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Cognitive function in multiple sclerosis and its modulation by cholinergic drugs

Sarah Cader

A thesis submitted for the fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Oxford

New College

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Short Abstract

In order to assess cognitive function in multiple sclerosis (MS) and the effect of cholinergic modulation, experiments were conducted using functional magnetic resonance imaging (fMRI) to assess the brain activation during cognitive tasks.

A study comparing the processing of verbal working memory with an N-back task found that patients showed smaller increase in activation than healthy controls with greater task difficulty, suggesting a reduced functional reserve. Controls and patients showed differences of correlations between brain regions activated. Interactions between prefrontal regions may provide an adaptive mechanism that could limit clinical expression of the disease distinct from recruitment of novel processing regions.

The effect of Rivastigmine on the cognitive processing in MS patients was tested in a longitudinal study, involving serial fMRI scans. Changes in the brain activation patterns were demonstrated with drug administration, without any changes in behavioural measures. Rivastigmine may act to increase the functioning of the normal neural network reducing the need for previously recruited compensatory mechanisms in MS patients.

A study on healthy subjects examined the effect of cholinergic inhibition on cognitive processing and brain activation. Changes in functional activation due to Hyoscine during verbal working memory were found analogous to that in MS patients without any changes in behavioural measures. Processes that potentially impair brain cognitive function may recruit similar compensatory functional adaptive mechanisms.

Studies on rats and MS patients explored the effect of Rivastigmine on the relationship of the BOLD fMRI signal with the underlying neural activity. Rivastigmine may be influencing the cortical excitability after direct cortical stimulation but showed only a small effect on the BOLD signal under more physiological neural activity. The neural activity in response to visual stimulation is slightly increased with Rivastigmine in MS patients, a change not detected with functional imaging. These studies suggest that changes in BOLD signal do represent sufficiently large changes of underlying neural activity in the presence of Rivastigmine.

The relationship of damage in MS to measures of connectivity was studied using diffusion tensor imaging (DTI). Correlation was found between measures of connectivity and callosal size, a measure of fibre loss. The distribution of lesions was spatially correlated with changes in connectivity due to MS. Thus DTI could be utilized to explore the connectivity changes associated with MS, and the relationship with changes in functional activation.

Table of Contents

i.	Abbreviations	iv
ii.	List of figures	vi
iii.	List of tables	viii
iv.	Acknowledgements	ix

Section 1: Cognitive impairment in multiple sclerosis

1.1	Multiple sclerosis	1
1.1.1	Introduction	1
1.1.2	Pathophysiology	2
1.1.3	Clinical features	3
1.1.3.1	Natural History	3
1.1.3.2	Symptoms	4
1.1.4	Clinical investigations	5
1.2	Cognitive impairment in multiple sclerosis	6
1.2.1	Patterns of cognitive dysfunction	7
1.2.1.1	Memory	7
1.2.1.2	Attention and information processing	8
1.2.1.3	Executive function	9
1.2.1.4	Other functions	10
1.2.2	Relationship of cognitive function with disability & imaging	10
1.2.3	Treatment of cognitive symptoms	11
1.3	Central Cholinergic systems and cognition	12
1.3.1	Pharmacology of acetylcholine	12
1.3.2	Cholinergic projections	13
1.3.3	Cholinergic modulation of neural activity	14

Section 2: Functional imaging and multiple sclerosis

2.1	Magnetic resonance imaging	15
2.1.1	Principles of magnetic resonance imaging	15
2.1.2	T ₂ -weighted images	16
2.1.3	T ₁ -weighted images	17
2.1.4	Magnetic resonance spectroscopy	17
2.2	Functional magnetic resonance imaging	18
2.2.1	Contrast in functional imaging	18
2.2.2	Brain energy metabolism	19
2.2.3	Metabolism and blood flow	20
2.2.4	Temporal resolution of the BOLD response	21
2.2.5	Spatial resolution of functional imaging	21
2.2.6	Functional imaging of multiple sclerosis	22
2.2.7	Functional imaging during drug administration	23

Section 3: Verbal working memory in multiple sclerosis		
3.1	Introduction and rationale	25
3.2	Methods	28
3.3	Results	37
3.4	Discussion	45
3.4.1	Reduced functional reserve for cognition in patients with MS	45
3.4.2	Consistent functional impairment in mesial prefrontal cortex	47
3.4.3	Altered functional connectivity in patients with MS	48
3.4.4	A new strategic focus for cognitive therapies in MS?	50
 Section 4: The effect of Rivastigmine on memory and concentration in multiple sclerosis patients		
4.1	Introduction and rationale	51
4.2	Methods	53
4.3	Results	61
4.4	Discussion	75
4.4.1	The effect of Rivastigmine on cognitive performance	75
4.4.2	Rivastigmine and the Stroop effect	76
4.4.3	Rivastigmine and the N-back task	77
4.4.4	Modulation of cognitive processing with Rivastigmine	78
 Section 5: The effect of Hyoscine on functional activation during cognition in healthy subjects		
5.1	Introduction and rationale	81
5.2	Methods	82
5.3	Results	85
5.4	Discussion	96
5.4.1	Hyoscine and the N-back task	96
5.4.2	Hyoscine and the Stroop task	98
5.4.3	Modulation of cognitive processing with Hyoscine	99
 Section 6: The effect of Rivastigmine on the BOLD fMRI signal		
6.1	Introduction and rationale	101
6.2	Methods	103
6.2.1	Direct cortical stimulation in a rat model	104
6.2.2	Photic stimulation in MS patients	106
6.3	Results	111
6.4	Discussion	116
6.4.1	The effect of Rivastigmine on directly stimulated cortex	117
6.4.2	The effect of Rivastigmine in the contralateral cortex	118
6.4.3	Steady-state visual evoked potential with Rivastigmine	119
6.4.4	Effect of Rivastigmine on visual activation	119
6.4.5	Rivastigmine and the BOLD response	120

Section 7: Diffusion and connectivity in Multiple Sclerosis	
7.1	Introduction and rationale 121
7.2	Methods 124
7.3	Results 128
7.4	Discussion 134
7.4.1	Mean fractional anisotropy 135
7.4.2	Fibre loss and diffusion 136
7.4.3	Axial and radial diffusivity 137
7.4.4	Lesion volume, disability and connectivity 138
Section 8: General conclusions and future directions	
8.1	Patterns of cognition related activations in multiple sclerosis 141
8.2	The effect of cholinergic enhancement in MS patients 142
8.3	The effect of cholinergic blockade in healthy subjects 144
8.4	Potential effects of cholinergic modulation on the BOLD signal 146
8.5	The potential role of tractography in the study of MS 147
8.6	Limitations of the study 149
8.7	Final conclusions 151
Section 9: References	153

i. Abbreviations

AcCoA	acetyl coenzyme A
AR	activation ratio
ATP	adenosine triphosphate
AUC	area under curve
BEDPOST	Bayesian estimation of diffusion parameters obtained using sampling techniques
BOLD	blood oxygenation level dependant
CBF	cerebral blood flow
ChAT	choline acyltransferase
CHO	choline
CNS	central nervous system
COPE	contrast of parameter estimates
Cr	Creatine
CSF	cerebrospinal fluid
DeoxyHb	deoxygenated haemoglobin
DTI	diffusion tensor imaging
DWI	diffusion weighted imaging
EDSS	expanded disability status scale
EEG	electroencephalography
EPI	echo planar imaging
FA	fractional anisotropy
FDT	FMRIB's diffusion toolbox
FEAT	FMRIB's expert analysis tool
FILM	FMRIB's improved linear model
FLAME	FMRIB's local analysis of mixed effects
FMRI	functional magnetic resonance imaging
FMRIB	Functional magnetic resonance imaging of the brain (Centre for)
FOV	field of view
Hb	Haemaglobin
L GFS	left superior frontal gyrus
LSF	least squares fitting
MCFLIRT	motion correction with FMRIB's linear image registration tool
MNI	Montreal neurological institute
MRI	magnetic resonance imaging
MS	multiple sclerosis
NAA	N-acetylaspartate
NO	Nitric Oxide
PASAT	paced auditory serial addition task
PET	positron emission tomography
R GFI	right inferior frontal gyrus
RF	radiofrequency
SDMT	symbol digit modalities task
SPART	spatial recall task

SPECT	single photon emission computed tomography
SRT	selective reminding task
SSVEP	steady state visual evoked potential
T ₂ -LV	T ₂ lesion volume
TE	echo time
TR	repetition time
VEP	visual evoked potential
WLG	word list generation

ii. List of figures

- 3.1 Explanation of the verbal N-back task
- 3.2 Performance according to task difficulty in patients compared with controls during the n-back task
- 3.3 Mean functional activation during the N-back task across difficulties
- 3.4 Regional differences in functional activation between patients and controls
- 3.5 Mean signal change across task-specific regions in patients and controls
- 3.6 Functional correlations between activations in task-specific regions for patients and controls.

- 4.1 Explanation of the Stroop task
- 4.2 Frequency of side effects in patients on and off rivastigmine
- 4.3 Performance of patients in the neuropsychological battery on and off rivastigmine
- 4.4 Stroop reaction times in patients on and off rivastigmine
- 4.5 Group mean activation during the Stroop task
- 4.6 Mean regional signal during the Stroop task in patients on and off rivastigmine
- 4.7 Performance of patients in the N-back task on and off rivastigmine
- 4.8 Group mean activation during the N-back task
- 4.9 Areas of activation greater in patients taking rivastigmine than not during the N-back task
- 4.10 Group mean incremental activation across difficulties in the N-back task
- 4.11 Areas of incremental activation greater in patients taking rivastigmine than not
- 4.12 Mean signal change across task-specific regions in patients on and off rivastigmine
- 4.13 Functional connectivity in patients on and off rivastigmine

- 5.1 Stroop reaction times in healthy subjects on and off hyoscine
- 5.2 Group mean activation during the stroop task
- 5.3 Mean regional signal during the Stroop task in healthy subjects on and off hyoscine
- 5.4 Performance of healthy subjects in the N-back task on and off hysocine
- 5.5 Group mean activation during the N-back task
- 5.6 Areas of activation greater in subjects taking placebo during the N-back task
- 5.7 Group mean incremental activation across difficulties in the N-back task
- 5.8 Areas of incremental activation greater in subjects taking placebo during the N-back task
- 5.9 Areas of incremental activation greater in subjects taking hyoscine during the N-back task
- 5.10 Mean signal change across task-specific regions in subjects on and off hysocine
- 5.11 Functional connectivity in subjects on and off hysocine

- 6.1 Time course of BOLD signal before and after rivastigmine in directly stimulated cortex of the rat
 - 6.2 Time course of BOLD signal before and after rivastigmine in the contralateral cortex of the rat
 - 6.3 Group mean activation during the visual checkerboard stimulus in MS patients
 - 6.4 Mean steady state visually evoked potentials during the visual stimulus in MS patients
 - 6.5 Areas of activation greater in patients off rivastigmine than on rivastigmine during the visual stimulus
 - 6.6 Mean regional signal on and off rivastigmine during visual stimulus in MS patients
-
- 7.1 Correlation between mean FA and mean connectivity in MS patients
 - 7.2 Correlation between callosal area and mean connectivity in MS patients
 - 7.3 Correlation between NAA and mean connectivity in MS patients
 - 7.4 Correlation between T₂ Lesion volume and mean FA in MS patients
 - 7.5 Spatial correlation of FA and lesion load in MS patients
 - 7.6 Correlation between EDSS and mean connectivity in MS patients

iii. List of tables

- 3.1 Disease profile of MS patients in verbal working memory study
- 3.2 Co-ordinates of activation during the N-back task in patients and controls
- 3.3 Co-ordinates of incremental activation across the N-back task in patients and controls
- 3.4 Correlations between activations in task-specific regions for patients and controls

- 4.1 Disease profile of MS patients in rivastigmine study
- 4.2 Baseline performance of patients in the neuropsychological battery
- 4.3 Change in performance of patients in the neuropsychological battery after rivastigmine
- 4.4 Co-ordinates of activation during the Stroop task in patients
- 4.5 Mean regional signal during the Stroop task in patients on and off rivastigmine
- 4.6 Co-ordinates of activation and incremental activation during the N-back task in patients
- 4.7 Co-ordinates of greater incremental activation in patients taking rivastigmine in the N-back task
- 4.8 Correlations between activations in task-specific regions for patients on and off rivastigmine

- 5.1 Co-ordinates of activation during the Stroop task in healthy subjects
- 5.2 Mean regional signal during the Stroop task in healthy subjects on and off hyoscine
- 5.3 Co-ordinates of activation and incremental activation during the N-back task in healthy subjects
- 5.4 Co-ordinates of significant activation differences between subjects on placebo and hyoscine during the N-back task
- 5.5 Correlations between activations in task-specific regions for patients on and off hyoscine

- 6.1 Disease profile of MS patients in the visual stimulation study
- 6.2 Co-ordinates of activation during the visual checkerboard paradigm

- 7.1 Diffusivity measures and callosal area across MS patients
- 7.2 Disease profile and NAA concentration across MS patients
- 7.3 Eigenvalues for axial and radial diffusivity across MS patients

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Section 1: Cognitive impairment in multiple sclerosis

Multiple sclerosis (MS) is a leading cause of disability in young adults, and often affects cognitive function. The mechanisms of how the brain may limit the effect of damage in MS and how this is reflected in the clinical manifestation of cognitive impairment is poorly understood. One of the main themes of this thesis is cognitive function in MS and how this function may be affected by cholinergic modulation. An overview of MS, cognitive impairment, and its treatment is provided in this section.

1.1 Multiple Sclerosis

1.1.1 Introduction

MS is one of the leading causes of neurological disability, typically presenting for the first time in those between the ages of 20 and 40, occurring twice as commonly in women than men (Acheson, 1977; Kenealy et al., 2003; Kurtzke et al., 1979). However, onset of the disease can occur at virtually any age (Duquette et al., 1987; Kurtzke et al., 1979; Noseworthy et al., 1983). Affecting between one and two million people worldwide, a high prevalence of approximately 0.1% is found in countries such as the United Kingdom, the United States and Canada, with a reduced incidence in Asian and African countries (World Health Organization: http://www.who.int/mental_health/neurology/neurogy_atlas_review_references.pdf).

The diagnosis of multiple sclerosis requires evidence of at least 2 attacks resulting in neurological symptoms and signs. These should be disseminated in time and involving two or more non-contiguous anatomical areas within the central nervous system (CNS). Initially made on clinical grounds, the introduction of laboratory

investigations has allowed development of criteria for the diagnosis of MS (Poser et al., 1983).

1.1.2 Pathophysiology

MS is characterised by repeated episodes of inflammation resulting in areas of damage that can occur in any part of the brain or spinal cord. Typically this damage is manifest as demyelination within the white matter, which is the hallmark of the disease, impairing the conduction of impulses along axons (Prineas, 1985). Although plaques may occur anywhere within the CNS, sites of predilection within the brain include tissue bordering the lateral and fourth ventricles, periaqueductal tissue, corpus callosum, optic pathways, and the corticomedullary junction (Brownell and Hughes, 1962). Lesions may be located within cortical grey matter, and in cases outnumber the lesions found in white matter (Kutzelnigg and Lassmann, 2005; Pagani et al., 2005). In keeping with the overall progressive course of the disease, in most longstanding cases it is usual to find at autopsy a mixture of old inactive lesions together with chronic lesions showing evidence of recent edge activity. Newly formed lesions located in previously unaffected white matter are rarely encountered in patients with long-standing disease, but common in those that have died within a few years from onset of symptoms (Prineas, 2001). Associated with the demyelination is injury to the axons and their loss, now considered also to be a significant pathological process in MS (DeLuca et al., 2004; Trapp et al., 1998). There may be a pronounced loss of axons within the old lesions (Barnes et al., 1991) and also within normal appearing white matter (Evangelou et al., 2000). Axonal loss may be significant for the irreversible functional deficits in MS (De Stefano et al., 1998). The distribution of plaques or lesions within the MS contributes to a clinically variable disease, and

pathological studies showing heterogeneity within lesions add further to the mixed presentations across MS patients (Lucchinetti et al., 2000).

1.1.3 Clinical features

1.1.3.1 Natural history

The course of MS is highly variable and 4 patterns of disease have been defined (Lublin and Reingold, 1996). In the majority (80-85%), the disease initially follows a relapsing pattern of acute attacks with periods of remission. This form of the disease is referred to as *relapsing remitting*, with many (50-60%) beginning to steadily worsen between attacks after some years - the *secondary progressive* form of the disease. Far less commonly in *primary progressive* multiple sclerosis, the deterioration in neurological symptoms occurs from the onset of disease and never manifest acute attacks. A small proportion of patients present initially as if they are going to have primary progressive disease but the course is interrupted by disease exacerbations in the *progressive-relapsing* form (Miller, 2001).

Typically, the initial attack of MS often resolves completely or nearly so, and the patient remains well until the next episode. Most do relapse within 3 years, but a minority may be relapse free for a long period of time. However, half of MS patients required assistance to walk by 15 years after onset of symptoms (Weinshenker et al., 1989). Predicting the course of MS in an individual patient is virtually impossible, but the course of the disease over the first five years provides some indication to the subsequent progression (Kurtzke et al., 1977). Those presenting initially with sensory symptoms have a better prognosis than those with early corticospinal or cerebellar dysfunction (Kraft et al., 1981). Age of onset also influences prognosis, with patients

that experience their first symptoms after the age of 40 tending to follow a more rapidly progressive course (Miller, 2001).

1.1.3.2 Symptoms

The ability of MS to affect any area within the central nervous system results in a wide range of possible symptoms (Miller, 2001). Motor weakness due to corticospinal tract involvement occurs in at least a third of patients during their initial attack and to a much greater degree in chronic disease. Symptoms vary from abnormal reflex activity, to heaviness and stiffness, and frequently progressing to severe spastic paraparesis. Legs are much more frequently involved than arms, and symptoms appear earlier in legs than arms. Sensory symptoms are often the earliest symptoms in MS, and common during the entire course of the disease. Any modality of sensation may be affected, though vibration sense appears to be lost more commonly than loss of joint position, pinprick, temperature sensation. Often there are complaints that are difficult to associate with objective neurological signs. A specific symptom of MS is L'hermitte's sign, in which there is a sudden electric-like sensation radiating down the spine or extremities, usually when the neck is flexed (Kanchandani and Howe, 1982). However, neuropathic pain may be present in the limbs or elsewhere, in addition to pain resulting from spasticity of muscles. Abnormalities of brainstem function are frequently manifested as impairments of ocular motility. Nystagmus, usually horizontal, is found in many patients. Internuclear ophthalmoplegia due to a lesion within the median longitudinal fasciculus is another common manifestation. The inability of the affected eye to adduct during abduction of the unaffected eye does not usually result in complaints of double vision unless particularly severe, as the eyes otherwise move normally. Other less common brainstem symptoms include dysarthria

and deafness. Optic neuritis is a common presenting manifestation. Visual loss is seldom total, even when initial loss is extremely severe as good recovery usually occurs (Slamovits et al., 1991). Colour vision may be affected without any discernable loss of acuity. Visual field evaluation often reveals a central scotoma. Cerebellar disturbances are also frequently encountered in MS. This can affect gait, speech, and co-ordination. True to the ability of MS to occur anywhere within the central nervous system, autonomic dysfunction is also commonly found. Urinary or faecal incontinence and retention can be amongst the most disabling features of MS. Although some symptoms are more commonly encountered than others, virtually any function attributed to the central nervous system can be affected.

1.1.4 Clinical Investigations

There are three main investigations that are undertaken in confirming the diagnosis of MS. The first method introduced was the examination of cerebrospinal fluid (CSF) usually obtained by lumbar puncture. The CSF can be entirely normal. However, amongst a variety of non-specific changes, qualitative changes of immunoglobulin in the form of oligoclonal bands are often found in the CSF whilst being absent in serum (Walsh and Tourtellotte, 1983).

Evoked potentials, the second method employed in the investigation of MS, represent electrical potentials evoked by brief sensory stimuli. Signals may become delayed or blocked when they cross through a demyelinated region. Most commonly visual evoked potentials (VEPs) are measured often with a checkerboard reversal stimuli, although somatosensory and auditory evoked potentials can also be measured (Waxman, 1981).

Magnetic resonance imaging (MRI), along with evoked potential recording, can be used as para-clinical evidence of one of the requisite lesions prior to diagnosis of MS (McDonald et al., 2001; Poser et al., 1983). Some of the underlying principles of MRI are discussed in section 2, with particular reference to functional imaging. The majority of MS patients have characteristic findings on MRI, allowing direct visualization of some of the lesions that are found with pathological studies (Bruck et al., 1997; Katz et al., 1993). MRI provides a range of techniques for visualizing the central nervous system in patients with MS. The most commonly used sequences are T₂-weighted images providing evidence of lesions whether active or chronic inactive, and T₁-weighted images allowing differentiation of acute lesions with the use of gadolinium enhancement as an exogenous contrast agent.

1.2 Cognitive impairment in multiple sclerosis

The prevalence of MS-related cognitive impairment and its potentially huge impact on every day function has become increasingly recognized. In the past, this may have been underestimated due to the difficulty in eliciting impairment and the general belief that cognitive dysfunction occurred only infrequently in MS and then usually in advanced disease. However, several studies suggest that 30-70% of MS patients are thought to have problems with cognitive function (Kujala et al., 1996; Peyser et al., 1980; Rao et al., 1991a). The functional impact of this can be striking, affecting employment, interpersonal relationships, independence in daily activities and the capacity to benefit from rehabilitation (Amato et al., 1995; Langdon and Thompson, 1999; Rao et al., 1991b).

1.2.1 Patterns of cognitive dysfunction

Impairment is more often confined to specific domains rather than a global deficit, with a predilection for certain domains over others.

1.2.1.1 Memory

Consequent to the frequent complaints of MS patients for memory problems, aspects of memory impairment have been amongst the most thoroughly investigated deficits in MS. Memory encompasses a broad range of functions. Most of the studies of memory-related impairment used standard clinical measures testing aspects of explicit memory, particularly of episodic memory. The ability to explicitly learn and subsequently recall new material is impaired in MS patients in general (Grant et al., 1984; Minden et al., 1990; Rao et al., 1984; Rao et al., 1989b). Typically less pronounced than deficits in free and cued recall, recognition memory may also be impaired (Thornton and Raz, 1997; Wishart and Sharpe, 1997). There is some debate as to whether retrieval (Rao et al., 1989b; Rao et al., 1993) as opposed to encoding and storage processes are disrupted in MS. More recent observations challenge a specific deficit in retrieval, and suggest that MS can disrupt acquisition as well. Delayed recall has been shown to be intact if additional trials are allowed to learn new material (DeLuca et al., 1994; DeLuca et al., 1998). The performance across trials on multi-trial learning tasks is less consistent in MS patient than that of controls (Beatty et al., 1996; Coolidge et al., 1996). MS patients are also less likely than controls to spontaneously apply systematic learning strategies (Arnett et al., 1997; Canellopoulou and Richardson, 1998).

Subjective complaints of word finding difficulty are common amongst MS patients suggesting problems with semantic memory, another aspect of explicit memory. Indeed deficits in the recall of object names in confrontation naming, famous people and events, and facts learned have been reported (Beatty et al., 1988; Beatty and Monson, 1989; Paul et al., 1997), but it appears to be rare in relapsing-remitting disease (Beatty et al., 1989). Semantic and phonemic fluency are often impaired in MS patients (Beatty et al., 1989; Beatty et al., 1988; Rao et al., 1989b). However, performance on these tasks require normal rapid information processing, which is often impaired in MS, in addition to efficient access to semantic memory.

In contrast to explicit memory, implicit memory appears to be relatively spared in MS. The ability to learn and remember without conscious awareness, as demonstrated by a change in task performance or behavior, has been reported in several studies. In priming tasks, MS patients perform comparably to controls and the performance of skill learning tasks is also intact in MS patients (Beatty and Monson, 1989; Fischer, 2001).

1.2.1.2 Attention and information processing

Deficits in information processing speed are commonly reported in studies of MS patients (Beatty and Monson, 1989; DeLuca et al., 1993; Kujala et al., 1994; Litvan et al., 1988a; Rao et al., 1989c), and is in keeping with frequent complaints by MS patients that they have difficulty in thinking quickly. Impairments in the transferring of information between hemispheres has been documented using appropriately specific tasks (Kujala et al., 1994). This generalized slowing of information processing has been likened to that seen in normal ageing, possibly due to a reduced

signal-to-noise ratio resulting from weakened neural signals or increased background noise (Kail, 1998).

Simple attentional performance is generally intact in MS patients. Attention span has been found to be normal in some studies (Amato et al., 1995; Heaton et al., 1985; Litvan et al., 1988a), though impairment may be more prominent in patients with progressive disease (Paul et al., 1998). MS patients perform reasonably on self-paced selective attention tasks (Franklin et al., 1988), unless particularly complex or speeded (Jennekens-Schinkel et al., 1988; van den Burg et al., 1987). Alternating and divided attention, more complex attentional processes, are frequently impaired (Franklin et al., 1988; Paul et al., 1998). These attentional processes overlap with the concept of working memory - a system responsible for the temporary storage of information and its controlled processing. MS patients exhibit deficits on tasks that tax the resources of the working memory system (Litvan et al., 1988b; Pelosi et al., 1997; Ruchkin et al., 1994). Working memory appears to be particularly important for everyday function in MS (Higginson et al., 2000).

1.2.1.3 Executive function

Unlike memory and attention, impairments in executive function are more likely to be apparent to others than the MS patient themselves.

As group, MS patients often have impaired ability to form concepts and for abstract reasoning. Perseveration frequently characterizes the performance of patients in these tasks. Most executive functions are affected by MS and may influence the potential benefit that patients could obtain from various rehabilitation techniques (Beatty and

Gange, 1977; Beatty and Monson, 1996; Canellopoulou and Richardson, 1998; Foong et al., 1997; Grigsby et al., 1993).

1.2.1.4 Other functions

Impairments of visual perception, both in facial and visual form perception are reasonably frequent in MS (Rao et al., 1991a), whilst visuospatial perception may be less common. Major abnormalities of language are rare, and determining the full extent of language deficit is difficult when memory impairments are co-existent. However, subtle abnormalities of language are probably more common than previously thought. A proportion of patients are cognitively intact, whilst others have different patterns of relatively isolated deficits, and a small proportion may have deficits in multiple domains, sometimes to a severe degree. MS-related cognitive impairment appears to be heterogenous, frequently involving circumscribed deficits in one or two domains. Only a small proportion of patients would meet criteria for dementia (Fischer, 2001).

1.2.2 Relationship of cognitive function with clinical disability and imaging

Both disease duration and the clinical disability as measured by the expanded disability status scale (EDSS) (Kurtzke, 1983) is a poor predictor of cognitive function (Beatty et al., 1990), though there may be a stronger relationship with working memory performance (Thornton and Raz, 1997). Similar to other spheres of function, disease activity affects cognitive function, and impairments during acute relapses show improvements during remission (Foong et al., 1998).

Cognitively impaired MS patients have been found in some studies to have larger T2 lesion burdens than patients without deficit, though not all patients with cognitive dysfunction had high T2 lesion volumes (Franklin et al., 1988; Rao et al., 1989a). Other measures of pathology such as sub-cortical lesion volume and atrophy may have better predictive value (Anzola et al., 1990; Damian et al., 1994; Franklin et al., 1988; Swirsky-Sacchetti et al., 1992). Associating specific cognitive deficits in MS with lesions in a given region has been problematic, particularly when there is widespread disease, and since performance on a task is likely subserved by distributed cognitive networks rather than a specific brain region. The potential role of functional imaging and connectivity analysis for the investigation of cognitive impairment in MS patients is discussed in section 2.

1.2.3 Treatment of cognitive symptoms

The treatment of cognitive symptoms in MS is unsatisfactory. Ideally the underlying cause of these symptoms would be amenable to treatment. However, disease-modifying medication for MS have limited impact on the disease and it is also not clear whether there is any benefit to cognitive function.

Although there are currently no licensed medications for the treatment of cognitive impairment in MS, a number of drugs have been introduced in the management of Alzheimer's and Lewy-body dementia. Drugs that reduce the breakdown of acetylcholine by inhibiting acetylcholinesterase have been shown to improve cognition and limit further decline in these conditions (Gottwald and Rozanski, 1999; Rosler et al., 1999; Wesnes et al., 2002). Similar drugs have recently been tried in MS in a number of small studies. Open-label trials of donepezil have shown some promise

for the improvement of cognitive function in MS patients (Greene et al., 2000; Krupp et al., 2004). Rivastigmine, another cholinesterase inhibitor, has been found to have a potentially beneficial effect on the functional brain activation during an attentional task in MS patients (Parry et al., 2003).

1.3 Central cholinergic systems and cognition

The rationale for the use of cholinesterase inhibitors in the treatment of cognitive symptoms stems from numerous studies in both humans and animals, which have demonstrated the importance of cholinergic systems for memory and particularly attentional performance (Freo et al., 2002; Gold, 2003; Thiel, 2003).

1.3.1 Pharmacology of acetylcholine

Acetylcholine is an important neurotransmitter found throughout the central and peripheral nervous system. It is synthesized in a reaction catalysed by choline acetyltransferase (ChAT) from acetyl-coenzyme A (AcCoA) and choline. The AcCoA is primarily synthesized in the mitochondria, while the choline is transported to the brain in free and phospholipid form. Much of the choline is recycled from the breakdown of acetylcholine and actively transported back into the presynaptic terminal.

Acetylcholine is broken down by cholinesterases, the predominant form being acetylcholinesterase. It is on these enzymes that the drugs used to improve cognition have been targeted. These anticholinesterases can cause reversible or irreversible (by the phosphorylation of the esteratic site) inhibition which increase the extracellular acetylcholine levels, thereby prolonging its action.

Acetylcholine mediates its action mostly via two classes of receptors, Muscarinic receptors, which show a relatively slow response time (100 to 250ms) being coupled to G proteins, but may then act directly on ion channels or utilize second messenger systems. Depending on the receptor subtype, cholinergic stimulation can lead to hyperpolarisation or depolarization. Nicotinic receptors are composed of subunits making up an ion channel that undergoes conformational change when stimulated by acetylcholine. The subunit composition of receptors varies depending on its location, and this imparts different properties on the ligand-gated ion channel. Both types of acetylcholine receptors have been identified within the central nervous system (Cooper et al., 1996).

1.3.2 Cholinergic projections

There are two main groups of neurons that show projections, in addition to the central cholinergic neurons that are within local circuits. From the brain stem, including laterodorsal and pedunculopontine tegmental nuclei, cholinergic neurons project primarily to the thalamus. Those originating from the basal forebrain show diffuse and widespread innervation of the cerebral cortex and hippocampus (Cooper et al., 1996). These projections appear to be important for the modulation of cognitive performance, specifically those requiring complex attentional processing. Loss of these afferent inputs is associated with a loss of performance in rats in tasks designed to assess divided attention (Sarter et al., 1999). Further experiments have demonstrated that the integrity of the cortical cholinergic input system is necessary for attentional performance (Bucci et al., 1998; Chiba et al., 1999; McGaughy et al., 2002; McGaughy and Sarter, 1998; Turchi and Sarter, 1997).

1.3.3 Cholinergic modulation of neural activity

A number of previous studies have demonstrated the ability of acetylcholine to modulate neural activity at a cellular level. In the sensory cortex, application of acetylcholine has a facilitatory effect, via increased neuronal spiking or an increased excitatory post-synaptic potential, on responses to visual, auditory and somatic stimuli (McKenna et al., 1988; Sato et al., 1987; Sillito and Kemp, 1983; Sillito et al., 1983; Tremblay et al., 1990). This appears to be mediated by muscarinic receptors resulting in depolarization (Caulfield et al., 1993; McCormick, 1992). There also appears to be a differential action depending on the source of incoming fibers. Acetylcholine has been found to exert a greater suppressive effect on intrinsic fibers than afferent fibers (Patil and Hasselmo, 1999) and so may effectively increase the signal-to-noise ratio of the afferent neural activity. Thus acetylcholine, may serve to shift the dynamics of the cortical networks into a state where afferent influence predominates over intracortical influence (Kimura, 2000), which may increase the sensitivity and functioning of the neural network recruited to perform a task. In a disease state such as dementia or MS, the additional use of drugs to enhance cholinergic transmission may help limit the possibly reduced signal-to-noise ratio hypothesized by Kail (Kail, 1998).

Section 2: Functional imaging and multiple sclerosis

Functional imaging has become a widely used method for brain mapping and studying the neural basis of human cognition. Functional imaging is employed in this thesis to investigate cognitive function in MS and the effect of drug modulation on this function. In this section, the principles underlying functional magnetic resonance imaging are discussed. Also briefly discussed is functional imaging in the context of multiple sclerosis and pharmacological manipulation.

2.1 Magnetic resonance imaging

2.1.1 Principle of magnetic resonance imaging

MRI is based on the principle that protons (or hydrogen atoms) have a detectable signal when placed in a magnetic field (Jezzard and Clare, 2001; Stone et al., 2001). Advantage is taken of the abundance of hydrogen atoms in intra- and extra-cellular water, lipids and other more complex molecules, all of which are found readily in MS lesions. Whilst in a magnetic field, protons align with the longitudinal axis of the field in a state of equilibrium. In a process known as precession, the protons wobble around this axis at a frequency specific for the hydrogen nuclei and proportional to the strength of the magnetic field. To perturb the nuclei from the equilibrium state, a radiofrequency pulse is applied at the Larmor frequency described above via the head coil of the scanner. When the RF pulse is turned off, there is a return of the longitudinal magnetization also known as the T1 relaxation time. The T2 relaxation time refers to the loss of transverse magnetization. The T1 and T2 constants reflect the local tissue environment of the protons. This provides contrast in the image to differentiate between tissue compartments. By varying the pattern and timing of the

RF pulse, in combinations with changes in the main magnetic field with the additional magnetic field gradients, one can impart spatial information to the signal to create a magnetic resonance image. The pulse sequence refers to the pattern and timing of RF and magnetic field gradient pulses, some of which are more commonly employed in the investigation of MS

2.1.2 T2-weighted images

The time between RF excitation and signal acquisition is known as the Echo Time (TE), and by allowing more time for T2 relaxation with a long TE, the MR images are T2 weighted. Thus signals from tissues with longer T2 values decay less and appear as hyperintense on T2-weighted images relative to tissues with shorter T2 relaxation times. Random tumbling of neighboring nuclei lead to low frequency random fluctuations in the local field at the molecular level resulting in the Larmor frequencies of different nuclei to vary. These slight increases or decreases cause a loss of bulk transverse magnetization and consequently a loss of signal. T2 processes are highly sensitive to both very slow molecular motions, and to motions at the Larmor frequency. Therefore, T2 is very sensitive to changes in tissue water content, and to the nature and concentration of tissue macromolecules. Typically cerebrospinal fluid (CSF) is bright, as are MS plaques, which leads to the practical problem in the precise definition of periventricular lesions. In addition, both active and chronic inactive lesions are visualized as hyperintense, but the advantage of the T2W image is that a large number of MS plaques are well seen.

2.1.3 T1-weighted images

By shortening the time between repetitions of the pulse sequence (TR), less time is allowed for the water to regain its equilibrium magnetization. More signal is found in tissues with a relatively short T1 relaxation time, as there is more recovery than those tissue with a long T1 during the shorter TR. The T1 relaxation rate is influenced only by components of molecular motions at the larmor frequency. In this way, greater specificity to pathological phenomenon is provided at the expense of sensitivity. Protons in CSF or tissue associated with oedema or loss of structural integrity have a long T1, and appear dark on these sequences. Injection of gadolinium increases the sensitivity of T1 for the detection of lesions (Rovaris and Filippi, 2000). The strong paramagnetic properties of this element results in effects on the local rate of water MR relaxation proportionate to its local concentration (Tofts and Kermode, 1991). This effect is more pronounced on T1 than T2 relaxation, but does not normally cross the blood brain barrier. When the integrity of this barrier is compromised, such as in active lesions, the gadolinium causes an increased T1 signal at these sites.

2.1.4 Magnetic resonance spectroscopy

Unlike the water-proton-based MRI techniques described above, in magnetic resonance spectroscopy (MRS), signals are recorded from the protons in various CNS metabolites (Rudkin and Arnold, 1999). The concentration of these metabolites is a fraction of tissue water, and visual images are not usually obtained in the same way as standard MRI. Instead, small volumes of interest are selected to provide chemico-pathological information with a spectrum of various compounds found in that region. In practise, using relatively long echo times (i.e. TE of 136 or 272 milliseconds) identifies four major resonance peaks: N-Acetylaspartate (NAA), choline (Cho),

creatine (Cr) and lactate. Lactate is generally not detectable, but can be associated with acute lesions (Clanet and Berry, 1998). The Cr peak is relatively constant and often used to normalize the signal intensities of the other metabolites. Cho measures membrane phospholipids, and an increase may represent increased membrane turnover with release of lipids and breakdown products of myelin (Arnold et al., 1998). NAA has been shown to be localized within neurons and neuronal processes (Moffett et al., 1991; Simmons et al., 1991) and a number of studies have linked axonal pathology with reduced NAA in MS patients (De Stefano et al., 2001; Fu et al., 1998).

2.2 Functional magnetic resonance imaging

2.2.1 Contrast in functional imaging

Most fMRI studies now carried out utilize the ability of blood to act as an endogenous contrast agent. Haemoglobin (Hb), an iron-containing molecule that carries the majority of the oxygen in the blood, can exist in either the deoxygenated or the oxygenated state. The iron gives Hb magnetic properties that depend on the state of oxygenation. Whilst oxygen is bound, the molecule is diamagnetic (having little effect on the environment), but with deoxygenation (deoxyHb) the molecules becomes highly paramagnetic (Pauling and Coryell, 1936) which can influence the MR signal (Brooks et al., 1975). The confinement of deoxyHb to the red blood cells results in magnetic susceptibility differences between deoxygenated blood and surrounding space, creating gradients across and near compartment boundaries. Pulse sequences designed to be sensitive to these differences generate signal alterations whenever the concentration of deoxyHb changes (Ogawa and Lee, 1990; Ogawa et al., 1990a; Ogawa et al., 1990b). This blood oxygenation level dependant (BOLD) mechanism

can be used to obtain functional imaging of underlying brain activity related to increases in blood oxygenation in humans (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992). Although a decrease in signal might be expected due to an increase in deoxyHb with activity, there appears to be overcompensation of the increase in cerebral blood flow (CBF) delivering an oversupply of oxygenated blood (Fox and Raichle, 1986; Fox et al., 1988).

2.2.2 Brain energy metabolism

Energy is required for the neural activity that takes place in the brain. Information is transferred within the brain through the propagation of altered membrane potentials and via the release of chemical neurotransmitters. Both processes involve work coupled to ion transport across the membranes of cells. These energy-utilizing processes are closely linked to energy-producing processes via adenosine triphosphate (ATP), and minimal changes in ATP are demonstrated even with as much as a 10-fold variation in cellular work (Detre et al., 1990a; Detre et al., 1990b; Matthews et al., 1981). The pathways responsible for the synthesis of ATP are regulated by factors related to changes in its utilization. ATP concentration is maintained by changes in hydrolysis of phosphocreatine (Wyss et al., 1992), and more sustained increases are provided by increased glycolysis and oxidative phosphorylation. Some of the sub-processes of glycolysis are near equilibrium state, and this allows for change to occur in the order of milliseconds, critical to maintaining bio-energetic homeostasis in response to rapid changes in cell energy utilization, such as neuronal depolarization (Gjedde, 2001). At steady state, the brain almost completely oxidizes glucose via aerobic glycolysis, so that almost all pyruvate produced by glycolysis undergoes oxidative phosphorylation in the mitochondria. Changes in neuronal membrane

potential are associated with energy utilization (Gjedde, 2001) and increased oxygen uptake (Erecinska et al., 1991). An increase in the glycolytic rate is found during functional brain activation, though with little change in the oxygen consumption (Fox and Raichle, 1986; Fox et al., 1988). However, other studies have shown that a significant increase is demonstrated with more complex situation suggesting that there is activation of cells with higher oxidative capacity than cells of low oxidative capacity normally activated (Gjedde, 2001; Vafae and Gjedde, 2000; Vafae et al., 1998).

2.2.3 Metabolism and blood flow

Cerebral blood flow is not related in a simple way to increased oxygen consumption, and although consumption may remain unchanged, increased blood flow can be demonstrated (Kuwabara et al., 1990). It is possible that transient accumulation of ions or molecules due to brain metabolism may trigger the haemodynamic response in parallel with regional activation (Logothetis and Pfeuffer, 2004). The excess potassium in glial cells, following the increased extracellular potassium during neuronal excitation, has been speculated to be released perivascularly, dilating the resistance vessels (Paulson and Newman, 1987). However, there appears to be a heterogenous response depending on the location within the brain (Caesar et al., 1999). Alternatively the responses may be driven by neurotransmitter-related signaling rather than the direct local needs of the brain (Attwell and Iadecola, 2002). This may be mediated via nitric oxide (NO), which causes vasodilation of brain blood vessels. Inhibitors of NO synthase reduce the dilatory effect of neurotransmitters injected into neocortex and hippocampus (Faraci and Breese, 1993; Fergus and Lee,

1997). The accumulation of lactate may also be driving the haemodynamic response (Laptook et al., 1988).

2.2.4 Temporal resolution of the BOLD response

Typically, there is a short delay (4-6 seconds) between the onset of a stimulus and the peak of the haemodynamic response. This in itself is not problematic if the shape of the haemodynamic response can be accurately estimated. However, this may be influenced by disease (Pineiro et al., 2002), and may vary between individuals (Aguirre et al., 1998), or cortical areas and task (Rajapakse et al., 1998). This variability in response latency, rather than the delay, limits the temporal interpretation of fMRI. The temporal resolution is dependant on the repetition time between signal acquisitions, and this needs to be sufficiently long enough to allow adequate recovery of the longitudinal magnetization before the next pulse. This is usually at least one second, but may be further limited by scanner hardware, as a short TR requires very rapid gradient switching. These upper limits of temporal resolution only generally apply to event-related experimental designs. In a block design, where the stimulus is maintained for relatively long periods of time, one assumes a constant response across the whole block. In this thesis, only conventional block designs are employed, and thus the temporal discrimination provided by fMRI is more than sufficient.

2.2.5 Spatial resolution of functional imaging

The theoretical limit to spatial resolution is determined by the size of the smallest vascular component able to act as an independent functional unit. Although it is not clear whether this is the capillary or the feeding arteriole, the actual resolution is determined by a number of other factors. Changes around large vessels are likely to be

of greater magnitude than changes in the microvasculature, which can skew spatial localization (Gati et al., 1997). This may be particularly problematic in studies with low signal-to-noise. The cerebral blood flow response may not be restricted to areas of increased excitatory neuronal activity. An optical imaging study of visual stimulation demonstrated that blood flow changes were not confined to the neuronal activity in columns, but extended over inactive areas (Malonek and Grinvald, 1996). These effects should be borne in mind when interpreting the BOLD signal.

2.2.6 Functional imaging of multiple sclerosis

fMRI allows patterns of brain activation associated with a certain task to be mapped, providing some indication to the networks involved. Differences in the patterns of activity have been demonstrated between various patient groups and healthy controls (Pineiro et al., 2001; Reddy et al., 2002). These differences may exist even in the absence of overt clinical disability (Bookheimer et al., 2000). Unsurprisingly a number of studies have also shown changes in the functional activation of MS patients compared to control subjects (Lee et al., 2000; Pantano et al., 2002). Although, this has been most clearly defined in, it is not limited to motor tasks. Similar changes in brain activation have been shown in the cognitive function of MS patients (Lazeron et al., 2004; Mainero et al., 2004; Wishart et al., 2004). Such alterations in brain activation have been interpreted as evidence of functionally adaptive reorganisation. However, there are complexities in interpreting differences in patterns of activation between subjects with pathology and healthy controls. Brain regions identified by fMRI are those associated with task performance but not those necessary (Johansen-Berg et al., 2002). Difference in patterns of activation may be associated with alternative strategies for the performance of a task (Cifelli and Matthews, 2002). More

fundamental processes may also be affected by pathology and can also be associated with alterations in brain activity, and may not be adequately controlled (Price and Friston, 1997). The degree to which MS might directly influence neurovascular coupling is unclear, though this is likely to occur in the same regions in which axonal loss and demyelination is occurring, but as alluded to earlier, the haemodynamic response does appear to be influenced by disease (Pineiro et al., 2002). Thus, it may be difficult to generalize responses from healthy subject populations to that of MS patients.

2.2.7 Functional imaging during drug administration

The interpretation of the BOLD signal under conditions of pharmacological manipulation is a potentially problematic issue that is can often be overlooked. However, given the complex relationship between the underlying neural activity and the generation of the BOLD response, the potential for this to be influenced by drug administration should be considered.

In one study of photic stimulation, activity-related changes in the visual cortex were reduced by the administration of acetazolamide, which increases the cerebral blood flow without changing neuronal metabolic activity (Bruhn et al., 1994). Indomethacin has a similar effect, but administration of acetylsalicylate did not (Bruhn et al., 2001). Another group found that the local cerebral blood flow in relationship to neuronal activity was not affected during altered experimental conditions (Rosengarten et al., 2002).

In this study, functional imaging is performed during cholinergic modulation. Cerebral blood flow is enhanced by the administration of nicotine, a phenomenon demonstrated in both animal and human studies (Hall, 1972; Skinhoj et al., 1973;

Uchida et al., 1997; Zubieta et al., 2005). This may be mediated by the stimulation of cholinergic receptors in the basal forebrain (Linville et al., 1993; Sato et al., 2004), and these increases in themselves do not indicate the degree to which there may be changes in cerebral blood flow independent of changes in neuronal metabolic activity. For instance, a study examining the effect of scopolamine (blocking the action of acetylcholine) on the change of blood flow due to somatosensory stimulation did not find any influence of the drug, but did find a change in the baseline cerebral blood flow (Nakao et al., 1999).

One of the aims of this thesis is, therefore, to examine the possible effect of rivastigmine on the BOLD response, with particular reference to MS patients.

Section 3: Verbal Working Memory in Multiple Sclerosis

A number of studies have explored brain activation during cognitive processing in MS patients. The effect of MS and to what extent the brain is able to compensate for areas of damage is not entirely clear. The overall aim of this thesis is to investigate mechanisms of functional adaptation of cognitive function in MS and how this may be influenced by cholinergic modulation. This first experiment was, therefore, designed to characterize more fully the patterns of cognitive-related activation in a group of MS patients in a task of increasing difficulty and the effect on interactions between regions involved.

3.1 Introduction and rationale

Cognitive dysfunction (estimated to affect between 30%-70% of patients) is now appreciated to be a characteristic feature of MS (Rao et al., 1991). The most commonly identified functional impairments affect attention, speed of information processing and memory (Achiron and Barak, 2003).

Some functional imaging studies already have attempted to define changes in brain activity during memory tests in patients with MS relative to healthy controls. Using the paced auditory serial addition task, Mainero and colleagues defined differences in activity in several regions of brain, including areas involved in the primary task (inferior and middle frontal and superior and middle temporal gyri and the inferior parietal cortex) (Mainero et al., 2004a). In a memory recall task there was overlap between regions recruited to a greater extent in patients who performed well on the task relative to healthy controls and the primary task-associated activations (Mainero et al., 2004a). Similar to findings for Alzheimer's disease, in which subjects in an

early, pre-clinical phase show relatively increased prefrontal cortical activation with memory deficits (Bookheimer et al., 2000), the findings in MS were interpreted as evidence for increased activity in relevant areas that is able to functionally compensate for injury associated with progression of disease. Correlations between activation changes and T2-hyperintense lesion burden were consistent with this. However, increased brain activity is not expected generally with progression of the disease. Previous positron emission tomography (PET) and Single Photon Emission Computed Tomography (SPECT) studies have emphasised reduced cerebral blood flow and metabolism in patients with established MS (Brooks et al., 1984; Paulesu et al., 1996; Sun et al., 1998). A recent report using arterial spin labeling confirms that grey matter hypo-perfusion is associated generally with secondary progressive and primary progressive MS (Rashid et al., 2004). These latter studies suggest that patients with MS may have limited cognitive “functional reserve”, the ability to match brain activity to cognitive demands.

Use of tasks with graded difficulty to assess the ability of brain regions to increase activity with increasing task demands offers a parametric approach to testing this notion directly. In initial work, Wishart and colleagues used 1- and 2-back tasks in a small group of MS patients to test for memory task related increases in brain activity (Wishart et al., 2004). They found that patients showed relatively less increased activity than healthy controls in regions associated with increasing task difficulty. Although interpretation of this study is limited by lack of direct measurement of performance during the fMRI task, these results suggest that early functional impairments in patients may be reflected as a reduced ability to recruit relevant brain regions.

However, it is important to appreciate the complexities of interpreting differences in patterns of activation across the brains of subjects with pathology relative to healthy controls. First, fMRI identifies brain regions in which activity is *associated* with task performance, not those that are *necessary* (Johansen-Berg et al., 2002). Secondly, alternative strategies for performance of a task can be associated with differences in patterns of activation without being able to be interpreted in a simple way as adaptive (Cifelli and Matthews, 2002). Finally, pathology may affect more fundamental processes, such as those for perception or action, that also can be associated with differences in brain activity and potentially may not be adequately controlled in the contrast (Price and Friston, 1997). Thus, interpretation of evidence for functional reorganisation (particularly for cognitive tasks) is complex.

Here data is presented from an n-back working memory task over three levels of task difficulty in order to parametrically assess changes in brain activity in MS patients relative to healthy controls. If adaptive functional reorganisation contributes to limiting clinical expression of pathology affecting cognition, then differences between patients and controls should be manifest even in early stages of the disease. Thus this experiment looks at a group of relapsing-remitting MS patients who do not clinically express memory deficits. Study of a patient group without clinically evident cognitive deficits also removes performance differences confounds. The hypothesis is that adaptive changes may be manifest as altered functional interactions between brain regions, as well as differences between the regions recruited. Therefore, the study is extended with a functional connectivity analysis to test for multi-variate differences in relations between activities in brain regions normally recruited for the task.

3.2 Methods

Subjects

Twenty-one right-handed patients (6 men, 15 women; median age 39 years, range 22-55 years) with clinically definite multiple sclerosis (all relapsing remitting; median duration 6 years, range 1-20 years) according to the Poser Criteria (Poser et al., 1983) performed a verbal working memory paradigm whilst undergoing fMRI scanning (Table 1).

Patient	EDSS	Disease duration	Time since last relapse	T ₂ -LV	Medial Frontal T ₂ -LV	Age
		<i>yrs</i>	<i>mths</i>	<i>mls</i>	<i>mls</i>	<i>yrs</i>
1	2.0	4	6	8.23	0.78	24
2	1.0	2	18	4.66	0	43
3	5.0	12	3	3.1	0.075	39
4	3.0	16	24	1.69	0.235	55
5	6.0	20	10	13.98	0	42
6	1.5	6	4	16.02	0.12	34
7	2.5	10	6	3.69	0	30
8	0.0	1	6	3.19	0	23
9	3.0	3	4	7.48	0.255	37
10	1.0	8	10	5.02	0.16	43
11	2.0	14	27	10.31	2.17	37
12	1.0	6	12	17.74	1.305	40
13	1.5	10	9	0.77	0.125	43
14	2.0	2	3	2.14	0.28	34
15	3.0	7	3	4.97	0.22	28
16	3.0	9	14	2.29	0.145	34
17	0.0	6	36	22.99	1.595	24
18	1.5	8	24	7.91	0.55	42
19	1.0	6	68	1.74	0.255	39
20	0	6	48	0.85	0.075	43
21	5	4	7	7.39	0.415	43

Table 3.1 Disease characteristics including the lesion volume (LV) in each patient.

Disability was assessed with Kurtzke Expanded Disability Status Scale (EDSS) (median EDSS 2, range 0-6) (Kurtzke, 1983) at the time of the fMRI scanning by experienced neurologists (AC, ZC). No patient subjects had clinically evident visual deficits, impairment of right-hand movement or symptoms related to altered cognition (memory, language, calculation or attentional deficits) on the basis of clinical histories obtained during routine follow-up or directed questioning at the initial interview for this study. None were on disease-modifying therapy. Subjects with a history of psychological and psychiatric problems, epilepsy, learning disability, alcoholism, head injury, episodes of loss of consciousness or severe ongoing stressors (such as bereavement, divorce, financial problems) were excluded. 16 right-handed, age-matched healthy control subjects were also studied (6 men, 10 women; median age 39 years, range 23-51 years). All subjects were advised to abstain from smoking and drinking caffeine-containing beverages for 4-6 hours before the scan. Written consent was obtained from all subjects. Ethical approval for this study was given by Oxford Regional Ethics Committee.

Sessions

The subjects were asked to attend the imaging center on two separate sessions, which were at least one day, but less than a week apart from each other. During the first session the subjects were screened for exclusion criteria, as stated above, and were invited to read typewritten instructions of the paradigm without any time limit. They then were asked whether they understood the task and any further questions were answered. At this stage the examiner suggested a strategy to carry out the task more efficiently: sub-vocally repeating the letters of the paradigm as they were presented,

in groups of 2, 3 or 4 (according to the condition type for n=1,2 or 3 back, respectively). A training paradigm different but of identical general design to that for the fMRI task then was administered with a desktop PC. After completion of the paradigm, the National Adult Reading Test (Nelson, 1991) and a hospital anxiety depression questionnaire (Zigmond and Snaith, 1983) were administered.

During a second session the subject was first reminded of the rules of the task and of the strategy, and then imaged while performing the task inside the scanner. The total duration of the imaging session varied between 60- 90 minutes. After scanning, all patients underwent a full neurological examination. Finally the Hospital Anxiety and Depression scale (Bjelland et al., 2002) was administered to patients and controls, and the Fatigue Severity Scale (Krupp et al., 1988; Krupp et al., 1989) to the patients only.

Subjects viewed the stimuli on a PC video display unit for the behavioural session and on a back-projection screen at the foot of the MRI couch during functional scanning. The screen was easily seen using prism glasses, which also allowed use of corrective lenses in case of refractive deficits.

Paradigm

The paradigm used was a sequential letter task that has been previously used in several neuroimaging studies of verbal working memory (VWM) (Braver et al., 1997; Cohen et al., 1997; Honey et al., 2002; Nystrom et al., 2000). Subjects were presented with a sequence of English alphabet consonants in either lower or upper case (subtending an angle of 6 degrees) appearing one at a time.

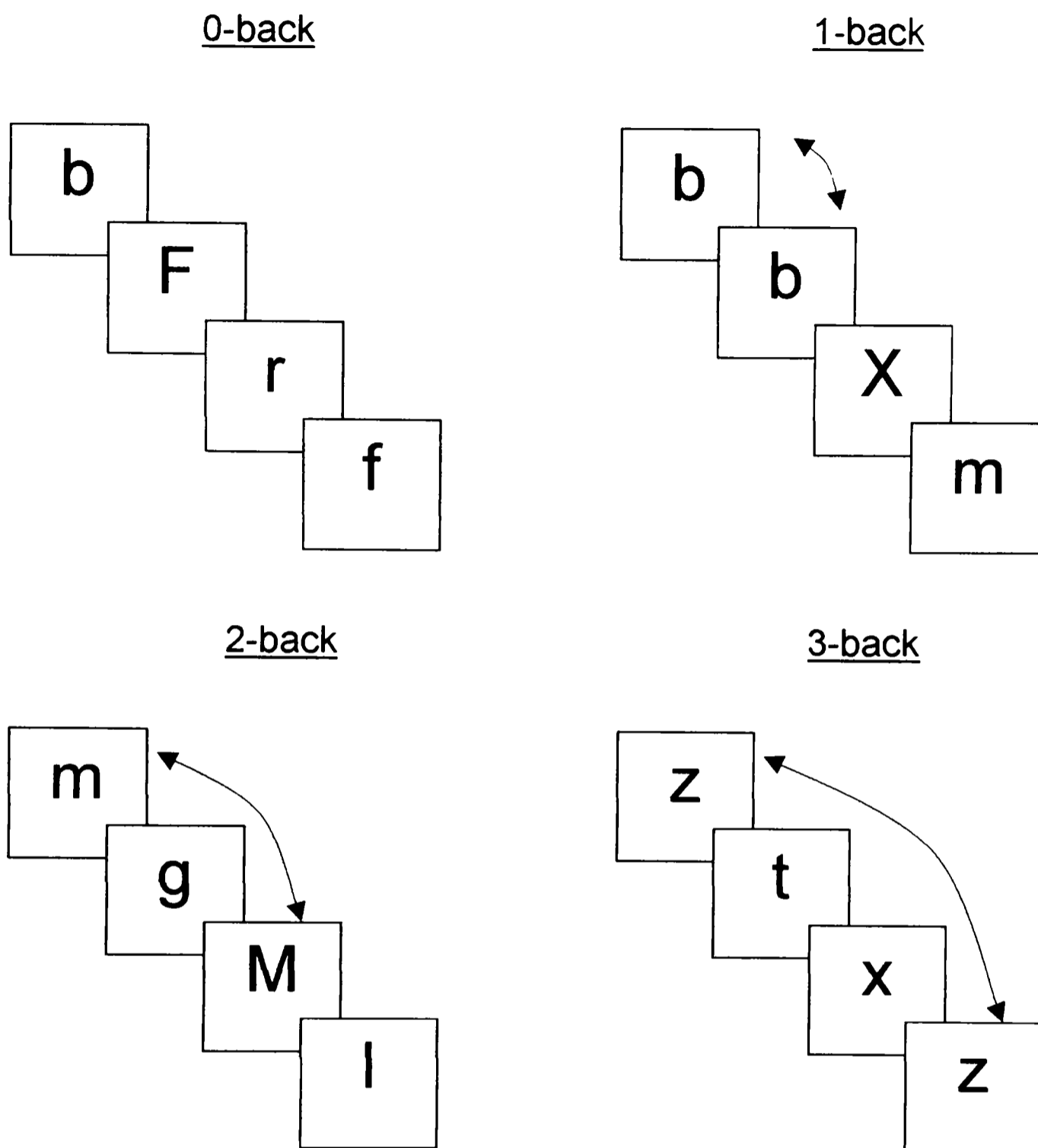


Figure 3.1 The Verbal N-back task. Letters are presented in sequence during each block of sixty seconds. There are three task conditions that require a button press when a letter is repeated from 1,2 or 3 letters back. This is contrasted with a 0-back condition in which a button press occurs with a target letter specified at the beginning of the block. Each of the four task conditions is presented in four blocks in a pseudorandom order.

In each session the task involved one control condition and three memory conditions of varying difficulty (Figure 3.1). Each task block lasted for 1 min with four blocks per condition arranged in a pseudo-random order. At the start of each block the

subjects would see 0-, 1-, 2- or 3-back displayed to indicate which task they would perform for that full block. In the 0-back task, a target letter was shown under the word 0-back at the start and the subject was required to press a button every time that letter appeared, regardless of case. For the other three memory conditions a button press was required whenever any letter was repeated (upper or lower case) the appropriate number of letters (n=1, 2 or 3) further down the sequence. For example, in the 1-back condition, the button was pressed when the same letter appeared immediately, but in the 3-back condition only if it occurred three letters further on. Each task condition was controlled for the number of target letters, and the letters were presented every 2 seconds. The number of responses correctly made for each task was recorded.

Imaging

All scans were performed using a 3.0 Tesla whole body scanner with a Varian Inova console and a quadrature birdcage radiofrequency head coil. An echo-planar imaging (EPI) sequence was used to acquire the fMRI data [24 x 8 mm coronal slices, TE = 30 ms, TR = 3000 ms, field of view (FOV) = 192 x 256 mm, matrix 64 x 64]. A T₁-weighted [64 x 3 mm coronal slices, TR = 15 ms, TE = 6.9 ms] and a T₂-weighted [34 x 5mm coronal slices, TR = 5000 ms, TE = 65 ms] anatomical scans were also acquired for each subject.

Data Analysis

Lesion volume quantification was measured manually using Jim, Version 3 (kindly provided in a demonstration package by Dr. Mark Horsfield, Xinapse Software,

Leicester University). This software uses a semi-automated threshold outlining approach to identifying lesion borders and allowed the total volume of lesions for each subject to be estimated from T₂-weighted images. The observer was blinded to other results. The mean intra-rater variability was 3.5% for use of the software. A regional analysis was performed in bilateral white matter of the medial halves of the prefrontal cortex (defined posteriorly by the central sulcus and inferiorly by the sylvian fissure). Cingulate cortex cross-sectional area in the coronal plane was measured using the same software by outlining the edge of the cingulate cortex, averaged over three separate slices at the level of the anterior commissure. These values were normalized for brain volume by the intracranial width at the same level, giving a ratio in units of mm.

Analysis of the fMRI data was carried out using FMRI Expert Analysis Tool, version 5 (FEAT) (www.fmrib.ox.ac.uk/fsl). The following pre-statistics processing steps were applied: motion correction using MCFLIRT (Jenkinson and Smith, 2001), spatial smoothing using a Gaussian kernel of full width half maximum (FWHM) 5 mm, mean-based intensity normalisation of all volumes by a constant factor and high-pass filtering (Gaussian-weighted LSF straight line fitting, with $\sigma=200.0$ s). Statistical analysis was carried out using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction (Woolrich et al., 2001). All probability values reported are corrected for multiple comparisons. The statistical images generated were related to the brain anatomy of each subject by registration with the individual T₁-weighted structural scan.

To identify brain activation during the verbal working memory task in patients and controls, analysis of mean activation at each level of task difficulty was performed. For patients and then for controls subjects separately, the three task conditions (1-, 2- and 3-back) were contrasted with the 0-back condition. The data was then analysed for areas showing increasing activation with increasing task difficulty by applying a linear contrast from 1-back through to 3-back. Two “between-group” analyses were performed: (i) patients-controls and (ii) controls-patients for this linear contrast.

All group analyses were performed using FMRIB’s Local Analysis of Mixed Effects (FLAME) model (Behrens et al., 2003) with Z (Gaussianized T) statistic images thresholded using clusters determined by $Z > 3.5$ and a (corrected) cluster significance of $p = 0.01$ (Forman et al., 1995; Friston et al., 1994; Worsley et al., 1992). The high-resolution T_1 -weighted images from the subjects were co-registered into standard space (Montreal Neurological Institute 152 Brain) and the group thresholded Z statistic images overlain. In the between-groups analysis, the T_1 -weighted images were averaged to produce a mean structural image on which the thresholded Z statistic image was overlaid. This allowed assessment of activation areas in terms of anatomical landmarks as well as reporting the co-ordinates of peak activations within each anatomically defined area.

Brain regions showing task-difficulty dependant increases in activation in controls as identified from the thresholded Z statistic group mean image of the linear contrast in controls were divided into ‘masks’. These masks were then applied as regions of interest on the functional image of each individual during 1-, 2- and 3-back tasks,

allowing a calculation of mean activation per subject in each region at each level of difficulty.

Functional connectivity

To look at the interactions between different regions involved in the verbal working memory task, a measure of functional connectivity was calculated. There are several approaches that have been suggested for functional connectivity analysis of fMRI data (Penny et al., 2004; Ramnani et al., 2004). We have adopted an approach relying on few assumptions, to minimize the potential bias in contrasts between the patient and control populations. Our analysis assesses multivariate correlations between incremental changes in brain activation with increasing task difficulty.

The analysis was confined to regions that demonstrated a monotonic increase in activity with task difficulty and corresponded to the same regions used above in the region of interest analysis. Within each region of interest the difference in mean signal from 1-back (the lowest level of difficulty) to 3-back (the highest level of difficulty) was calculated to determine the relative activation increase corresponding to increasing task demand.

The correlations between activation changes in directly anatomically connected regions were calculated for each of the MS patients and for the healthy control subjects. To do this, each region was considered separately as being dependent on activity across all of the other regions of interest. A linear regression of signal changes from anatomically connected areas on the signal change in each defined dependant

region was performed. For example, signal change in the cingulate, superior medial frontal, left prefrontal and right parietal regions were applied to the linear regression analysis of signal change in the right prefrontal region. To account for co-linearity between regions, the independent contribution of each region was assessed by the partial correlation co-efficient. The significance of each correlation was tested against the null hypothesis that there was no contribution as part of the linear regression (SPSS 12.0.1). A corrected, two-tailed $p < 0.05$ was considered statistically significant. Any correlations that were found to be significant in only patients or control subjects were indicated and a direct comparison between the groups was done using a Fisher's Z-r transformation (Rao, 1973). The Z value from this transform was compared against a normal distribution to obtain a p value. Together, this approach allows identification of regions of potential functional connectivity, and also any regions where the magnitude of connectivity differs between groups.

Statistics

A non-parametric, Kruskal-Wallis Test was used to test for differences in behavioural performance. Using a repeated measures analysis, the effects of group (patient or control), task difficulty (1-, 2- or 3-back), region, and their interactions on mean activations were determined. The analysis considered mean signal changes in 6 regions of interest (bilateral dorsolateral prefrontal cortices, bilateral parietal cortices, anterior cingulate and superior medial frontal gyri). Pearson correlation was used to assess any relationship of lesion volume or cingulate cross-sectional area with functional activation measures. Before applying any analysis that assumes normal distribution, checks were made that the data approximated normality. All probability values were corrected for multiple comparisons. All statistical analyses were performed using SPSS for Windows (version 12.0.1).

3.3 Results

Performance on the n-back task A comparable level of performance on the n-back task was found between the relapsing-remitting MS patient and control groups during the fMRI study (Figure 3.2). Both patients and healthy volunteers demonstrated a similar decline in performance with increasing task difficulty.

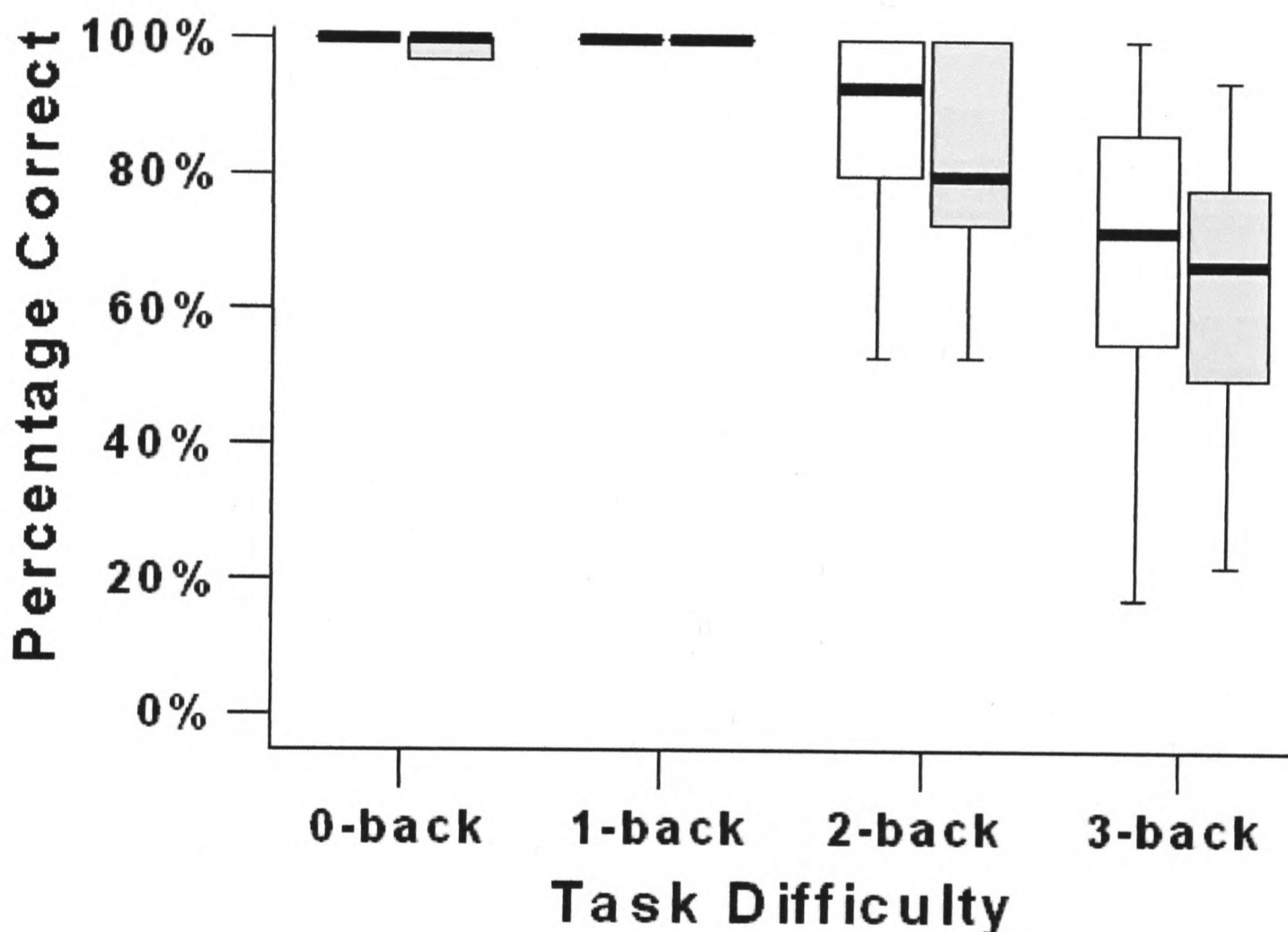


Figure 3.2 Performance according to task difficulty in patients ($n=21$, shown in grey) and controls ($n=16$, shown in white) during the 'n-back' verbal working memory task. There were no significant difference in performance between patients and controls for any level of difficulty.

fMRI activation during the n-back task In the control group, the task specific activation was found in regions corresponding to medial frontal areas and bilaterally in posterior parietal cortices, inferior frontal gyri and dorsolateral prefrontal cortices ($Z>3.5$, corrected $p<0.01$). These areas were identified at each level of task difficulty (Figure 3.3a-c, Table 3.2).

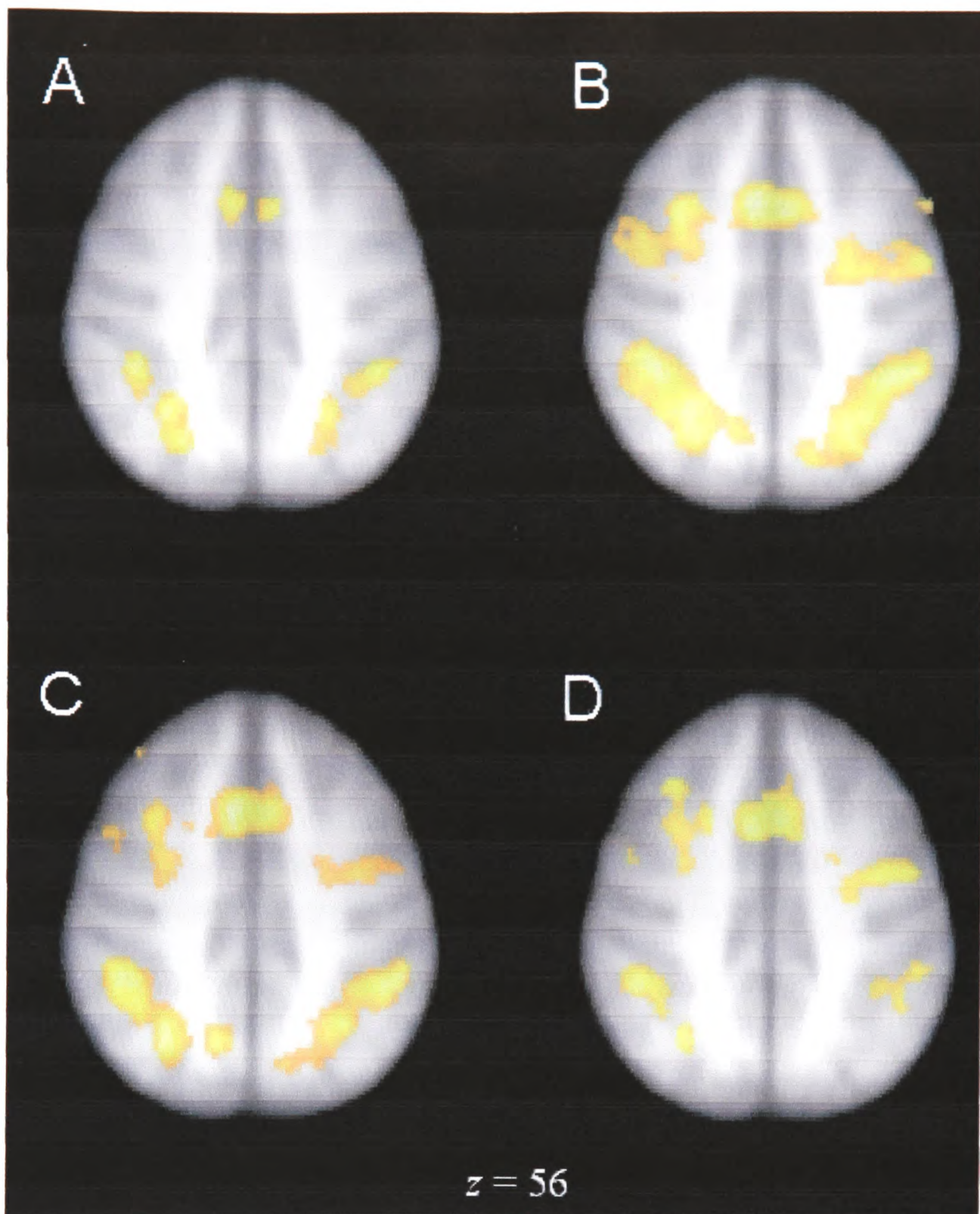


Figure 3.3 Group mean activation during the 'n-back' verbal working memory task, in healthy subjects ($n=16$), corresponding to the task conditions (a) 1-back, (b) 2-back, (c) 3-back ($Z>3.5$, corrected $p<0.01$). (d) Regions of significant linear increase in activation with increasing task difficulty ($Z>3.5$, corrected $p<0.01$). MNI standard space co-ordinates, and maximum Z-scores for each region for (b) 2-back and (d) linear increase are shown in tables 3.2 and 3.3. Group mixed effects Z-maps are superimposed onto a mean structural image.

Patients showed similar areas of activation to the control group at each level of task difficulty ($Z>3.5$, $p<0.01$) (Table 3.2 and 3.3). No significant differences were found

for a simple contrast between patient and controls groups for the main effect of task at n=1, 2 or 3.

Anatomical region	Co-ordinates of max Z scores in MNI space (mm)			Maximum Z score
	x	y	Z	
	Simple Contrast (1-, 2-, 3-back)			
<i>Controls</i>				
Right Dorsolateral Prefrontal	40	40	28	5.12
Left Dorsolateral Prefrontal	-28	-12	44	5.23
Right Inferior Frontal	-30	18	-14	4.24
Left Inferior Frontal	-32	22	-6	4.25
Right Posterior Parietal	34	-56	50	5.08
Left Posterior Parietal	-44	-40	50	4.58
Superior Frontal	8	14	44	5.47
Anterior Cingulate	6	32	30	4.61
<i>Patients:</i>				
Right Dorsolateral Prefrontal	36	30	24	4.53
Left Dorsolateral Prefrontal	-48	12	18	4
Right Inferior Frontal	44	20	-10	4.32
Left Inferior Frontal	-34	22	-6	4.25
Right Posterior Parietal	56	-38	40	5.52
Left Posterior Parietal	-44	-40	50	4.58
Superior Medial Frontal	-8	-2	58	3.63
Anterior Cingulate	14	28	30	5.05

Table 3.2 Areas of activation during the verbal working memory task in a mixed effects group cluster based analysis ($Z > 3.5$, $p < 0.01$) for the simple contrast for healthy controls and for relapsing-remitting MS patients. They are displayed separately for both patient and control groups who demonstrate similar areas of activation. MNI standard space co-ordinates of the peak Z score within each region of activity is given.

Regions identified with the main effect of task also demonstrated significant monotonic increases with task difficulty, modeled as a linear increase in activity in a second level analysis from n=1-3 ($Z > 3.5$, corrected $p < 0.01$) (Figure 3.3d, Table 3.3).

Anatomical region	Co-ordinates of max Z scores in MNI space (mm)			Maximum Z score
	x	y	z	
	Contrast of linear increasing activation (1 to 3 back)			
<i>Controls</i>				
Right Dorsolateral Prefrontal	40	34	28	5.16
Left Dorsolateral Prefrontal	-48	-2	36	4.95
Right Inferior Frontal	-34	20	-10	4.61
Left Inferior Frontal	-34	22	-8	4.69
Right Posterior Parietal	34	-58	50	4.92
Left Posterior Parietal	-44	-40	48	4.55
Superior Medial Frontal	8	10	56	4.54
Anterior Cingulate	4	16	34	5.28
<i>Patients</i>				
Right Dorsolateral Prefrontal	38	38	30	3.94
Left Dorsolateral Prefrontal	-30	22	30	4.12
Right Inferior Frontal	50	18	-8	4.14
Left Inferior Frontal	-34	18	-10	3.71
Right Posterior Parietal	48	-48	42	4.28
Left Posterior Parietal	-50	-48	52	3.92
Anterior Cingulate	0	16	28	3.53

Table 3.3 Areas of activation during the verbal working memory task in a mixed effects group cluster based analysis ($Z > 3.5$, $p < 0.01$) for the linear contrast for healthy controls and for relapsing-remitting MS patients. Patient and control groups who demonstrate similar areas of activation. MNI standard space co-ordinates of the peak Z score within each region of activity is given. Similar areas are found for both the simple contrast (1, 2 or 3-back) and for the linear contrast (see also table 3.2).

Effect of disease on incremental activation on increasing task difficulty To test the hypothesis that there may be impairment in functional reserve in patients, we tested for activity related changes in activation for the n-back task. Significant monotonic increases in activation were found with greater task difficulty (across $n=1-3$) in patients ($Z < 3.5$, $p < 0.01$) (Table 3.3). However, contrasting patients with controls in a mixed-effects analysis showed significant between-group differences. Controls showed significantly greater increases in activation in the superior medial frontal

gyrus than patients ($Z > 3.5$, $p < 0.01$) (Figure 3.4). There were no areas in which patients showed greater incremental activation than controls.

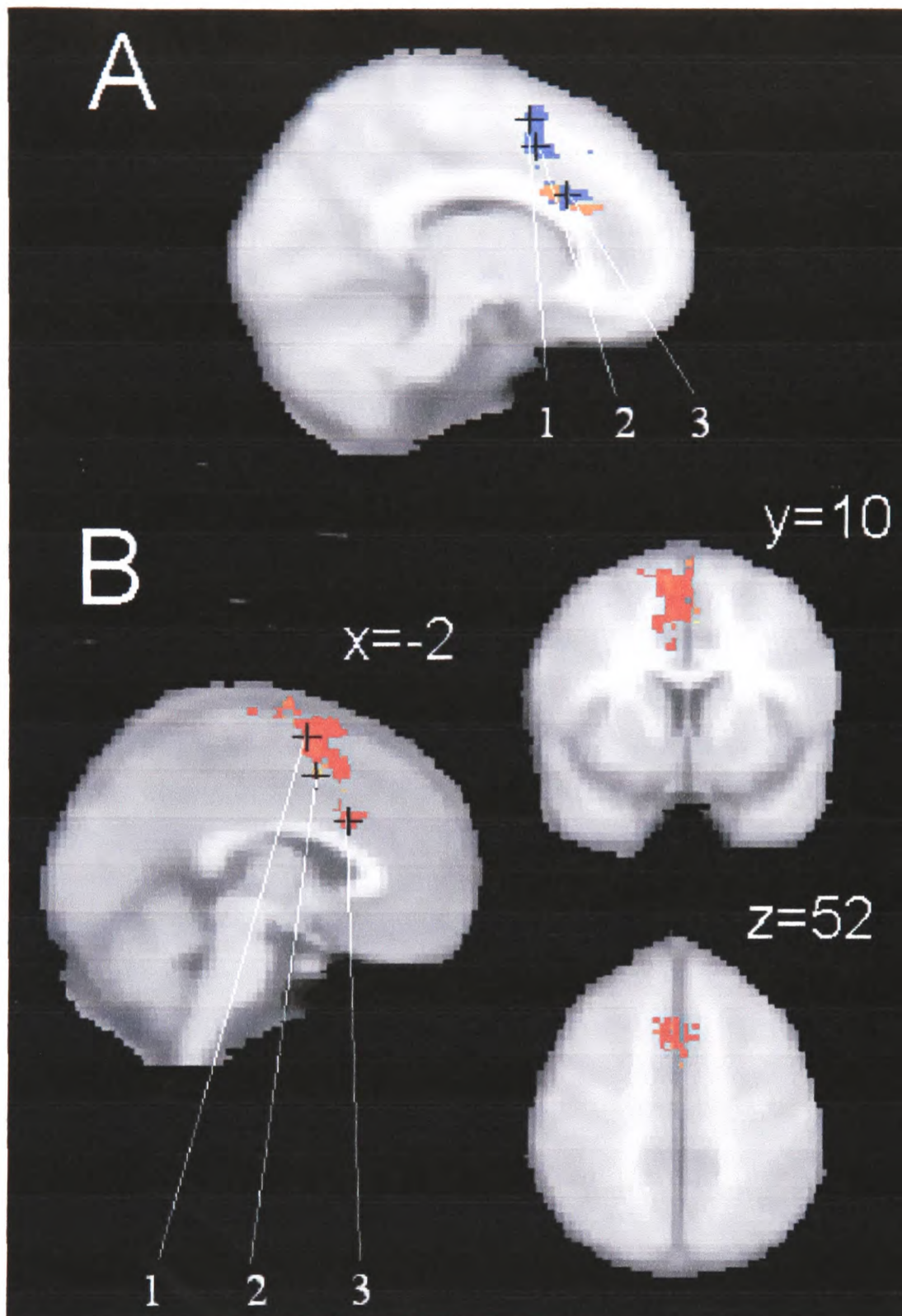


Figure 3.4 Regional differences between patients ($n=21$) and controls ($n=16$). (a) Medial frontal regions of patients (orange) and controls (blue) overlaid and the coordinates for the contrast analysis are indicated by (1), (2) and (3) (b) Mixed effects analysis of controls minus patients ($Z > 3.5$, $p < 0.01$), the MNI co-ordinates of the three main areas of activation are (1) $x=-2$ $y=2$ $z=64$, $Z=4.34$ (2) $x=-2$ $y=6$ $z=48$, $Z=5.31$ (3) $x=-2$ $y=18$ $z=30$, $Z=5.12$. Z maps are superimposed on a mean structural image.

Effect of increasing task difficulty in a region-of-interest analysis To better understand these group analysis results, the monotonically increasing activation

changes with increasing task difficulty were assessed in each subject for regions of interest including the medial superior frontal gyrus and the anterior cingulate cortex. In the control group, 13/16 subjects showed significant activation in these regions, while only 8/21 MS showed significant relative activation in the anterior cingulate and 3/21 in the superior frontal gyrus (Chi-square, $p < 0.001$). No significant difference in cranial-size-normalized cross-sectional area of the anterior cingulate (measured at the same level) was found between the patients (mean, 2.17 ± 0.56 mm) and healthy controls (mean, 2.27 ± 0.35 mm) to account for the differences in activation. No correlation between cross-sectional area and activation was found for patients in the MS group ($r = -0.17$, $p = 0.46$).

The region of interest analysis was extended to assess increases in activity associated with increasing task difficulty across each region of interest defined in the main effect of task. The mean relative signal intensity for each of the regions (Table 3.2) was calculated at each level of task difficulty for each subject across $n = 1, 2$ or 3 relative to the 0-back task. Patients showed consistently lower increases in activation with increasing task difficulty relative to healthy controls in all regions showing a significant main effect of the task (Figure 3.5, corrected $p < 0.001$). There was also a significant interaction between region and group for the increase in activation with increasing difficulty ($p < 0.001$).

Effect of T_2 lesion volume and disease duration There was no significant correlation between the global T_2 -hyperintense lesion load, the T_2 -lesion volume in the medial frontal cortex, or disease duration with activations for the individual contrasts for main effect or the monotonic increase in activation with increasing task difficulty. (See table 3.1 for disease characteristics across subjects)

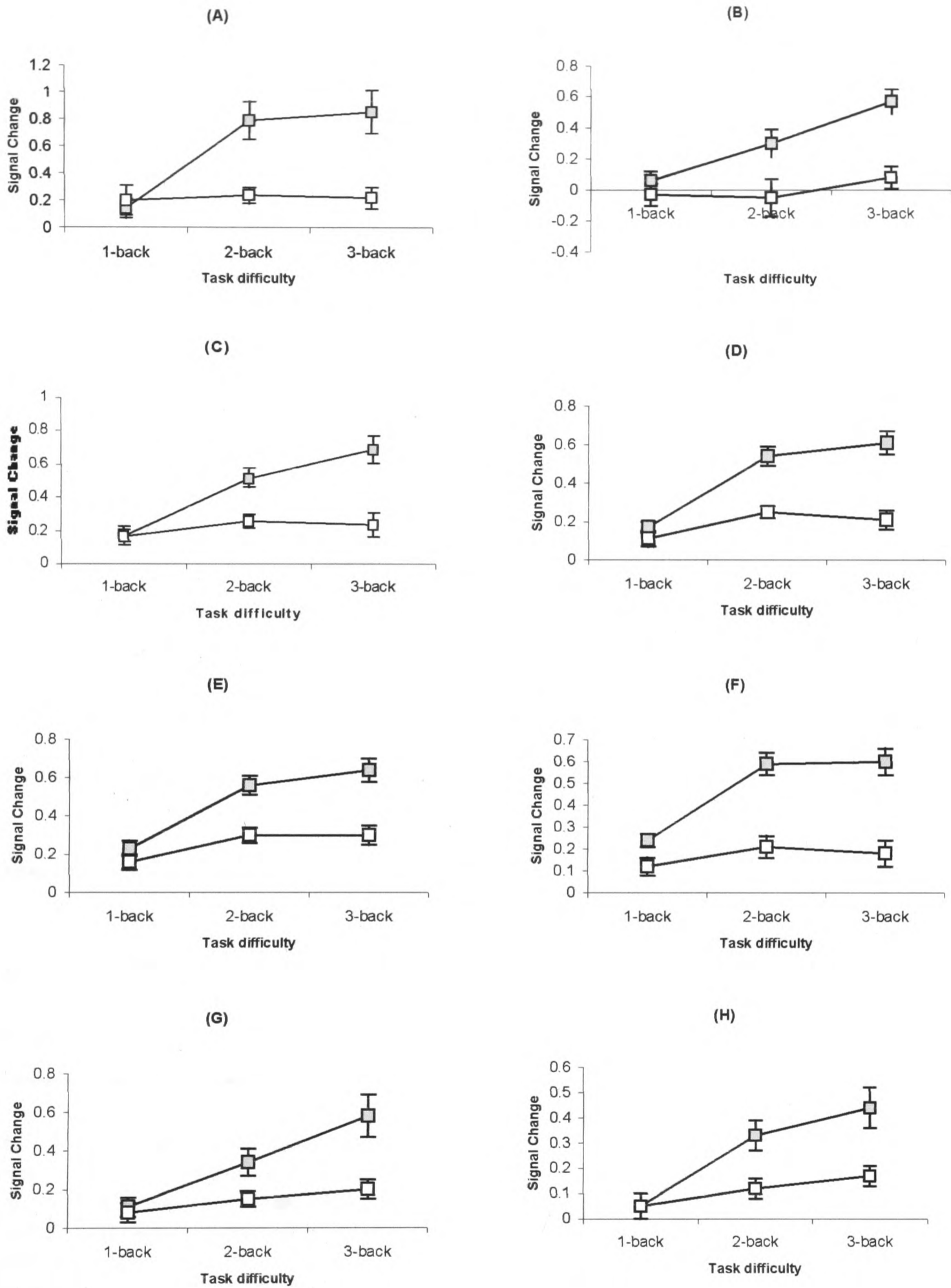


Figure 3.5 Mean signal change in (a) superior medial frontal, (b) anterior cingulate, (c) right dorsolateral prefrontal, (d) left dorsolateral prefrontal, (e) right posterior parietal, (f) left posterior parietal, (g) right inferior frontal, and (h) left inferior frontal cortex. Significance ($p < 0.001$) between patients (white squares) and controls (grey squares) was demonstrated on a repeated measures analysis. Standard error is indicated using error bars.

Functional interactions between brain regions during the n-back task To test the hypothesis that changes in interactions between brain regions might mediate adaptive functional changes with disease progression, a functional connectivity analysis was performed as a multivariate test for group differences in correlations between regional activities with increasing task difficulty. Individual correlations between changes of activity in anatomically directly connected regions were identified (Table 3.4).

Dependant Region	Tested Region	Patients	Controls
Right Prefrontal	Left Prefrontal	0.710*	0.579
	Right Parietal	0.211	0.629
	Superior Medial Frontal	0.226	0.772*
	Anterior cingulate	-0.073	-0.325
Left Prefrontal	Right Prefrontal	0.806*	0.677
	Left Parietal	-0.322	0.06
	Superior Medial Frontal	-0.018	-0.136
	Anterior cingulate	-0.260	-0.473
Anterior Cingulate	Right Prefrontal	0.595	0.788[†]
	Left Prefrontal	-0.222	-0.508
	Superior Medial Frontal	-0.369	-0.435
Superior Medial Frontal	Right Prefrontal	0.331	0.607
	Left Prefrontal	0.025	-0.364
	Anterior Cingulate	-0.369	0.378
Right Parietal	Right Frontal	0.715*	0.719*
	Left Parietal	0.073	0.392
Left Parietal	Left Frontal	0.034	0.312
	Right Parietal	0.345	0.368

Table 3.4 The partial correlation coefficients between regions tested for patients and controls. The dependant regions are shown in the first column, and independent regions that were regressed against this are shown in the second column. Only regions that were potentially anatomically connected directly were considered. Significant ($p < 0.05$ after correction for multiple comparisons) correlations are indicated *. One further correlation showed a trend to significance [†].

We then tested for differences in these correlations between the healthy controls and patients. The control group showed a significant correlation between the right dorsolateral prefrontal cortex and the superior frontal gyrus (corrected $p < 0.05$) that

was not found in the patient group (figure 3.6b). Patients showed a significant correlation between activity changes in the right and left dorsolateral prefrontal regions (figure 3.6c, corrected $p < 0.05$) not demonstrated in the control subjects.

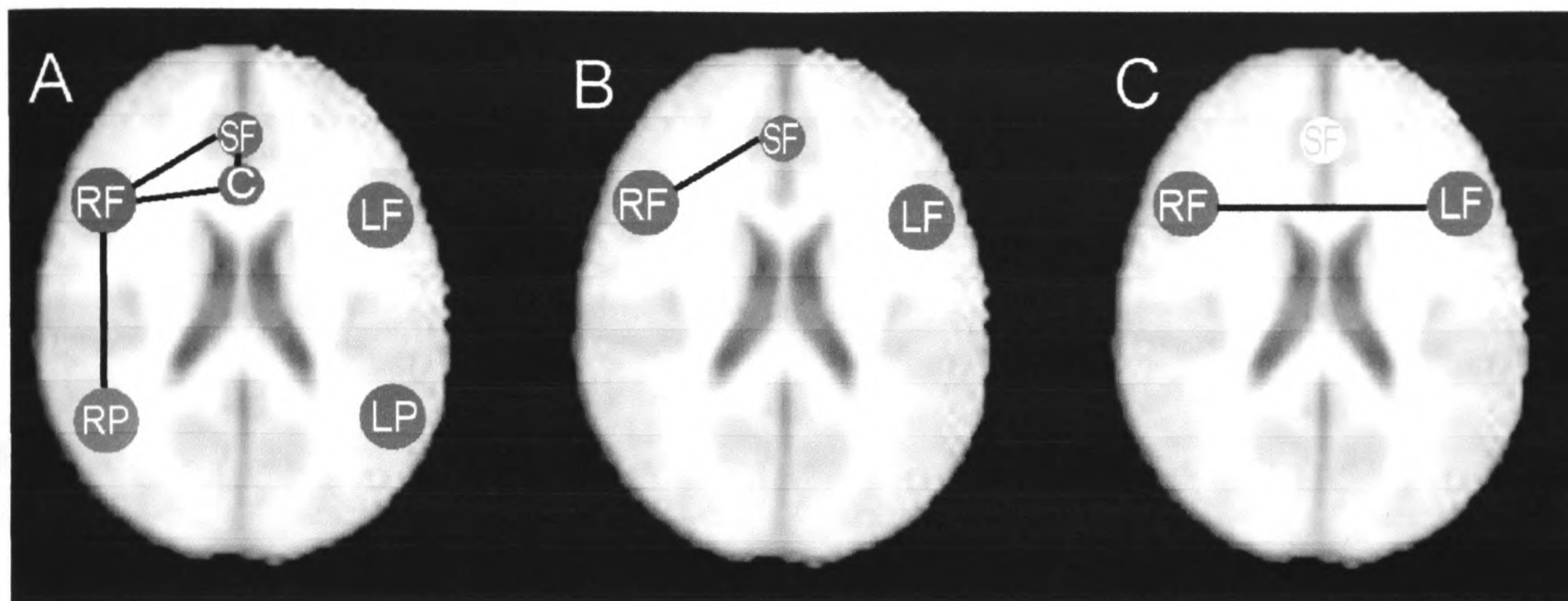


Figure 3.6 The most significant correlations between activation in regions (a measure of functional connectivity) involved in processing increasing task demand are indicated in (a). The image (b) shows connections that were more significant in controls than patients ($p < 0.05$). In (c) are those connections only significant for patients ($p < 0.05$). C=cingulate, SF=Superior Medial Frontal, RF=Right Dorsolateral Prefrontal, LF= Left Dorsolateral Prefrontal, RP=Right Parietal, LP=Left Parietal.

3.4 Discussion

This experiment investigated the differences in verbal working memory processing between MS patients and age-matched healthy controls. The study revealed that despite similar performance and similar regions of activity, a reduced functional capacity was found in MS patients. Changes in functional connectivity may limit the effects of the reduced reserve thereby maintaining behavioural performance. This is discussed in more detail below.

3.4.1 Reduced functional reserve for cognition in patients with MS

The first major finding of this study was that, relative to healthy control subjects, patients with MS had reduced increments in functional activation with increasing

difficulty of a verbal working memory task, a task assessing neuropsychological domains commonly impaired in MS. Decreased activation for patients was found despite similar performance on the task for patients and controls. This suggests changes in brain processing for the task and can be interpreted as an indication of reduced *functional reserve* for cognition (Lazeron et al., 2004). This would be consistent with previous metabolic imaging studies suggesting relative brain hypometabolism in patients with MS (Brooks et al., 1984; Paulesu et al., 1996; Rashid et al., 2004; Sun et al., 1998) resulting in areas less able to meet cognitive demands particularly as they increase. The differences between healthy controls and the patients reflect the consequences of pathology, although this was sub-clinical at the stage that the patients were studied. The sensitivity of behavioural measures in this context is insufficient to detect the effects of pathology found with functional imaging.

Differences in activation could be due to differences in cognitive strategy. However, this is unlikely. All subjects were limited from using spatial cues by including both upper and lowercase letters as targets, forcing reliance on verbal cues. All subjects also were trained in a specific strategy involving sub-localisation of letters throughout the paradigm. The notion that subjects used a common cognitive strategy for the task is supported by the observation that functional activation patterns for the main effect of task were similar for both patients and control subjects.

Although some previous studies have identified potentially adaptive recruitment of novel brain regions in patients with MS during cognitive tasks (Mainero et al., 2004a; Penner et al., 2003), this study emphasized that controls and patients both activated

anatomically similar regions. Consistent with prior studies of verbal working memory, activations were most significant in the posterior parietal, lateral prefrontal and medial prefrontal cortices. These observations are not without precedent. Lazeron and colleagues (Lazeron et al., 2004) failed to find novel regions of brain recruitment with a 'Tower of London' task, although the fMRI context was not controlled for differences in task performance. The failure to identify novel adaptively recruited brain regions in this experiment may be explained in part by differences in the tasks used and differences in the organization of the distributed networks responsible for cognition related to task performance. Perhaps also significant, however, is that performance in the patient and control groups was well matched and training subjects with a preferred strategy controlled the cognitive strategy. Control for the motor responses required for the task also may have limited differences, as patients with MS show altered patterns of motor-related activation at an early stage in the disease (Pantano et al., 2002; Reddy et al., 2000).

3.4.2 Patients show most consistent relative functional impairment in mesial prefrontal polymodal cortex

A second major finding was that, while differences between patients and controls in task related activation increases were widespread, the most consistent difference in task-related activation between patients and controls was found in the superior frontal and anterior cingulate gyri. These regions both include polymodal neocortex involved in processing cognitive functions including attention (Lenartowicz and McIntosh, 2005). Demands for coherence of input for this polymodal processing may make function of this region particularly vulnerable to pathology from MS. It also is

possible that this region is *selectively* involved by neocortical pathology with MS. Bo and colleagues had described a particularly high neocortical lesion load in the anterior cingulate (Bo et al., 2003). However, the relative selectivity of differences in this region may simply be quantitative. It is possible that a large group of healthy controls might confer greater sensitivity to detection of a wider range of changes in the patient group.

3.4.3 Potentially adaptive, altered functional connectivity between medial and lateral prefrontal cortex in patients with MS

Despite the evidence for reduced cognitive functional reserve, MS patients were able to maintain working memory performance similar to controls, suggesting potentially compensatory increases in efficiency of use or of interactions between interacting regions. Consistent with this, the third major finding was of altered task-related functional connectivity in patients compared with controls. Previous work concentrating on consequences of axonal injury in the white matter in patients with MS has emphasised disruption of frontal connectivity as an expression of pathology and neuropsychological deficits with MS have been interpreted in terms of disconnection models (Mainero et al., 2004a). However, analysis of functional connectivity suggests a more complex situation. Based on an effective connectivity analysis of PASAT working memory task, Audoin et al recently reported that different interactions show either decreased or *increased* connectivity in patients with multiple sclerosis (Audoin et al., 2003). This suggests that with lower susceptibility to injury or pathological involvement, some pathways show *enhanced* function to compensate (at least partially) for impaired functional connectivity within a processing network.

There are several approaches to measuring functional connectivity (Ramnani et al., 2004). For this experiment, we have used a highly data-driven approach constrained only by basic anatomical information limiting the possible connections to those for which there is evidence for large direct tracts. An advantage of this approach is that it has less potential model-based bias. Care was taken in the correlation analysis also to factor out co-linearities.

Not unsurprisingly, healthy controls showed significantly stronger functional connectivity between the superior medial frontal region and the right prefrontal cortex. Patients showed significantly reduced activation of the medial frontal regions relative to the controls. The functional connectivity and univariate activation analyses together therefore both suggest functional pathology limiting interactions between the lateral and medial prefrontal cortex in the patients. However, the functional connectivity analysis also defined changes in patients that could not be inferred directly from a univariate analysis: patients showed relatively increased functional connectivity between the right and left prefrontal cortices, a functional relationship not significant in healthy control subjects. Recruitment of homologous regions in the two hemispheres as a compensatory mechanism is consistent with observations in the motor system for patients with MS, in whom increased recruitment of premotor cortex ipsilateral to the hand moved is among the most consistent differences in MS patients relative to healthy controls (Cifelli and Matthews, 2002). The increased functional connectivity between the two lateral prefrontal cortices could be speculated to be an adaptive mechanism that contributes to limiting expression of pathology with this cognitive task.

3.4.4 A new strategic focus for cognitive therapies in MS?

In summary, the results have shown that even early in the progression of disease and before clinical expression of neuropsychological deficits, MS patients have evidence for reduced cognitive functional reserve by fMRI. The consistency of fMRI changes in the patients was striking, suggesting that it provides a sensitive measure of disease-related change. Associated with the primary activation changes are differences in functional connectivity involving increased direct interactions between lateral prefrontal cortices in the two hemispheres. This suggests unmasking of a direct inter-hemispheric pathway (not used in healthy control subjects that may in part compensate for relative dysfunction in medial prefrontal regions. The reduced functional reserve in patients could lead to failure of processing with increasing cognitive demands in the patients at lower levels of task demands than for healthy controls. As a functional expression of pathology, it may provide a measure sensitive to change with an increasing burden of disease over time. Strategies to enhance the functional reserve and limit its rate of loss or to enhance potentially compensatory functional connectivity provide new targets for therapy in MS. One possible approach might be to enhance conduction or prolong neuronal summation times (Mainero et al., 2004b).

Some of these alternative possibilities are explored in the following sections. Sections 4 and 5 assess the effect of cholinergic modulation on cognitive processing in MS patients and healthy volunteers respectively. Section 6 assesses the effect of rivastigmine on the relationship between BOLD fMRI signal and the underlying neural activity. Section 7 explores the potential of in-vivo anatomical connectivity measures in understanding the effect of MS.

Section 4: The effect of rivastigmine on memory and concentration in multiple sclerosis patients

Cognitive impairment is a significant problem for patients with MS. The previous section demonstrated differences in the brain activation during processing of verbal working memory between MS patients and healthy controls. Cholinergic therapy has a potential role in improving cognitive symptoms. In this section, the effect of rivastigmine on neuropsychological performance and underlying brain activation is considered.

4.1 Introduction and rationale

Impairment of cognitive function in MS patients has been widely reported (Rao et al., 1991a) and can cause a significant impact on the quality of life (Rao et al., 1991b). In the previous section, sub-clinical deficits in verbal working memory as demonstrated by functional activation were present before any apparent clinical impairment. Thus the prevalence of impaired cortical cognitive processing may be even more widespread than reported. Whilst treatment is available for other symptoms of MS, there is little to offer patients suffering from memory or concentration problems (Bagert et al., 2002). Acetylcholinesterase inhibitors have been introduced to treat cognitive symptoms in Alzheimer's and Lewy-Body dementia, demonstrating a significant benefit to patients (Gottwald and Rozanski, 1999; Rosler et al., 1999; Wesnes et al., 2002). More recently, clinical studies of acetylcholinesterase inhibitors have been undertaken in groups of MS patients (Greene et al., 2000; Krupp et al., 2004). Rivastigmine is one such acetylcholinesterase inhibitor, increasing the amount of acetylcholine available at cortical synaptic junctions (Jann, 2000).

A potentially beneficial adaptive response in functional activation during the Stroop task was found using single-dose rivastigmine in a small study of MS patients. This study demonstrated that the abnormal activation pattern found in MS patients during the Stroop task 'normalised' to the pattern seen in healthy controls after administration of rivastigmine (Parry et al., 2003). Memory and attention are domains particularly affected in MS (Achiron and Barak, 2003). Increasing the amount of acetylcholine available might be expected to enhance the cortical processing of these functions as it has been shown to be important for both attentional performance and memory (Gold, 2003; Sarter et al., 2003). Effects may be mediated by amplifying the processing of task-relevant stimuli (Furey et al., 2000; Sarter et al., 1999; Turchi and Sarter, 1997). Facilitation of cortical responses may be relevant to MS where a reduction in the functioning of the normal cortical network could occur as consequence of axon loss or reduced temporal summation due to demyelination.

In this study, a Stroop task and an N-back task is applied to assess changes in brain activity in MS patients when taking rivastigmine relative to the same patients off rivastigmine. The stroop task is a task that assesses attentional performance, requiring rapid resolution of response conflict due to competing sources of incoming information (Bush et al., 1998). If rivastigmine improves the cortical processing of attention and memory then changes should be demonstrated in the functional activation during the tasks. The previous section describes a reduced functional reserve in MS patients in the verbal working memory task, which may be improved with rivastigmine without necessarily changing behavioural performance in the task. The hypothesis is that cognitive symptoms are ameliorated by the use of acetylcholinesterase inhibitors. Therefore, in addition, data is presented from a

neuropsychological battery in order to assess the changes in neuropsychological performance with the administration of rivastigmine in the group of MS patients. If memory and attention are enhanced by rivastigmine, then test scores in the battery should be improved.

4.2 Methods

Subjects

Patient	Disease Type	EDSS	Disease duration yrs	Age yrs	T ₂ -LV mls
1	RR	2	3	38	1.82
2	RR	3	13	49	1.97
3	RR	2	10	36	0.69
4	SP	6	12	53	0.39
5	RR	2	9	48	25.40
6	RR	0	7	44	4.51
7	RR	2	15	43	7.97
8	SP	4	14	53	12.65
9	RR	4	9	37	10.29
10	RR	1	1	33	4.94
11	RR	3	21	47	16.89
12	SP	3	3	45	7.90
13	RR	3	8	39	2.31
14	RR	3	9	47	15.63
15	RR	2	4	32	5.32

Table 4.1 Disease characteristics including the lesion volume (LV) in each patient.

Twenty-one patients were recruited and fifteen right handed patients (5 men, 10 women; mean age 43 years, range 32-53 years) with clinically definite multiple sclerosis (3 secondary progressive, 12 relapsing remitting; mean duration 9 years, range 1 –21 years) according to the Poser Criteria (Poser et al., 1983) completed a randomised crossover single-blind study of rivastigmine (Table 4.1). Disability was

assessed with the EDSS (Kurtzke, 1983) at the time of initial screening (median EDSS 2.5, range 0-6). None of the patients had suffered from a relapse within 3 months of starting the study. Of the six patients that withdrew from the study, two patients withdrew immediately after screening, two patients withdrew due to the occurrence of a relapse during the trial and two patients withdrew because of unacceptable nausea. All patients had subjective complaints of altered cognition (memory, language, calculation or attentional deficits) but had no clinically evident visual deficits or impairment of right-hand movement on the basis of clinical histories obtained during initial interview for this study. Subjects with a history of psychological and psychiatric problems, epilepsy, learning disability, alcoholism, head injury, episodes of loss of consciousness or severe ongoing stressors (such as bereavement, divorce, financial problems) were excluded. All subjects were advised to abstain from drinking caffeine-containing beverages for 4-6 hours prior to the scan. No subjects smoked regularly, and those that smoked occasionally were advised not to smoke during the duration of the study. Written consent was obtained from all subjects and Oxford Regional Ethics Committee gave ethical approval for the study.

Sessions

Subjects were required to attend the imaging centre on three separate occasions, four to six weeks apart. During the first session, subjects were screened for exclusion criteria and read typewritten instructions for the paradigms without any time limit. They were asked whether they understood the task and any further questions were answered. Training paradigms different but of identical general design to that for the fMRI task was then administered with a desktop PC. After completion of the paradigm, the National Adult Reading test (Nelson, 1991) and a hospital anxiety and

depression questionnaire (Zigmond and Snaith, 1983) were administered. A baseline brief repeatable battery of neuropsychological tests was given to the subject. Suitable subjects were then randomised to receive either rivastigmine with domperidone (as an anti-emetic) or just domperidone (to control for the effect of this tablet) in a manner blinded to investigators. Drugs were prescribed from pharmacy within the John Radcliffe Hospital that held a copy of the randomisation code for rivastigmine administration. An experienced neurologist (EL) was available for advice if any difficulties arose to avoid unintentional unblinding of the investigators.

For those subjects randomised to receive rivastigmine, a slowly escalating dosage regime was implemented. Starting at one 1.5-milligram capsule twice a day for the first two weeks, this was increased to two capsules twice a day after two weeks. At four weeks, the patients were asked to take three capsules twice a day. The slow dose increase was to minimise the incidence of side effects and is based on the regime routinely used clinically in patients with dementia. Thus, subjects would be on 9 milligrams of rivastigmine a day when they attended for their next visit. One 10-milligram tablet of domperidone was to be taken twice a day in all subjects whether taking rivastigmine or not.

During the second session, scheduled for after the patient reached the required dose of 9 milligrams a day of rivastigmine, the subjects first repeated the neuropsychological battery. Following reminding of the rules of the fMRI tasks, subjects were imaged whilst performing them inside the scanner. Subjects were asked to fill a pro-forma indicating the incidence of side effects. Subjects previously on domperidone only

were then prescribed rivastigmine and vice versa. In this way all subjects received rivastigmine for half the duration of the study in a crossover design.

The third session, approximately 4-6 weeks from the second session, followed the same pattern as the second session. Any unused medication was returned directly to the pharmacy.

Brief Repeatable Battery of Neuropsychological tests.

This battery consists of five tests, the selective reminding (SRT), 10/36 spatial recall (SPART), symbol digit modalities (SDMT), paced auditory serial addition (PASAT) and word list generation (WLG) tests which can be administered within 30 minutes (Bever et al., 1995; Boringa et al., 2001).

The SRT measures verbal learning during a list-learning task over six trials. The list consists of 12 words that were matched for commonality between the three sessions that the subject attended. Subjects are instructed to recall all the words, and on every consecutive trial, only missed words from the preceding trial are given. After administration of the PASAT, patients are asked to recall the list. A word recalled on two consecutive trials is considered to have entered long-term storage on the first of the trials and scored for all subsequent trials. The total sum of words in long-term storage is noted. If words are consistently recalled then it is scored as in consistent long-term retrieval, and the total sum of words noted. Finally the total number of words in the delayed recall is also noted. The SRT addresses short-term and long-term components of verbal memory (Buschke and Fuld, 1974).

In the SPART, 10 checkers are placed on a 6x6 checkerboard to assess visuospatial learning. The board is shown for 10 seconds and the subject is asked to reproduce the position of the checkers on an empty board. This is repeated twice and after the delayed SRT. The total number of correct responses across the three trials and the delayed trial is recorded (Bever et al., 1995).

Sustained attention and concentration are examined by the SDMT. Nine geometric symbols are labelled 1 to 9. During 90 seconds, the subject substitutes symbols in a row by the corresponding number and responds verbally. The score is the number of correct substitutions (Smith, 1982).

The PASAT is a measure of sustained attention and information processing speed by adding pairs of digits. Each number is added to the one that immediately precedes it, and the subject responds verbally, over 60 pairs of digits. The digits are presented first at 3 seconds apart then 2 seconds. The number of correct responses at each rate of speed is taken (Gronwall, 1977).

The semantic verbal fluency test of the WLG evaluates the spontaneous production of names in a given category within 90 seconds. The subject is asked to give as many names of 'vegetables and fruits', 'animals' and 'sports' across the three sessions (Bever et al., 1995).

Raw data scores for each test were converted to Z scores by referring to normative data. If there were sub-tests, then these were averaged to give the Z score for that test.

Paradigms

Two paradigms were used during functional scanning. The first was the N-back task (Braver et al., 1997), employed in a manner identical to that described in section 3.2 (Figure 3.1). In addition, the study also used a Counting Stroop task (Bush et al., 1998) previously used to assess attentional performance. The advantage of this design is that it avoids the need for a verbal response or complex coding in measuring behavioural response. In addition, it removes the potential confound of colour perception difficulties that can occur in MS. This task consists of two conditions, and subjects are asked to press a button with their right hand corresponding to the number of words shown on screen. In the neutral condition, the words ('cat', 'dog', bird or 'mouse') displayed do not relate to the number of words shown. However, in the incongruent condition, the words ('one', 'two', 'three' or 'four') are not in agreement with the number of words shown (Figure 4.1).

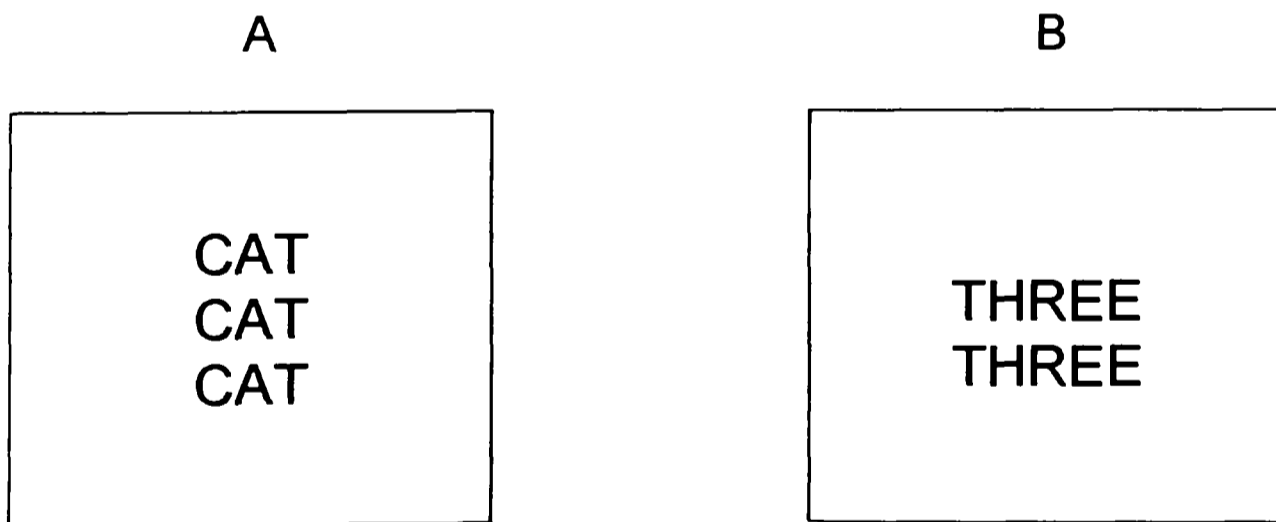


Figure 4.1 In the Counting Stroop task, subjects are required to press button 1,2,3 or 4 depending on the number of words presented on the screen. There are two conditions, (A) neutral, and (B) incongruent. The Stroop effect is the difference in response time between these two conditions.

The sets of between one and four words are presented on a screen as a vertical list every 1.5 seconds. The task conditions were presented in alternating blocks of thirty seconds over 8 minutes. A 30 second rest period was included at the start and at the end. The reaction times for each button press and the number of correct responses was recorded.

Subjects viewed the stimuli on a back projection screen at the foot of the MRI couch during functional scanning. The screen was seen using prism glasses, allowing the use of corrective lenses in case of refractive deficits.

Imaging

All scans were performed using a 3.0 Tesla whole body scanner with a Varian Inova console and a quadrature birdcage radiofrequency head coil. An echo-planar imaging (EPI) sequence was used to acquire the fMRI data [24 x 6 mm axial slices, TE = 30 ms, TR = 3000 ms, FOV = 192 x 256, matrix 64 x 64]. A T₁-weighted [64 x 3 mm axial slices, TR = 15 ms, TE = 6.9 ms] and a T₂-weighted [17 x 8mm axial slices, TR = 5000 ms, TE = 65 ms] anatomical scans were also acquired for each subjects.

Data Analysis

Lesion volume was measured manually using MRICro, version 1.37. Identified lesions were highlighted and the total lesion volume calculated from the T₂-weighted images. The mean intra-rater variability was 3.2% for the use of this software.

Analysis of the fMRI data and the group analysis were carried out using the same tools described in section 3.2.

Identification of brain activation during the verbal working memory task was performed in the same manner as in section 3.2, including the region-of-interest and functional connectivity analysis, using the same masks as previously applied.

To identify functional activation during the Stroop task, the two explanatory variables, 'Neutral' and 'Incongruent' were entered to model each subject's fMRI data on a voxel-by-voxel basis. The principle contrast of interest was 'Incongruent minus Neutral' to determine the brain regions activated specifically during the Stroop conflict task. This contrast was applied to analyse the group mean activation across the subjects. Two fMRI analyses were performed to examine the effect of rivastigmine: (i) group analysis and (ii) region of interest analysis.

(i) Group analyses

To test whether there are differences in fMRI activation between patients taking rivastigmine and patients not taking rivastigmine, the following paired between subject group analysis were performed:

(a) On minus off rivastigmine

(b) Off minus on rivastigmine

(ii) Region-of-interest analysis

Parry and colleagues have previously identified two regions, the right inferior frontal gyrus (R GFI) and the left superior frontal gyrus (L GFS), that are associated with differences between MS patients and healthy controls. A decrease in the maximum

signal intensity was found in the L GFS and an increase in R GFI. In this study, binary masks of these regions (defined anatomically) were applied to each subject's individual contrast of parameter estimate image (COPE) for the incongruent minus neutral contrast, to determine the maximum COPE value in these regions under both test conditions (on or off rivastigmine).

Statistics

Paired t-tests were employed to determine the effect of rivastigmine on frequency of side effects, performance of neuropsychological tests, performance on the Stroop and N-back tasks, and the regional activation during the Stroop and N-back tasks. The difference in the number of patients showing improved performance in the neuropsychological battery between actual results and expected results assuming the null hypothesis was determined using the chi-squared test. Pearson correlation was used to assess any relationship between lesion volumes or behavioural measures with functional activation. A Fisher's Z-r transformation was used to compare correlations in the functional connectivity analysis (see section 3.2). A corrected, two-tailed $p < 0.05$ was considered statistically significant. Before applying any analysis that assumes normal distribution, checks were made that the data approximated normality. All probability values were corrected for multiple comparisons. All statistical analyses were performed using SPSS for windows (version 12.0.1).

4.3 Results

Adverse effects of rivastigmine The frequency of side effects was significantly greater in patients when taking rivastigmine compared to when they were not ($p < 0.01$) (Figure 4.2). Side effects included nausea, vomiting, diarrhoea and light-headedness and varied in severity.

Baseline scores on neuropsychological battery (Table 4.2) There was a range of performance across the patients in the neuropsychological tests. Four out of the fifteen patients showed more than 1 S.D difference across all the tests. The other patients demonstrated either milder deficit or above average performance. Overall patients showed the worst performance in the word list generation task.

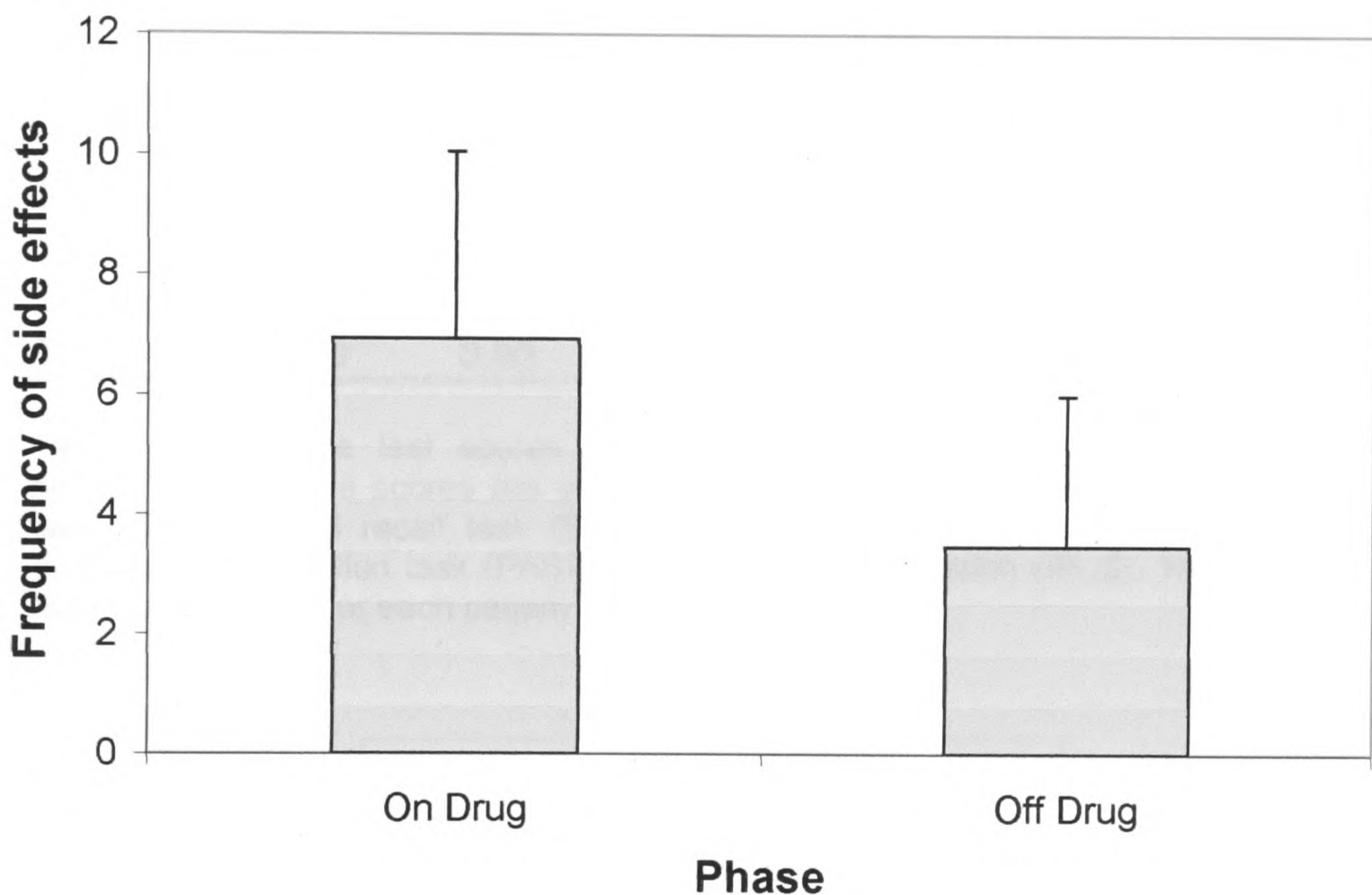


Figure 4.2 The frequency of side effects in MS patients whilst on and off drug. There was a two-fold increase in frequency whilst on drug ($p < 0.01$)

Effect of rivastigmine on the performance of neuropsychological tests in MS patients

There was a trend to increased performance across the tests (Figure 4.3) but this was not statistically significant on a paired t-test for the average change in performance ($p = 0.07$). 11 out of the 15 patients showed an overall small improvement in test scores, while 4 patients showed a decline (Table 4.3). This was not significant on chi-square test ($p = 0.07$) when compared to the null hypothesis.

Subject	Baseline test scores					Average
	SRT	SPART	SDMT	PASAT	WLG	
1	0.36	-0.16	0.40	-0.22	-1.35	-0.20
2	-1.70	-1.49	-0.90	0.06	-1.23	-1.05
3	-0.15	1.12	1.28	1.02	-0.34	0.59
4	0.20	0.01	-0.17	0.01	-0.72	-0.13
5	-0.36	-0.74	-1.86	-1.19	-0.85	-1.00
6	-0.12	-0.79	0.56	1.06	1.05	0.35
7	-0.72	0.57	0.07	-0.55	-0.09	-0.15
8	-1.56	-1.32	-1.54	-2.21	-1.61	-1.65
9	-0.64	-0.38	0.15	0.60	-0.97	-0.25
10	-1.22	-0.99	-0.09	-0.13	-2.37	-0.96
11	1.35	-0.63	2.49	0.46	0.42	0.82
12	0.11	1.12	-0.90	-0.59	-1.23	-0.30
13	0.79	0.54	0.31	-0.69	-1.35	-0.08
14	0.63	-1.10	-1.94	-2.26	-1.35	-1.21
15	-1.36	0.90	0.15	1.06	-0.22	0.11

Table 4.2 Baseline test scores were converted into Z-scores using published normative data. The scores are shown for each patient in the selective reminding task (SRT), spatial recall task (SPART), symbol digit modalities (SDMT), paced auditory serial addition task (PASAT) and word list generation (WLG). The average across these tests for each patient is also shown.

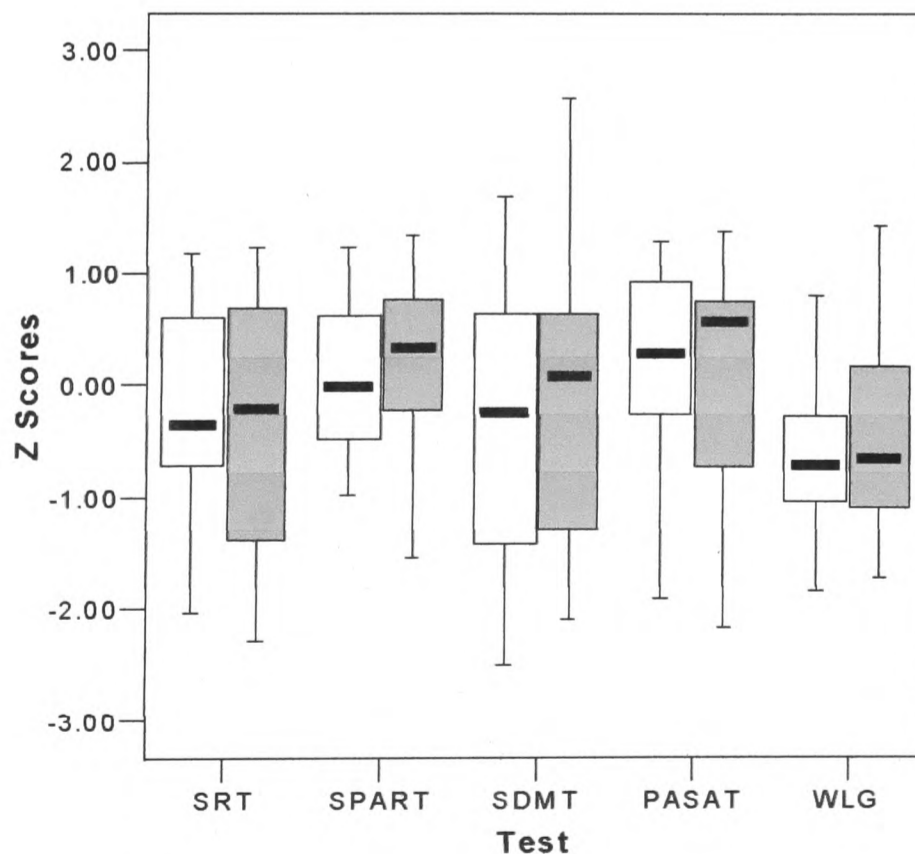


Figure 4.3 Performance off (white) and on (grey) rivastigmine in the spatial recall task (SRT), spatial recall task (SPART), symbol digit modalities task (SDMT), paced auditory serial addition task (PASAT) and word list generation (WLG). There was a trend to increased performance ($p=0.07$).

Subject	On – Off Rivastigmine test scores					Average
	SRT	SPART	SDMT	PASAT	WLG	
1	0.16	1.06	-0.40	-0.18	0.51	0.23
2	-0.24	-0.44	0.16	0.04	0.00	-0.10
3	0.28	-0.22	-0.65	-0.41	1.77	0.16
4	-0.48	0.22	0.40	0.78	-0.63	0.06
5	0.43	0.03	0.40	0.28	-0.25	0.18
6	0.35	-0.44	-0.32	-0.18	1.01	0.08
7	-0.24	0.35	-0.31	-0.20	-0.80	-0.24
8	-0.55	0.69	0.32	0.69	-0.63	0.11
9	2.15	0.36	-0.48	0.05	-0.13	0.39
10	-1.24	-0.06	0.08	0.18	0.25	-0.16
11	0.05	-0.47	0.89	0.37	0.00	0.17
12	-0.20	0.00	-0.48	0.09	1.90	0.26
13	-0.11	0.00	1.85	-0.15	-0.63	0.19
14	-1.36	-0.56	-0.16	-0.24	-0.25	-0.51
15	-0.89	1.00	0.24	0.09	0.89	0.27

Table 4.3 Test scores were converted into Z-scores using published normative data. The change in test scores with the administration of rivastigmine are shown for each patient in the selective reminding task (SRT), spatial recall task (SPART), symbol digit modalities (SDMT), paced auditory serial addition task (PASAT) and word list generation (WLG). The average across these tests for each patient is also shown.

Behavioural performance during the Stroop task There was a trend to slower reaction times in patients taking rivastigmine compared to when not on rivastigmine ($p=0.07$). Patients showed similar size of Stroop effect on and off rivastigmine (Figure 4.4).

Functional activation during the Stroop task In the simple contrast between Stroop task minus the neutral condition, areas identified as being significantly active ($Z>3.5$, $p<0.01$) include right posterior parietal, left posterior parietal, right lateral prefrontal, left lateral prefrontal and medial frontal regions (Figure 4.5). The MNI co-ordinates of the maximum Z score for each of the discrete clusters are shown in Table 4.4.

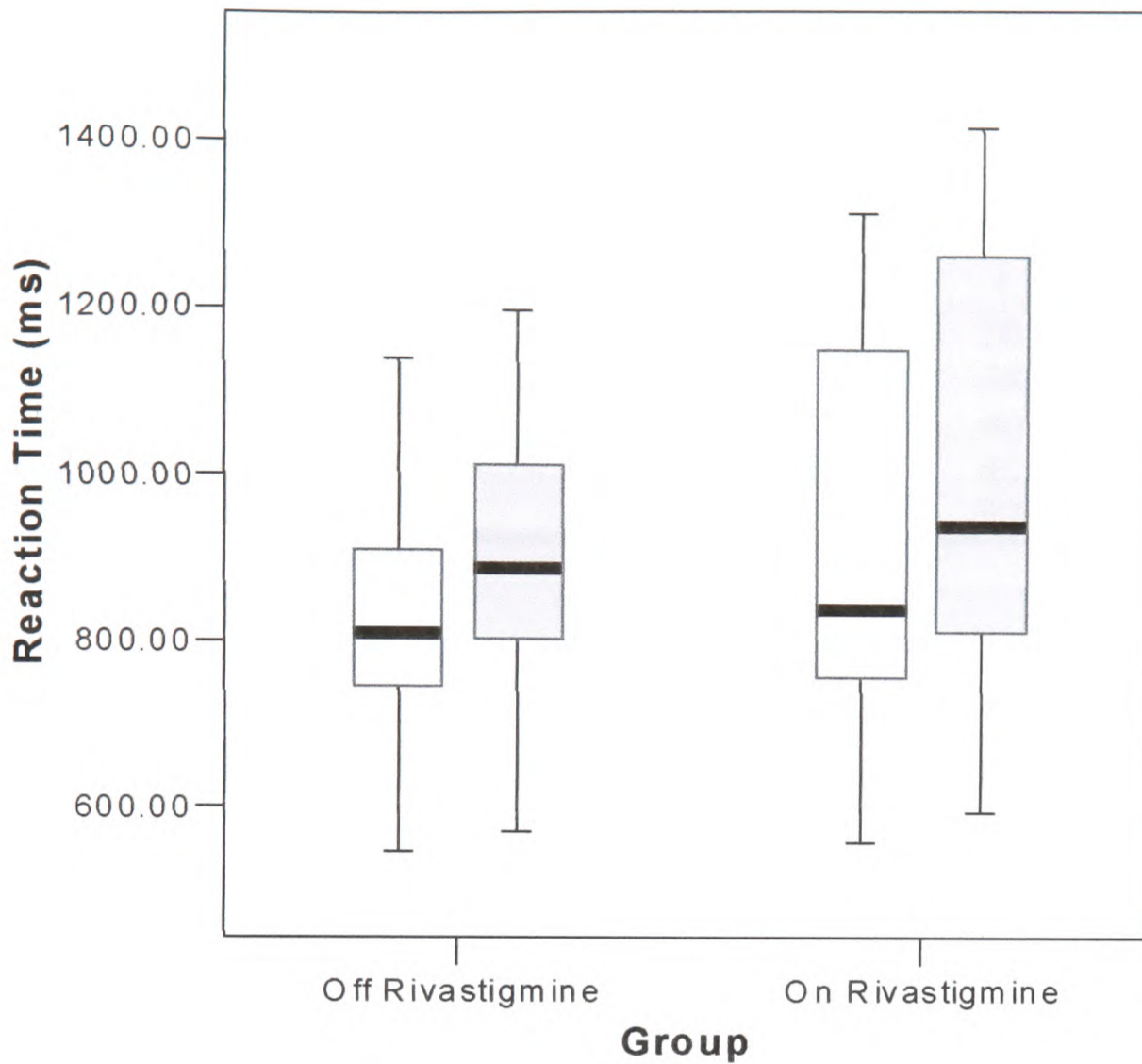


Figure 4.4 Reaction times in MS patients on and off rivastigmine. There was a non-significant trend ($p=0.07$) for slower reactions times during the neutral (white) and incongruent (grey) Stroop conditions. A similar Stroop effect size was seen on and off drug.

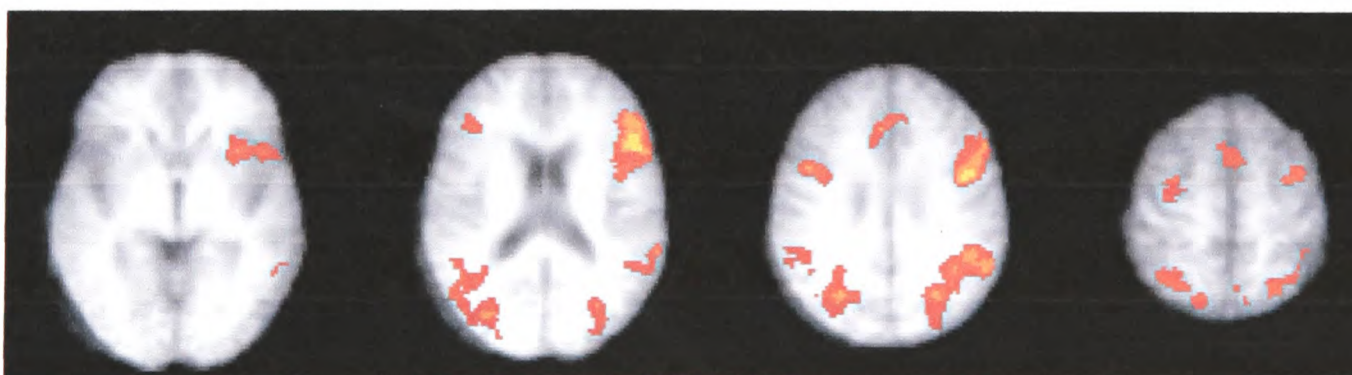


Figure 4.5 Regions activated during the Stroop task superimposed on the mean structural image ($Z>3.5$, $p<0.01$) The MNI co-ordinates of the maximum Z scores for each of the discrete clusters are shown in table 4.4.

Anatomical region	Co-ordinates of max Z scores in MNI space (mm)			Maximum Z score
	x	Y	z	
	Right Lateral Prefrontal	44	6	
Left Lateral Prefrontal	-44	2	34	4.78
Right Posterior Parietal	30	-66	40	4.65
Left Posterior Parietal	-46	-44	40	5.31
Medial Frontal	0	12	52	3.91

Table 4.4 Regions identified during the Stroop task on fMRI activation ($Z > 3.5$, $p < 0.01$).

Effect of rivastigmine on functional activation in MS patients In a mixed-effects group analysis, there were no areas of significant difference between patients on and off rivastigmine.

The effect of rivastigmine on region-of-interest activation in MS patients To explore more powerfully any activation changes in MS patients with the administration of rivastigmine, a region-of-interest analysis was carried out. The maximum contrast of parameter estimate was measured in the left superior frontal and right inferior frontal gyri, identified previously (Parry et al., 2003) as being areas potentially sensitive to the effects of rivastigmine.

A significant increase in activation ($p < 0.05$) in patients on rivastigmine compared with patients off rivastigmine was found within the right inferior frontal gyrus (Figure 4.6, Table 4.5). There were no significant differences in the mean signal intensity changes within the left superior frontal gyrus or in the ratio of activation between

right and left regions. There was no correlation of activation changes with changes in behavioural measures.

Subject	On Rivastigmine			Off Rivastigmine		
	L GFS	R GFI	AR	L GFS	R GFI	AR
1	57	209	0.27	142	102	1.39
2	80	147	0.55	60	107	0.56
3	41	145	0.28	63	69	0.91
4	97	169	0.57	52	65	0.80
5	104	230	0.45	24	89	0.27
6	52	51	1.01	46	74	0.62
7	293	351	0.84	224	96	2.34
8	45	77	0.58	63	68	0.93
9	55	75	0.73	80	123	0.65
10	106	135	0.78	44	85	0.52
11	99	89	1.11	153	102	1.50
12	30	103	0.29	42	104	0.41
13	74	128	0.57	24	59	0.41
14	146	40	3.63	100	78	1.28
Mean	91	139	0.83	80	87	0.90
(S.D)	(66)	(82)	(0.8)	(57)	(19)	(0.6)

Table 4.5 Maximum contrast of parameter estimates (COPE) in the left superior frontal gyrus (L GFS) and right inferior frontal gyrus (R GFI). The ratio of activation (AR) between the two regions of interest is not significantly different between patients on and off rivastigmine. A significant increase in activation is found within the R GFI ($p < 0.05$).

Behavioural performance during the N-back task There was no significant difference of performance during the working memory task in MS patients on and off rivastigmine (Figure 4.7). Patients showed a similar decline of performance with increasing task difficulty regardless of trial phase.

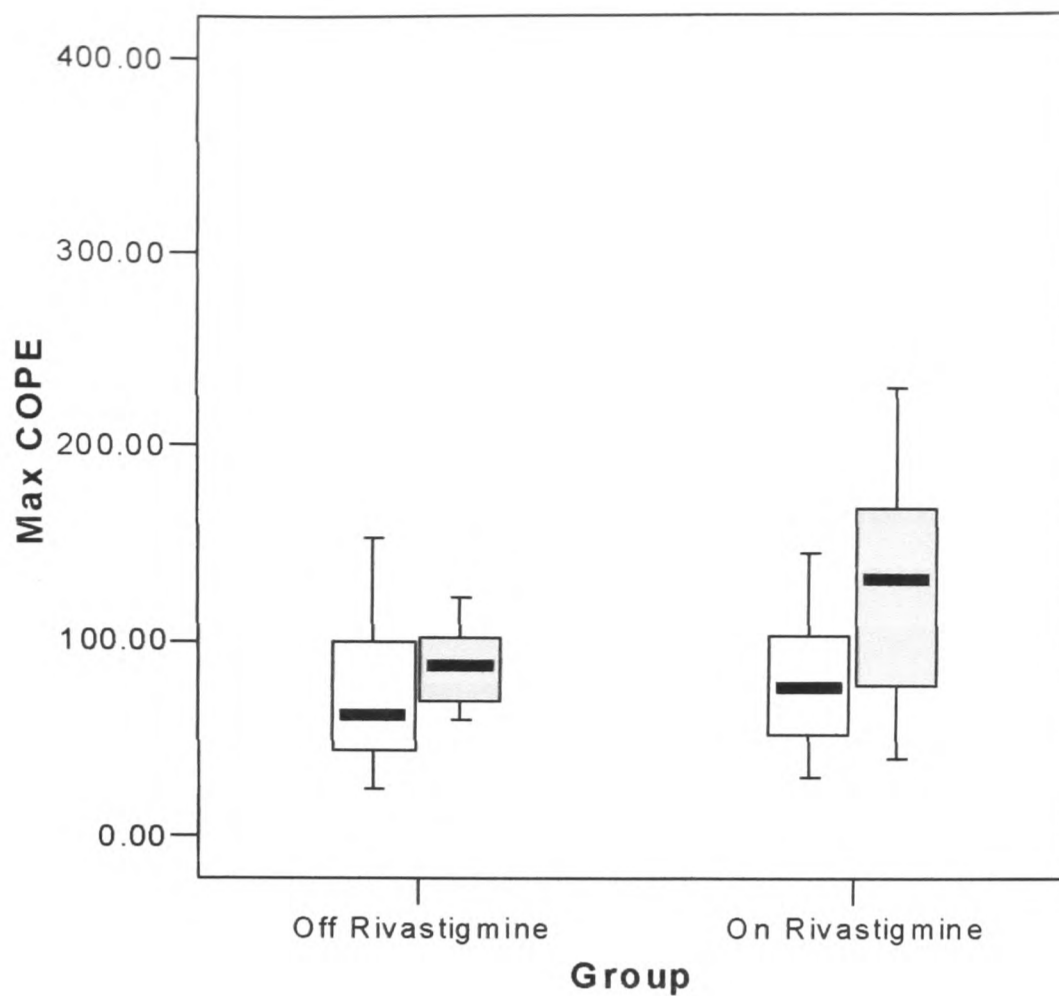


Figure 4.6 The maximum contrast of parameter estimate (COPE) in the left superior frontal gyrus (white) and in the right inferior frontal gyrus (grey). There is a significant increase in activation within the right inferior frontal gyrus in MS patients taking rivastigmine compared with when off rivastigmine ($p < 0.05$)

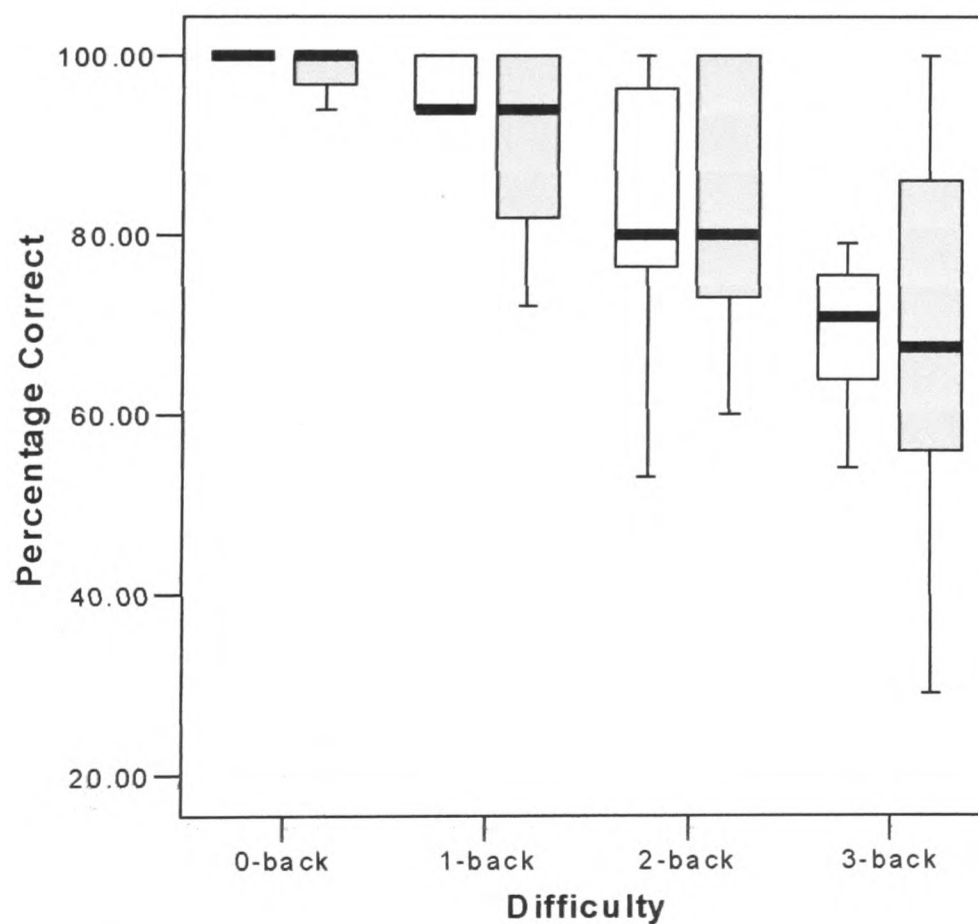


Figure 4.7 Performance of the N-back task in patients on (grey) and off (white) rivastigmine. There is no significant difference in performance at any level of task difficulty.

Functional activation during the N-back task in MS patients In the simple contrast of each level of n-back (1-, 2 and 3-back) minus 0-back, similar areas are identified as being significantly active ($Z > 3.5$, $p < 0.01$) and include right posterior parietal, left posterior parietal, right lateral prefrontal, left lateral prefrontal and medial frontal regions (Figure 4.8). The MNI co-ordinates of the maximum Z score for each of the discrete clusters are shown in Table 4.6.

Anatomical region	Co-ordinates of max Z scores in MNI space (mm)			Maximum Z score
	x	y	z	
	Simple Contrast (1-, 2-, 3-back) Right Lateral Prefrontal Left Lateral Prefrontal Right Posterior Parietal Left Posterior Parietal Medial Frontal	26 -52 22 -46 10	4 2 -66 -40 24	
Linear Contrast of linear activation (1- to 3-back) Right Lateral Prefrontal Left Lateral Prefrontal Right Posterior Parietal Left Posterior Parietal Medial Frontal Cerebellum	34 -46 54 -36 -6 -34	30 14 -48 -44 10 -74	30 28 34 28 44 -44	3.77 3.82 5.02 5.28 5.14 4.59

Table 4.6 MNI co-ordinates of significant ($Z > 3.5$, $p < 0.01$) discrete clusters of activation during the N-back task for the simple contrast (1-, 2, and 3-back) and linear contrast (1- to 3-back).

The effect of rivastigmine on activation during the 1-, 2- and 3- back tasks In a mixed effects group analysis contrasting the patients on rivastigmine minus patients off rivastigmine found one area of significant activation within the cerebellum (Figure

4.9) across all three task conditions ($Z=4.18$, $p<0.01$, $x=38$, $y=-64$, $z=-54$). There were no areas of significantly greater activation in patients off rivastigmine compared with patients on rivastigmine.

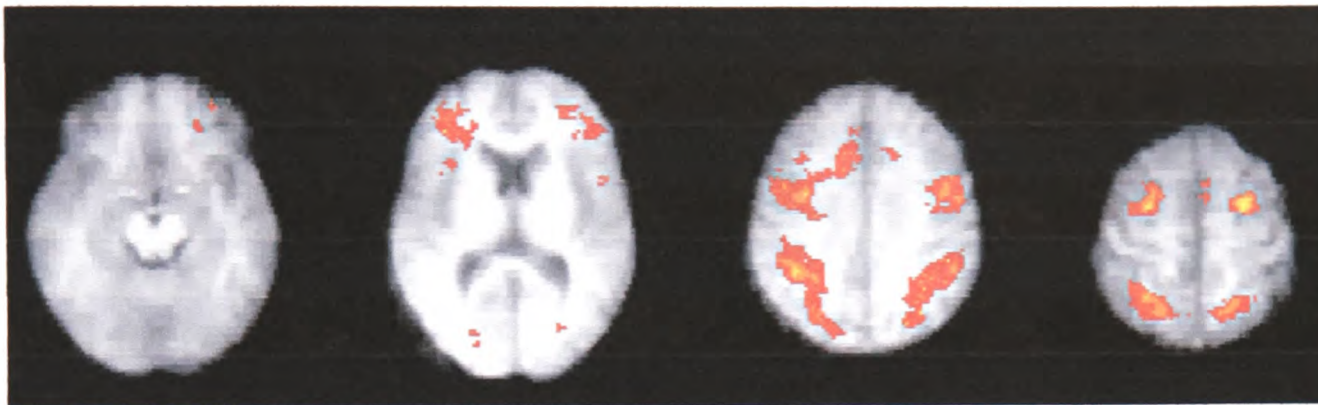


Figure 4.8 Areas of significant activation ($Z>3.5$, $p<0.01$) during the 1-back task superimposed on the mean structural image. Similar regions are found in the 2- and 3-back levels of difficulty, but with increased cluster sizes and Z-values. MNI co-ordinates for the maximum Z score at each of the discrete cluster are shown in table 4.6.

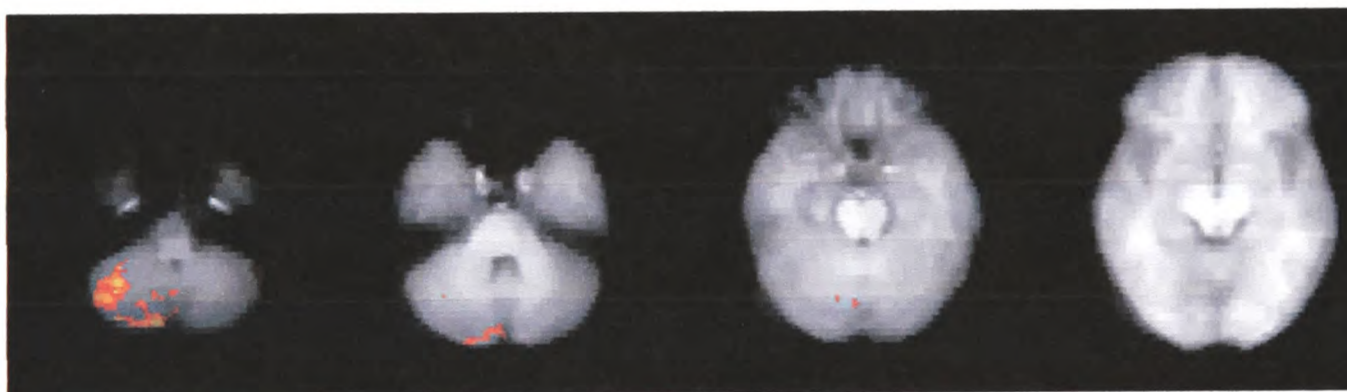


Figure 4.9 Area of significantly greater activation in patients on rivastigmine compared with off rivastigmine ($Z>3.5$, $p<0.01$) during the 3-back task. The maximum Z-score of 4.18 is at MNI co-ordinates of $x=38$, $y=-64$, $z=-54$.

The effect of increasing task difficulty on the functional activation during the N-back task in MS patients Regions identified as showing significant increasing activation ($Z>3.5$, $p<0.01$) with increasing task difficulty were similar to those found at each level and included the right posterior parietal, left posterior parietal, right

lateral frontal, left lateral frontal, cerebellum and medial frontal regions (Figure 4.10, Table 4.6).

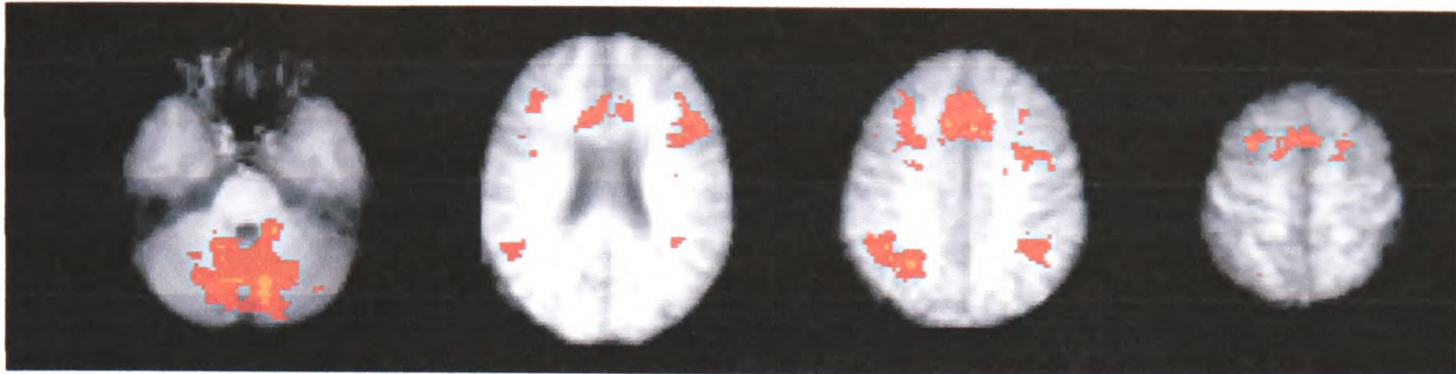


Figure 4.10 Regions of significantly increasing activation with increasing task difficulty ($Z > 3.5$, $p < 0.01$) superimposed on the mean structural image. The MNI co-ordinates of the maximum Z score for each of the discrete clusters are shown in table 4.6.

The effect of rivastigmine on the incremental activation of increasing task difficulty

On a mixed effects group analysis of patients on rivastigmine minus patients off rivastigmine, there was a significant ($Z > 3.5$, $p < 0.01$) increase in activation in the right posterior parietal, left posterior parietal, left frontal and occipital pole (Figure 4.11, Table 4.7). There were no areas of greater incremental activation in patients off rivastigmine compared with on rivastigmine.

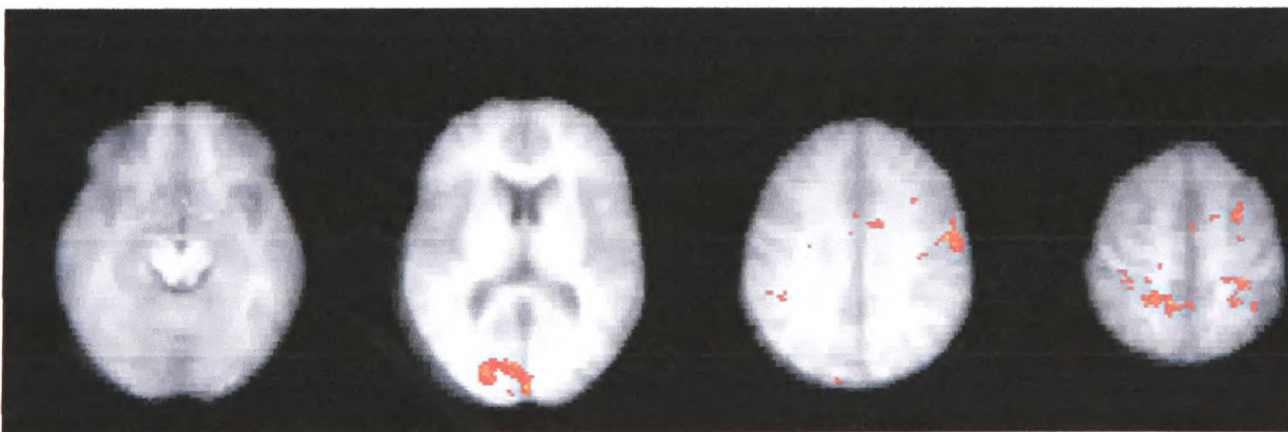


Figure 4.11 Regions of significantly greater incremental activation in patients taking rivastigmine ($Z > 3.5$, $p < 0.01$) superimposed on the mean structural image. The MNI co-ordinates of maximum Z scores for each cluster are shown in table 4.7.

Anatomical region	Co-ordinates of max Z scores in MNI space (mm)			Maximum Z score
	x	y	z	
	Left Posterior Parietal	-26	-42	
Right Posterior Parietal	22	-48	58	3.70
Occipital Pole	0	-94	8	4.22
Left Frontal	-50	-6	36	3.89

Table 4.7 MNI co-ordinates of significant ($Z > 3.5$, $p < 0.01$) clusters of greater incremental activation in patients taking rivastigmine compared with patients off rivastigmine.

Region-of-interest analysis on the effect of rivastigmine in MS patients To further explore the effect of rivastigmine on activation during the N-back task, the mean signal was measured in regions previously identified as involved in the task at each level of task difficulty. There was a significant decrease in the mean signal at the 1-back level ($p < 0.01$) and a significant increase in the incremental activation ($p < 0.01$) in patients on rivastigmine compared with patients off rivastigmine (Figure 4.12). No correlation was found between behavioural measures and the functional activation.

Effect of rivastigmine on the functional connectivity in MS patients for the N-back task Using the same regions of interest as previously used, the functional connectivity was assessed in patients on and off rivastigmine. There were significant differences in functional connectivity between patients on and off rivastigmine (Table 4.8, Figure 4.13a-b). There was a significantly greater connectivity in patients taking rivastigmine between left to right frontal, anterior cingulate to left frontal, and right parietal to right frontal regions (figure 4.13b) on using Fisher's Z-r transformation ($p < 0.05$). There were no areas of significantly greater connectivity in patients off rivastigmine.

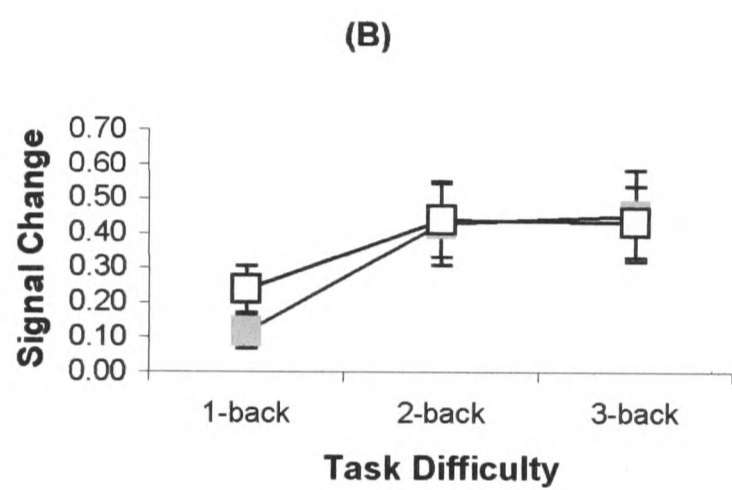
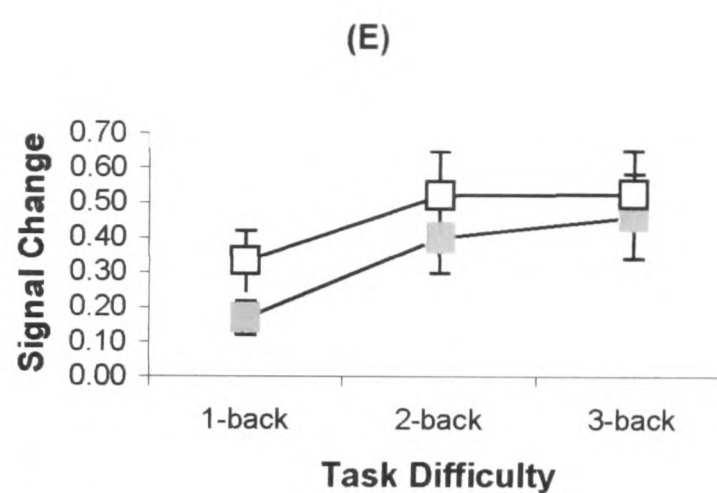
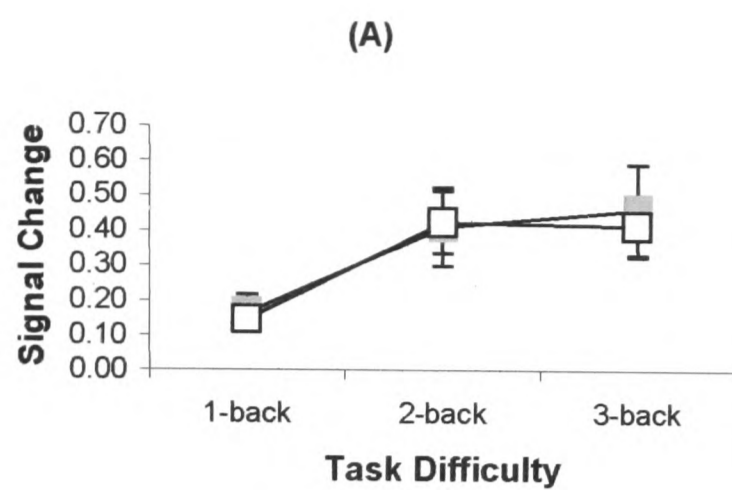
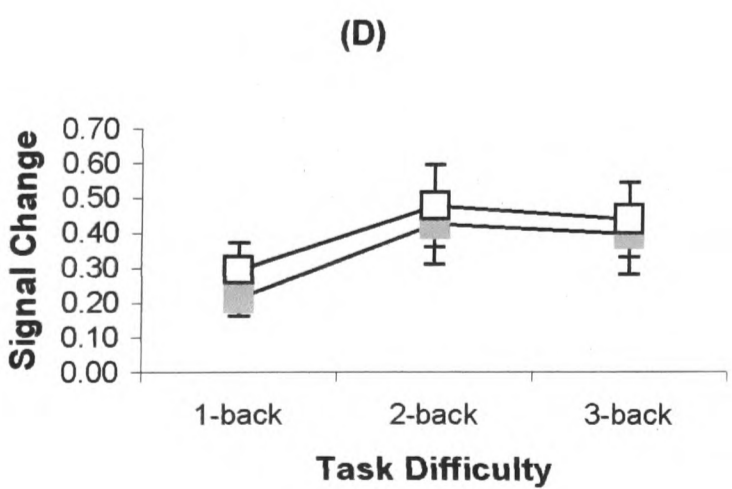
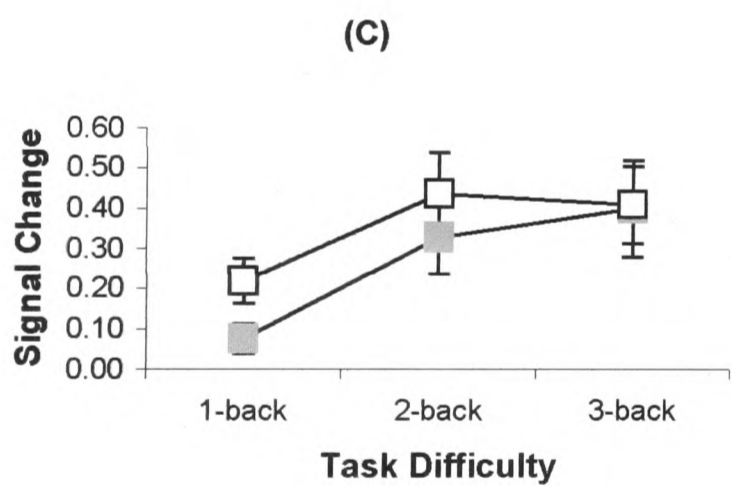


Figure 4.12 Mean signal at each level of task difficulty for the medial frontal (A), left dorsolateral frontal (B), right dorsolateral (C), left parietal (D), and right parietal (E) regions on (grey) and off (white) rivastigmine. There was a significantly lower activation in the 1-back task ($p < 0.01$), and a significantly greater incremental activation ($p < 0.01$) in patients taking rivastigmine.



Dependant Region	Tested Region	Rivastigmine	
		On	Off
Right Prefrontal	Left Prefrontal	0.737*	0.413
	Right Parietal	0.500	-0.047
	Superior Medial Frontal	-0.289	0.115
	Anterior cingulate	-0.139	0.488
Left Prefrontal	Right Prefrontal	0.764*	0.059
	Left Parietal	0.032	0.661
	Superior Medial Frontal	-0.173	-0.036
	Anterior cingulate	0.712	0.229
Anterior Cingulate	Right Prefrontal	-0.477	0.569
	Left Prefrontal	0.728*	-0.081
	Superior Medial Frontal	0.615	0.318
Superior Medial Frontal	Right Prefrontal	0.404	0.352
	Left Prefrontal	-0.167	0.254
	Anterior Cingulate	0.615	0.318
Right Parietal	Right Frontal	0.747*	0.093
	Left Parietal	0.239	0.442
Left Parietal	Left Frontal	0.471	0.758*
	Right Parietal	0.188	0.375

Table 4.8 The partial correlation coefficients between regions tested. The dependant regions are shown in the first column, and independent regions that were regressed against this are shown in the second column. Only regions that were potentially anatomically connected directly were considered. Significant ($p < 0.05$ after correction for multiple comparisons) correlations are indicated.

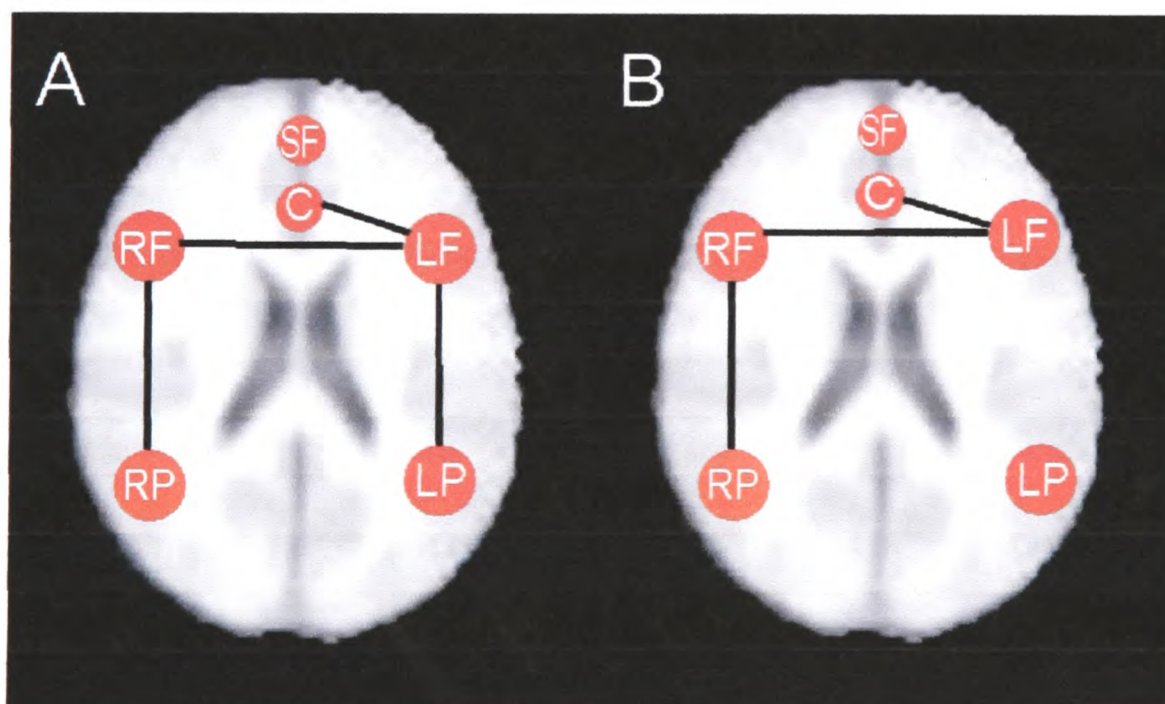


Figure 4.13 The functional connectivity in MS patients during the N-back task (A) and the significantly increased connectivity ($p < 0.05$) found in patients taking rivastigmine (B) compared with patients off rivastigmine. C=cingulate, SF=superior medial frontal, RF=right dorsolateral frontal, LF=left dorsolateral frontal, RP=right parietal, LP=left parietal

T₂ Lesion volume The mean T₂ lesion volume was 7.91 ml (S.D = 7.4), (Table 4.1). There was no correlation of lesion volume with any measure of behaviour or functional activation.

4.4 Discussion

This study investigated the effects of rivastigmine on behavioural measures of cognitive function and functional activation of cognitive processing. There was no significant effect of rivastigmine on behavioural tests. However, an effect was seen on the functional activation in the Stroop and N-back tasks. This is discussed in more detail below.

4.4.1 The effect of rivastigmine on cognitive performance

On average, small increases were seen in test performance with the administration of rivastigmine in MS patients. A positive effect of rivastigmine is in keeping with previous clinical studies of cholinesterase inhibitors in MS (Greene et al., 2000) and dementias (Bilikiewicz et al., 2002; McKeith et al., 2000). Although, more patients showed an increase in performance than a decline, this was not significantly different from the null hypothesis. This non-significant trend was also reflected in the change in performance across the patients. Another study with a cholinesterase inhibitor did not show a beneficial effect on cognition (Moller et al., 1999). The results of this experiment could be interpreted as a lack of efficacy for rivastigmine in MS patients. However, the baseline neuropsychological tests reveal a heterogeneous group of subjects entering the study. Clinical symptoms in MS are variable reflecting the variation in disease severity, lesion location (Charil et al., 2003) and any adaptive mechanisms that may be utilised (Cifelli and Matthews, 2002; Reddy et al., 2000). In

order that task performance was not affected by motor or sensory difficulties, subjects were recruited on the basis of having relatively mild multiple sclerosis. Patients were required to have subjective complaints of poor memory or concentration. Nevertheless, the remaining heterogeneity amongst the patients recruited may be enough to mask a positive effect of rivastigmine on those patients that may potentially show benefit. Many of the patients express minimal baseline cognitive dysfunction despite symptomatic complaints. Due to a ceiling effect there may be limited scope for rivastigmine to improve cognition in these patients. Further to this there may be limitations in the neuropsychological testing. The brief repeatable battery of neuropsychological was devised to provide a convenient means to test cognitive function in MS patients (Bever et al., 1995). Given the relatively mild cognitive deficits, the sensitivity of the battery may be insufficient to detect an effect in this context.

4.4.2 Rivastigmine and the Stroop effect

The experiment showed that rivastigmine increased activation within the inferior frontal gyrus, a region identified previously as being associated with normal processing in healthy control subjects (Parry et al., 2003). The observed change in the activation within the inferior frontal gyrus is likely a consequence of treatment with rivastigmine, rather than a change in the level of performance, given the absence of any significant change in, or correlation with, behavioural measures of the Stroop task. However, the increased activation found may represent increased response suppression associated with the inferior frontal cortices (Kemmons et al., 2005) in the MS patients that could be reflected in the non-significant trend to slower reaction times. The change in the activation ratio identified by Parry and colleagues as being a

normalisation of functional activation back to that seen in their group of healthy subjects (Parry et al., 2003) was not replicated in this study. Differences in the group of MS patients studied could be responsible for the variation. Nevertheless, in both studies, patients had relatively mild disease, and the degree of cognitive impairment was similar on baseline tests. Alternatively, despite the intention to follow the same protocol, the experimental design and analysis may not have been sufficiently similar to detect the same effect. It is also possible that the difference in study outcome may reflect the differences in mechanisms of action between acute and more chronic dosing (Uzum et al., 2004). Longer-term treatment with rivastigmine can lead to changes in receptor levels already demonstrated for nicotinic receptors (Wang and Sun, 2005) that would not occur after single doses. Indeed, not finding a different effect between acute and chronic dosing would have been surprising and with possible desensitisation, a smaller response to chronic than single dose rivastigmine could be predicted.

4.4.3 Rivastigmine and the N-back task

One of the principle findings of this experiment was the greater incremental activation with increasing task difficulty after the administration of rivastigmine. This was largely reflected in the reduced activation during the 1-back task. Similar to the findings of healthy controls demonstrating a greater functional reserve for increasing task difficulty, in section 3.3, the MS patients have a greater capacity to increase activation when on rivastigmine. This was found despite a similar performance in patients on and off drug, suggesting an increased functional reserve for cognition with rivastigmine. The region-of-interest analysis demonstrates that this is a widespread effect in all task relevant areas, but the mixed-effects whole brain analysis indicate

that some areas are particularly sensitive to the drug effect. These may represent areas of less damage within the normal neural network and so have greater potential to respond to drug-enhancing effects. Regions already affected greatly by pathology would only have limited ability for any further adaptive changes. One must be careful, however, in the interpretation of these potentially contradictory changes. One potential explanation is that this merely reflects random variation around similar means. There is a moderate variability within behavioural performance of the n-back tasks that might be translated into variability of bold response. Given the relatively small numbers of this study, the results found in this study may not be replicated in a larger study. One of the potential compensatory mechanisms for pathology suggested in section 3 was the increased functional connectivity between task relevant areas. The increased functional connectivity found in patients taking rivastigmine suggests that the drug may be acting to improve cortical processing function of the normal neural network. At all levels of difficulty, increased cerebellar activation was found in patients taking rivastigmine. Enhanced cerebellar activation has been identified with increasing memory load (Smith and Jonides, 1997), and the increase found with cholinergic modulation has been suggested to be beneficial for performance (Kumari et al., 2003).

4.4.4 Modulation of cognitive processing with rivastigmine

The modulation by rivastigmine on the activity of cortical brain regions recruited is likely to be mediated by an increase in the amount of acetylcholine available in relevant cortical synaptic junctions. The widespread innervation throughout the brain (McGaughy et al., 2000) supports the role of acetylcholine as a modulator of regional cortical activity. The enhanced activation seen within the right inferior frontal gyrus

during the Stroop task with the administration of rivastigmine appears in contrast to the reduced activation found across relevant brain regions in the 1-back task. However, the differential effects of acetylcholine, increasing activity in some pathways and decreasing in others (Kimura et al., 1999) could explain this. At a cellular level, the modulation can either act to suppress or facilitate signal propagation depending on the input source, thereby shifting the dynamics of the cortical network (Kimura, 2000). Thus acetylcholine may increase the sensitivity and functioning of the neural network recruited to perform the tasks, by improving the signal-to-noise ratio of relevant neural activity. Whilst increasing the efficiency of neural processing within the task-specific region and so reducing the level of necessary activity particularly at low demand such as the 1-back task, some regions may show increased activity associated with increased propagation of signal typically at higher level of demand. Temporal and spatial summation, phenomena needed for effective propagation of signal, would be adversely affected in the context of demyelination and axonal loss that occurs in MS. Enhancing the signal in intact or less damaged neurones may be sufficient to overcome the summation requirements that could be provided by increasing the acetylcholine available in cortical synaptic junctions, similar to other studies of drug modulation enhancing conduction (Mainero et al., 2004). No significant improvement was observed within the patient group on drug compared to off drug in any of the behavioural outcome measurements. The drug may act to increase functioning of the normal neural network recruited to perform the task reducing the requirement for previously recruited compensatory mechanisms, and so may not result in behavioural changes. Rivastigmine could reduce further decline in function by maximising any potential functional adaptation and so maintaining cognitive function as disease progresses. However, in a study of control subjects, a

cholinesterase inhibitor was shown to enhance behavioural measures of working memory whilst reducing task-specific activity on PET scans (Furey et al., 1997). As discussed earlier, the chronic dosing in this study may have led to changes in acetylcholine receptor levels resulting in smaller effects than those found in acute dosing studies. Future studies are needed to shed more light on the complex effects of this drug.

Section 5: The effect of hyoscine on functional activation during cognition in healthy subjects

A number of studies have shown the importance of acetylcholine in cognition. Enhancing acetylcholine neurotransmission may improve performance of certain cognitive functions. The previous section demonstrated a potentially beneficial alteration in brain activation during the Stroop and N-back task in MS patients with rivastigmine. To further understand how cholinergic modulation influences brain activation, an acetylcholine antagonist is given to healthy volunteers during working memory and attentional tasks. The effect on functional activation is considered in this section.

5.1 Introduction and rationale

The role of central cholinergic systems in cognitive processing has been supported by several studies (Freo et al., 2002; Gold, 2003; Thiel, 2003). Studies of nicotine acting directly on acetylcholine receptors have shown enhanced attention and improved learning (Levin and Simon, 1998; Rezvani and Levin, 2001). Acetylcholine antagonism, in contrast, impairs cognitive performance (Parrott, 1986) and can be reversed by cholinergic agents (Bejar et al., 1999; Wesnes and Revell, 1984). In support of this, functional imaging studies have demonstrated changes in brain activity and improved behavioural performance with nicotine (Kumari et al., 2003) and cholinesterase inhibitors (Furey et al., 1997) in healthy subjects. Functional imaging studies of anti-cholinergic agents during cognitive tasks show impairment of normal cortical processes (Bahro et al., 1999; Thiel et al., 2002).

However, acetylcholine seems to be more involved in attentional processes (Sarter et al., 2003) and the modulation of working memory processes (Everitt and Robbins, 1997). As described previously, the Stroop and N-back tasks assess continual attentional performance and manipulation of data within working memory (sections 3.1 and 4.1). These tasks allow the probing of functional activation for these specific processes during administration of an anti-cholinergic agent.

If hyoscine impairs the cortical processing of attention or working memory, thereby affecting the neural activity, differences should be found in the functional activation during the Stroop or N-back tasks. In the previous study of rivastigmine in MS patients, a potentially beneficial change in functional activation was demonstrated. This change occurred in the absence of any significant increase in behavioural performance. Hyoscine, by blocking the action of acetylcholine, might be speculated to cause changes in brain activation that are opposite to those seen with rivastigmine. This is undertaken in healthy subjects with no complaints of cognitive impairment or any evidence of neurological disease, avoiding any confounding factors. Thus data is presented from the two cognitive tasks to assess changes in brain activity in healthy volunteers with the administration of hyoscine.

5.2 Methods

Subjects

13 right-handed healthy volunteers (6 men, 7 women; mean age 29 years, range 23-37 years) completed a randomized double-blind crossover study of hyoscine. Subjects with a history of psychological and psychiatric problems, epilepsy, learning disability,

alcoholism, head injury, episodes of loss of consciousness or severe ongoing stressors (such as bereavement, divorce, financial problems) were excluded. Subjects were advised to abstain from drinking caffeine-containing beverages for 4-6 hours prior to the scan. No subjects smoked regularly, and those that smoked occasionally were advised not to smoke during the duration of the study. Written consent was obtained from all subjects and Oxford Regional Ethics Committee gave ethical approval for the study.

Sessions

Subjects attended on two separate sessions, which were at least one day apart. During the first session the subjects were screened for exclusion criteria, as stated above, and were invited to read typewritten instructions for the paradigms without any time limit. They were asked whether they understood the tasks and any further questions were answered. Training paradigms different but of identical general design to that for the fMRI tasks were then administered with a desktop PC. Following this, the subjects were imaged while performing the tasks inside the scanner.

During a second session the subject was first reminded of the rules of the task and then performed alternate versions of the tasks during imaging again.

Trial Design

Suitable subjects were randomized into Group A or Group B (Group A: visit 1 = drug, visit 2 = placebo; Group B: visit 1=placebo, visit 2 = drug). The drug was 0.3 mg of Hyoscine and the placebo was a flavorless 100mg Vitamin C tablet. Subjects took the placebo or drug under medical supervision and were told to report any side effects.

The fMRI scan was approximately 30 minutes after ingesting the tablet (to coincide with the time to maximum effect of the drug). At the end of each fMRI session, subjects were asked whether they thought they had taken placebo or hyoscine.

Paradigms

The paradigms (N-back and Stroop tasks), stimulus presentation and performance outcome measures were identical to those described in the previous study (sections 3.2 and 4.2).

Imaging

All scans were performed using a 3.0 Tesla whole body scanner with a Varian Inova console and a quadrature birdcage radiofrequency head coil. An EPI sequence was used to acquire the fMRI data [24 x 6 mm axial slices, TE = 30 ms, TR = 3000 ms, FOV = 192 x 256, matrix 64 x 64]. A T₁-weighted [64 x 3 mm axial slices, TR = 15 ms, TE = 6.9 ms] anatomical scan was also acquired for each subject.

Data Analysis

Analyses of the fMRI data was undertaken with tools already described (section 3.2) and identification of the brain activation during the tasks were carried out using the same contrasts (sections 3.2 and 4.2).

To identify the effect of hyoscine, the following paired between group analysis were performed for both the Stroop and N-back tasks:

- (a) Hyoscine minus placebo
- (b) Placebo minus Hyoscine

Statistics

To assess the effect of hyoscine on performance and regional brain activation during the Stroop and N-back tasks, paired t-tests were used. Pearson correlation was used to test any relationship between performance and functional activation. Fisher's Z-r transformation was employed to compare correlations in the functional connectivity analysis (see section 3.2). A corrected, two-tailed $p < 0.05$ was considered statistically significant. Before applying any analysis that assumes normal distribution, checks were made that the data approximated normality. All probability values were corrected for multiple comparisons. All statistical analyses were performed using SPSS for windows (version 12.0.1).

5.3 Results

Adverse effects of Hyoscine There was no significant difference in the frequency of side effects in subjects taking hyoscine compared to taking placebo.

Behavioural performance during the Stroop task There was no significant difference in the reaction times of subjects on hyoscine or placebo. Subjects showed similar size of Stroop effect on and off hyoscine (Figure 5.1).

Functional activation during the Stroop task In the simple contrast between Stroop task minus the neutral condition, areas identified as being significantly active ($Z > 3.5$, $p < 0.01$) include right posterior parietal, left posterior parietal, right lateral prefrontal, left lateral prefrontal and medial frontal regions (Figure 5.2). The MNI co-ordinates of the maximum Z score for each of the discrete clusters are shown in Table 5.1.

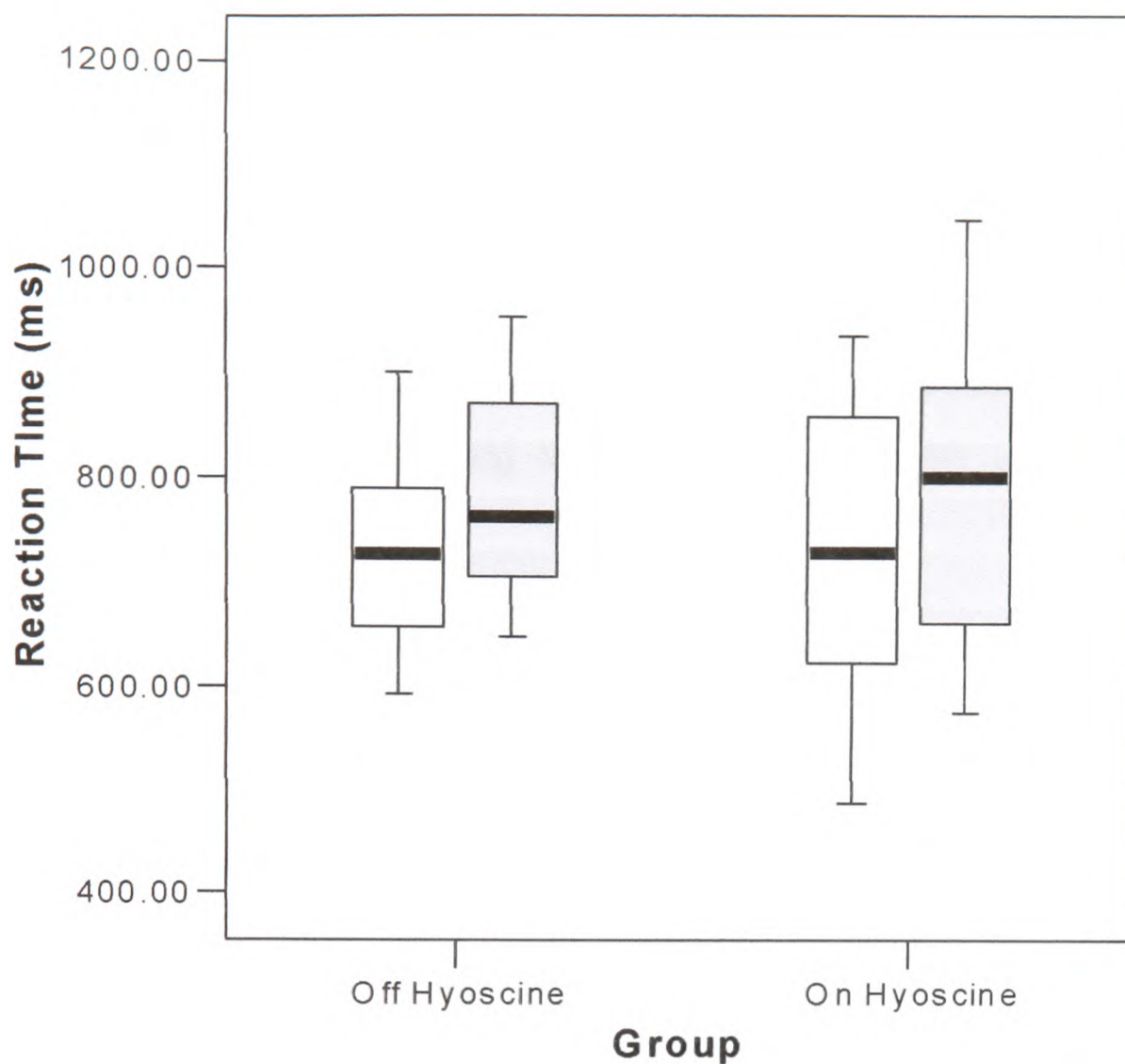


Figure 5.1 Reaction times in healthy subjects on hyoscine and on placebo. There was no significant difference in reactions times during the neutral (white) and incongruent (grey) Stroop conditions. A similar Stroop effect size was seen with hyoscine and placebo.

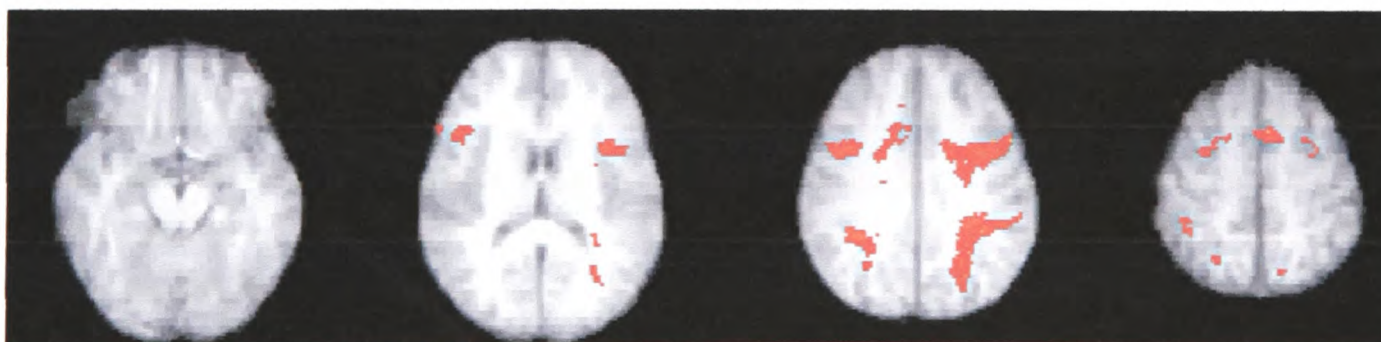


Figure 5.2 Regions activated during the Stroop task superimposed on the mean structural image ($Z > 3.5$, $p < 0.01$) The MNI co-ordinates of the maximum Z scores for each of the discrete cluster are shown in table 5.1.

Anatomical region	Co-ordinates of max Z scores in MNI space (mm)			Maximum Z score
	x	y	z	
	Right Lateral Prefrontal	50	10	
Left Lateral Prefrontal	-42	10	30	5.21
Right Posterior Parietal	46	-42	50	3.76
Left Posterior Parietal	-30	-56	40	5.20
Medial Frontal	2	10	48	3.86

Table 5.1 Regions identified during the Stroop task on fMRI activation ($Z > 3.5$, $p < 0.01$).

Effect of hyoscine on functional activation in healthy subjects In a mixed-effects group analysis, there were no areas of significant difference between subjects on hyoscine and placebo.

The effect of hyoscine on region-of-interest activation in healthy subjects A region-of-interest analysis was carried out to explore further any potential changes in functional activation in healthy subjects with the administration of hyoscine. As with the patients taking rivastigmine, the maximum contrast of parameter estimate was measured in the left superior frontal and right inferior frontal gyri.

There were no significant differences in the mean signal within the left superior frontal gyrus, the right inferior frontal gyrus or in the ratio of activation between right and left regions (Table 5.2, Figure 5.3). There was no correlation of activation changes with changes in behavioural measures.

Subject	On Hyoscine			Off Hyoscine		
	L GFS	R GFI	AR	L GFS	R GFI	AR
1	16	42	0.38	130	103	1.26
2	67	114	0.59	36	64	0.57
3	73	39	1.88	60	33	1.80
4	28	56	0.50	11	36	0.30
5	58	84	0.70	17	74	0.23
6	78	106	0.73	54	72	0.75
7	28	94	0.30	39	64	0.61
8	84	48	1.74	94	52	1.82
9	26	107	0.25	56	107	0.53
10	148	69	2.15	51	111	0.46
11	154	178	0.86	66	264	0.25
12	76	155	0.49	138	163	0.85
13	54	44	1.23	37	61	0.60
Mean	68	87	0.91	61	92	0.77
(S.D)	(43)	(44)	(0.64)	(39)	(62)	(0.54)

Table 5.2 Maximum contrast of parameter estimates (COPE) in the left superior frontal gyrus (L GFS) and right inferior frontal gyrus (R GFI). The ratio of activation (AR) between the two regions of interest is not significantly different between subjects on hyoscine or placebo.

Behavioural performance during the N-back task There was no significant differences of performance during the working memory task in subjects on hyoscine and on placebo. There was a similar decline of performance with increasing task difficulty regardless of trial phase (Figure 5.4).

Functional activation during the N-back task in MS patients In the simple contrast of each level of n-back (1-, 2 and 3-back) minus 0-back, similar areas are identified as being significantly active ($Z > 3.5$, $p < 0.01$) and include right posterior parietal, left posterior parietal, right lateral prefrontal, left lateral prefrontal and medial frontal regions (Figure 5.5). The MNI co-ordinates of the maximum Z score for each of the discrete cluster are shown in Table 5.3.

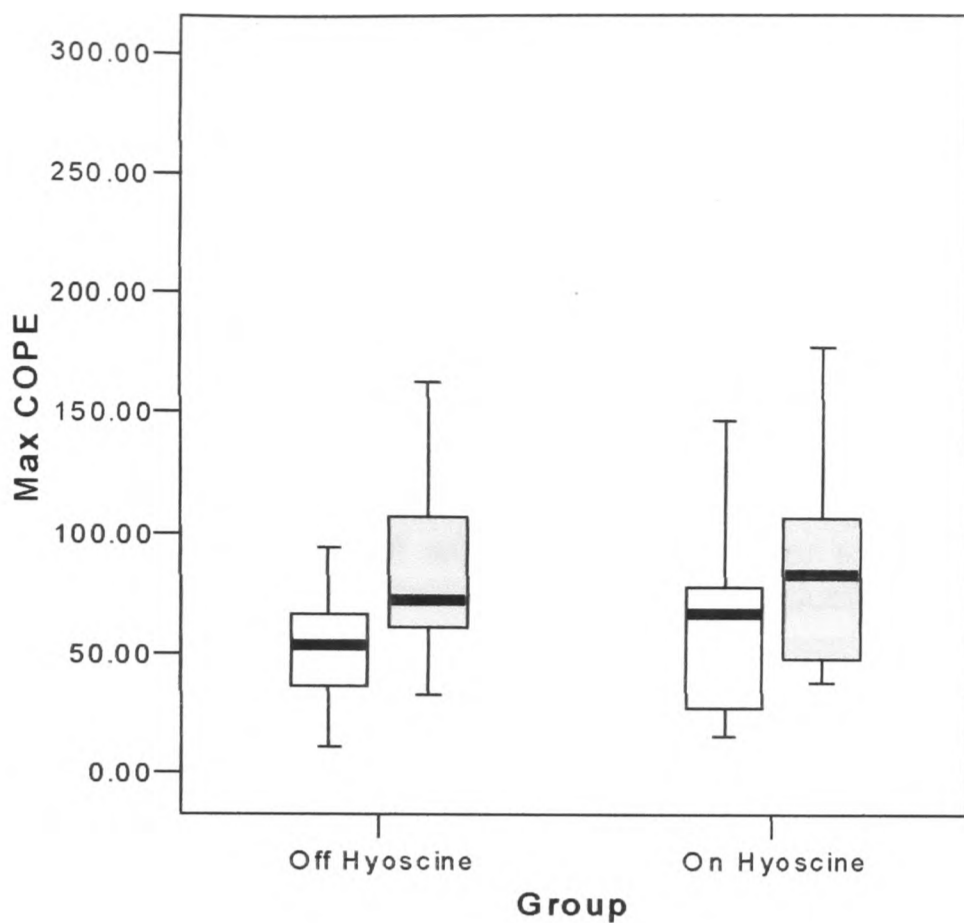


Figure 5.3 The maximum contrast of parameter estimate (COPE) in the left superior frontal gyrus (white) and in the right inferior frontal gyrus (grey). There is no significant change in activation within either region.

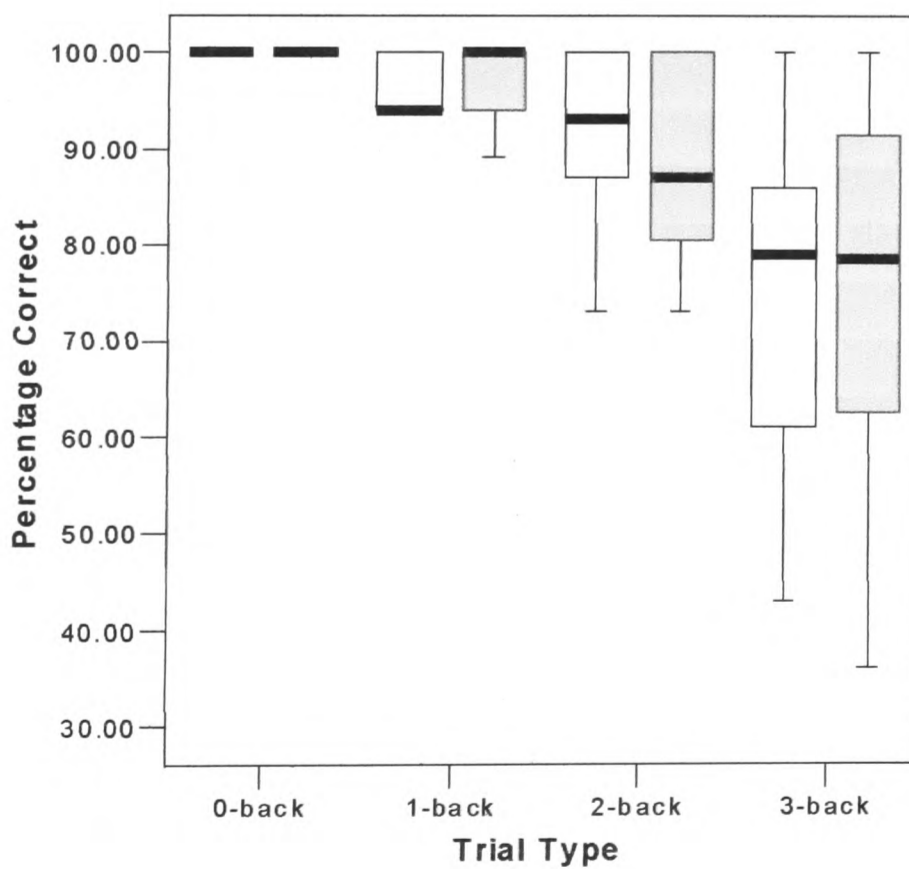


Figure 5.4 Performance of the N-back task in healthy subjects on hyoscine (white) and on placebo (grey). There is no significant difference in performance at any level of task difficulty.

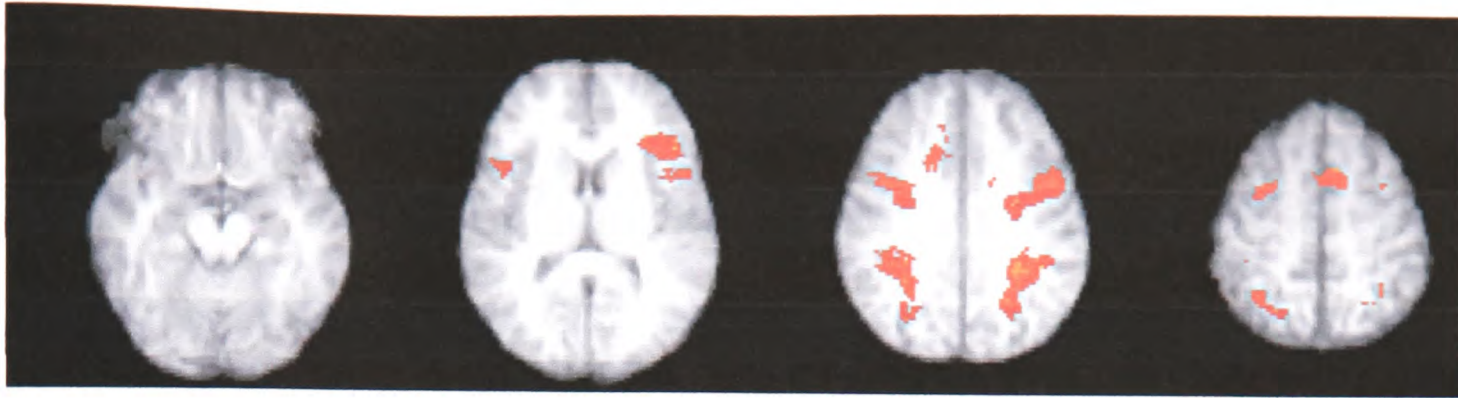


Figure 5.5 Areas of significant activation ($Z > 3.5$, $p < 0.01$) during the 1-back task superimposed on the mean structural image. Similar regions are found in the 2- and 3-back levels of difficulty, but with increased cluster sizes and Z-values. MNI co-ordinates for the maximum Z score at each discrete cluster is shown in table 5.3.

Anatomical region	Co-ordinates of max Z scores			Maximum Z score
	in MNI space (mm)			
	X	y	z	
Simple Contrast (1-, 2-, 3-back)				on 1-back contrast
Right Lateral Prefrontal	52	24	12	4.59
Left Lateral Prefrontal	52	14	24	4.15
Right Posterior Parietal	36	-44	42	4.75
Left Posterior Parietal	-48	-36	46	5.31
Medial Frontal	-2	8	48	3.83
Linear Contrast of linear activation (1- to 3-back)				
Right Lateral Prefrontal	24	16	54	6.09
Left Lateral Prefrontal	-50	4	36	7.79
Right Posterior Parietal	46	-36	56	6.49
Left Posterior Parietal	-36	-44	46	6.86
Medial Frontal	-8	6	58	6.18
Cerebellum	30	-58	-58	6.42

Table 5.3 MNI co-ordinates of significant ($Z > 3.5$, $p < 0.01$) discrete clusters of activation during the N-back task for the simple contrast (1-, 2-, and 3-back) and peaks of activation within a large merged cluster linear contrast (1- to 3-back).

The effect of hyoscine on activation during the 1-, 2- and 3- back tasks At the 1- and 2-back levels of difficulty, there was not significant difference between subjects on hyoscine or on placebo in a mixed effects group analysis. There were significant areas of activation for the 3-back task in subjects on placebo minus subjects on hyoscine (Table 5.4). Two discrete clusters were identified, one encompassing the right prefrontal region and extending medially and the other in the left prefrontal region extending medially (Figure 5.6). No areas of significance were found for the contrast of subjects on hyoscine minus subject on placebo.

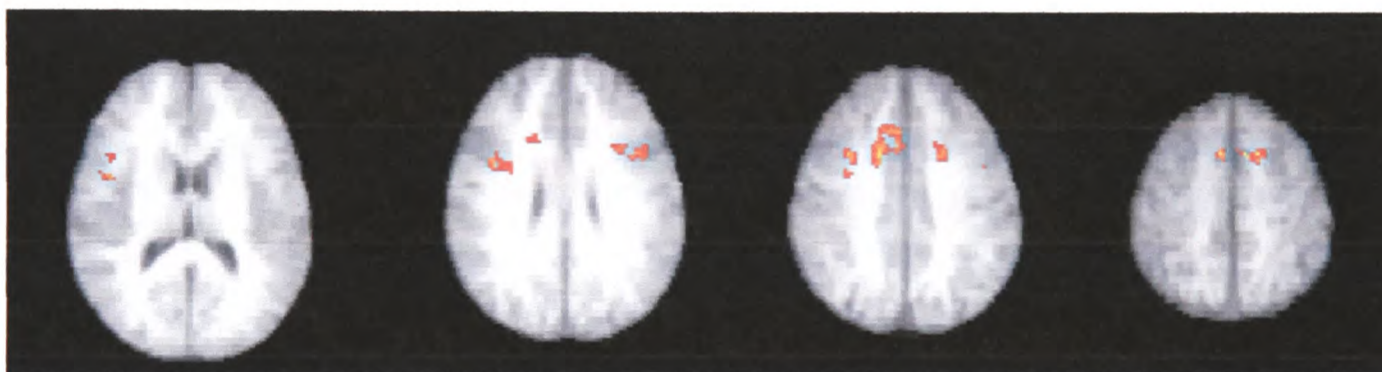


Figure 5.6 Regions of significantly greater activation in subjects on placebo compared with hyoscine ($Z > 3.5$, $p < 0.01$) during the 3-back task. The MNI coordinates of the maximum Z values are shown in table 5.4.

The effect of increasing task difficulty on the functional activation during the N-back task in healthy subjects With increasing task difficulty, incremental activation was found in regions across the brain ($Z < 3.5$, $p < 0.01$) and peaks of activation corresponded to the right posterior parietal, left posterior parietal, right lateral frontal, left lateral frontal, cerebellum and medial frontal regions (Figure 5.7, Table 5.3).

The effect of hyoscine on the incremental activation of increasing task difficulty On a mixed effects group contrast of subjects on placebo minus subjects on hyoscine, there was a significant ($Z > 3.5$, $p < 0.01$) increase in activation in the right posterior parietal, left posterior parietal, left dorsolateral frontal, right dorsolateral frontal, right inferior frontal, bilateral basal ganglia, left occipital pole, and cerebellum (Figure 5.8, Table 5.4). In the contrast of subjects on hyoscine minus subjects on placebo significant areas of increased activation were found in the posterior cingulate, orbitofrontal gyrus and the parieto-occipital sulcus (Figure 5.9, Table 5.4).

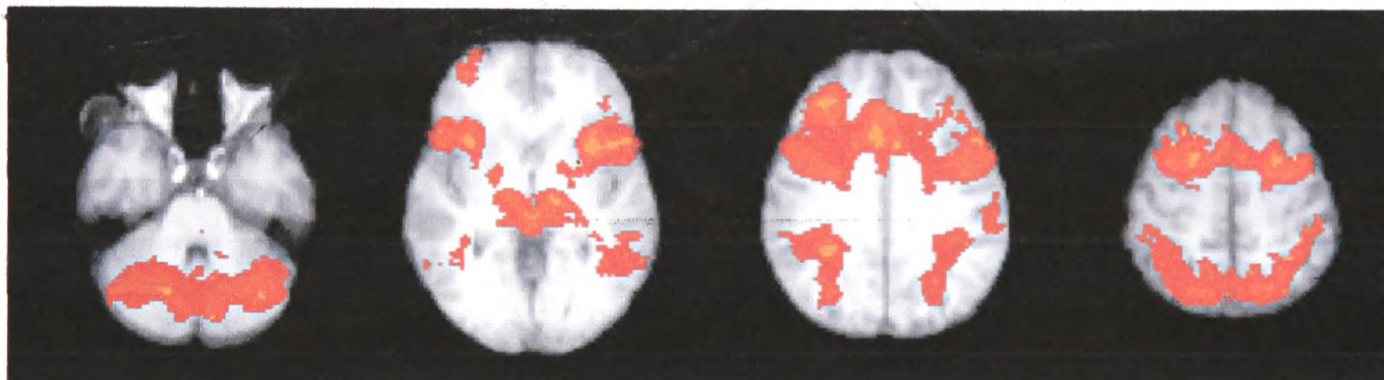


Figure 5.7 Regions of significantly increasing activation with increasing task difficulty ($Z > 3.5$, $p < 0.01$) superimposed on the mean structural image. The MNI co-ordinates of the maximum Z score for each of the peaks of activation are shown in table 5.3.

Region-of-interest analysis on the effect of hyoscine in healthy controls The mean signal was measured in the same regions-of-interest as explored previously in the previous chapters for each level of difficulty. There was a significant increase in the mean signal at the 2-back ($p < 0.01$) and decrease at the 3-back ($p < 0.05$) level and a significant decrease in the incremental activation ($p < 0.001$) in subjects on hyoscine compared with on placebo (Figure 5.10). No correlation was found between behavioural measures and the functional activation.

Anatomical region	Co-ordinates of max Z scores in MNI space (mm)			Maximum Z score
	x	y	z	
	Simple Contrast (3-back task) <i>Placebo minus Hyoscine</i>			
Right prefrontal	40	2	36	3.8
Left prefrontal	-16	10	54	3.76
Linear contrast (1- to 3-back) <i>Placebo minus Hyoscine</i>				
Right dorsolateral prefrontal	38	2	58	3.61
Left dorsolateral prefrontal	-56	2	44	4.08
Right posterior parietal	38	16	0	4.49
Left posterior parietal	-48	-26	60	3.72
Right inferior frontal	34	16	0	4.49
Medial frontal	2	8	40	4.08
Left cerebellum	-38	-56	-54	4.81
Right cerebellum	32	-44	-52	4
Left occipital pole	-24	-84	8	4.52
Basal ganglia	-17	-16	4	4.6
<i>Hyoscine minus placebo</i>				
Orbito-frontal gyrus	-14	42	-6	5.15
Posterior cingulate	0	-60	28	4.33
Left parieto-occital sulcus	-44	-66	32	4.14

Table 5.4 MNI co-ordinates of significant ($Z > 3.5$, $p < 0.01$) clusters of activation changes in patients taking hyoscine compared with placebo.

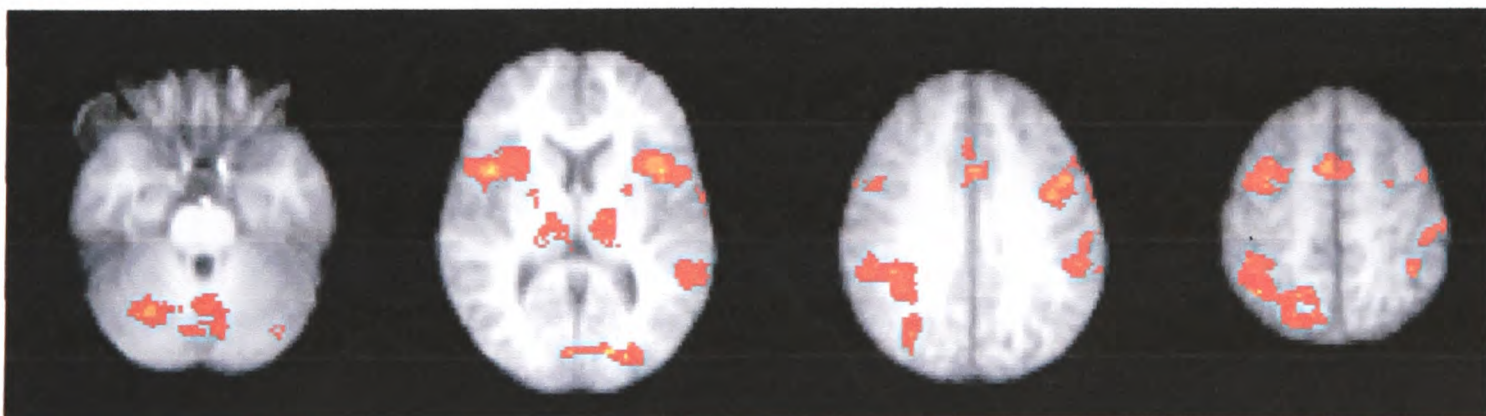


Figure 5.8 Regions of significantly greater incremental activation in subjects taking placebo minus hyoscine ($Z > 3.5$, $p < 0.01$) superimposed on the mean structural image. The MNI co-ordinates of maximum Z scores for each cluster are shown in table 5.4.

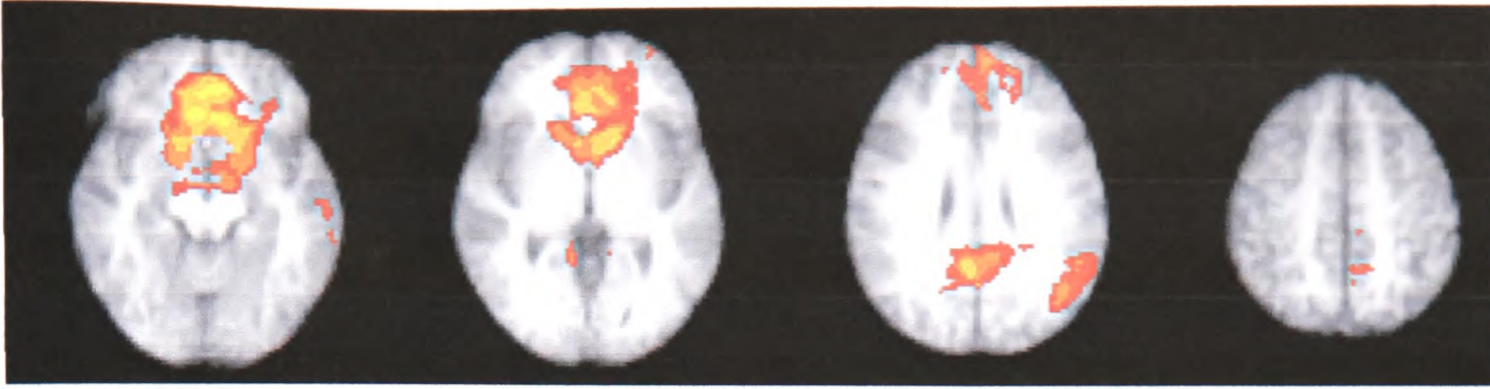


Figure 5.9 Regions of significantly greater incremental activation in subjects taking hyoscine minus placebo ($Z > 3.5$, $p < 0.01$) superimposed on the mean structural image. The MNI co-ordinates of maximum Z scores for each cluster are shown in table 5.4.

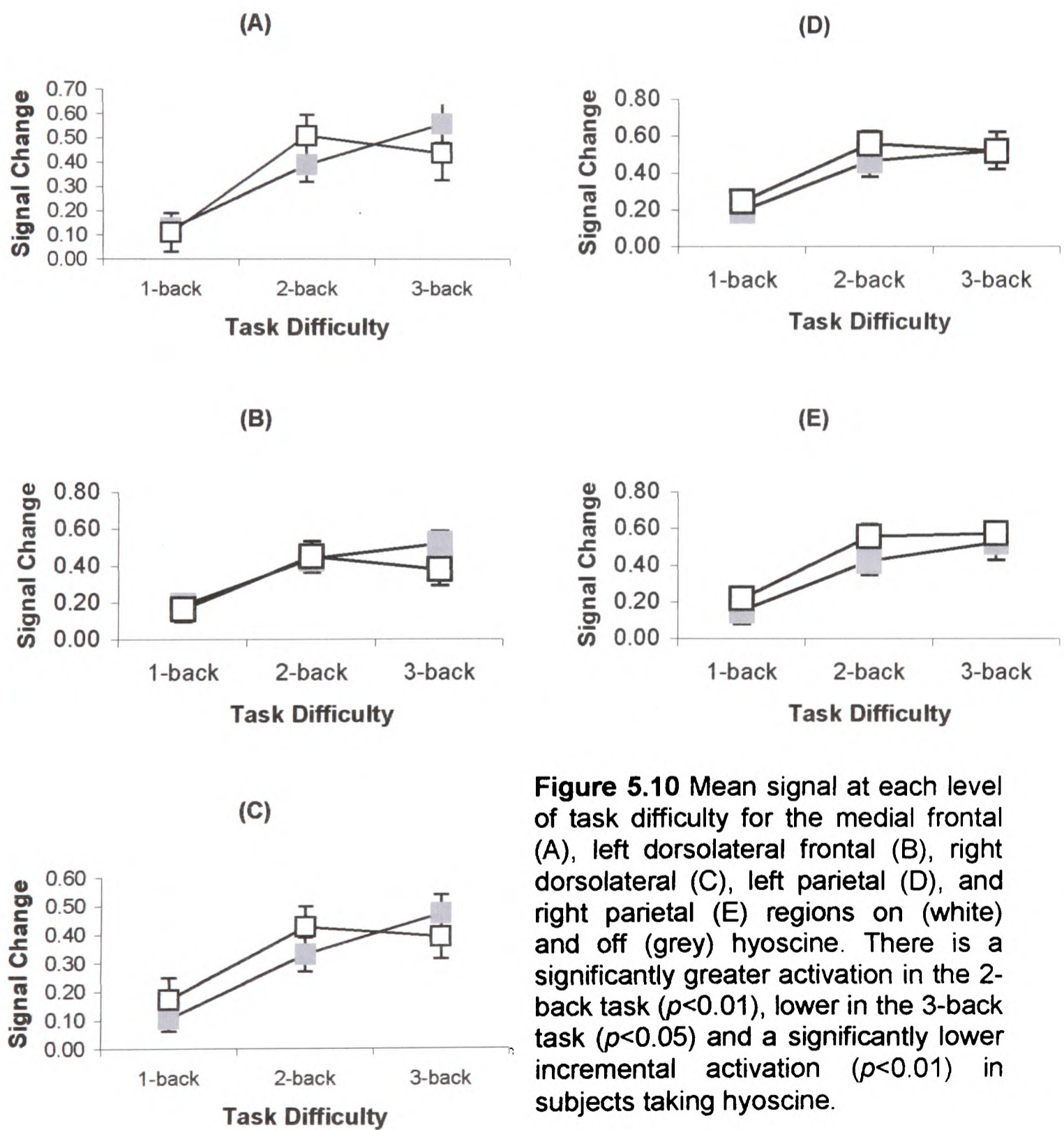


Figure 5.10 Mean signal at each level of task difficulty for the medial frontal (A), left dorsolateral frontal (B), right dorsolateral (C), left parietal (D), and right parietal (E) regions on (white) and off (grey) hyoscine. There is a significantly greater activation in the 2-back task ($p < 0.01$), lower in the 3-back task ($p < 0.05$) and a significantly lower incremental activation ($p < 0.01$) in subjects taking hyoscine.

Effect of hyoscine on the functional connectivity in healthy subjects for the N-back task Using the same regions of interest as previously used, the functional connectivity was assessed in subjects on hyoscine and placebo. There were significant differences in functional connectivity between subjects on hyoscine and placebo (Table 5.5, Figure 5.11a-b). On Fisher's Z-r transformation, there was a significantly greater connectivity ($p<0.01$) in subjects taking hyoscine than placebo between left and right frontal regions (Figure 5.11a). The connectivity between the right prefrontal and superior medial frontal regions was significantly greater ($p<0.05$) in subjects taking placebo than hyoscine (Figure 5.11b).

Dependant Region	Tested Region	Hyoscine	
		On	Off
Right Prefrontal	Left Prefrontal	0.588	0.097
	Right Parietal	-0.284	0.502
	Superior Medial Frontal	0.241	0.600
	Anterior cingulate	0.312	0.508
Left Prefrontal	Right Prefrontal	0.803*	-0.218
	Left Parietal	0.830*	0.567
	Superior Medial Frontal	0.088	0.482
	Anterior cingulate	-0.256	0.145
Anterior Cingulate	Right Prefrontal	0.232	0.325
	Left Prefrontal	0.118	-0.175
	Superior Medial Frontal	0.320	0.161
Superior Medial Frontal	Right Prefrontal	0.232	0.755*
	Left Prefrontal	0.424	0.530
	Anterior Cingulate	0.320	0.161
Right Parietal	Right Frontal	0.344	0.498
	Left Parietal	0.665	0.106
Left Parietal	Left Frontal	0.652	0.685*
	Right Parietal	0.394	0.224

Table 5.5 The partial correlation coefficients between regions tested. The dependant regions are shown in the first column, and independent regions that were regressed against this are shown in the second column. Only regions that were potentially anatomically connected directly were considered. Significant ($p<0.05$ after correction for multiple comparisons) correlations are indicated*.

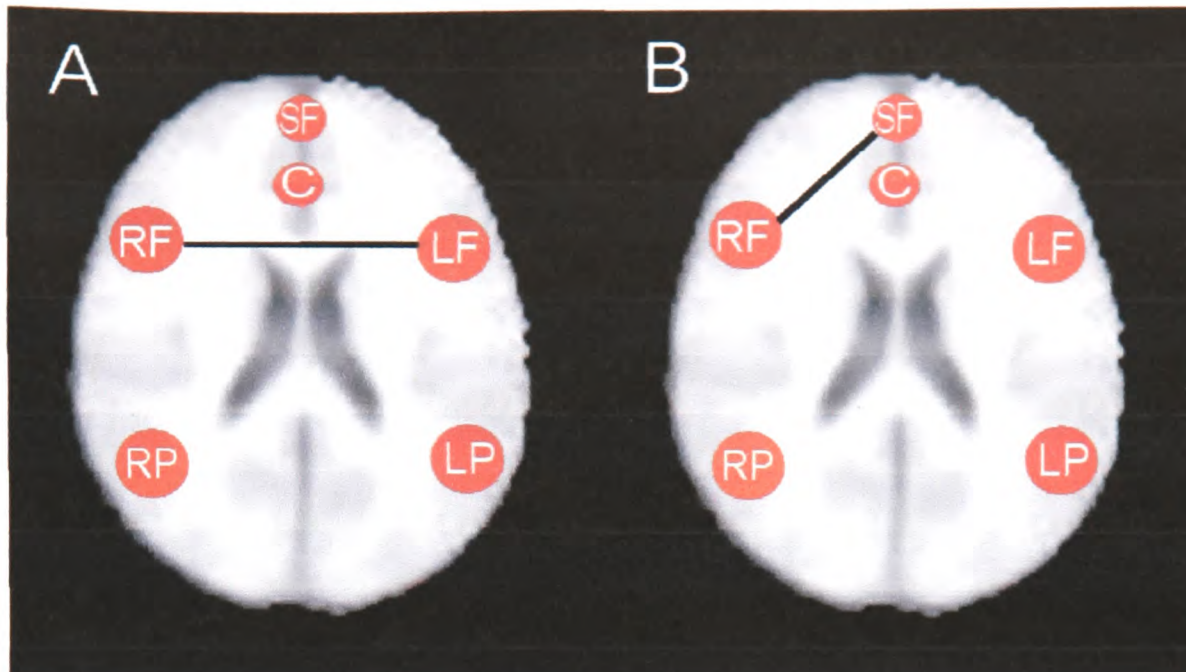


Figure 5.11 The functional connectivity in healthy subjects during the N-back task showing (A) greater connectivity in subjects on hyoscine ($p < 0.01$) and the (B) greater connectivity found in subjects taking placebo ($p < 0.05$). C=cingulate, SF=superior medial frontal, RF=right dorsolateral frontal, LF=left dorsolateral frontal, RP=right parietal, LP=left parietal

5.4 Discussion

The effects of hyoscine on the functional activation during the Stroop and N-back task were investigated in this study. There was no significant effect of hyoscine on behavioural performance in either task or in the functional activation during the Stroop task. An effect was seen on the functional activation of the N-back task. This is discussed in more detail below.

5.4.1 Hyoscine and the N-back task

The main finding of this experiment was the altered functional activation found during the N-back task in subjects taking hyoscine compared with subjects taking placebo. The whole brain analysis revealed a significantly greater activation within prefrontal areas in subjects on placebo at the 3-back level, but not at lower levels of difficulty. The absence of any significant change in behavioural performance at the 3-back or other levels of difficulty suggests that the observed reduction in the activation

within the prefrontal regions is a result of treatment with hyoscine, rather than a change in the level of performance. A caveat to the lack of any observable behavioural change is that only the error rate of the task is measured. Time urgent responses are not a feature of the task setup, but a study of nicotine on the N-back task has shown an increase in reaction times at the 3-back level (Kumari et al., 2003).

The effects of hyoscine on the n-back task are further apparent in the whole brain contrast of incremental activation. Increased activation in the cerebellum has been identified in this and previous studies (Smith and Jonides, 1997) to be associated with increasing memory load. The reduced incremental activation in the cerebellum with the administration of hyoscine might be detrimental to performance that may be apparent if memory load were to increase further. Reduced incremental activation was not confined to the cerebellum. Greater incremental activation with placebo over hyoscine was found across many regions usually involved in the processing of verbal working memory. This was found on both the whole brain group analysis and on the region-of-interest analysis. Analogous to the reduction in functional reserve in MS patients demonstrated in section 3, hyoscine may reduce the capacity of normal neural networks recruited during the task. In contrast, the administration of hyoscine increased the incremental activation in regions not typically involved. This increased incremental activation may be due to additional areas being recruited to compensate for reductions elsewhere. This would explain the similar performance in subjects taking placebo or hyoscine, despite the expected detrimental effect of hyoscine. Alternatively hyoscine may be suppressing the normal shift in functional processing that occurs when attending to tasks of increasing cognitive demand. In this case, the maintained performance would have to be explained by the residual functional reserve

in the task-specific areas that would only reach their limit with even higher task demand.

In section 3, differences in functional connectivity were found between MS patients and matched healthy controls. The key findings were greater connectivity between right prefrontal and superior medial frontal regions in the control subjects, whilst patients demonstrated increased connectivity between right and left prefrontal regions. In this study, healthy subjects showed a greater connectivity between right prefrontal and superior medial frontal when on placebo compared with on hyoscine. When subjects were taking hyoscine, an increased connectivity between right and left prefrontal regions was found. Functional adaptive mechanisms may follow similar patterns regardless of the cause of compromise to the existing pathways for the cognitive task in question. This is supported by functional studies showing similar mechanisms of functional reorganisation in different diseases (Lee et al., 2000; Pineiro et al., 2001).

5.4.2 Hyoscine and the Stroop task

In contrast to the N-back task, hyoscine appeared to have little effect on the processing of the Stroop task. A study examining the effects of scopolamine showed a reduction in performance during the Stroop task which was reversed by nicotine (Wesnes and Revell, 1984). Numerous studies have suggested the importance of acetylcholine in attentional function (Blokland, 1995; Sarter et al., 2003). Inhibiting cholinergic function would be expected to impair the cortical processing of the Stroop task, a paradigm testing this function. It is surprising then that this study was unable to find an effect of hyoscine in either the task performance or the functional activation.

This might be explained by insufficient dosage in the experiment as the dose (0.3mg) was lower than that used by Wesnes and Revell (1.2mg) (Wesnes and Revell, 1984). Indeed one study demonstrated that the 0.3mg dose had minimal behavioural effects on cognitive performance (Nuotto, 1983). Other studies, however, found a dose-related effect of hyoscine on cognition (0.15 – 1.2mg), but still effective at the 0.3mg dose (Parrott, 1986). The literature on the effects of hyoscine on functional activation of healthy human subjects during the Stroop task is sparse. However, Parry and colleagues found no effect of increasing the acetylcholine by the administration of rivastigmine in healthy control subjects on the functional activation during the Stroop task despite seeing an effect in MS patients (Parry et al., 2003). The cholinergic modulation of Stroop cognitive processing, and particularly the patterns of brain activity, appears to be complex and further study with higher doses of hyoscine may be useful.

5.4.3 Modulation of cognitive processing with hyoscine

The changes in activity of brain regions involved in the N-back task are likely due to the blockade of acetylcholine mediated by hyoscine. One explanation of this is that modulation of acetylcholine leads to changes in the cortical processing. The putative role of acetylcholine in switching cortical dynamics to facilitate afferent inputs would support this (Kimura, 2000). The widespread projections of cholinergic neurones include distribution to areas involved in working memory and attention (Everitt and Robbins, 1997; Thiel, 2003). Correspondingly, the cortical processing of attention and memory function may be enhanced under the influence of acetylcholine and impaired during acetylcholine antagonism. Thus the alteration in cortical brain activity may represent real changes in the task-specific processing of the N-back task.

Although acetylcholine may mediate its effect by altering the pattern of neuronal firing, alternatively the fMRI effects could occur as a consequence of changes in blood flow caused by a direct drug effect, or by alterations in the nature of the neurovascular coupling mechanism. In this and the previous study, the pattern of fMRI activation during the tasks was contrasted against a control task rather than a rest task. Global increases in blood flow or constant changes in the neurovascular coupling by the drug would be expected to occur across task conditions equally.

Interpretation becomes difficult when the BOLD response no longer becomes a reliable indicator of the underlying neural activity due to a variable effect of drug on the neurovascular coupling across task conditions (Bruhn et al., 2001). There is no evidence to suggest that this is the case for cholinergic agents and not all drugs cause dynamic changes in neurovascular coupling (Meno et al., 2005; Rosengarten et al., 2002). At present, it is reasonable to propose that the alterations in functional activation during the N-back task represent real changes in the cortical processing of verbal working memory due to hyoscine.

Section 6: The effect of rivastigmine on the BOLD fMRI signal

The BOLD fMRI signal is an indirect measure of the underlying neural activity that depends on the consistency of neurovascular coupling. The previous sections found an alteration of functional activation during cognitive tasks with cholinergic modulation. The interpretation of these findings depends, in part, on how the BOLD signal relates to underlying neural activity under drug conditions. In this section, the effect of rivastigmine on the BOLD signal during direct cortical stimulation in a rat model and also during visual stimulation in MS patients is considered.

6.1 Introduction and rationale

Most fMRI studies, including those in this thesis, are based on blood oxygenation level dependant (BOLD) contrast, in which the paramagnetic deoxyhaemoglobin acts as an endogenous contrast agent (Ogawa et al., 1990). Cortical and subcortical neural activity leads to changes in local cerebral blood flow, which causes changes in the blood oxygenation (Logothetis, 2003).

The administration of acetazolamide, which increases cerebral blood flow without changing neuronal metabolic activity, reduces activity-related MRI signal changes in the visual cortex in response to photic stimulation (Bruhn et al., 1994). The same group demonstrated a similar effect with indomethacin but not with acetylsalicylate (Bruhn et al., 2001). In contrast, the dynamic neurovascular coupling - the relationship between

local cerebral blood flow and neuronal activity during altered experimental conditions, is unaffected by Glyceryl Trinitrate (Rosengarten et al., 2002).

Animal and human studies have shown that administration of nicotine increases cerebral blood flow (Hall, 1972; Skinhoj et al., 1973; Uchida et al., 1997; Zubieta et al., 2005), possibly mediated by the stimulation of nicotinic receptors in the basal forebrain (Linville et al., 1993; Sato et al., 2004). However, Nakao and colleagues found that scopolamine did not influence the change in blood flow with somatosensory stimulation despite changes in baseline cerebral blood flow (Nakao et al., 1999). It is not clear the degree to which increases in cerebral blood flow occur independently of changes in neuronal metabolic activity with cholinergic modulation. Any alteration of coupling introduces a potential confound for the interpretation of BOLD signal change.

Here data is presented from an experiment examining the effect of rivastigmine on the BOLD signal during direct cortical stimulation in a rat model. If rivastigmine alters the haemodynamic response to underlying neural activity, then differences should be seen in the BOLD signal response to the direct cortical stimulation. The neural activity in directly stimulated cortex is potentially subject to modulation by acetylcholine causing an increase in cortical excitability (Gu, 2002). This may be more pronounced in the prolonged BOLD response from direct stimulation, but may not exert a significant effect in the contralateral cortex, which reflects more physiological activity (Austin et al., 2003). Thus, the effect of rivastigmine on the BOLD signal is examined in both cortices.

Since the effect of rivastigmine in MS patients is of particular interest, the influence of rivastigmine during photic stimulation in a group of MS patients is also studied. One previous fMRI study found no effect of nicotine on the BOLD signal response to photic stimulation (Jacobsen et al., 2002). However, there was no measurement of the underlying neural activity and instead it was assumed that this would stay constant despite the administration of nicotine. Cholinergic receptors are present in the visual cortex, and thus acetylcholine is likely to influence neural activity in this region (Bentley et al., 2004; Laplante et al., 2005). To address this, steady state visual evoked potential (SSVEP) in response to visual stimulation is measured in addition to the BOLD fMRI signal. If rivastigmine does not alter the dynamic neurovascular coupling, then similar effects should be seen in SSVEPs and BOLD signal. Establishing the degree to which changes in BOLD signal change independently from any neural activity during rivastigmine administration will help in the interpretation of relevant fMRI studies.

6.2 Methods

6.2.1 Direct cortical stimulation in a rat model

This study was performed in collaboration with Drs N Sibson and V Austin (Department of Biochemistry, Oxford University), who held home office approval for the work carried out on the animal subjects. The methodology employed followed a reproducible protocol already established by Austin and Colleagues (Austin et al., 2003).

Animal preparation

Sprague Dawley rats (n=7), weighing 253 ± 25 g, were anaesthetised with 1.5% halothane in 40% O₂ : 60% N₂O mixture, tracheotomized and artificially ventilated. One femoral artery was cannulated for monitoring of mean arterial blood pressure (MABP). An intraperitoneal line was inserted for the administration of rivastigmine. Temperature was maintained at 37°C using a rectal thermostat probe and a heated water blanket.

Electrodes were prepared and placed in a manner previously described (Austin et al., 2003). During electrode placement, halothane was maintained at 2.0 - 2.5% halothane. Using a stereotaxic frame, holes were placed in the skull above the region corresponding to the left hindpaw motor cortex, 2.5 mm posterior and 1.5 mm and 3.5 mm lateral to the bregma. The electrodes were inserted anterior along the surface of the brain and fixed in place with cyanoacrylate. Extra insulation with a layer of cyanoacrylate and gauze was used to prevent electrical conduction to local musculature and potential motion artefacts.

Cortical Stimulation

Rectangular pulses of 0.3 ms duration were applied at 300 Hz in 50 ms trains repeated five times per second. The amplitude during functional imaging was at 120% of the threshold required for visible hindpaw movement (approximately 2 mA). The paradigm involved a 2.5 second period of stimulation and 102.5 seconds of rest, repeated for 10 blocks. 10 minutes prior to stimulation, anaesthesia was reduced to 0.8% halothane. One of the rats was studied on the bench in a mock fMRI experiment (including the

rivastigmine bolus). On completion of each experiment, the animal was euthanized by anaesthetic overdose followed by cervical dislocation.

Drug administration

Rivastigmine was administered via an intraperitoneal line. No intravenous formulation was currently available, but the oral solution (2mg/ml) was developed from the original intravenous solution (Novartis Pharma) with the addition of sodium benzoate (preservative) and quinoline yellow (dye). The dosage for each rat was based on weight at 3mg per kg and was given as a bolus following the first control fMRI experiment with an equivalent volume of saline for infusion. The fMRI protocol was repeated at 40 minutes and 1 hour 40 minutes following the bolus.

Imaging

MRI measurements were performed on a 7.0 T horizontal bore magnet, with actively shielded gradients and a Varian Inova spectrometer. The RF pulses were applied using a Aldermann-Grant resonator covering only the head of the animal. For functional imaging, single slice T_2^* -weighted images were acquired 7 mm posterior to the rhinal fissure (15° flip angle, TE = 12 ms, TR = 20.5 ms, FOV 60 x 60 mm. Matrix 128 x 84, in-plane voxel 0.47 x 0.71 mm, 1.5 mm slice thickness) in an image time of 1.75 seconds.

Data analysis

Analysis was carried out using FEAT. The following pre-processing steps were applied to the data: Spatial smoothing with a gaussian kernel of 1 mm FWHM and a non-linear

high-pass temporal filter to remove low frequency artefacts. Statistical analysis was carried out using a semi-model free analysis (Clare et al., 1999). Z statistic images were thresholded at p value = 2.5×10^{-5} , equivalent to a corrected statistical significance of $p=0.01$, to identify regions for time course analysis. The time course of the BOLD response for the directly stimulated and contralateral cortex (mean of all voxels in the activated regions) was determined for all animals. Signal intensities were converted to percentage change from baseline values. An average BOLD response time course for the entire period of the run was determined for left and right motor cortices over all runs. The mean time course for a single stimulation period (block) was calculated by averaging the blocks at each time point (pre-rivastigmine, 40 minutes, and 1hr 40 minutes). Paired T-tests were used to test for significance between these time points, in each hemisphere. Before applying any analysis that assumes normal distribution, checks were made that the data approximated normality. All probability values were corrected for multiple comparisons.

6.2.1 Photic stimulation in MS patients

Subjects

7 patients (4 men, 3 women; mean age 43 years, range 32-53 years) with clinically definite multiple sclerosis (1 secondary progressive, 6 relapsing remitting; mean duration 12 years, range 4 –21 years) according to the Poser criteria (Poser et al., 1983) completed a randomised crossover single blind study of rivastigmine (Table 6.1). Disability was assessed with the EDSS (Kurtzke, 1983) at the time of initial screening (median EDSS 3.0, range 2-6). None of the patients had suffered from a relapse within 3 months of starting the study. No patients had clinically evident visual deficits on the basis of clinical

histories obtained during the initial interview for this study. Subjects with a history of psychological and psychiatric problems, epilepsy, learning disability, alcoholism, head injury, episodes of loss of consciousness or severe ongoing stressors (such as bereavement, divorce, financial problems) were excluded. All subjects were advised to abstain from drinking caffeine-containing beverages for 4-6 hours prior to scan. No subjects smoked. Written consent was obtained from all subjects and Oxford Regional Ethics Committee gave ethical approval for the study.

Patient	Disease Type	EDSS	Disease duration <i>yrs</i>	Age <i>yrs</i>
1	RR	3	13	49
2	SP	6	12	53
3	RR	2	9	48
4	RR	2	15	43
5	RR	3	21	47
6	RR	3	8	39
7	RR	2	4	32

Table 6.1 Disease characteristics in each patient.

Sessions

Subjects were asked to attend on three separate occasions, four to six weeks apart. During the first session, subjects were screened for exclusion criteria. Suitable subjects were then randomised into two groups (Group 1: Phase 1 - Rivastigmine and Domperidone, Phase 2 - Domperidone alone; Group 2: Phase 1 – Domperidone alone, Phase 2 – Rivastigmine and Domperidone). The protocol for drug administration was identical to that followed in section 4.2.

During the second session, 4-6 weeks after the initial visit, subject underwent functional imaging whilst viewing a flashing checkerboard visual paradigm. At the same visit, subjects also underwent visual evoked potential recording during the same visual stimulation.

The third session, another 4-6 weeks later, followed the same pattern as the second session.

Paradigm

This experiment used a flashing checkerboard paradigm, that provides robust visual activation (Jacobsen et al., 2002). The stimulus consisted of a computer generated black and white checkerboard pattern (checker size was 2 cm x 2 cm) flashing at 8 Hz for 30 seconds alternating with 30 seconds of a central cross hair set against a black background (rest). The total length of the paradigm was 10 minutes (10 blocks of flashing checkerboard and 10 blocks of rest).

Imaging

Scans were performed using a 3.0 Tesla whole body scanner with a Varian Inova console and a quadrature birdcage radiofrequency head coil. An echo-planar imaging (EPI) sequence was used to acquire the fMRI data [24 x 6 mm axial slices, TE = 30 ms, TR = 3000 ms, FOV = 192 x 256, matrix 64 x 64]. A T₁-weighted [64 x 3 mm axial slices, TR = 15 ms, TE = 6.9 ms] anatomical scan was also acquired for each subject.

Image analysis

Analysis of the fMRI data and the group analysis were carried out using the tools described in section 3.2. To identify brain activation during the visual stimulation, the flashing checkerboard condition was contrasted with the rest condition. This contrast was applied to analyse the group mean activation across the subjects. Two fMRI analyses were performed to examine the effect of rivastigmine: (i) group analysis (ii) region of interest analysis.

(i) Group analysis

To test whether there are differences in fMRI activation between patients taking rivastigmine and patients not taking rivastigmine, the following paired between subject group analyses was performed:

- (a) On minus off rivastigmine
- (b) Off minus on rivastigmine

(ii) Region of interest analysis

A mask of the occipital region was defined anatomically on the mean structural image. This was applied to each subject's functional image to obtain the maximum contrast of parameter estimate on and off rivastigmine.

Steady-state visual evoked potential (SSVEP)

Electroencephalography (EEG) was used to record activity during the same visual stimulation as presented during functional imaging. EEG recordings were taken from a

limited number of electrodes to cover the visual cortex (PO7, PO3, O1, Oz, O2, PO4, PO8) with reference and ground electrodes. This was performed in a dark room whilst subjects watched a monitor in the room. A trigger was sent from the computer generating the paradigm to the recording software (Neuroscan version 4.2) every 1 second during the flashing checkerboard. This allowed the analysis software (Neuroscan version 4.3) to epoch the data into 1-second windows. Epochs that contained deflections attributed to eye-blinks were rejected (if deflections were greater than twice the average maximum/minimum deflections in the entire stimulus “on” data). This accounted for approximately 15% of the data. These epochs were averaged into a single dataset over 1 second for each electrode measured which had 8 peaks corresponding to the frequency. The magnitude of signal was calculated by rectifying the data, and calculating the area under the curve (AUC) across this 1-second averaged epoch (Singh et al., 2003). This allowed a comparison between the magnitudes of signal in the two test conditions (on and off rivastigmine).

Statistics

Paired t-test was used to assess the effect of rivastigmine on the steady state visual evoked potential and on the region-of-interest analysis of functional activation. Any correlation between these measures was determined by Pearson correlation. A *p*-value <0.05 was considered statistically significant. Before applying any analysis that assumes normal distribution, checks were made that the data approximated normality. All probability values were corrected for multiple comparisons. All statistics were performed using SPSS for Windows (Version 12.0.1).

6.3 Results

The effect of rivastigmine on directly stimulated rat motor cortex A significant increase in BOLD signal (figure 6.1) was seen after the administration of the rivastigmine both at 40 minutes post-rivastigmine ($p=0.02$) and 1 hr 40 minutes post-rivastigmine ($p<0.001$). There is also a significant difference between 40 minutes and 1 hr 40 minutes post-rivastigmine ($p=0.04$).

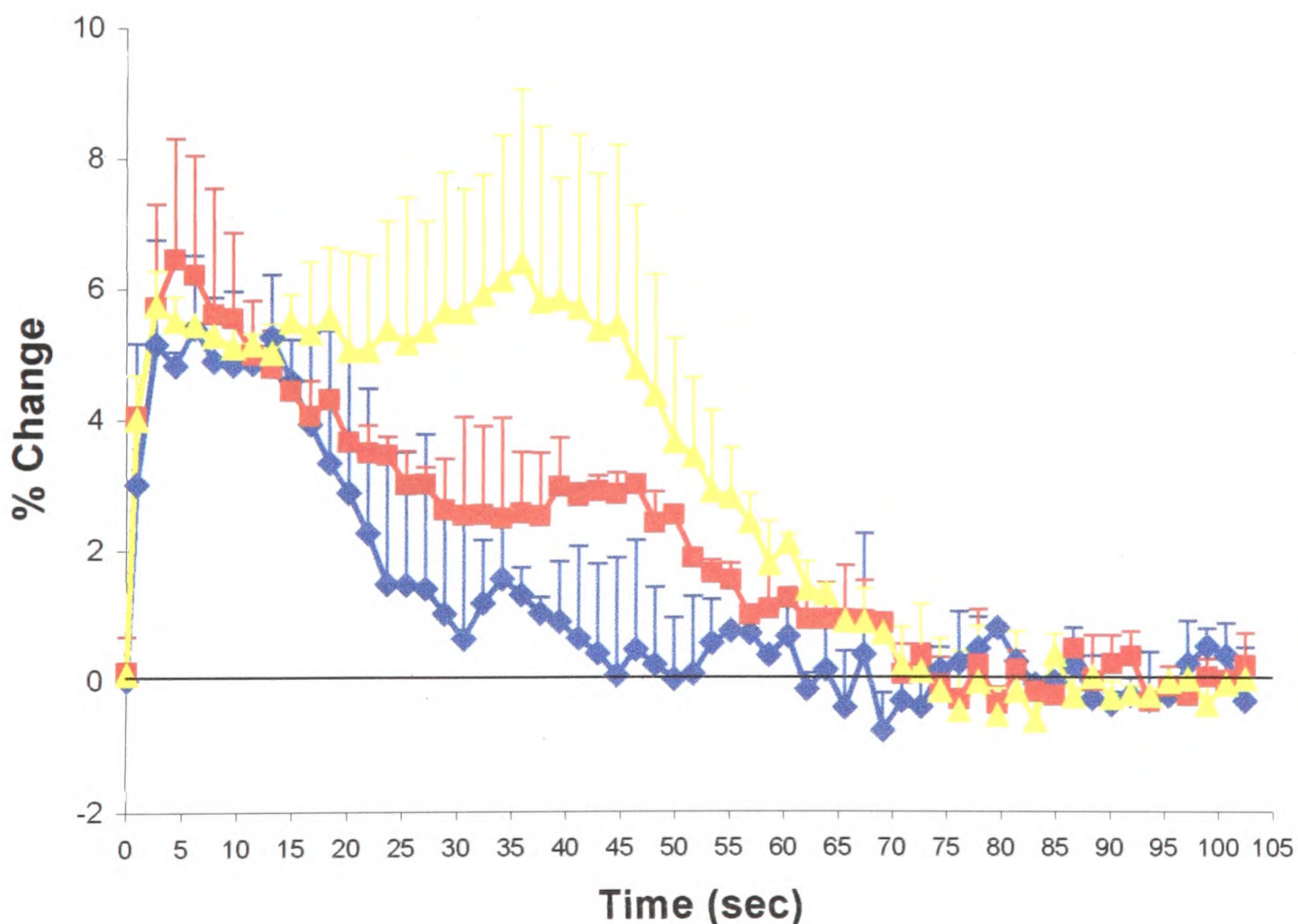


Figure 6.1 The averaged time course of the BOLD signal after direct stimulation of the motor cortex for all animals [pre-rivastigmine (blue), 40 min post-rivastigmine (red) and 1hr 40min post-rivastigmine (yellow)]. There is a significant prolongation of the BOLD signal especially after 1 hr 40 min. ($p<0.001$)

The effect of rivastigmine on the rat contralateral motor cortex A significant increase in the BOLD signal was seen at 40 minutes post-rivastigmine ($p < 0.01$) compared with pre-rivastigmine, in the latter half of the response. There was a trend to a similar change in BOLD signal at 1 hr 40 minutes ($p = 0.08$), but no significant difference between 40 minutes and 1 hr 40 minutes post-rivastigmine (Figure 6.2).

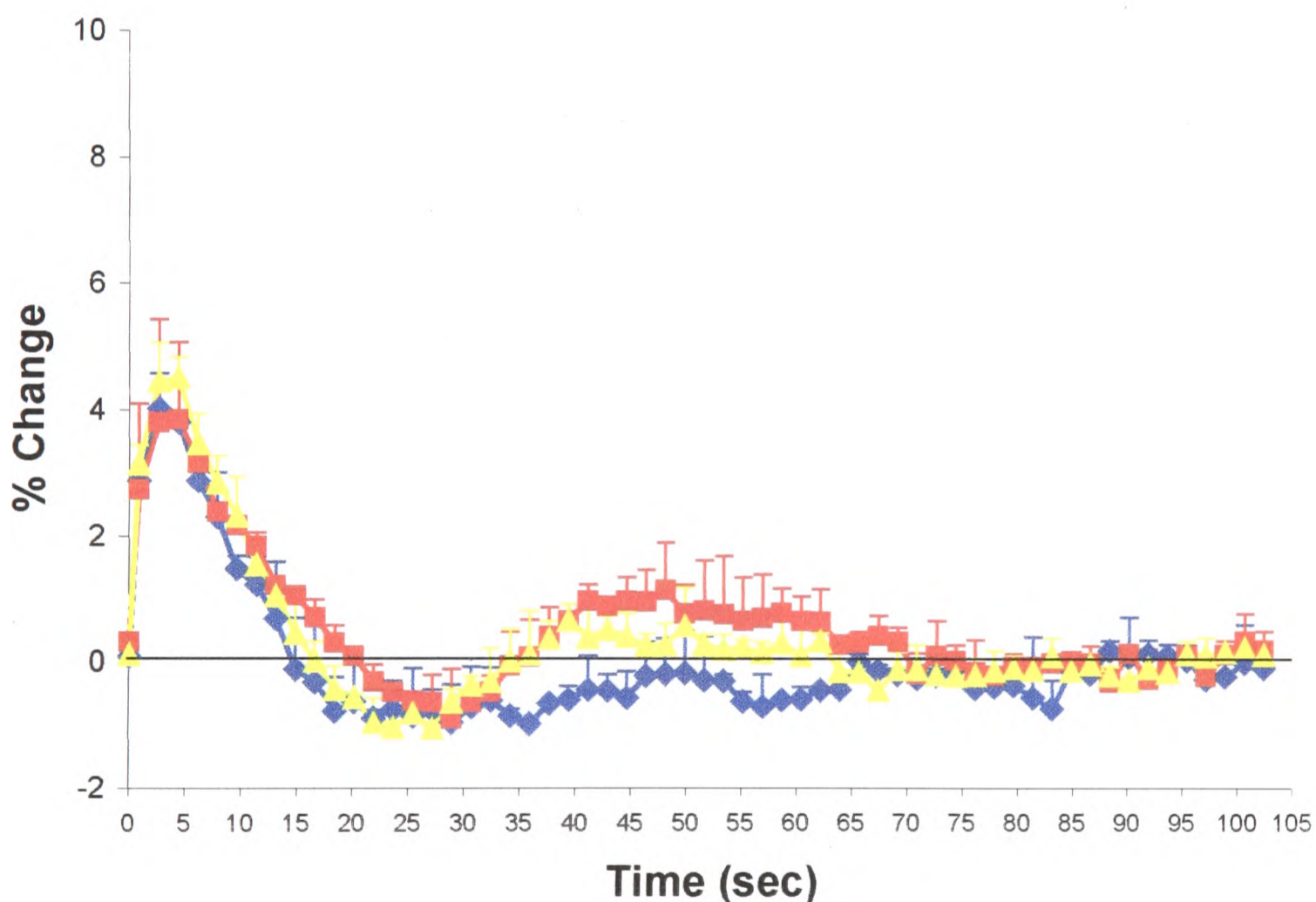


Figure 6.2 The averaged time course of the BOLD signal in the contralateral cortex after direct stimulation of the motor cortex for all animals [pre-rivastigmine (blue), 40 minutes post-rivastigmine (red) and 1hr 40 min post-rivastigmine (yellow)]. There was a significant change at 40 mins post-rivastigmine compared to pre-rivastigmine ($p < 0.01$).

FMRI activation during visual checkerboard presentation in MS patients Task specific activation was found in regions corresponding to bilateral occipital areas ($Z > 3.5$, $p < 0.01$).

There was also activation found in other regions to a lesser extent (Figure 6.3, Table 6.2). These included activation in the right and left lateral geniculate, right and left middle frontal, insular and precentral regions.

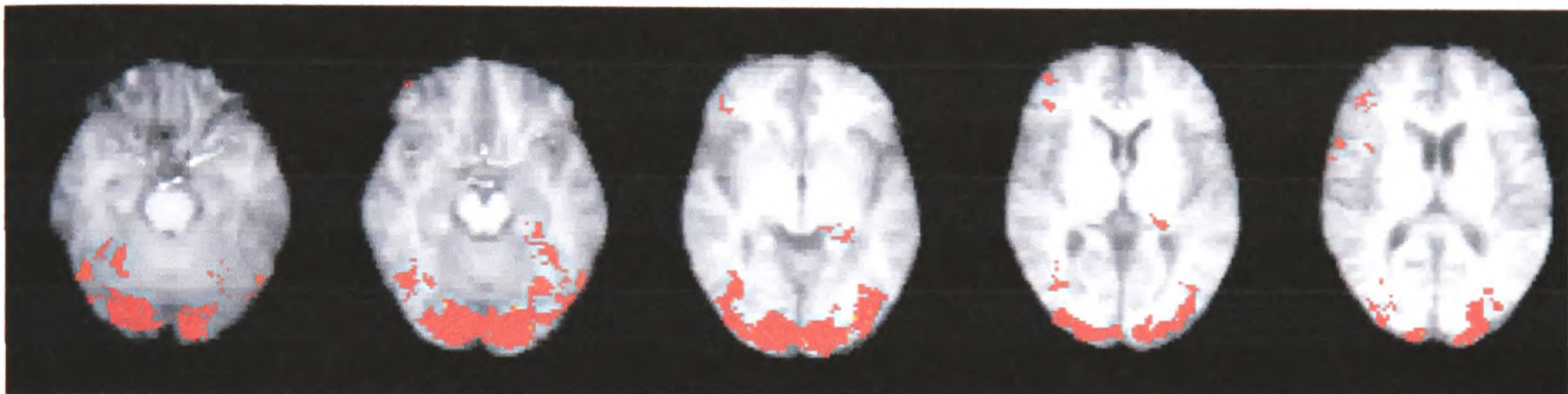


Figure 6.3 Activation during presentation of the visual checkerboard ($p < 0.01$, $Z > 3.5$). Functional activation is overlaid on the mean structural image. The co-ordinates of maximum Z scores are shown in table 6.2.

Region	MNI Co-ordinates			Maximum Z Value
	x	y	z	
Occipital (Bilateral)	6	-98	-16	19.6
Right lateral geniculate	24	-30	-10	6.08
Left lateral geniculate	-22	-30	-6	5.79
Right middle frontal	40	36	18	6.06
Left middle frontal	-52	24	16	6.03
Right Insular	44	-2	22	6.34
Right precentral sulcus	38	-6	34	5.77
Right precentral gyrus	54	-6	40	6.77

Table 6.2 Regions of significant activation during the visual checkerboard ($Z > 3.5$, $p < 0.01$). Co-ordinates are indicated in MNI standard space. Also see figure 6.3.

The effect of drug on SSVEPs during visual checkerboard presentation in MS patients

There was a significant increase in the mean area under the curve (AUC) across the seven electrodes recorded ($p < 0.01$) in the patients taking rivastigmine compared with the patients off rivastigmine (Figure 6.4)

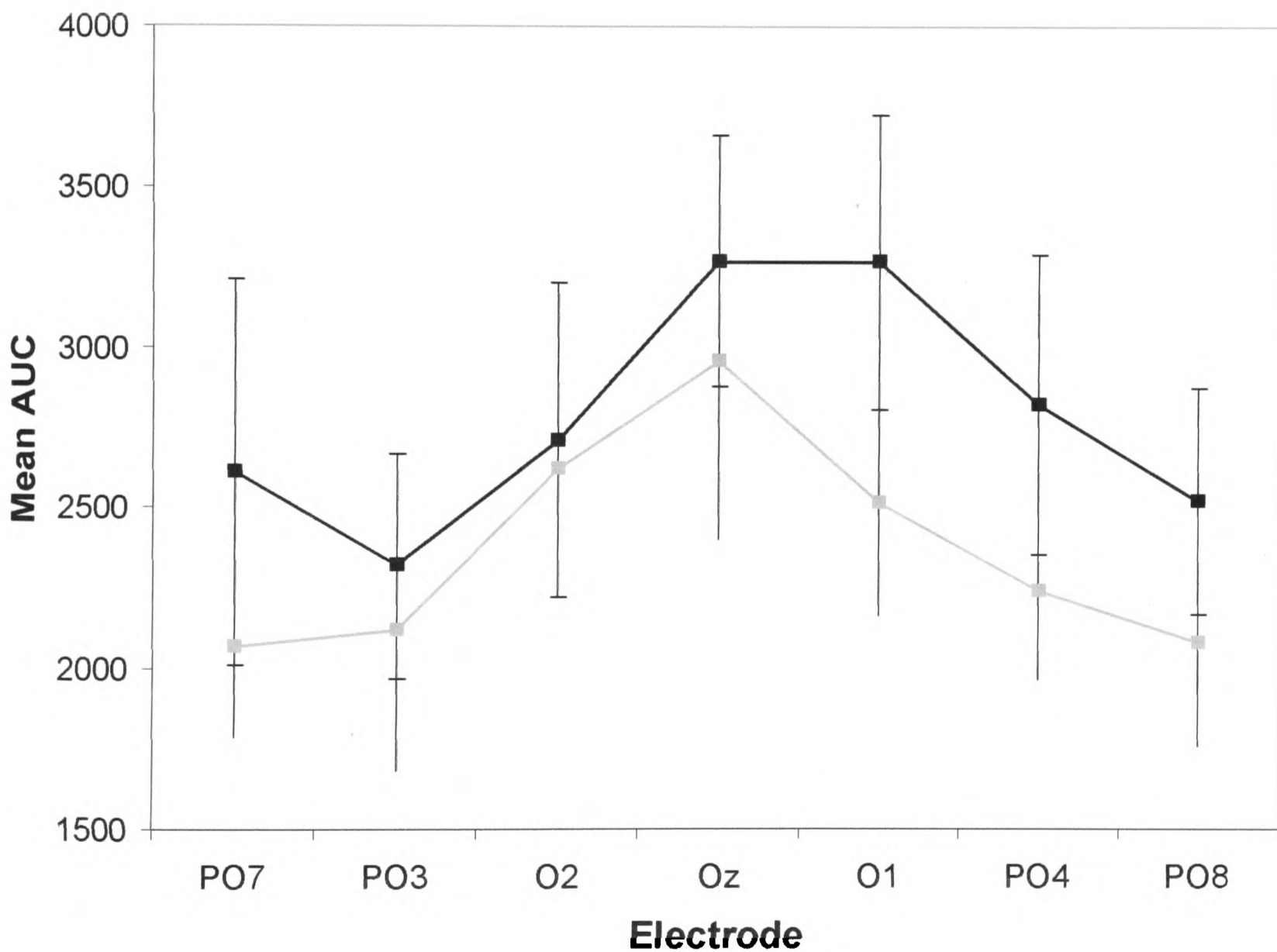


Figure 6.4 The mean activity during steady state visual evoked potential recording as quantified by the area under the curve (AUC) across the electrodes whilst on rivastigmine (black line) and off rivastigmine (grey line). A significant change ($p < 0.01$) was demonstrated on a paired t-test.

The effect of drug on fMRI activation A significantly greater area of activation was found in patients off rivastigmine minus patients on rivastigmine ($Z > 2.3$, $p < 0.01$) restricted to a small cluster in the inferior portion of the occipital pole extending into the cerebellum (maximum Z score = 7.75, at $x = -20$, $y = -86$, $z = -30$) (Figure 6.5). There were no significant areas of activation in patients taking rivastigmine minus patients off rivastigmine.

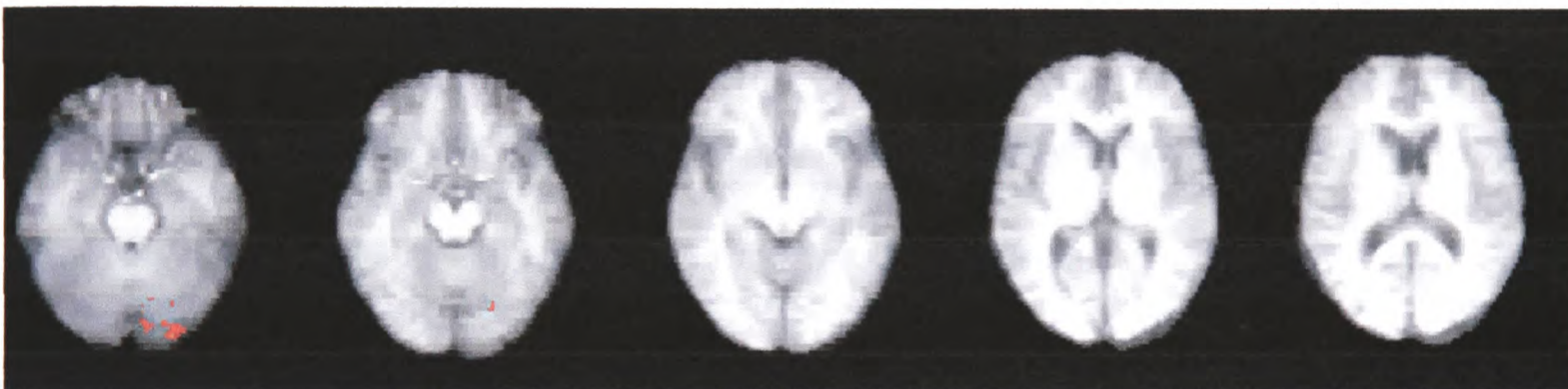


Figure 6.5 Significant areas of activation on a mixed effects contrast of patients off rivastigmine minus patients on rivastigmine ($p < 0.01$, $Z > 2.3$). The maximum Z score of 7.75 at $x = -20$, $y = -86$, $z = -30$. There were no significant areas of activation for on minus off rivastigmine. Functional activation is overlaid on the mean structural image.

The effect of drug on the functional activation in a region-of-interest analysis To clarify the signal changes found in the group fMRI analysis, the maximum contrast of parameter estimate within the occipital region was measured for each subject on and off rivastigmine. The region of interest was defined on the mean structural image of all the subjects. There was no significant change in the maximum COPE value between the patients taking rivastigmine and patients off rivastigmine in a paired t-test (Figure 6.6). A negative correlation between the changes in fMRI signal and in AUC was found but this was not significant ($r = 0.669$, $p = 0.10$)

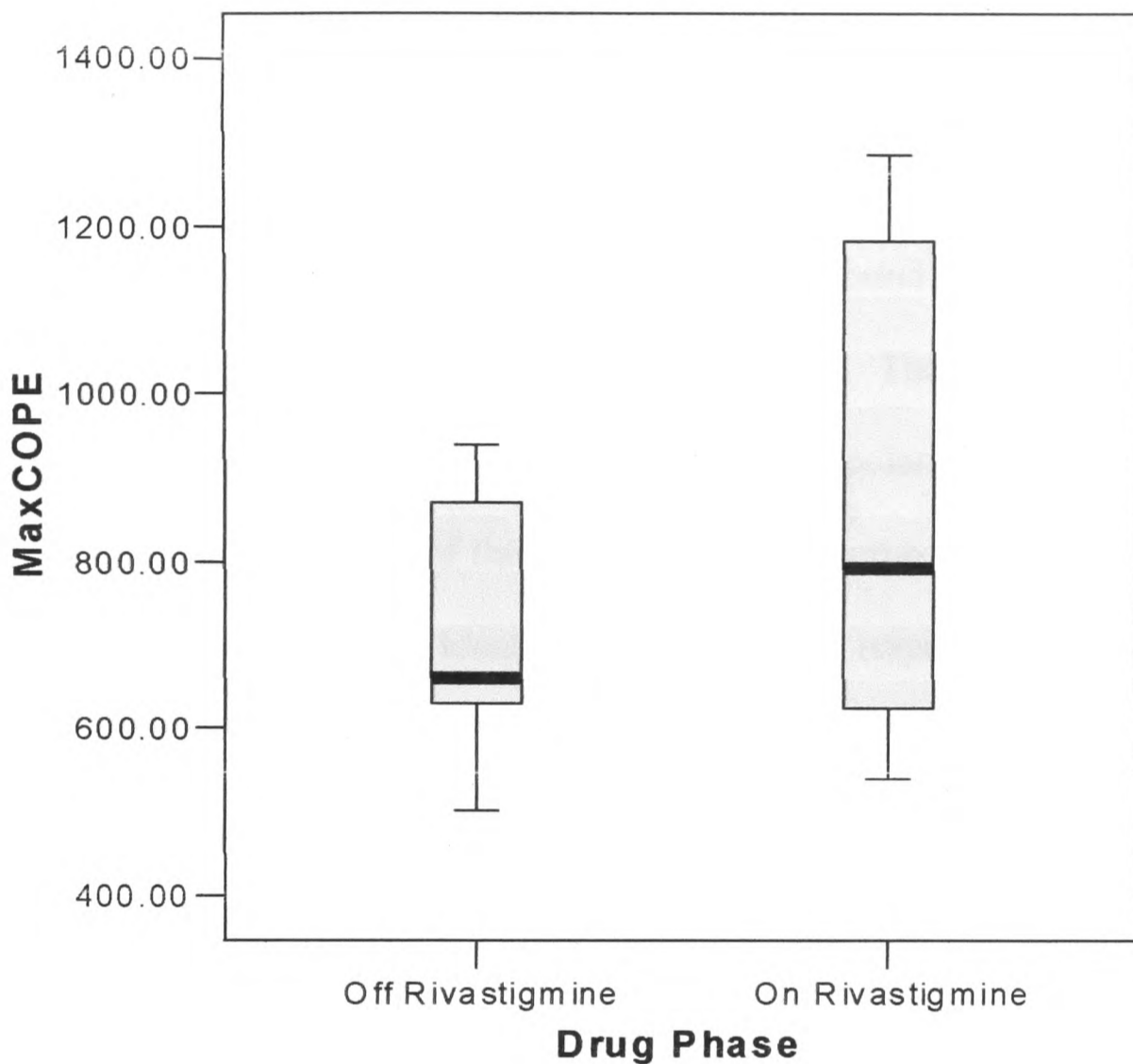


Figure 6.6 Maximum contrast of parameter estimate image (COPE) value in the occipital lobe during presentation of the visual checkerboard. There is no significant difference between the patients while on rivastigmine compared when off rivastigmine.

6.4 Discussion

This study examined the effect of rivastigmine on the relationship between the BOLD response measured in fMRI and the underlying neural activity. There was a significant change in the BOLD signal measured during the direct cortical stimulation. Rivastigmine significantly increased the neural activity during visual stimulation measured by EEG, which was not found with fMRI. The details of these results are discussed below.

6.4.1 The effect of rivastigmine on directly stimulated cortex

The first finding of this study was the increase in BOLD signal after the administration of rivastigmine during direct cortical stimulation of the motor cortex. A prolongation in the haemodynamic response after the initial peak in signal was found at both time points after the administration of rivastigmine compared with before. The direct, suprathreshold electrical stimulation is likely to result in extensive depolarisation of all neuronal populations throughout all layers of the underlying motor cortex (Akgoren et al., 1996). Austin and colleagues had initially identified that the BOLD response extends beyond the stimulation period in the directly stimulated motor cortex that may be due to after-discharges evoked by the high-frequency stimulation (Austin et al., 2003). It might be speculated that the administration of rivastigmine alters the neurovascular coupling during direct cortical stimulation explaining the increase in signal. However, acetylcholine is a powerful modulator of the excitability of neocortical neurons (Gu, 2002). Increases in motor cortical excitatory processes and decreases in intracortical inhibition have been found with the administration of tacrine, an acetylcholinesterase inhibitor (Korchounov et al., 2005). This would be consistent with a delay before a reduction in signal rather than a change in the peak signal. The prolongation of signal found with rivastigmine administration may therefore be the consequence of an increase of motor cortical excitability. Additionally, some of the differences might be explained by an order effect. The nature of the experiment precluded the use of a crossover design, and the possibility that the prolongation of BOLD signal was simply due to the persistent stimulation cannot be excluded. Over time, there may be a sensitization over the cortex to

the stimulation reflected in a greater BOLD signal. To address this, a further experiment using an animal proceeding through the entire protocol with only saline infusions might be considered.

6.4.2 The effect of rivastigmine in the contralateral cortex

In contrast to the directly stimulated cortex, the effect of rivastigmine on the contralateral cortex was less marked and significant change was only found at forty minutes after drug administration. The signal change decreased rapidly after the end of stimulation unlike the directly stimulated cortex, which reflects the origin of the neural activity measured here. The activation is unlikely to have occurred via spread of the electrical stimulus across the cortical surface and the signal profile likely reflects a more physiological stimulation than the directly stimulated cortex (Austin et al., 2003). Any neural activity in the contralateral cortex is likely to arise through interhemispheric connections from the directly stimulated cortex. A strong interhemispheric synchronization between the motor cortices has been described with connected neuronal cells firing with only milliseconds delay (Innocenti et al., 1995). The small changes seen in the latter part of the BOLD signal after the administration of rivastigmine most probably represent the propagation of the increased excitability seen in the directly stimulated cortex. Given the possible modulation of underlying neural activity, it is difficult to determine if any small changes in neurovascular coupling have occurred. However, the peak signal and early part of the haemodynamic response remain unaltered after rivastigmine administration. Further to this, the 3mg/kg dose administered is at levels much higher than that given to human subjects, and enough to cause systemic effects such as increased blood pressure. Thus it

is a reasonable assumption that the signal measured in human fMRI experiments is less likely to be affected, allowing more confident interpretation of any changes in processing attributed to rivastigmine.

6.4.3 Steady-state visual evoked potential under the influence of rivastigmine

A small increase in signal as measured by SSVEPs across the occipital region was found in patients taking rivastigmine compared with the patients not taking rivastigmine. As described previously, the widespread projections of cholinergic neurones include the visual system and the same mechanisms that might modulate cortical processing elsewhere have been shown to act in the visual system (Roberts et al., 2005). Thus the increased signal in response to the visual stimulation detected by SSVEPs is likely to reflect enhanced cortical processing following an increase in acetylcholine.

6.4.4 Effect of rivastigmine on the functional activation during the visual checkerboard

The flashing visual checkerboard paradigm resulted in a robust functional activation across the occipital region, as well as less marked functional activation in some other regions. Despite the finding of increased activity with rivastigmine in the SSVEPs, a significantly greater activation was found in patients not taking rivastigmine in a small area of the inferior occipital lobe extending into the cerebellum. This region was not directly measured with SSVEPs, and thus any potential decrease in signal would be missed. Whilst rivastigmine may increase signal in some areas particularly relevant to attending the flashing visual checkerboard, other areas may show a decrease in activity

and hence signal. This is in keeping with the concept that cholinergic modulation results in dynamic shift in the cortical network (Kimura, 2000). Confining the analysis to an area corresponding to the regions covered by the SSVEPs measurement did not demonstrate any differences in patients taking or not taking rivastigmine. One study showed a linear correlation between BOLD fMRI and EEG across a range of frequencies in healthy controls (Singh et al., 2003). Given that the change in neural activity due to rivastigmine as measured by the SSVEPs was relatively small, it is possible that sensitivity of the BOLD fMRI signal is not sufficient to detect the same degree of change.

6.4.5 Rivastigmine and the BOLD response

The interpretation of the effect of any drug on the functional activation during a specified task depends on the consistency of neurovascular coupling under drug and non-drug conditions. That rivastigmine appears to have minimal effects on the BOLD response to underlying neural activity makes this interpretation considerably easier. In the case of rivastigmine, and thereby the effect of acetylcholine, it has been shown in the rat study that the haemodynamic response is only affected to a small degree under the more physiological conditions (Figure 6.2) at doses much higher than that administered to humans. Further, the study in humans has demonstrated that, although a small increase in neural activity in response to visual stimulation is found with rivastigmine, there was not a marked global or regional increase in the BOLD signal. Therefore, any significant alterations in the functional activation during task processing are likely to represent a real modulation of the neural processing.

Section 7: Diffusion and connectivity in Multiple Sclerosis

The demyelination and axon loss in MS can disrupt the normal neural network. In section 3, differences in functional connectivity were demonstrated that might be a possible adaptive mechanism for this disruption. Diffusion tensor imaging could provide a means to explore changes in anatomical connectivity in-vivo. In this last experiment, the relationship of diffusion and connectivity to underlying markers of disease-related damage is studied in a group of MS patients. This would assist in interpreting how changes in anatomical connectivity might lead to changes in patterns of brain activation and clinical disability.

7.1 Introduction and rationale

Diffusion weighted imaging (DWI) uses the random movement of water molecules in tissue to generate image contrast (Rowley et al., 1999). However, the diffusion of water in brain tissue white matter is not free in all directions due to the organisation of cell cytoarchitecture and membranes. Water diffusion is generally greater in the direction parallel to axons than perpendicular to them, showing diffusion anisotropy (Chenevert et al., 1990; Moseley et al., 1990). By measuring the diffusion in several directions, diffusion tensor imaging (DTI) is able to detect the extent of directional bias of diffusion to distinguish regions where fibres are highly aligned from those whose fibres are less coherent (Le Bihan et al., 2001; Westin et al., 2002).

The fractional anisotropy (FA) gives a scalar metric of how much of the diffusion is in the principle direction of diffusion (Pierpaoli and Basser, 1996; Wiegell et al., 2000). For highly anisotropic diffusion, the FA index is close to 1. Both axonal loss (Ferguson et al., 1997; Filippi et al., 2003) and demyelination (Franklin, 2002) found in MS can potentially lead to a loss of tissue integrity that can be detected by DTI as a loss of tissue anisotropy (Dong et al., 2004; Le Bihan et al., 1986). Recent studies have demonstrated lower FA values in MS lesions than in normal appearing white matter (Bammer et al., 2000; Tievsky et al., 1999), which in turn were lower than that found in healthy controls (Ciccarelli et al., 2001).

Explicit information about the connection between regions is not provided by DTI, even though directional information at the voxel level is provided. Information regarding the orientation of fibres within a voxel can be used in tractography methods to determine the pathways of cerebral connections in vivo (Behrens et al., 2003b). A non-invasive method to determine anatomical connectivity has great potential for increasing our understanding of MS, in which connections between regions may be disrupted. Ideally, tractography techniques will be able to define quantitative and reproducible parameters for measuring anatomical connectivity in the context of MS. However, understanding how measures of diffusion and connectivity relate to established markers of disease-related damage in MS is needed.

The corpus callosum is an area of the brain connecting homologous regions of the right and left hemispheres. It is an easily identified large fibre tract with high fibre

directionality. Findings of reduced FA values in the corpus callosum of MS patients have been suggested to be due to loss of axons (Hasan et al., 2005). This is consistent with the correlation of diffusion changes with total cerebral lesion volume (Ge et al., 2004) and local lesion load (Ciccarelli et al., 2003). Pathological studies have demonstrated a significant reduction in the number of axons traversing the corpus callosum, which is reflected in the cross-sectional area (Evangelou et al., 2000). Thus, the corpus callosum is an ideal region to assess the effect of fibre loss on connectivity and diffusion.

Axonal dysfunction or loss can be estimated with magnetic resonance spectroscopy (MRS), based on the signal intensity of N-Acetylaspartate (NAA). This metabolite is localised almost exclusively in neurones and neuronal processes (Moffett et al., 1991; Simmons et al., 1991). Modest correlations between FA and the NAA: creatine (Cr) ratio have been demonstrated in normal appearing corpus callosum of MS patients (Oh et al., 2004).

Whilst axon loss will be a major contribution to the reduction of tissue anisotropy, demyelination may also potentially influence diffusion and tract mapping. The mean diffusivity of water can be considered as two components, the axial diffusivity relating to diffusivity parallel to the principle direction and the radial diffusivity that is the average of the diffusion perpendicular to the principle direction (Song et al., 2002). Animal studies have suggested that decreases in axial diffusivity may reflect axonal loss (Song et al., 2003) while increases in radial diffusivity may indicate demyelination (Song et al., 2005).

Here data is presented from a DTI study in a group of MS patients, looking at two different quantitative measurements along the tracts generated by the probabilistic tractography across the splenium of the callosum: mean FA and mean connectivity in the reconstructed tracts. In this region, there should be a robust correlation between these two measures, the callosum is an area previously shown to have highly reproducible values of connectivity (Ciccarelli et al., 2003). If fibre loss has a large influence on diffusion, then there should be a strong correlation of the callosal area with mean FA and mean connectivity. To gain further insight of the tractography measures in MS, T₂-weighted lesion volume and NAA are also measured. The axial and radial components of diffusivity in the generated tracts may provide more information regarding the relative contribution of axon loss or demyelination. If it can be shown that MS pathology results in a predictable change in connectivity, then tractography could provide a potential method to interrogate the disruption of regional brain connections, its relationship to brain activation and clinical disability in MS.

7.2 Methods

Subjects

Fifteen patients (7 men, 8 women; median age 43 years, range 27-52 years) with clinically definite multiple sclerosis (13 relapsing remitting, 2 secondary progressive; median duration 8 years, range 2-14 years) according to the Poser criteria (Poser et al., 1983) underwent scanning. Disability was assessed with the EDSS (median EDSS 2.5,

range 1-4) (Kurtzke, 1983). Written consent was obtained from all subjects. Ethical approval for this study was given by the Oxford Regional Ethics Committee.

Imaging

All scans were performed using a Siemens 1.5 Tesla whole body scanner with a standard quadrature head coil, except the MRS scan which was performed on a 3.0 Tesla whole body scanner with a Varian Inova console and a quadrature birdcage radiofrequency head coil. The following scans were performed for each subject:

- (i) Magnetic resonance spectroscopy using a localised PRESS sequence (Bottomley, 1987) [TE=26 ms, TR = 5000 ms] and a WET water suppression scheme (Ogg et al., 1994) in an approximate 1cm³ volume of interest in the splenium of the corpus callosum, and avoiding any MRI-visible lesions.
- (ii) T₂-weighted anatomical scan [35 x 5 mm axial slices, TR = 5000 ms, TE = 65 ms]
- (iii) T₁-weighted anatomical scan [176 x 1mm axial slices, TR = 15 ms, TE = 6.9 ms]
- (iv) Diffusion weighted imaging [53 slice of isotropic 2.5 mm x 2.5 mm x 2.5 mm, TR = 7300 ms, TE = 87 ms. Two data sets were obtained consisting of 60 diffusion-weighted images acquired with a b-value 1000 smm⁻², and four volumes were acquired with no diffusion weighting. Diffusion gradients were uniformly distributed through space.

Optimised cardiac gating was used to minimize artefacts from cerebrospinal fluid pulsatile flow (Wheeler-Kingshott et al., 2002)]

Data analysis

(i) Absolute NAA concentrations were estimated using brain tissue water as a concentration reference with MRUI software and the VARPRO method (Naressi et al., 2001). The relative volumes of water in cerebrospinal fluid and tissue compartments within the voxel were estimated with bi-exponential fitting of the water signal decay T_2 decay [TR=15 s, TE = 26, 42, 72, 92, 132, 282, 512, 612, 812, 1012, 1212 ms].

To confirm the results of metabolite concentrations, LCModel was employed to check for similar results (Provencher, 1993).

(ii) Lesion volume quantification was measured manually using MRICro, version 1.37. Identified lesions were highlighted and the total lesion volume calculated from the T_2 -weighted images. The mean intra-rater variability was 3.2% for the use of this software. A binarised map of the lesions for each subject was included in a group image to create a lesion probability map (Narayanan et al., 1997).

(iii) The cross-sectional area of the corpus callosum was averaged over the three middle sagittal slices of the T_1 -weighted images. The infundibulum of the pituitary was used as an anatomical landmark to define the midline. The anterior-posterior diameter of the inside of the skull was used to correct for variation in brain size, as routinely used in the FMRIB Centre, Oxford University.

(iv) The diffusion weighted volumes were averaged to give a single four-dimensional data set. The diffusion in each voxel was modelled using BEDPOST (Bayesian estimation of diffusion parameters obtained using sampling techniques) (www.fmrib.ox.ac.uk/fsl), to provide the diffusion tensor image. The diffusion tensor image was compared with the lesion probability map to provide a composite image of the spatial correlation (Smith et al., 2005).

To perform the probabilistic diffusion tractography, a 'tracker' function within the FMRIB's diffusion toolbox (FDT) was employed (Behrens et al., 2003a; Behrens et al., 2003b; Smith et al., 2004). A single voxel was selected as a seed in the left side of the corpus callosum within the splenium, using the same anatomical landmarks in each subject. The software then generated a tract from this point, based on the probability of diffusion. The tracking was programmed to stop if any seed particles went outside the corpus callosum. Thus only the potential tract within the corpus callosum was considered.

Within this generated tract, the FA, axial diffusivity and radial diffusivity value for each voxel was measured, and then averaged over the whole volume of the generated tract to provide a mean value for each patient. To obtain a measure of the connectivity, the number of seed particles reaching each voxel within the generated tract was retrieved, and also averaged to give a mean connectivity across the corpus callosum.

This was repeated from right to left side, and the average was calculated across the two generated tracts. To avoid potential confound, seeds were placed to avoid generating tracts through MRI-visible lesions.

Statistics

Pearson correlations were used to assess the relationship between the mean FA, connectivity, callosal area, NAA concentration, EDSS, lesion volume and disease duration. A p -value <0.05 after correction for multiple comparisons was considered significant. Before applying any analysis that assumes normal distribution, checks were made that the data approximated normality. All statistics were performed using SPSS for Windows (version 12.0.1).

7.3 Results

Subject	Mean Connectivity	Mean FA	Corrected Callosal Area (mm)
1	511.78	0.26	2.94
2	2979.69	0.65	4.51
3	1457.56	0.39	3.81
4	1119.13	0.33	3.12
5	2317.67	0.61	4.09
6	2017.09	0.52	3.49
7	2412.23	0.65	3.65
8	1635.02	0.44	3.64
9	100.68	0.18	2.54
10	1337.73	0.40	3.10
11	361.64	0.24	2.60
12	2624.27	0.62	2.86
13	2570.34	0.56	4.17
14	1417.63	0.42	2.52
15	3108.10	0.65	4.77

Table 7.1 Mean connectivity, fractional anisotropy (FA) and callosal area in the MS patients. Callosal area is corrected for internal anterior-posterior skull dimension.

Diffusion in the corpus callosum of MS patients Table 7.1 shows the mean connectivity, fractional anisotropy and corrected callosal area in the MS patients. The average connectivity in MS patients was 1731.4 (S.D = 947.9) and the fractional anisotropy was 0.46 (S.D = 0.16). A strong correlation ($r=0.98$, $p<0.001$) was found between the mean connectivity and fractional anisotropy across the patient group (Figure 7.1).

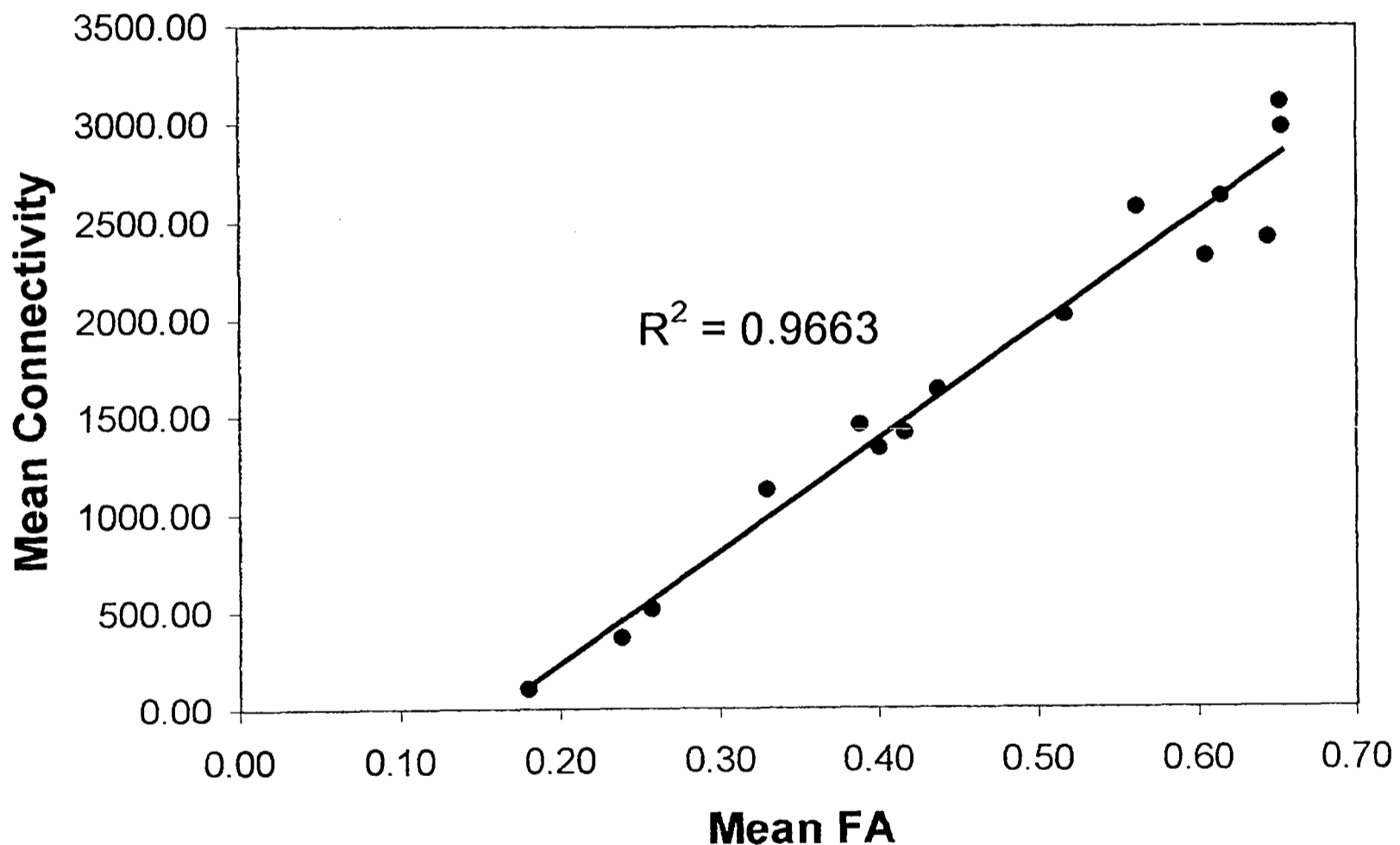


Figure 7.1 Correlation between mean connectivity and mean fractional anisotropy across the posterior section of the corpus callosum ($p<0.001$, $r=0.98$).

The relationship of callosal area and measures of diffusion There was a significant correlation of the corrected callosal area with fractional anisotropy ($r=0.73$, $p=0.001$) and with connectivity ($r=0.78$, $p<0.001$), (Figure 7.2)

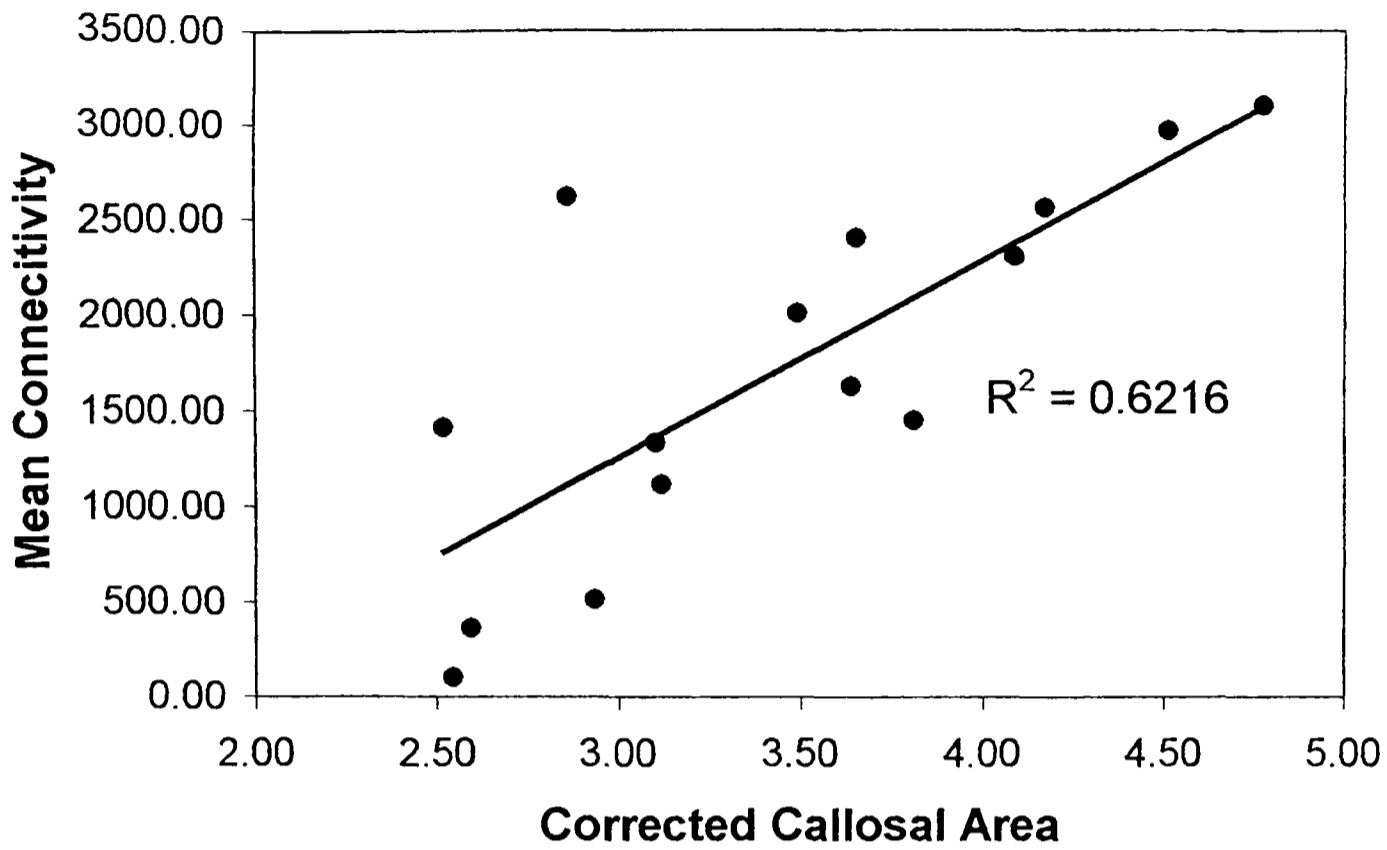


Figure 7.2 Correlation between mean connectivity and corrected callosal area ($r=0.78$, $p<0.001$)

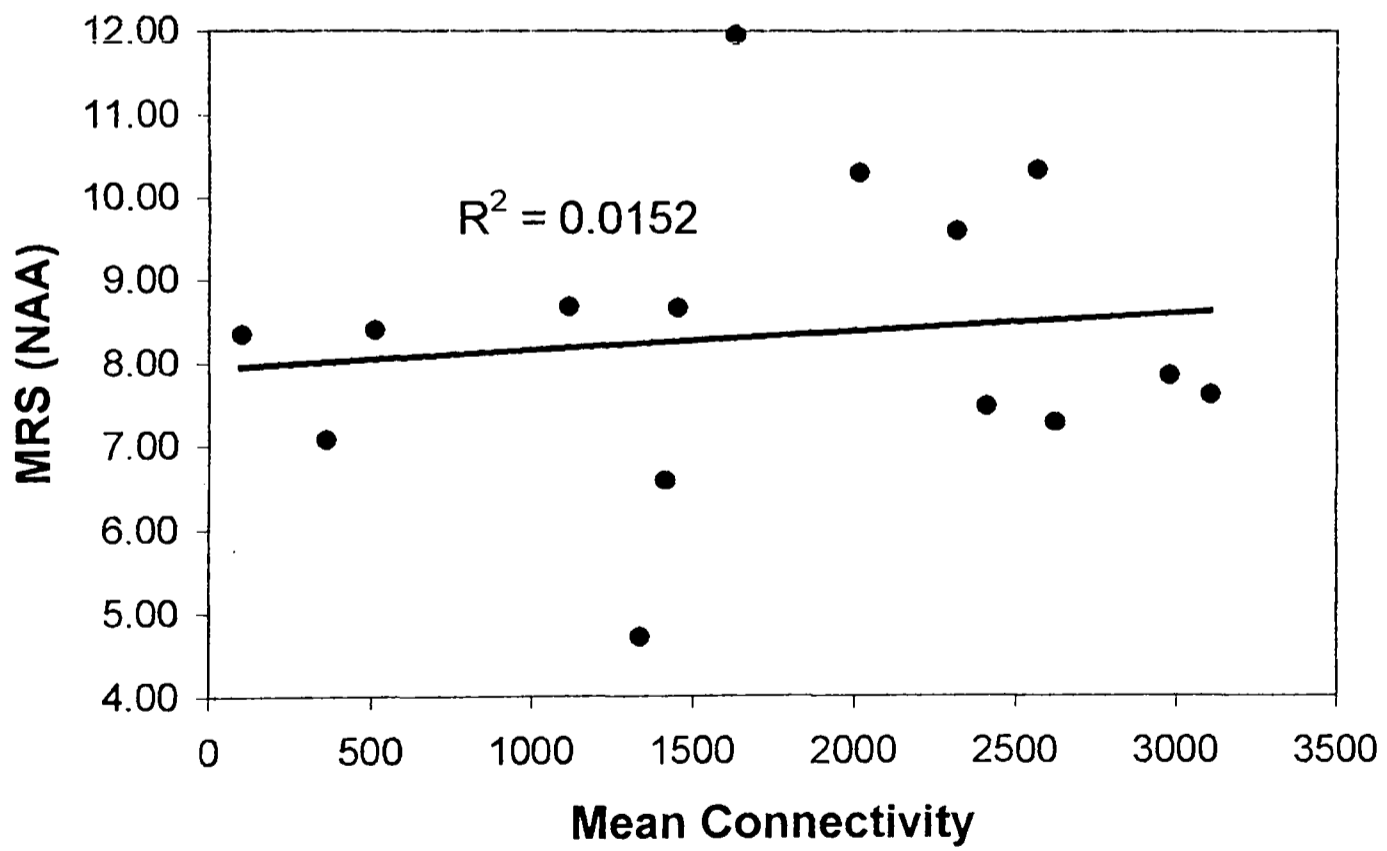


Figure 7.3 No significant correlation was found between Absolute NAA and mean connectivity.

N-Acetylaspartate and measures of diffusion Absolute concentrations of NAA (Table 7.2) did not show any correlation with either mean connectivity ($r=0.12$) or with fractional anisotropy ($r=0.09$), (Figure 7.3).

Subject	NAA (ppm)	EDSS	Duration (years)	T ₂ Lesion Volume
1	8.39	3.5	11	5.71
2	7.85	2	6	3
3	8.65	3.5	10	18.21
4	8.67	2.5	11	10.65
5	9.60	2.5	14	2.88
6	10.30	1	2	3.72
7	7.48	2.5	6	0.79
8	11.96	3.5	9	10.06
9	8.34	4	13	17.3
10	4.71	1.5	6	8.65
11	7.07	4	6	40.51
12	7.29	1.5	8	4.22
13	10.34	1	13	1.02
14	6.58	3	4	10.9
15	7.62	3	3	7.69

Table 7.2 Spectroscopy, EDSS, disease duration and T₂ lesion volume across the MS patients

The axial and radial components of diffusivity To further understand the relationship of fractional anisotropy to other brain changes in MS patients, the components of fractional anisotropy were measured. The axial component, relating to the principal direction of the diffusion, and the radial component of diffusivity, relating to the perpendicular plane, was calculated for each subject in the area across the posterior corpus callosum (Table 7.3). The callosal area did not correlate with the axial component ($r=-0.27$, $p>0.357$) but

did show a negative correlation with the radial component ($r=-0.65$, $p<0.01$). There was no significant correlation of either component with the concentration of NAA.

<i>Subject</i>	Axial Eigenvalue	Radial Eigenvalue
1	0.0022	0.0015
2	0.0019	0.0006
3	0.0022	0.0013
4	0.0021	0.0013
5	0.0019	0.0006
6	0.0017	0.0007
7	0.0017	0.0005
8	0.0017	0.0009
9	0.0023	0.0018
10	0.0020	0.0012
11	0.0021	0.0016
12	0.0019	0.0006
13	0.0020	0.0008
14	0.0016	0.0009
15	0.0018	0.0005

Table 7.3 Axial and radial components of diffusion across the corpus callosum in MS patients

Diffusion and T_2 lesion volume The mean global T_2 lesion volume was 9.7 mls (S.D = 10.1 mls). There is a significant correlation of global lesion volume with both connectivity ($r=-0.66$, $p<0.01$) and fractional anisotropy ($r=-0.67$, $p<0.01$), (figure 7.4). There is a significant spatial correlation ($p<0.01$) between lesion load and fractional anisotropy (Figure 7.5)

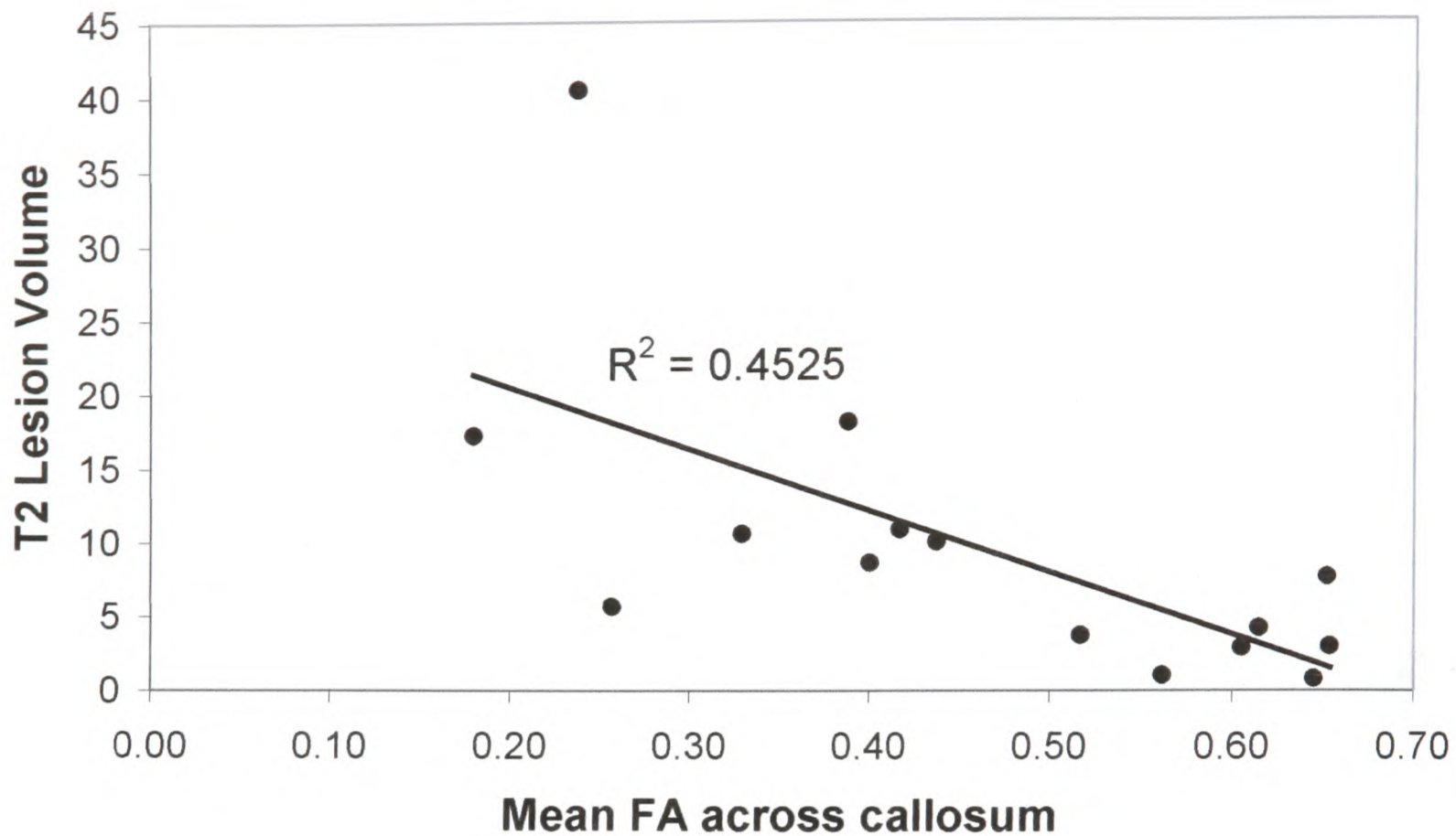


Figure 7.4 The correlation of T₂ lesion volume and fractional anisotropy ($r=-0.67$, $p<0.01$)

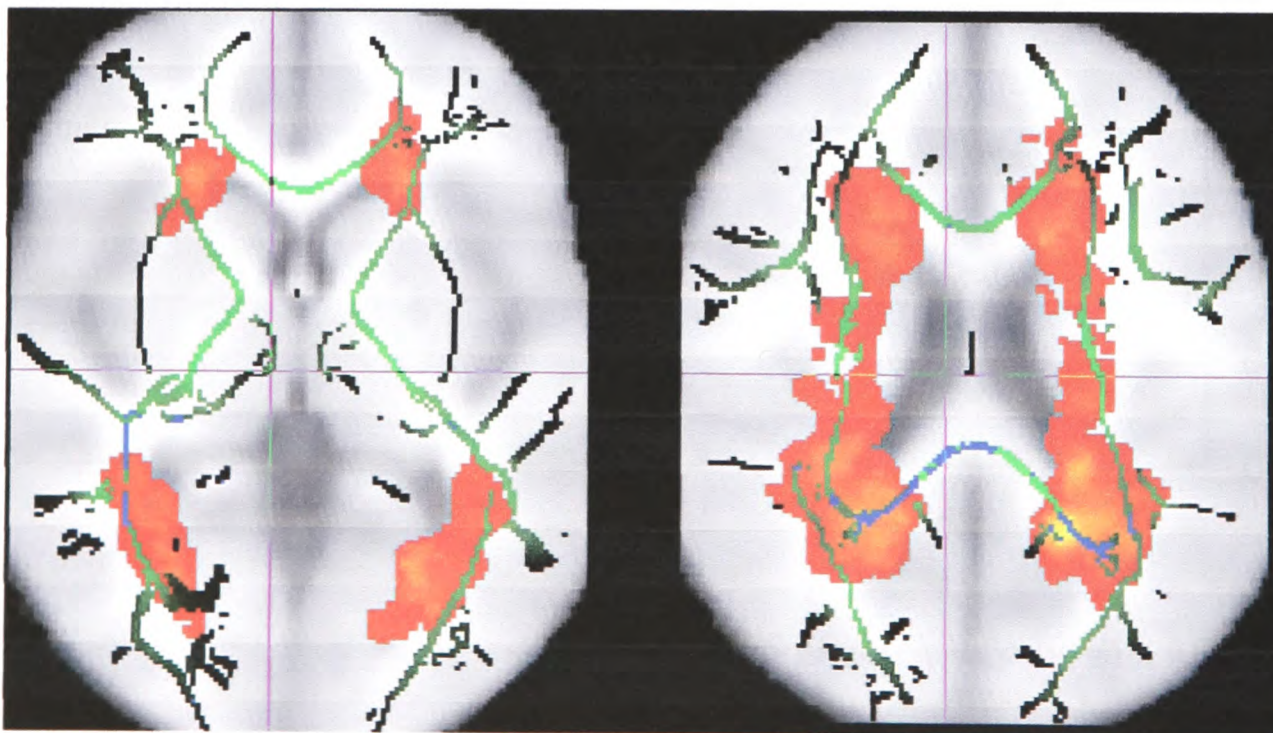


Figure 7.5 The spatial correlation of fractional anisotropy with T₂ lesion load in MS patients ($p<0.01$). The blue indicates the areas of highest negative correlation. This correlation is overlaid on the lesion probability map (red) of the patients.

Clinical disability and diffusion There was a modest correlation of EDSS with connectivity ($r=0.62$, $p=0.01$), (figure 7.6) and with fractional anisotropy ($r=0.61$, $p=0.01$). There was no correlation of disease duration with measures of diffusion.

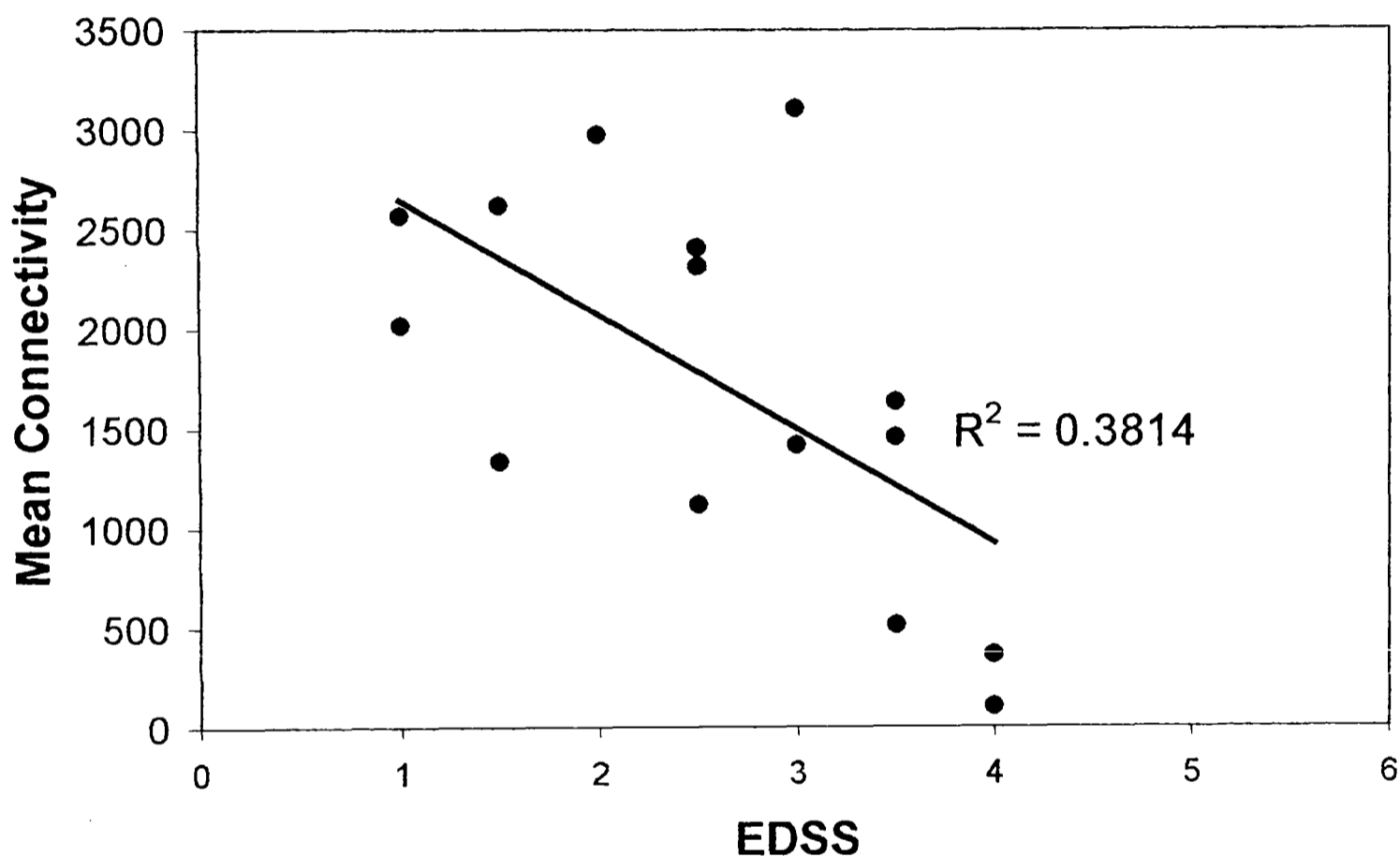


Figure 7.6 The correlation of connectivity with EDSS in MS patients ($r=0.62$, $p=0.01$).

7.4 Discussion

In this study, the relationship of connectivity measured by diffusion tensor imaging in the corpus callosum to markers of disease-related damage in MS is examined. A strong relationship of connectivity with callosal area was found, and some correlation with clinical disability. Global lesion volume showed a modest correlation with connectivity

that was supported by a strong spatial correlation. N-Acetylaspartate measured in the posterior callosum did not show significant correlation with any other measures. These results are discussed in more detail below.

7.4.1 Mean fractional anisotropy and connectivity

Two quantitative measures of diffusion are employed in this study that are found to correlate strongly with each other. Information about the orientation of diffusion at each voxel allows the probabilistic tractography technique (Behrens et al., 2003b) to generate a tract between the two sides of the corpus callosum. The mean fractional anisotropy within this tract gives an indication of how directional the diffusion was across the MS subjects. Studies have already indicated that FA is a reliable index of the directional information at the voxel level (Chenevert et al., 1990). However, if one is to determine the pathways of cerebral connections in vivo, then information about the connections between neighbouring voxels is needed. Finding that changes in connectivity closely reflect changes in FA suggest that this connectivity does provide a reproducible method to interrogate the integrity of a fibre tract. The mean connectivity provides a measure of the probability that diffusion will occur from one voxel to neighbouring voxels, using a newly established tracking method (Smith et al., 2004). If fibres tracts are entirely intact, then the mean connectivity will be close to the maximum value, with minimal spread of diffusion and a small volume of tract generated. However, as fibres are disrupted by disease, the spread of diffusion will occur over a greater volume of tract, and the connectivity across this larger volume will fall. In the corpus callosum the fibres run predominantly from side to side, and minimally in other directions. The reproducibility of

tractography may vary in other regions of the brain, where there are lower levels of fibre organisation (Ciccarelli et al., 2003). However, the use of probabilistic tractography in healthy subjects has demonstrated accurate mapping of pathways between cortex and thalamus (Behrens et al., 2003a) that would not have the same high degree of organisation as the callosum. We have found that the probabilistic tractographic method appears to reflect the underlying fractional anisotropy across this group of MS patient with varying disability and disease-related damage. Thus, accurate mapping of pathways, or their disruption, should be possible in MS patients.

7.4.2 Fibre loss and diffusion

One of the main findings of this study is the robust correlation of both mean FA and connectivity with the cross-sectional area of the corpus callosum. Independent studies have shown reductions in axon numbers associated with smaller callosal area (Evangelou et al., 2000) and lower FA values (Hasan et al., 2005) in the callosum of MS patients. Thus the loss of fibres is likely to be a major factor in the reduced connectivity within the callosum of MS patients.

No correlation was found with the concentration of NAA in the callosum, however. A number of studies have linked axonal pathology with reduced NAA in MS patients (De Stefano et al., 2001; Fu et al., 1998). Other studies have indicated that changes in NAA concentration may be reversible (De Stefano et al., 1995; De Stefano et al., 1999). Given this, the fall in NAA cannot merely express axon loss, but may also reflect a reversible axonal dysfunction (Davie et al., 1994). In a study of primary progressive MS, brain

volume changes did not correlate with changes in NAA (Fu et al., 1998). Metabolic depression has been described in areas distant but anatomically linked to regions of acute focal injury (Baron, 1985; Bowler et al., 1995). Although no subjects had suffered any clinical relapse within two months of the study, the metabolic changes may take several months to resolve (De Stefano et al., 1999) and there may be some ongoing sub-clinical injury. Decreases in NAA have been demonstrated in association with this metabolic depression both in vivo (Fulham et al., 1994) and in vitro (Rango et al., 1995). These potentially reversible elements may not influence the diffusion of water molecules, and so would not be detected with DTI. However, if the changes are not permanent then their relevance to chronic disability is minimal, and does not significantly limit the potential of DTI in mapping neural networks in MS patients.

7.4.3 Axial and radial diffusivity

Diffusional anisotropy depends on the degree of diffusion in the predominant direction (axial diffusivity) and also the amount of diffusion in the plane perpendicular to the principle direction (radial diffusivity). Changes in these components may potentially be used to differentiate myelin loss versus axonal injury (Song et al., 2002). This study has found stronger correlations of radial than axial diffusivity with callosal area. Demyelination may, therefore, be responsible for some of the reductions in callosal cross-sectional area (Lycklama a Nijeholt, 2005). However, axonal atrophy and loss is considered to make a greater contribution to atrophy (Ganter et al., 1999; Losseff et al., 1996). A similar finding to this study was found by Henry and colleagues. This group found that the reduction in tissue anisotropy represented an increase in diffusion

transverse to fibres without significant change in diffusion along the fibres. This was concluded to reflect the signature of wallerian degeneration of neurone rather than demyelination (Henry et al., 2003). The interpretation of these components of diffusivity appears to be complex, and further elucidation is needed. A potential avenue may be the analysis of the short T2 component in determining the amount of myelin present in a tissue (Webb et al., 2003). Areas of demyelination have been detected in pathological specimens of MS brains using this approach (Moore et al., 2000). Thus, investigating the relationship of connectivity and diffusion to underlying changes of demyelination in MS patients is possible in vivo.

7.4.4 Lesion volume, disability and connectivity

This study has demonstrated a modest correlation of T₂-weighted lesion volume with connectivity. Both demyelination and axonal loss are associated with MS lesions, but while demyelination is likely to have only local effects, wallerian degeneration can result in the loss of axons in distant but connected regions (Evangelou et al., 2000; Moore et al., 2000). This is in keeping with previous studies that have found reductions in FA associated with the total cerebral lesion volume (Ge et al., 2004) and with local lesion load (Ciccarelli et al., 2003). A correlation does not necessarily imply a causal relationship, but a strong spatial association between connectivity and MRI-visible lesion was found in this study. MS lesions are likely to be responsible for changes in connectivity. MS can cause widespread pathology, and different processes may be independent of each other, a hypothesis supported by a study demonstrating that axonal pathology measured by whole brain NAA was independent of MRI-visible inflammation.

(Filippi et al., 2003). In addition, a number of studies have shown a weakness in the association between T₂-weighted lesions and clinical disability (Khoury et al., 1994; Paty et al., 1994). Similarly, in this study only a modest correlation was found between disability as determined by EDSS and the imaging findings. However, Wilson and colleagues found that whilst the integrity of pyramidal tracts in MS patients showed some correlation with EDSS, a much stronger relationship was found with a specific pyramidal functional score (Wilson et al., 2003). Thus, establishing the integrity of relevant pathways in MS can potentially provide a great deal of insight into the clinical disability and the differences found in brain activation of specific functions.

Section 8: General conclusions and future directions

The aim of this thesis was to investigate activation, modulation and reorganisation of the brain in multiple sclerosis during cognitive processing. This was largely motivated by the huge impact that cognitive impairment has on a large number of people suffering from multiple sclerosis. A better understanding of the extent and significance of adaptive or modulatory mechanisms in MS would allow more successful treatment, but also in itself provide greater insight into mechanisms underlying cognitive dysfunction. The experiments in this thesis was therefore designed with the following specific aims:

- *To more fully characterize the differences in working memory-related activation in MS patients compared to controls (Section 3)*
- *To assess the effect of cholinergic enhancement on the altered functional activation of MS patients, and how this relates to changes in behavioural measures (Section 4)*
- *To assess the effect of cholinergic blockade on the functional activation in healthy populations (Section 5)*
- *To explore the potential confounding effect of cholinergic modulation on the functional imaging signal (Section 6)*
- *To examine the potential of tractographic techniques in mapping connectivity in MS patients (Section 7)*

The conclusions of these investigations, and suggestions for future research, are discussed below.

8.1 Patterns of cognition-related activations in multiple sclerosis

Section 3 gave the results from a study designed to characterize the patterns of cognitive-related fMRI activation in patients with MS, specifically for verbal working memory.

Decreased activation for patients was found despite similar performance on the task for patients and controls. Previous studies have pointed to a reduced *functional reserve* for cognition (Lazeron et al., 2004), resulting in areas less able to meet cognitive demands particularly as they increase. The differences between healthy controls and the patients reflect the consequences of pathology, although this was sub-clinical at the stage that the patients were studied. The sensitivity of behavioural measures in this context is insufficient to detect the effects of pathology found with functional imaging.

This study emphasized that controls and patients both activated anatomically similar regions, although some previous studies have identified novel brain regions in patients with MS during cognitive tasks (Mainero et al., 2004; Penner et al., 2003). Lazeron and colleagues (Lazeron et al., 2004) failed to find novel regions of brain recruitment with a 'Tower of London' task, although the fMRI context was not controlled for differences in task performance.

The most consistent difference in task-related activation between patients and controls was found in the superior frontal and anterior cingulate gyri, though differences between patients and controls in task related activation increases were widespread. These regions both include polymodal neocortex involved in processing cognitive

functions including attention (Lenartowicz and McIntosh, 2005). This region may be particularly vulnerable to pathology from MS due to the demands for coherence of input in polymodal processing. It also is possible that this region is *selectively* involved by neocortical pathology with MS, consistent with a study finding a particularly high neocortical lesion load in the anterior cingulate (Bo et al., 2003).

Despite the evidence for reduced cognitive functional reserve, MS patients were able to maintain working memory performance similar to controls, suggesting potentially compensatory increases in efficiency of use or of interactions between interacting regions. Consistent with this, altered task-related functional connectivity in patients compared with controls was found. The functional connectivity and univariate activation analyses together therefore both suggest functional pathology limiting interactions between the lateral and medial prefrontal cortex in the patients. Patients showed relatively increased functional connectivity between the right and left prefrontal cortices, a functional relationship not significant in healthy control subjects. The increased functional connectivity between the two lateral prefrontal cortices could be speculated to be an adaptive mechanism that contributes to limiting expression of pathology with this cognitive task. Recruitment of homologous regions in the two hemispheres as a compensatory mechanism is consistent with observations in the motor system for patients with MS, in whom increased recruitment of premotor cortex ipsilateral to the hand moved is among the most consistent differences in MS patients relative to healthy controls (Cifelli and Matthews, 2002). Based on an effective connectivity analysis of PASAT working memory task, Audoin et al recently reported that different interactions show either decreased or *increased* connectivity in patients with multiple sclerosis (Audoin et al., 2003). This suggests that with lower

susceptibility to injury or pathological involvement, some pathways show *enhanced* function to compensate (at least partially) for impaired functional connectivity within a processing network.

The reduced functional reserve in patients could lead to failure of processing with increasing cognitive demands in the patients at lower levels of task demands than for healthy controls. As a functional expression of pathology, it may provide a measure sensitive to change with an increasing burden of disease over time. Strategies to enhance the functional reserve and limit its rate of loss or to enhance potentially compensatory functional connectivity provide new targets for therapy in MS.

8.2 The effect of cholinergic enhancement in MS patients

Cholinergic enhancement can be provided with the blockade of cholinesterases. Section 4 described a longitudinal study of a cholinesterase inhibitor, Rivastigmine, in MS patients examining the functional activation and behavioural performance during cognitive tasks.

The experiment showed that rivastigmine increased activation within the inferior frontal gyrus, a region identified previously as being associated with normal processing in healthy control subjects (Parry et al., 2003), during the Stroop task. In the N-back task, greater incremental activation with increasing task difficulty after the administration of rivastigmine. This was largely reflected in the reduced activation during the 1-back task. The MS patients have a greater capacity to increase activation when on rivastigmine. The region-of-interest analysis demonstrates that this is a widespread effect in all task relevant areas, but the mixed-effects whole brain analysis indicate that some areas are particularly sensitive to the drug effect.

The increased functional connectivity found in patients taking rivastigmine suggests that the drug may be acting to improve cortical processing function of the normal neural network. At all levels of difficulty, increased cerebellar activation was found in patients taking rivastigmine. Enhanced cerebellar activation has been identified with increasing memory load (Smith and Jonides, 1997), and the increase found with cholinergic modulation has been suggested to be beneficial for performance (Kumari et al., 2003).

Consistently across all tasks, both within the scanner and in the neuropsychological battery, no measurable changes in performance were found. The observed change in the activation is thus likely a consequence of treatment with rivastigmine, rather than a change in the level of performance, given the absence of any significant change in, or correlation with, behavioural measures. The drug may act to increase functioning of the normal neural network recruited to perform the task reducing the requirement for previously recruited compensatory mechanisms, and so may not result in behavioural changes. Acetylcholine may increase the sensitivity and functioning of the neural network recruited to perform the tasks, by improving the signal-to-noise ratio of relevant neural activity. Whilst increasing the efficiency of neural processing within task-specific region and so reducing the level of necessary activity particularly at low demand such as the 1-back task, some regions may show increased activity associated with increased propagation of signal typically at higher level of demand. Temporal and spatial summation, phenomena needed for effective propagation of signal, would be adversely affected in the context of demyelination and axonal loss that occurs in MS. Enhancing the signal in intact or less damaged neurones may be

sufficient to overcome the summation requirements that could be provided by increasing the acetylcholine available in cortical synaptic junctions.

However, the lack of behavioural change suggests that, although rivastigmine may potentially be beneficial for cognitive processing, clinical use of such drugs would be limited. Studies in more cognitively disabled MS patients or longitudinal studies examining deterioration in cognition may be able to shed further light into the potential role of cholinergic enhancement.

8.3 The effect of cholinergic blockade in healthy subjects

Section 5 addressed how cholinergic antagonism influences the brain activation during cognitive tasks.

The whole brain analysis revealed a significantly greater activation within prefrontal areas in subjects on placebo at the 3-back level, but not at lower levels of difficulty in the N-back task. There was also an effect on the whole brain contrast of incremental activation. The reduced incremental activation in the cerebellum with the administration of hyoscine might be detrimental to performance that may be apparent if memory load were to increase further. Reduced incremental activation was not confined to the cerebellum. Greater incremental activation with placebo over hyoscine was found across many regions usually involved in the processing of verbal working memory. This was found on both the whole brain group analysis and on the region-of-interest analysis. The administration of hyoscine, in contrast, increased the incremental activation in regions not typically involved. This increased incremental

activation may be due to additional areas being recruited to compensate for reductions elsewhere. Alternatively hyoscine may be suppressing the normal shift in functional processing that occurs when attending to tasks of increasing cognitive demand.

Healthy subjects showed a greater connectivity between right prefrontal and superior medial frontal when on placebo compared with on hyoscine. When subjects were taking hyoscine, an increased connectivity between right and left prefrontal regions was found. This followed a similar pattern to that seen in MS patients compared with controls in section 3. Functional studies have shown similar mechanisms of functional reorganisation in different diseases (Lee et al., 2000; Pineiro et al., 2001) that would be consistent with this finding.

The role of cholinergic modulation in cognitive processes, particularly that of complex attentional tasks, appears to be important both in health and disease. Mechanisms underlying the impairment of processing with cholinergic blockade appear to be similar to mechanisms that may improve processing in states of cognitive impairment due to multiple sclerosis. This is consistent with studies demonstrating opposite effects on cognitive function (Wesnes and Revell, 1984). Alternatively, disease processes in MS may disrupt cholinergic pathways, preventing their potentially beneficial modulatory effect. Even if cholinergic pathways are not disrupted, cholinergic therapy may not have an effect, if this modulation has already been maximized to compensate for disease-related damage elsewhere. The experiments in this thesis suggest that this is not the case in the cohort of MS patients studied.

8.4 Potentially confounding effects of cholinergic modulation on the BOLD signal

The potential effects of rivastigmine on the functional imaging signal were examined in section 6 using two approaches. The rat model studied allowed a more direct testing of the BOLD response to a controlled stimulation. The visual stimulation experiment allowed an indirect testing of the BOLD response in MS patients.

An increase in BOLD signal was found after the administration of rivastigmine during direct cortical stimulation of the motor cortex with a prolongation in the haemodynamic response after the initial peak in signal. The direct, suprathreshold electrical stimulation is likely to result in extensive depolarization of all neuronal populations throughout all layers of the underlying motor cortex (Akgoren et al., 1996). Austin and colleagues had initially identified that the BOLD response extends beyond the stimulation period in the directly stimulated motor cortex that may be due to after-discharges evoked by the high-frequency stimulation (Austin et al., 2003). However, acetylcholine is a powerful modulator of the excitability of neocortical neurons (Gu, 2002). Increases in motor cortical excitatory processes and decreases in intracortical inhibition have been found with the administration of tacrine, an acetylcholinesterase inhibitor (Korchounov et al., 2005). This would be consistent with a delay before a reduction in signal rather than a change in the peak signal. The prolongation of signal found with rivastigmine administration may therefore be the consequence of an increase of motor cortical excitability. The signal change in the contralateral cortex decreased rapidly after the end of stimulation unlike the directly stimulated cortex, which reflects the origin of the neural activity measured here. The effect of rivastigmine on the contralateral cortex was less marked and significant

change was only found at forty minutes after drug administration. The peak signal and early part of the haemodynamic response remain unaltered after rivastigmine administration.

A small increase in signal as measured by SSVEPs across the occipital region was found in patients taking rivastigmine compared with the patients not taking rivastigmine. The increased signal in response to the visual stimulation detected by SSVEPs is likely to reflect enhanced cortical processing following an increase in acetylcholine (Roberts et al., 2005). There was no clear corresponding change in BOLD fMRI signal, suggest a lack of sensitivity compared with electrical recording. Rivastigmine appears to have minimal effects on the BOLD response to underlying neural activity makes this interpretation considerably easier. Therefore, any significant alterations in the functional activation during task processing are likely to represent a real modulation of the neural processing.

8.5 The potential role of tractography in the study of MS

In the final experiment, diffusion weighted imaging is utilized to explore the potential of tractographic techniques to map connectivity in MS, that might be used in the future to understand better the functional activation during tasks and any manifested clinical disability.

One of the main findings of this study is the robust correlation of both mean FA and connectivity with the cross-sectional area of the corpus callosum. Independent studies have shown reductions in axon numbers associated with smaller callosal area

(Evangelou et al., 2000) and lower FA values (Hasan et al., 2005) in the callosum of MS patients. Thus the loss of fibers is likely to be a major factor in the reduced connectivity within the callosum of MS patients. A modest correlation of T₂-weighted lesion volume with connectivity was demonstrated. Both demyelination and axonal loss are associated with MS lesions, but while demyelination is likely to have only local effects, wallerian degeneration can result in the loss of axons in distant but connected regions (Evangelou et al., 2000; Moore et al., 2000). This is in keeping with previous studies that have found reductions in FA associated with the total cerebral lesion volume (Ge et al., 2004) and with local lesion load (Ciccarelli et al., 2003). A correlation does not necessarily imply a causal relationship, but a strong spatial association between connectivity and MRI-visible lesion was found in this study. MS lesions are likely to be responsible for changes in connectivity. Wilson and colleagues found that whilst the integrity of pyramidal tracts in MS patients showed some correlation with EDSS, a much stronger relationship was found with a specific pyramidal functional score (Wilson et al., 2003). Thus, establishing the integrity of relevant pathways in MS can potentially provide a great deal of insight into the clinical disability and the differences found in brain activation of specific functions.

How much these connectivity measures are influenced by demyelination still needs to be determined with the accurate measures of any demyelination that may be present. This may be possible by determining the short T₂ component in brain imaging that might relate to the myelin content (Moore et al., 2000; Webb et al., 2003).

Further experiments may be able to demonstrate the relationship of anatomical connectivity measured by diffusion tensor imaging to functional activation and functional connectivity between networks involved in specific tasks.

8.6 Limitations of the study

There are a number of potential limitations that should be considered.

Multiple Sclerosis by its nature is a heterogeneous condition. There is variability in the clinical picture as well as the lesion distribution within the brain. When studying such a group, the responses of the brain may reflect this variation, and this can limit the ability to detect important changes. This may be counteracted in part, by being much more selective with the patients recruited to take part in the study. In any future study, limiting the study to those that fulfill narrow clinical criteria in with clearly defined mild-moderate cognitive impairment, would provide a greater sensitivity for detecting any potential beneficial effect of cholinergic enhancement on both fMRI and behavioral measures.

The sample sizes in this study are relatively small. Although power calculations were performed prior to and during the study, these were based on the numbers needed to detect differences of activation within fMRI. The behavioral measures were not of sufficient sensitivity to detect any changes. A future study with a larger number of subjects may help overcome the lack of statistical power in the behavioral endpoints.

The assessment of drug effect on the BOLD response is always a difficult one. The study of this in the rat model showed only small, but significant changes. There is a

limit to the extent that this can be generalized to humans and MS patients. The small, but significant change suggests that one must be cautious in interpretation of any functional activation during drug administration. In the VEP study, a small significant change detected on electrical recording was not detected by fMRI. This is also important to consider. Further study with a larger group of patients may improve the sensitivity. An alternative may be to apply simultaneous recording of EEG with fMRI, so that one can be certain that task conditions remain identical for each modality.

8.7 Final Conclusions

The experiments presented in this thesis attempted to investigate the activation, modulation and reorganisation of the brain in multiple sclerosis during cognitive processing. Although previous studies have demonstrated different patterns of cognitive-related brain activity in MS patients, the nature of these differences is unclear. Studies on patients further characterized the nature of the activation changes, and the extent to which they could be modulated by cholinergic enhancement. A clear deficit of cognitive processing was shown for verbal working memory that may be compensated by increased functional connectivity, limiting any decline in performance. Abnormal patterns of activation seen in MS, were modulated by rivastigmine, although no behavioural improvements were noted. Studies on normal subjects were used to investigate the potential effect of cholinergic blockade on the same cognitive tasks. The administration of hysocine resulted in alterations of brain activity patterns similar to that found in MS patients. Experiments in a rat model of direct cortical stimulation demonstrated small changes in the BOLD response with rivastigmine administration, and allowing more confident interpretation of the functional imaging results. The visual stimulation study suggested that functional

imaging might be less sensitive to drug effects than electrical recording. Section 7 demonstrated that probabilistic tractography may be useful in mapping connectivity in MS patients in particular to functional activation changes.

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