes essential to P3b generation, such as orientating and signal detection. However, for posterior sources, MS patients had a greater decrease in alpha power, normally associated with enhanced cognitive function, which may reflect a compensatory mechanism in response to the compromised early cognitive processing.

Citation: Kiiski H, Reilly RB, Lonergan R, Kelly S, O'Brien MC, et al. (2012) Only Low Frequency Event-Related EEG Activity Is Compromised in Multiple Sclerosis: Insights from an Independent Component Clustering Analysis. PLoS ONE 7(9): e45536. doi:10.1371/journal.pone.0045536

Editor: Friedemann Paul, Charité University Medicine Berlin, Germany

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Funding: This study was partly funded by an Enterprise Ireland grant to R.B. Reilly (eBiomed: eHealthCare based on Biomedical Signal Processing and ICT for Integrated Diagnosis and Treatment of Disease, http://www.enterprise-ireland.com/en/), by a Science Foundation Ireland grant to R.B. Reilly (Research Frontiers Program, http://www.sfi.ie), and by an Irish Research Council for Science, Engineering & Technology (www.ircset.ie) grant to H. Kiiski. No additional external funding was received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: whelanrob@gmail.com

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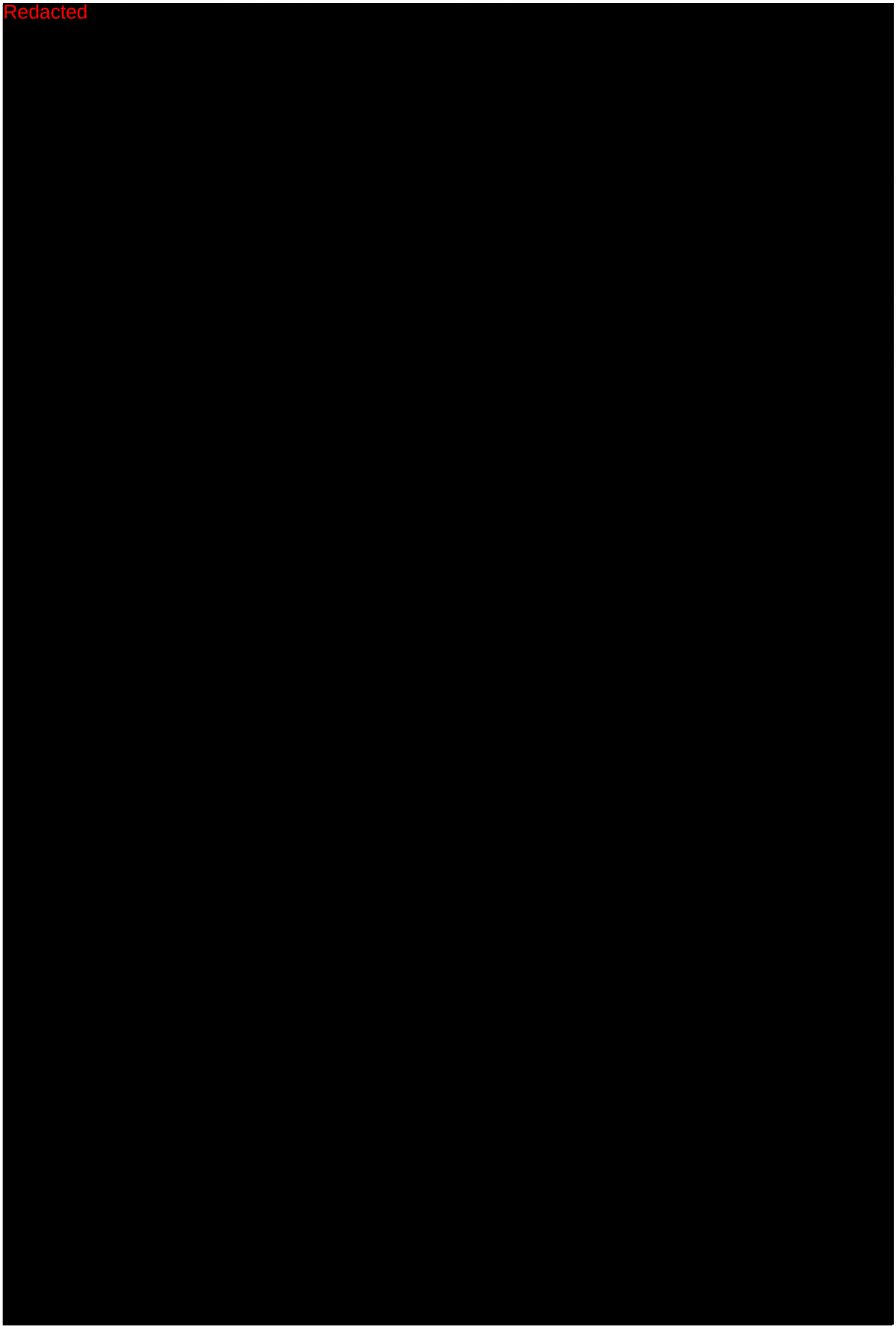
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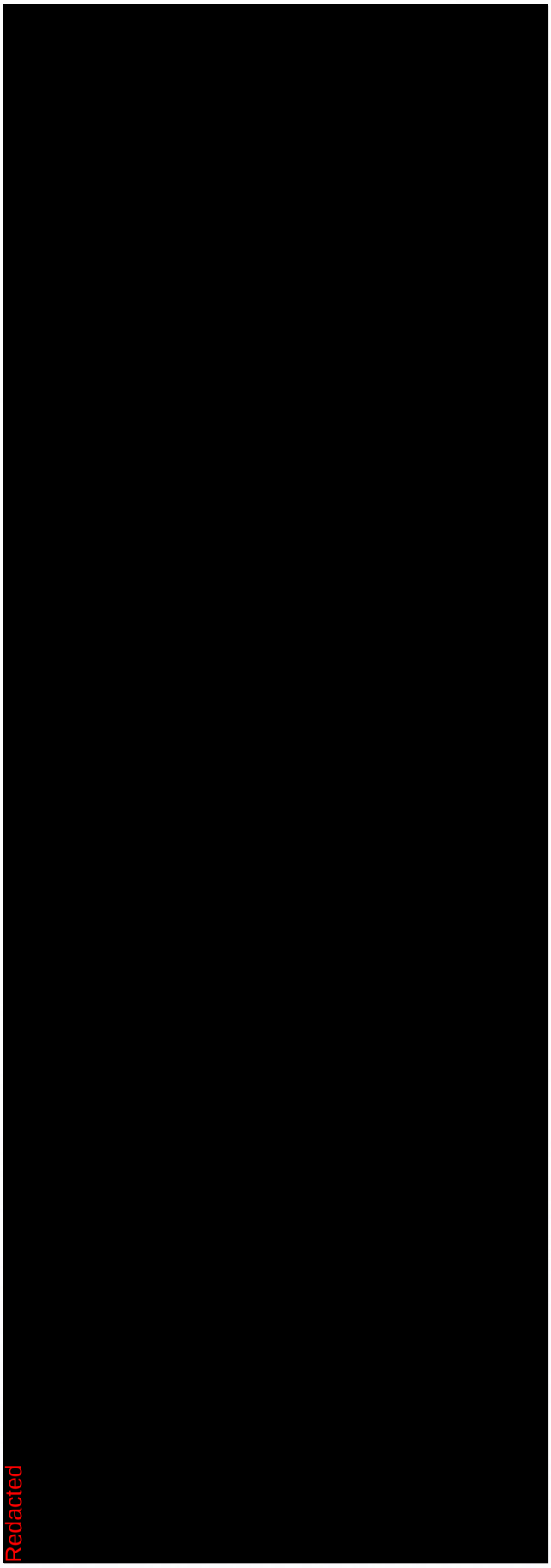
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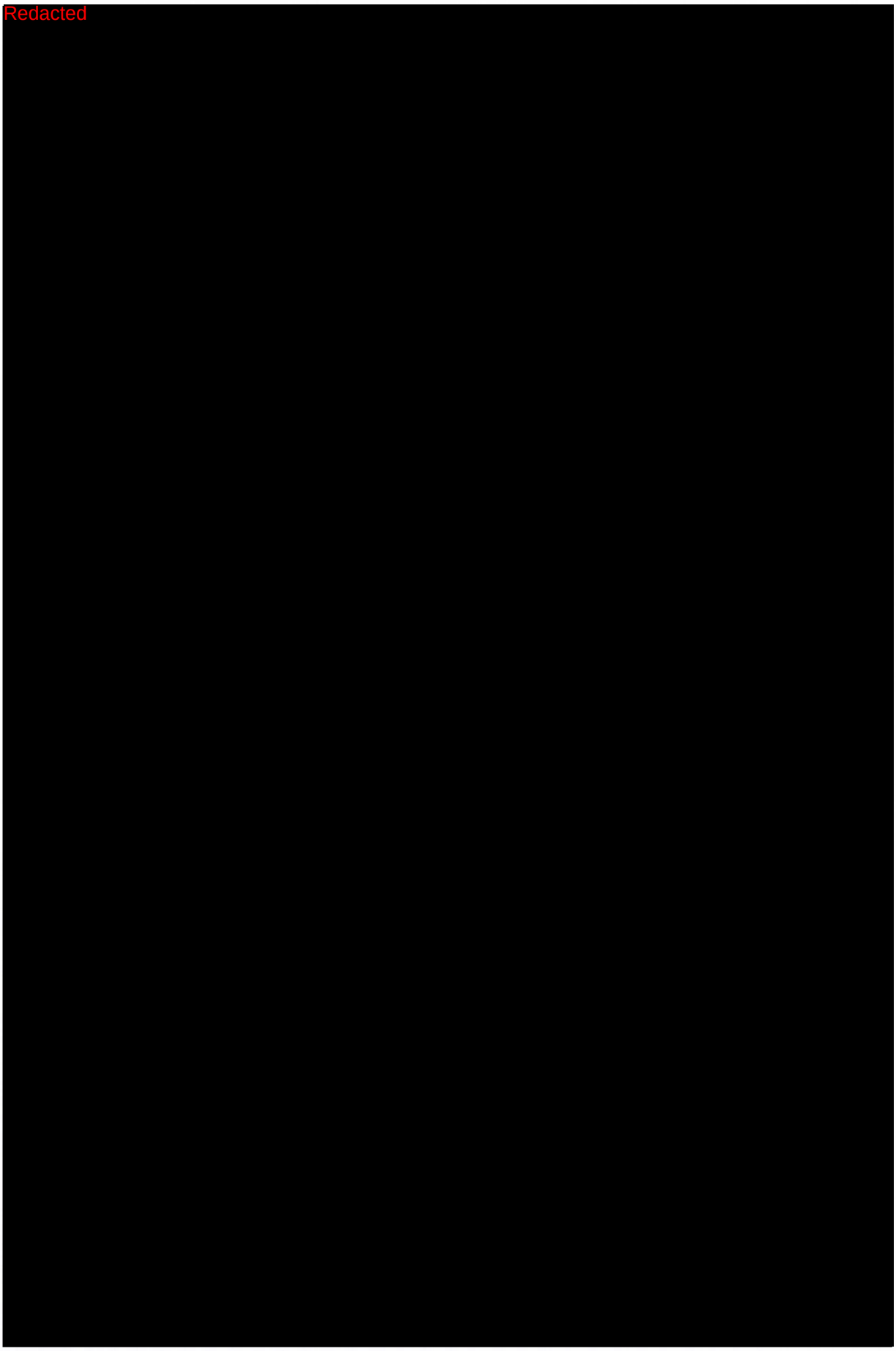

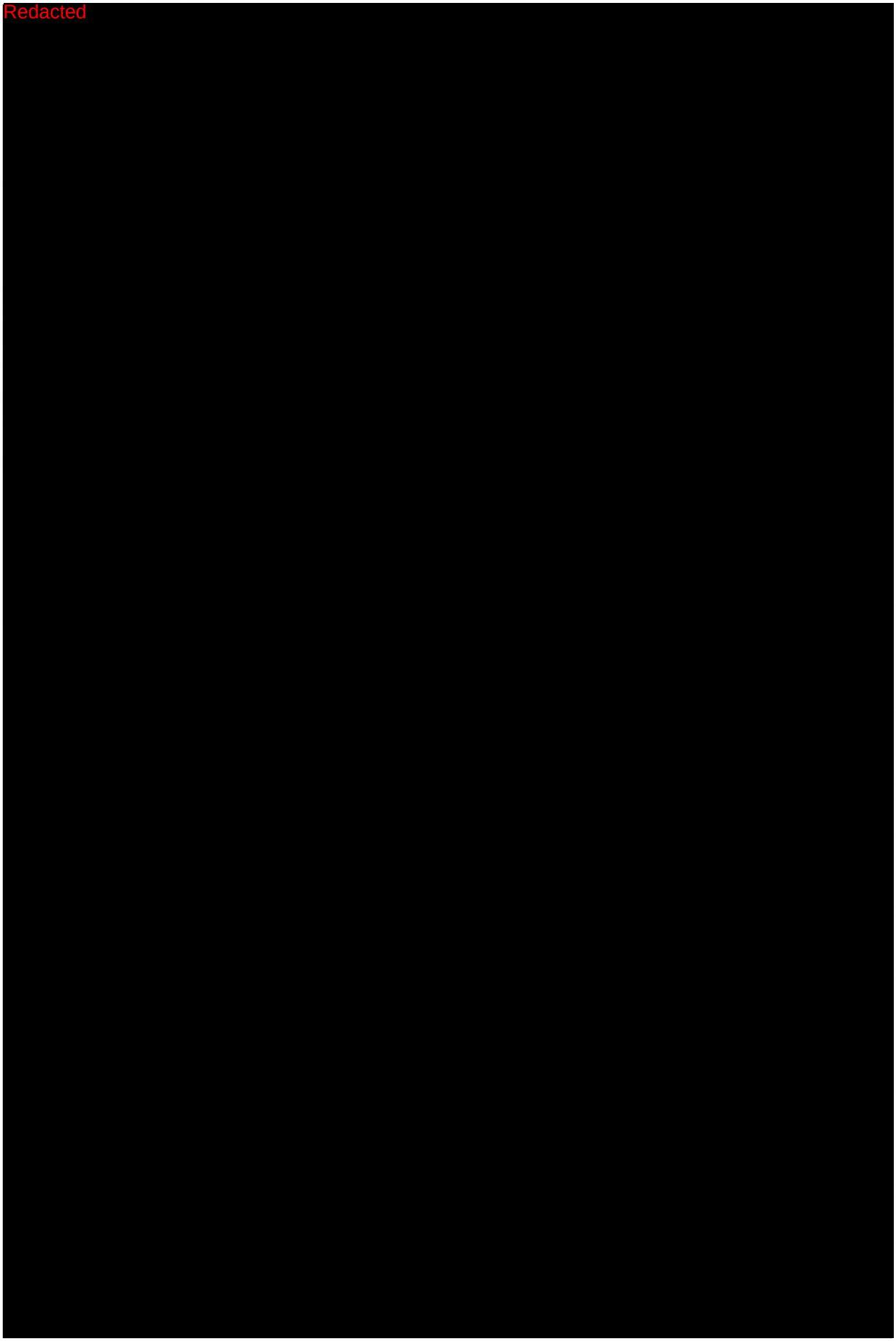
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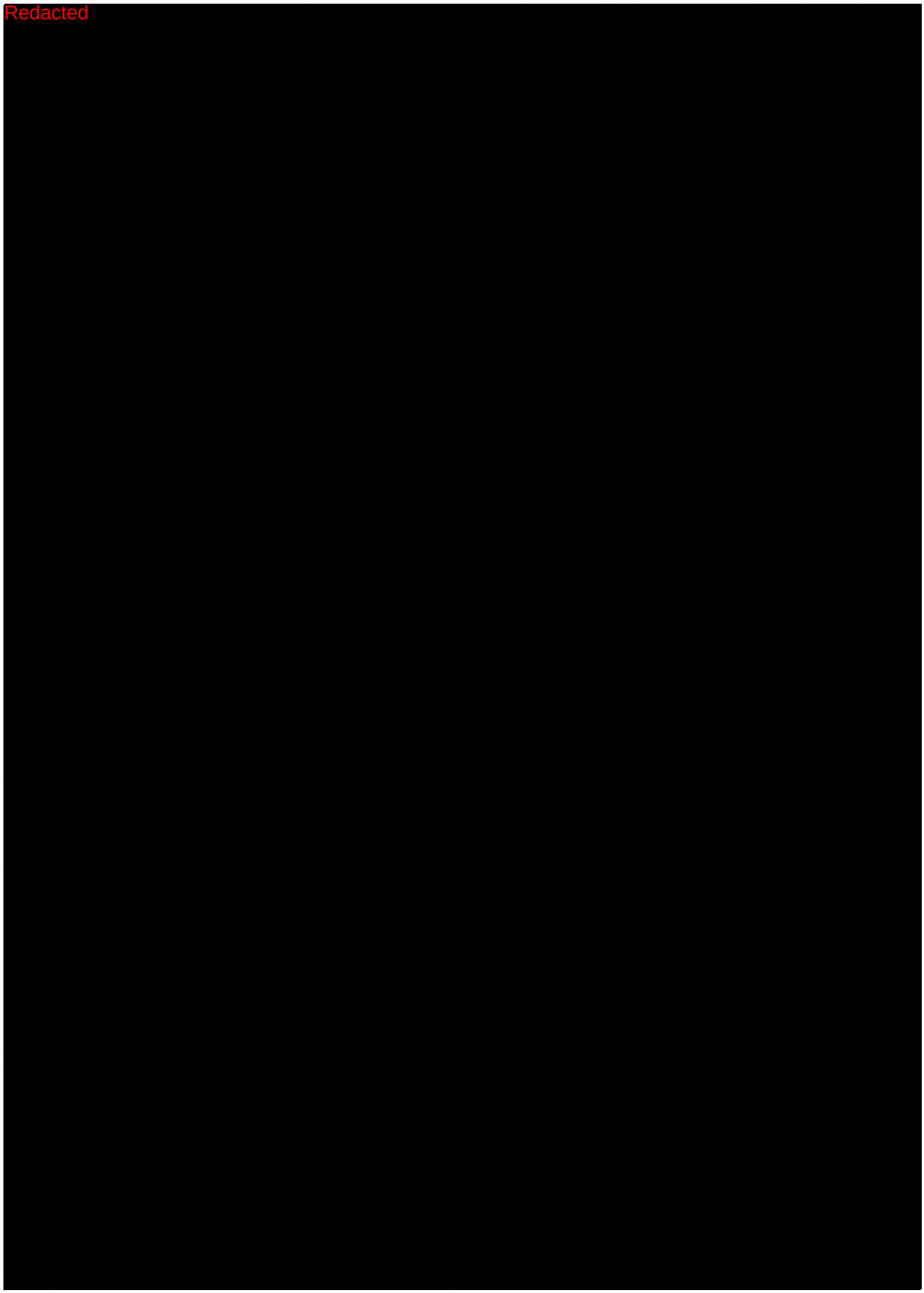

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Appendix 7. Journal paper examining P3 ERP activations and PASAT performance in multiple sclerosis over 12-month period.

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Change in PASAT performance correlates with change in P3 ERP amplitude over a 12-month period in multiple sclerosis patients

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ABSTRACT

Objective: To examine the correlation between the change in PASAT and the change in P3 event-related potentials (ERPs) over a 12-month period in multiple sclerosis (MS) patients, and to compare the 12-month change in the P3 ERP between MS patients and controls.
Methods: Forty-four subjects (27 MS patients, 17 controls) completed visual and auditory two-stimulus oddball and three-stimulus oddball tasks at Month 0 and Month 12. Data were recorded from a 128-scalp channel electroencephalography array. Data from scalp channels were converted into continuous interpolated images (incorporating the entire scalp and time). Amplitude, topographical differences and correlations were then tested using statistical parametric mapping.
Results: The change in visual and auditory P3a correlated significantly with the change in PASAT score ($r = 0.56$, $p < 0.001$ and $r = 0.48$, $p = 0.003$, respectively). Visual P3b and P3a showed greater decrease in 12 months in MS patients relative to controls. Visual P3b, auditory P3b and auditory P3a amplitudes had significantly decreased in MS patients after 12-month period.
Conclusions: Change in visual and auditory P3a ERP amplitudes correlate with change in PASAT scores in MS patients. Visual modality is more sensitive to changes in P3 ERP amplitudes over 12-month period.
Significance: P3 ERPs may have utility in monitoring the change in cognitive functioning in MS.
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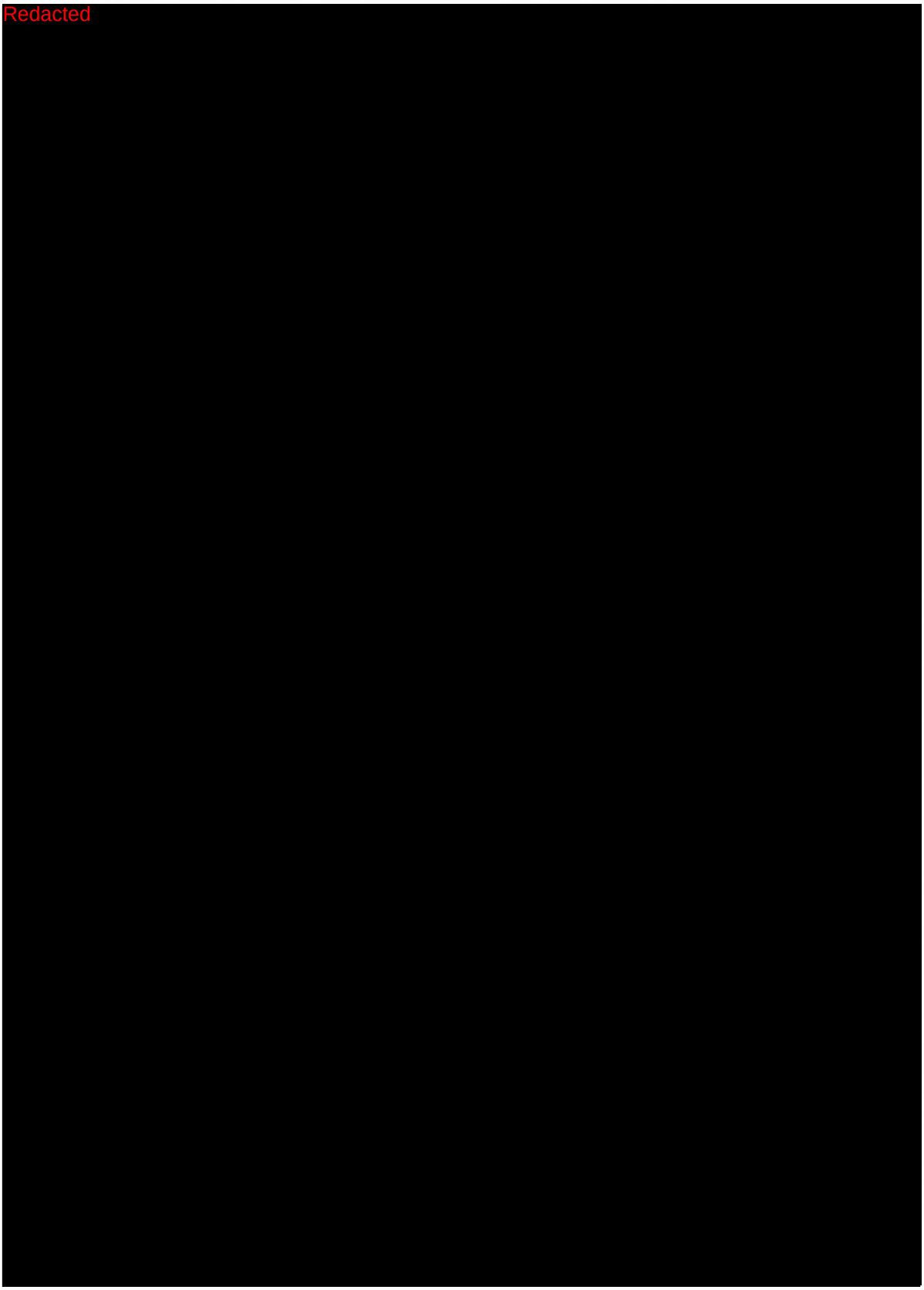
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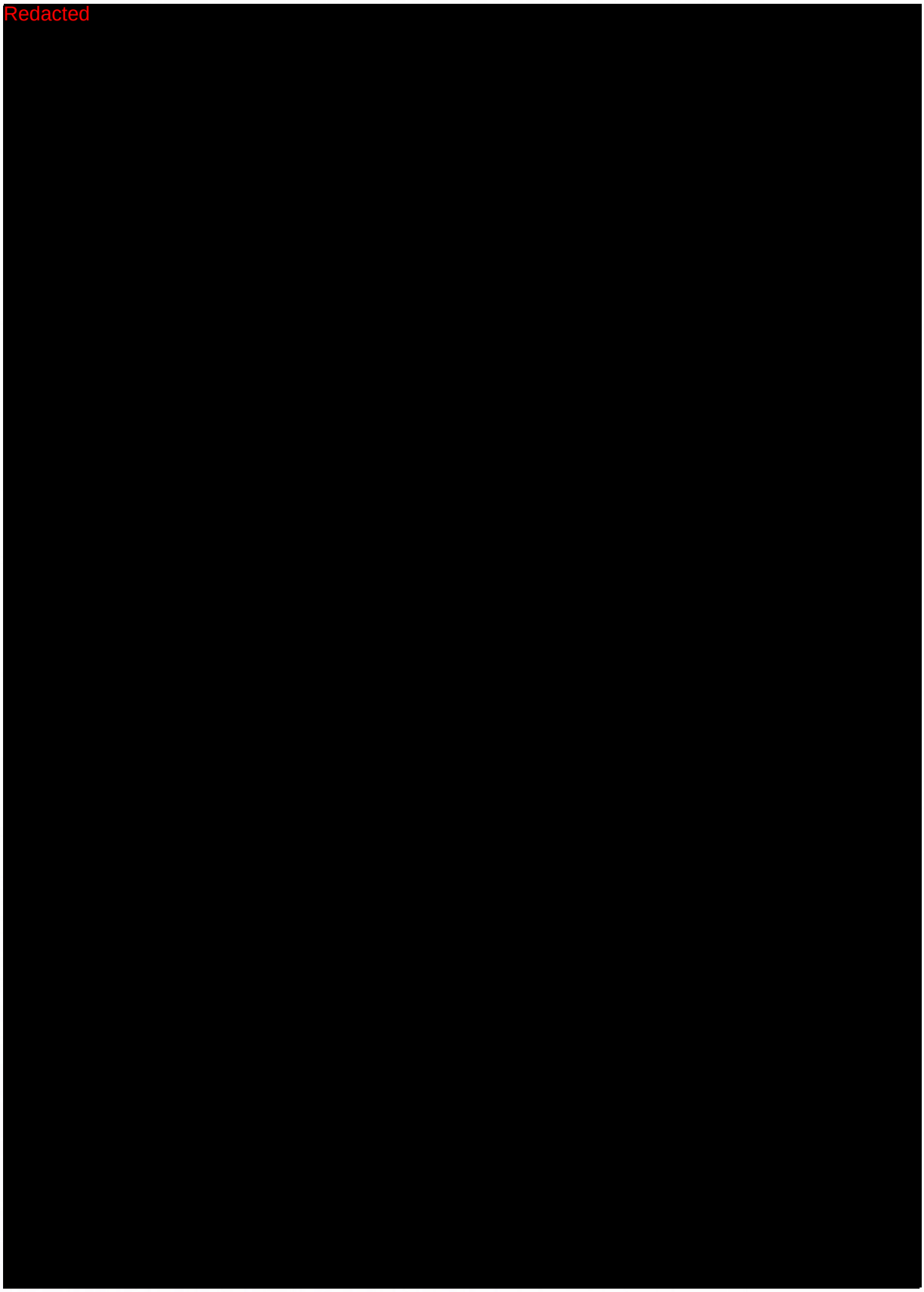

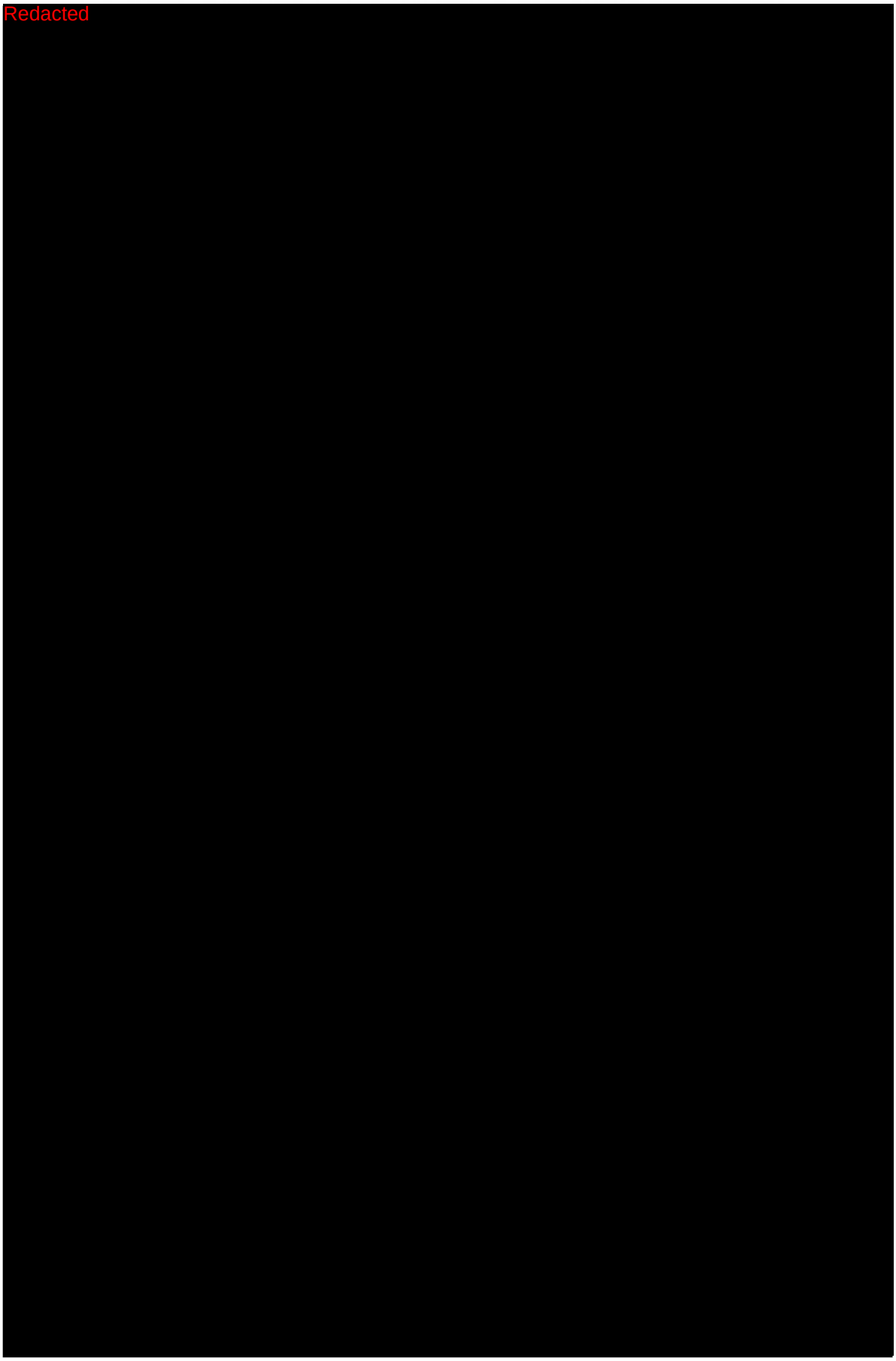
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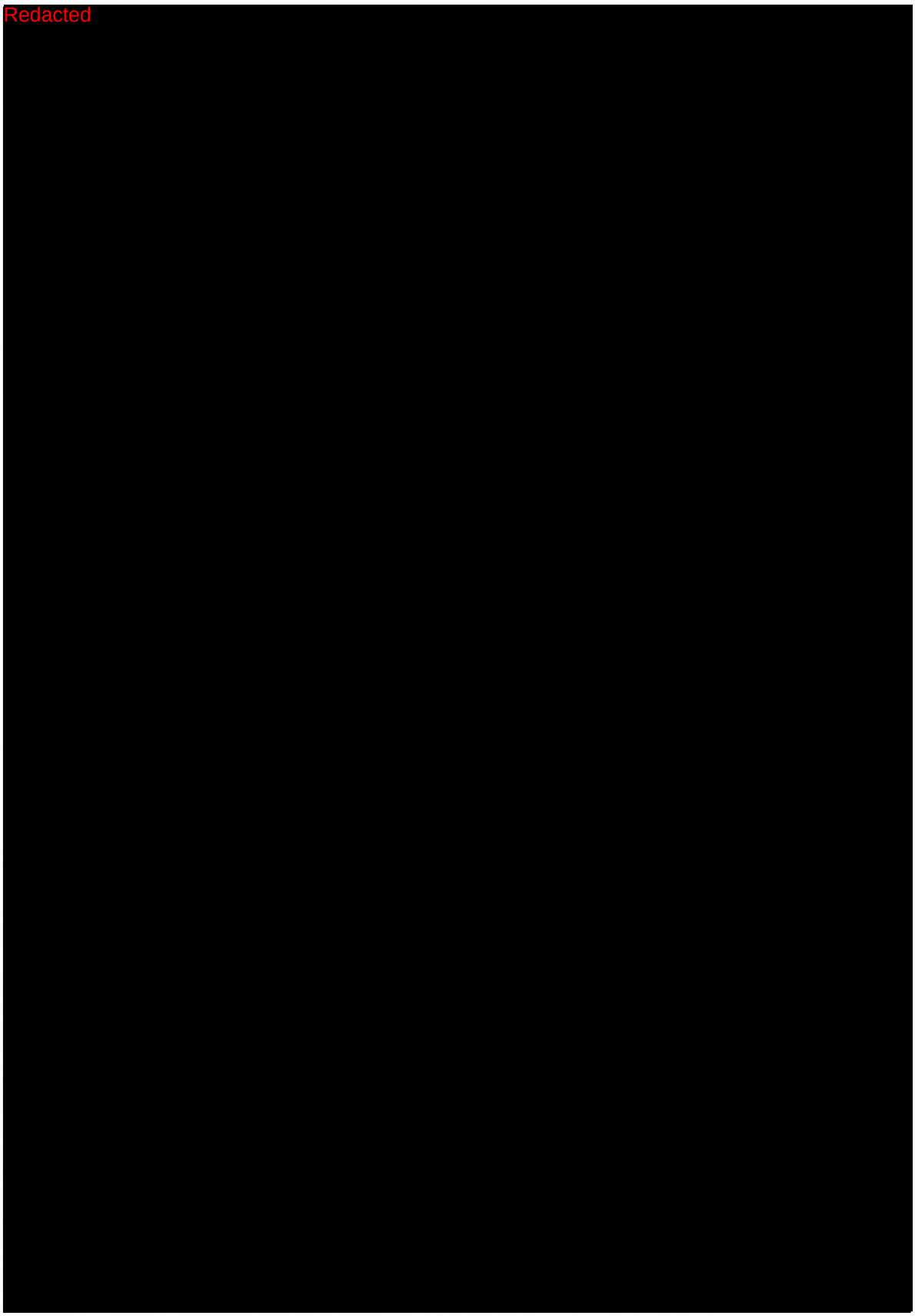
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