Functional MRI Studies of Memory, Attention and Treatment Response in Prodromal Alzheimer's Disease.

Thomas M. Dannhauser, MBChB, MRCPsych.
Department of Mental Health Sciences
University College London

Supervisors:
Dr Zuzana Walker
Dr Sukhwinder S. Shergill

Thesis submitted for the degree of Doctor of Philosophy (Ph.D)
at University College London

January 2011

"Physically speaking, we humans are rather unimpressive specimens, as organisms go….We have come to dominate the earth only by grace of one rather important specialization – the brain"

- Isaac Asimov

"Recent studies like those of Pearson in England, Mach in Germany, and Henri Poincaré in France, agree rather with Hume than with Kant: all science, even the most rigorous mathematics, is relative in its truth. Science itself is not worried about the matter; a high degree of probability contents it."

- Will Durant

I, *Thomas M Dannhauser*, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Previously published materials have been reproduced with permission from the copyright holders and where information has been derived from other sources, it has been appropriately indicated.

Abstract

BACKGROUND: Alzheimer's disease (AD) is characterised by initial episodic amnesia followed by deficits in divided attention. Amnestic mild cognitive impairment (AMCI), considered prodromal for AD, is characterised by isolated amnesia but attentional deficits have also been observed. Amnesia in AD is ascribed to medial temporal lobe pathology; however, attention deficits are likely due to lesions in other areas that demonstrate early AD neuropathology e.g. prefrontal cortex and basal forebrain cholinergic system. These lesions could contribute to episodic amnesia and causes attention deficits in prodromal AD. Acetylcholinesterase inhibitor drugs are used as treatment in AD and their effects on memory and attention remain to be determined in AMCI.

METHODS: Functional magnetic resonance imaging (fMRI) was used to study divided and selective attention, and verbal episodic memory in AMCI (n=20) by comparison to controls (n=10). AMCI were followed to identify those that progressed to AD. A controlled treatment trial was conducted to study the effects of the acetylcholinesterase inhibitor rivastigmine.

RESULTS: The AMCI sample had a high rate of progression to AD (n=12). AMCI demonstrated slower reaction times and altered cortical activation during divided attention. Reaction times correlated inversely with default mode network activation. Cortical activation was altered during selective attention. Verbal amnesia was associated with semantic deficits that correlated with altered cortical activation. Rivastigmine improved reaction times on divided attention but did not affect memory.

CONCLUSIONS: Divided attention is impaired and selective attention processing altered in prodromal AD. Verbal amnesia appears partly related to executive failure. Furthermore, correlations between memory and attention deficits suggest that executive failure contributes to deficits across cognitive domains in prodromal AD. Activation changes in prodromal AD during memory and attention processing suggest impaired regulation and failure of cortical resources. Rivastigmine improves divided attention via enhanced cortical processing but it does not benefit verbal episodic memory.

Key words: Alzheimer's disease, amnestic mild cognitive impairment, episodic memory, divided attention, prefrontal cortex, executive functioning, rivastigmine, fMRI.

Contents

List of Figures

List of Tables

Abbreviations

ACEI	Acetylcholinesterase inhibitor
Ach	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimer's dementia
AllAMCI	AMCI participant group containing all AMCI patients
AMCI	Amnestic mild cognitive impairment – single domain
ANOVA	Analysis of variance
APOE	Apolipoprotein E
BA	Brodmann area
BFCS	Basal forebrain cholinergic system
BOLD	Blood oxygen level dependent
Br	Bias rate
CA	Control attention condition
CoAMCI	AMCI group comprised of all patients that progressed to AD
CSF	Cerebrospinal fluid
DA	Divided attention condition
DLPFC	Dorsolateral prefrontal cortex

FAR	False alarm rate
FMRI/fMRI	Functional magnetic resonance imaging
HR	Hit rate
IPL	Inferior parietal lobe
MCI	Mild cognitive impairment
MMSE	Mini Mental State Examination
MTL	Medial temporal lobe
NART	National Adult Reading Test
NFT	Neurofibrillary tangle
NxAMCI	Untreated AMCI group
PET	Positron emission tomography
PFC	Prefrontal cortex
Pr	Corrected recognition rate
r	Pearson's correlation coefficient
R^2	Coefficient of determination
RivAMCI	AMCI group treated with rivastigmine
RT	Reaction time
§	Section

SD	Standard deviation
VLPFC	Ventrolateral prefrontal cortex

Original Papers

I. Dannhauser TM, Walker Z, Stevens T, Lee L, Seal M, Shergill SS. The functional anatomy of divided attention in amnestic mild cognitive impairment. Brain 2005; 128: 1418-1427

II. Dannhauser TM, Shergill SS, Stevens T *et al*. An fMRI study of verbal episodic memory encoding in amnestic mild cognitive impairment. Cortex 2008; 44: 869-880

Conference Proceedings

Dannhauser, Shergill, Walker, Seal, Stevens, Lee (2005), "Divided attention in Amnestic Mild Cognitive Impairment", paper given at British Neuropsychiatry Annual Conference, London, 10/2/2005.

Dannhauser, Walker, Shergill (2005), "The functional anatomy of divided attention in amnestic mild cognitive impairment", poster presented at EPOS workshop "Control in Attention and Action", Amsterdam.

Dannhauser, Shergill, Walker (2006), "An fMRI study of verbal episodic memory in amnestic mild cognitive impairment", poster presented at the International Conference on Alzheimer's Disease, Madrid, 15/7/2006

Dannhauser, Shergill, Dharendra, Walker (2008), "Cholinesterase inhibitor treatment improves speed but not accuracy in prodromal AD, an fMRI study", poster presented at the annual DeNDRoN conference, Newcastle, 14/10/2008.

Acknowledgments

This work would not have been possible without the contributions of the people who were kind enough to get involved and assist in carrying it out. I extend my deepest gratitude to everyone who helped to make this possible. I would especially like to thank:

Zuzana Walker, my supervisor at UCL, for her friendship and for giving me the opportunity to get involved in this research, for teaching, supporting and encouraging me.

Sukhwinder Shergill, my co-supervisor at the Institute of Psychiatry, for his friendship, support, advice, humour and for providing an alternative view which allows depth of vision.

Vincent Giampietro, for always making time to help, for his friendship and for teaching me the finer points of XBAM.

Michael Brammer, for enlightening me about the potential and limitations of statistical analyses and for his interest and contribution to our work.

Marc Seal for his patience, paradigm and for showing me the ropes at the IOP.

Rodney Walker for his friendship, interest and precise editing.

All the *patients and volunteers* who took part in the studies for their interest and efforts.

Staff at the Derwent Memory Clinic especially *Tim Stevens* and *Lean Lee* for their friendship and support.

The staff at the *MRI Unit at the Institute of Psychiatry* and Centre for Neuroimaging studies, especially *Chris Andrew, Fernando Zelaya, Heather Hippwell* and the *radiographers*.

My family in London, *Faye, Lucas, Cara, Jeff and Elaine* for giving me their love, understanding and support.

My parents, for their love and support and for providing the environment that stimulated my interest in science.

This research was made possible by the cooperation of two academic institutions, *University College London* and *The Institute of Psychiatry at King's College London*, and The North Essex Partnership Foundation NHS Trust which provided logistic support and from where subjects were recruited.

The study was partly funded by an unrestricted grant from *Novartis plc*.

1. Introduction

The aims of the work reported in this thesis were to study the neural correlates of verbal episodic memory, selective and divided attention, and drug treatment response in prodromal *Alzheimer's dementia* (AD). We therefore employed *functional magnetic resonance imaging* (fMRI) to study these cognitive processes and *acetylcholinesterase inhibitor* (ACEI) treatment responses in patients with *amnestic mild cognitive impairment* (AMCI), a prodromal stage of AD.

- Section 1.1 introduces AD and considers the merits and methods of identifying preclinical and prodromal AD.
- Section 1.2 introduces AMCI and concludes with a discussion on the validity of considering AMCI as prodromal for AD.
- Section 1.3 examines controversies in *distinguishing AD and AMCI from normal ageing* and theories accounting for cognitive decline in ageing are discussed.
- In Section 1.4, *episodic memory*, the brain networks that underpin it and relevant neuropsychological concepts are introduced. This is followed by a discussion of episodic amnesia in AMCI and of findings from functional neuroimaging studies of verbal and picture encoding tasks.
- Section 1.5 introduces *attention* by looking at relevant neuropsychological aspects and research findings in AMCI. This is followed by a discussion on the functional anatomy of attention in AMCI.
- In Section 1.6, cognitive regulation is introduced by looking at the functional relationships between the *central executive, basal forebrain cholinergic system, prefrontal cortex, brainstem locus coeruleus, memory and attention*.

- Section 1.7 discusses the role of *acetylcholine* in attention and memory, *acetylcholinesterase inhibitor treatment*, and the status of acetylcholinesterase inhibitor treatment in AD and AMCI.

1.1 Alzheimer's dementia

Alzheimer's dementia is currently the leading cause of dementia, engendering huge personal and financial costs to individuals and society. This section summarises the epidemiology, clinical characterisation, aetiology and neuropathology of AD, with the aim of providing the necessary background on which the concept of AMCI as a high-risk and often prodromal state for developing AD will be considered.

1.1.1 Epidemiology and Clinical Characterisation.

Dementia is characterised by acquired cognitive deficits sufficient to impair activities of daily living. Globally, more than 25 million people are affected by dementia and this is set to rise to 40 million by 2020, doubling in prevalence every 20 years (Ferri et al., 2005). The prevalence of dementia is 1% in individuals aged 60-64 years but it increases almost exponentially with age leading to a prevalence of more than 20% in people aged over 85 (Blennow et al., 2006). AD remains the leading cause of dementia accounting for 50-60% of cases while other common causes of dementia include vascular pathology and diffuse Lewy body disease. AD is therefore common and the associated human and financial costs make it a major health problem. Figure 1.1 illustrates the predominance of AD as underlying dementia aetiology and also the relative prevalence of the high-risk states for developing AD.

The first symptom of AD is forgetfulness (amnesia) and this is followed by minor difficulties in everyday functioning (dyspraxia), speech (dysphasia), and object recognition (agnosia). AD is clinically characterised by an initial amnestic syndrome followed by the gradual emergence of deficits in other cognitive domains. Amnesia is usually followed by deficits in attention, then abstract reasoning, language and visuospatial ability (Grady et al., 1988; Hodges and Patterson, 1995). Progressive cognitive impairment causes functional and behavioural decline leading to severe disability and eventually death (McKhann et al., 1984).

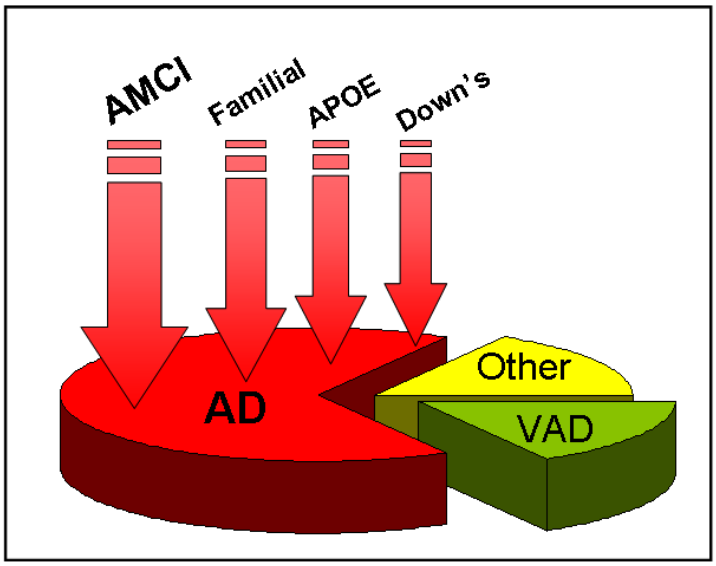


Figure 1. Dementia epidemiology and the high-risk states that contribute to AD.

AD remains the leading cause of dementia followed by vascular and other causes; however, isolated neuropathology of any specific type occurs rarely and AD is most often accompanied by vascular neuropathology. AMCI represents the most prevalent identifiable high-risk group where prodromal AD can be studied and is associated with a 10 – 15 % annual conversion rate (Blennow et al., 2006; Petersen et al., 1999).

1.1.2 Aetiology and Neuropathology

Although there is a wealth of information on AD, the aetiology of the sporadic form accounting for 99% of cases remains unclear. Risk factors for AD include ageing, decreased brain reserve, and factors associated with vascular disease including hypercholesterolemia, ischaemic heart disease, hypertension, obesity, smoking, atherosclerosis, and diabetes (Mayeux, 2003). It is unclear at this stage which of the risk factors contribute directly to the onset and progression of AD and which renders the brain more vulnerable to the effects of AD neuropathology thereby precipitating the clinical syndrome. The early onset familial form of AD is autosomal dominant and is caused by mutations in the genes for the amyloid precursor protein, presenilin 1 and 2. Presenilin 1 mutations account for 60-70% of cases (Blennow et al., 2006).

Genetic factors have been identified that play a role in the sporadic form of AD. An increased risk and earlier age of onset is found in carriers of the apolipoprotein Є4 (APOE4) allele (Farrer et al., 1997). The risk appears dose-dependent with the lowest risk associated with no APOE4 allele whilst the risk is 3 times higher in heterozygotes and 15 times higher in homozygotes for APOE4. The risk is also increased in first-degree relatives of those with sporadic late onset-AD (relative risk 3.2) (Fratiglioni et al., 1993). The frequency of APOE4 is double (47%) in familial late-onset cases of AD compared to the sporadic form thereby accounting for some but not all of these cases (Lannfelt et al., 1994). It therefore appears that there are still some undiscovered heritability factors accounting for the remainder of familial cases.

The *aetiology of the sporadic form of AD* remains unclear and the three leading hypothesis are related to the typical neuropathological lesions namely *amyloid or*

senile plaques, *neurofibrillary tangles* and *vascular lesions*. We will look at each of them in turn.

Amyloid

The *amyloid hypothesis* of AD neuropathology proposes that extracellular accumulation of *beta-amyloid peptide* (Aβ), as both the soluble Aβ fibrils and insoluble Aβ plaques, contributes to neurotoxicity and disrupts neuronal functioning resulting in the clinical syndrome of AD (Hardy and Selkoe, 2002). This is supported by genetic studies, which indicate that familial types of AD relate to mutations of the amyloid precursor protein (precursor to Aβ) and the generating enzyme (presenilin). Further support comes from findings of early Aβ plaque formation in the trisomy of Down's syndrome where an extra copy of the amyloid precursor protein gene is present, which may lead to increased protein production thereby increasing the substrate for Aβ (for a review see (Blennow et al., 2006)).

Tauopathy

On the other hand, recent neuropathological studies indicate *tauopathy* as the initial lesion in AD. Tauopathy is the hyperphosphorylation of microtubule-associated tau protein that results in intracellular accumulation of toxic tau and disassembly of microtubules leading to *neurofibrillary tangle* (NFT) formation. NFTs impair axonal transport and function. The earliest NFTs appear in the basal forebrain cholinergic system (BFCS), which is comprised of the nucleus basalis of Meynert, diagonal band of Broca and medial septal nucleus (Mesulam et al., 2004). The BFCS provides almost all cholinergic (acetylcholine) input to the cerebral cortex, entorhinal cortex, amygdala and hippocampus, the later being crucial for the formation of episodic

memory which is characteristically impaired in AD (Squire and Zola, 1996). NFT density and spread in entorhinal and hippocampal regions show inverse correlation with hippocampal volume and cognitive performance (MMSE scores) in AD (Jack et al., 2002). NFT density in the *ventromedial temporal lobe* (entorhinal cortex, hippocampus area CA1, amygdala and subiculum) and *inferior parietal lobe* distinguishes between normal, AMCI and AD subjects, and episodic memory (delayed word recall) shows significant negative correlation with the NFT counts in entorhinal cortex (r=-0.44) and hippocampus (r=-0.50) (Markesbery et al., 2006; Mitchell et al., 2002). NFT density and pre-tangle tau accumulation in *nucleus basalis of Meynert neurones* similarly distinguish these groups and correlates negatively with delayed word recall (Mesulam et al., 2004). There are therefore very significant associations between tauopathy in BFCS and medial temporal lobe (MTL) structures, episodic memory impairment and global cognitive functioning spanning the transition from normal ageing to AMCI and AD. These findings add weight to the claim the tauopathy is the leading neuropathological lesion in AD. Impaired cholinergic neurotransmission due to BFCS pathology and the strong associations between memory and cholinergic function provide the rationale for treating AD with compounds that prolong the effects of acetylcholine in synapses. Furthermore, the apparent gradual accumulation and presence of NFTs in the BFCS and the correlations with memory function provides the rationale for similar treatment in AMCI.

Figure 1.2 shows extracellular neuritic plaques and intraneuronal tangles visible in the same slice demonstrating that these two pathological features co-occur in established AD. Neuritic plaques, also know as senile plaques, consist of a central extracellular core of amyloid surrounded by swollen abnormal neuroglia and

neurones. NFTs consist of intracellular paired helical filaments of tau protein that eventually occupies almost the entire cell volume.

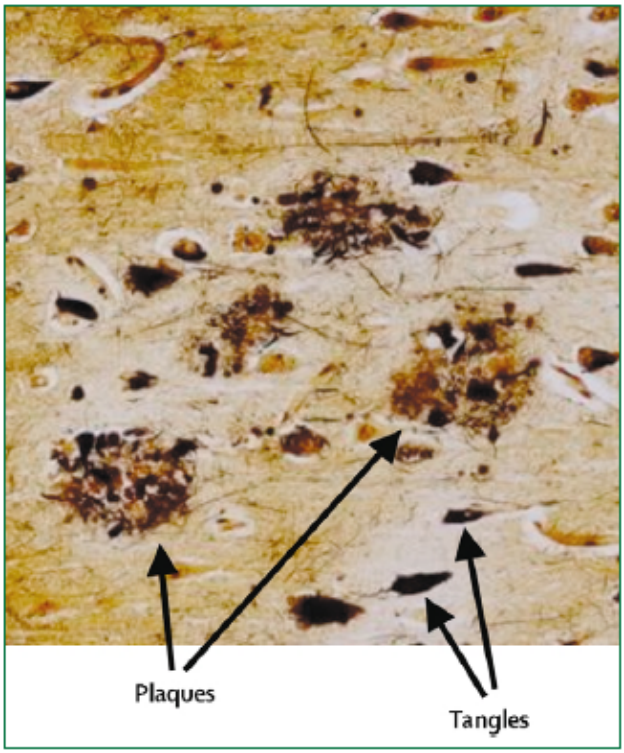


Figure 2. Neuritic plaques and neurofibrillary tangles in AD.

The light microscopy preparation depicted above has been stained to reveal plaques and tangles in AD in the cerebral cortex. Aβ forms extracellular plaques surrounded by neuroglia. Tangles, formed of hyperphosphorelated tau, are visible intracellular in neurones (Blennow et al., 2006). Reproduced from The Lancet, with permission from Elsevier.

Neurovascular pathology

Strong associations exist between AD and neurovascular risk factors such as hypercholesterolemia, ischaemic heart disease, hypertension, obesity, smoking, atherosclerosis, and diabetes (Mayeux, 2003). Furthermore, ischaemic stress due to

cerebrovascular disease and hypotension results in increased amyloid precursor protein expression and Aβ deposition in the cerebral artery boundary zones that are most vulnerable to hypoxia. These findings have lead to the *neurovascular hypothesis* of AD (Jendroska et al., 1995). A recent study reported increased hippocampal Aβ deposition and apolipoprotein expression following MTL ischaemia related to nearby focal cerebral infarct in adults not meeting histopathological criteria for AD (Qi et al., 2007). Ischaemia also increases the expression of pre-tangle tau epitomes in neurones and neuroglia in animal models and human brains after occlusive stroke events; however, NFTs have not been observed and this may be related to the degree and duration of hypoxia or activation of different chemical cascades (Uchihara et al., 1995; Uchihara et al., 2000). These and similar studies have lead some authors to argue that cerebral hypoxia may play a pivotal role in AD (Castellani, 2007).

The respective contributions to the initiation and progression of AD from these three leading neuropathological processes have not been clearly established and all three appear together in cortical areas in established AD. Other possible neuropathological mechanisms include inflammatory and infectious agents. Neither NFT nor Aβ is specific to AD as the former also occurs in other neurodegenerative disorders and the latter in normal ageing. Recent studies of brain amyloid deposition indicate that not everyone with increased amyloid load progress to AD but that most individuals with AD have increased amyloid (Jack et al., 2009; Kemppainen et al., 2007). However, tauopathy with NFT formation causes dementia in the absence of amyloid in non-AD neurodegenerative disorders including fronto-temporal dementia, cortico-basal degeneration, Pick's disease and progressive supranuclear palsy (Iqbal et al., 2005; Iqbal and Grundke-Iqbal, 2008). Furthermore, NFT density in the MTL lobes shows

inverse correlation with episodic memory performance in normal ageing, AMCI and AD, therefore apparently accounting for memory changes in all these states (Guillozet et al., 2003). The relevance of these findings to the work reported in this thesis, is that tauopathy appears to accumulate gradually in the BFCS and MTL and correlates with cognitive functioning thereby providing a neuropathological background for distinguishing different stages in the progression of AD and for acetylcholinesterase inhibitor treatment trials in predementia stages. In the following section, we discuss the necessity of early diagnosis and intervention in AD and the validity of AMCI as a prodromal stage of AD.

1.1.3 Identifying Preclinical and Prodromal AD

1.1.3.1 The case for reliable biomarkers in AD

Identifying sensitive and specific biomarkers is essential in the fight against AD. The low efficacy of currently available treatments for AD drives the search for reliable preclinical biomarkers to aid the development of interventions that arrest disease progression early in the course of AD. Efforts to develop such disease arresting treatments are complicated by the lack of specific biomarkers and at present a neuropathological diagnosis of AD at autopsy remains the gold standard (McKhann et al., 1984). Even the gold standard has limitations as *specificity* is reduced by the presence of significant cerebrovascular disease in around 50% of clinically diagnosed, autopsy positive cases, and as few as 30% of AD cases have isolated AD neuropathology. Cerebrovascular disease is an important comorbid condition because it contributes to cognitive decline and dementia and its typical symptoms overlap with AD (Kalaria, 2002; Roman, 2003). Ischaemic interruption of subcortical-prefrontal

circuits in cerebrovascular disease impairs executive control of working memory, attention, language, affect, motivation, and complex organisational and construction skills resulting in forgetfulness, changes in speech, affect and mood, similar to what can occur in AD (for a review see (Roman et al., 2002).

The *sensitivity* of the neuropathological diagnosis for AD is also poor as 20-40% of cognitively normal elderly cases are autopsy positive for AD. This highlights the current lack of understanding of the contributions made to the clinical presentation of AD by the two leading neuropathological lesions. These findings have lead to calls for the diagnostic criteria of AD to be revised and to include biomarkers (Blennow et al., 2006). Suggested biomarkers include structural neuroimaging (MTL volume), functional and molecular neuroimaging (positron emission tomography (PET) of cortical glucose metabolism and beta-amyloid) and cerebrospinal spinal fluid markers. Although these biomarkers may improve the sensitivity and specificity of diagnostic criteria for AD, the problem of identifying and quantifying the contributions from comorbid disorders remains. This in turn complicates the design of treatment studies, such as the work reported here, and limits the conclusions that can be drawn.

1.1.3.2 Neuropathological disease progression in AD

Preclinical and prodromal AD stages may exist because AD neuropathology appears to accumulate gradually thereby providing opportunities for early investigations and interventions. The functional brain changes and the progression of symptoms in AD could be ascribed to the presence and accumulation of AD neuropathology. This is supported by the strong inverse correlations between cognitive performance and neuropathology load (Arnold et al., 1991; Samuel et al., 1994; Terry and Katzman, 2001). For example, the strong inverse correlation between dementia severity and

synapse density in frontal cortex in autopsy confirmed AD suggests, by extrapolating backwards, that there is lesser but still significant reductions in synapse density in the prodromal and possibly preclinical stages. Further support for this comes from studies examining the basal forebrain cholinergic system (BFCS) where AD neuropathology seems to appear first. Autopsy studies have reported negative correlations between NFT density and pre-tangle tau accumulation in nucleus basalis of Meynert neurones and episodic memory (Mesulam et al., 2004). Similar negative correlation has been found between the low affinity p75 neurotrophin receptor (a marker of cholinergic neurones in the nucleus basalis) and measures of global cognition, working memory and attention in mild cognitive impairment and mild AD (Mufson et al., 2002). Furthermore, brain derived neurotrophic factor (important for nucleus basalis neurone survival) and its precursor (proBDNF) are reduced in the cortex of patients with MCI and AD, leading to their conclusion that "from a neurotrophic pathobiologic perspective, MCI is already early AD" (Mufson et al., 2007).

Taken together these results appear to indicate that AD is preceded by a preclinical phase where cognitive functioning appears normal but functional brain activation is altered due to accumulating AD neuropathology.

1.1.3.3 The role of functional neuroimaging in studying prodromal AD

Functional neuroimaging may aid in identifying and studying prodromal AD. Neuropathology accumulates gradually over many years in AD and functional neuroimaging has been used to determine if brain metabolism is altered in the preclinical phase of AD (Braak and Braak, 1997; Ohm et al., 1995). The potential for functional neuroimaging to reveal brain changes that precede the onset of symptoms is based on findings of altered activation in the face of maintained behavioural

performance in disease states and normal ageing (Becker et al., 1996; Jaeggi et al., 2007; Reuter-Lorenz et al., 2001). Less efficient cortical processing is associated with increased and/or decreased activation in task related cortical areas and with altered activation in functionally connected areas. Decreased activation in task related areas is thought to indicate *less efficient processing* whilst increased activation reflects *reallocation of neural resources*, *functional reorganisation* (where new areas take on the role of diseased areas), or deployment of *alternative cognitive strategies* (Han et al., 2009). Greater activation in task related areas, correlating with better performance, have been reported in several studies of normal adults. In healthy older adults, there is a shift from lateralised towards bilateral activation and this is often associated with better performance. These findings form the basis of the "Hemispheric asymmetry reduction in older adults" or HAROLD model (Cabeza, 2002). A study specifically designed to examine the causes of additional activation in contralateral areas was conducted using older adults who were matched on memory task performance by varying the number of words in a list that had to be encoded in order to achieve 75% accuracy. The task was parameterised and increased activation, in line with predictions based on the HAROLD model, was found during the more difficult condition, leading the authors to concluded that *increased activation in additional areas was related to extra attentional and monitoring demands required for superior performance* (Anderson et al., 2002).

Taken together, these findings indicate that functional neuroimaging can be used to demonstrate compensatory functional brain changes associated with maintained behavioural performance and activation changes associated with behavioural deficits. These methods therefore appear potentially useful in studying populations at high-risk

for diseases and disorders that have a long preclinical and/or prodromal stage, such as AD, where brain function is compromised but performance maintained preclinically due to automatic compensatory functions. We next look at the results from functional neuroimaging studies in high-risk, asymptomatic AD groups.

1.1.3.4 Functional neuroimaging studies of high-risk states for AD

Functional neuroimaging studies have been conducted on cognitively healthy *middle-age* adults at increased genetic risk of AD in order to look for evidence of compensatory brain activation that may indicate the presence of early pathology. Subjects at risk by virtue of a positive family history of AD (at least one first-degree relative with clinical AD) and APOE4 status (at least one APOE4 allele) demonstrated reduced activation in mid- and posterior inferior temporal areas during the recall of items from both working and long-term memory whilst still matching the performance of controls (Smith et al., 1999). The authors suggested that attenuated activation in task related areas was the consequence of local or distant neuropathology that reduced neural inputs to these areas. In contrast, greater extent and magnitude of activation in left hippocampal, parietal and prefrontal areas were reported in a similar high-risk group (APOE4 carriers) during the encoding of word pairs (Bookheimer et al., 2000). The authors concluded that the additional activation most likely reflects compensatory recruitment of extra neurones or increased firing rates of neurones required to match the performance of controls. This conclusion accords with the neural efficiency hypothesis, which posits that cortical activation correlates inversely with experience and cortical specialisation on a given task, and the findings therefore imply failing resources and/or loss of specialisation in the high-risk group (Haier et al., 1988; Neubauer and Fink, 2009). A recent large cross sectional study examined

the contributions of first-degree family history of AD and APOE4 carrier status on brain activation during encoding in middle aged adults (Johnson et al., 2006b). Activation was reduced in the MTL in APOE4 carriers without a family history and further reduced in carriers with a positive family history. The authors suggest that a yet unidentified inheritance factor contributes to the effects of APOE4 on brain activation during encoding. The findings from these studies in middle-aged cognitively healthy but high-risk groups reveal altered cortical activation when matching the performance of controls. This suggests that cortical resources may already be affected by early neuropathology in the preclinical stages of AD resulting in compensatory efforts in the face of failing resources. The findings of increased and decreased activation also highlights the fact that changes in regional brain activity should preferably be correlated with behavioural measures because our current understanding allows only limited interpretation of activation changes in isolation.

Studies in *older adults* have also been conducted with similar findings. A large cross sectional study of asymptomatic offspring of autopsy confirmed, late-onset familial AD cases (n=95, age range 50-75 years) demonstrated greater activation in frontal and temporal cortex during a memory encoding task compared to age matched controls (n=90) (Bassett et al., 2006). These findings occurred independently of APOE4 status. The effects of APOE4 carrier status on brain activation in older adults were demonstrated by a study comparing groups with (n=10) and without (n=10) APOE4. Similar findings of increased brain activation during memory encoding were reported, suggesting that the influence of APOE4 on activation appeared independent of age-related memory deficits or atrophy (Bondi et al., 2005).

The findings from these studies in cognitively healthy middle-aged and older adults appear to indicate that functional brain changes precede the symptomatic expression of AD.

1.1.3.5 Prodromal syndromes in the study of disease progression

Clinical syndromes, consisting of hallmark or characteristic initial symptoms of the disorder or disease of interest, are frequently used to diagnose and study prodromal disease stages. Interest in the transition between normal ageing and dementia has increased and so has the number of suggested prodromal syndromes spanning this gap. In the presence of underlying disease, normal cognitive functioning may initially be followed by *subjective cognitive impairment* where early neuropathology is overcome by compensatory mechanisms resulting in maintained cognitive function but increased cognitive effort (Rodda et al., 2009). This may in turn be followed by the onset of the first objective symptoms such as episodic amnesia, thereby meeting the criteria for a diagnosis of *amnestic mild cognitive impairment* (AMCI) which is discuss in detail in the following section (§1.2). Subjective cognitive impairment is likely to be more heterogeneous than AMCI but both are predictors of late-onset AD (Heun et al., 2006b; van Oijen et al., 2007).

Prodromal stages have also been used to study other neuropsychiatric disorders including schizophrenia where a prodromal stage signified by psychosis has been identified. The use of structured interviews to identify high-risk individuals results in high inter-rater reliability and accuracy for distinguishing schizophrenia from relevant differential diagnoses and from controls. High-risk subjects identified with these methods have conversion rates of around 50% at one-year follow-up (Miller et al., 2002; Woods et al., 2009; Woods et al., 2001). Functional neuroimaging

studies of such high-risk groups have demonstrated deficits in frontotemporal connectivity similar to that found in schizophrenia (Broome et al., 2009; Crossley et al., 2009). Functional neuroimaging has also been used to study the effects of atypical antipsychotic medication in a high-risk group. Treatment effects were found in task related cortical areas, thereby demonstrating the potential of these methods to study the cortical effects of pharmacological interventions (Fusar-Poli et al., 2007). It is therefore apparent that both prodromal stages and treatment responses can be examined using functional neuroimaging. In this thesis, similar methodology was employed to identify and study AMCI as prodromal for AD.

Summary

AD neuropathology accumulates gradually and appears to affect cortical processing in preclinical and prodromal stages. High-risk asymptomatic stages are associated with altered brain activation changes as demonstrated by functional neuroimaging. Altered brain activation in these high-risk stages is likely related to failing resources, damaged by AD neuropathology, and to compensatory efforts that maintain cognitive performance. Studying brain activation in high-risk populations can improve our understanding of disease progression in AD and may reveal the effects of treatment on brain function. These approaches are necessary, as our current understanding of AD has not enabled the development of satisfactory preventative, curative or symptomatic treatments. In the next section, we consider the validity of AMCI as a high-risk and prodromal stage of AD.

1.2 Amnestic Mild Cognitive Impairment

The mild symptomatic phase of AD that precedes the fully developed dementia syndrome, with implicit loss of personal autonomy, does not have any official clinical standing (Dubois and Albert, 2004). *Amnestic mild cognitive impairment* (AMCI) has emerged as one of the syndromes that span the gap between normal ageing and AD. AMCI diagnosed with sufficient clinical rigor can be highly predictive of AD. It is a refinement of *mild cognitive impairment* (MCI) which was initially conceived to span the gap between normal ageing and dementia in general (Flicker et al., 1991). In this section, we consider the construct validity of AMCI as prodromal AD by examining its epidemiology, clinical characterisation and neuropathology.

1.2.1 Clinical Characterisation and Epidemiology

AMCI denotes a prodromal stage of AD and it has been used to study disease progression in AD as it represents a high-risk state for conversion to AD. It stands to reason that the clinical utility of identifying a prodromal disease stage is in allowing early intervention, and the accuracy with which a prodromal stage is identified and distinguished from other confounding disorders depends on the sensitivity and specificity of the disease markers employed. AMCI thought to be prodromal for AD has recently been further refined to *AMCI-single domain*, which is characterised by isolated episodic memory impairment in the absence of other obvious cognitive or behavioural deficits and not related to pre-existing physical or emotional disorders. The current most widely used *diagnostic criteria for AMCI include:*

1. Memory complaint corroborated by an informant.
2. Objective memory impairment for age.
3. Essentially preserved general cognitive function.

4. Largely intact functional activities.
5. Not meeting criteria for dementia (Petersen, 2004).

Several further clarifications in conjunction with these criteria have been suggested to improve the construct validity of *MCI* as prodromal for dementia (Werner and Korczyn, 2008). These additional clarifications appear equally important in improving the construct validity of AMCI as prodromal AD. First, other causes of cognitive impairment such as vitamin B12 deficiency should be excluded along with dementia per se, as mentioned in the criteria. Second, the cognitive impairment should be of recent onset in order to exclude impairments associated with brain trauma and mental retardation. Third, it should be made clear to those diagnosed with AMCI that, although they are at high-risk of progressing to dementia, it is not a certainty. The first two additional clarifications were already incorporated into suggested criteria for identifying MCI that progress to AD (Dubois and Albert, 2004).

The other subtypes of AMCI include *AMCI-multiple domains*, *non-AMCI-single domain* and *non-AMCI-multiple domains*; each subtype respectively considered a prodromal stage for AD or vascular dementia; dementia with Lewy bodies or frontotemporal dementia; dementia with Lewy bodies or vascular dementia (Petersen, 2004). These were previously included under MCI and explains the aetiological heterogeneity of MCI.

AMCI appears to represent a high-risk and prodromal state for AD with a 10-15% annual conversion rate compared to 1-2% in healthy elderly. The most compelling argument for AMCI as a high-risk prodromal state comes from longitudinal studies which report a 80% dementia conversion rate after 6 years of follow-up (Fischer et al., 2007; Ganguli et al., 2004; Petersen, 2004; Petersen et al.,

2001; Petersen et al., 1999). Despite this high conversion rate, not all AMCI patients convert but it appears to be a reliable and prevalent risk indicator (Fischer et al., 2007).

The prevalence for AMCI varies between 3% and 19% in individuals aged over 65. Not surprisingly, higher prevalence rates have often been reported in cross-sectional studies, where AMCI had been diagnosed after interview and examination, compared to cohort studies where criteria had been applied retrospectively to data collected from general questionnaires and cognitive tests (Ganguli et al., 2004; Gauthier et al., 2006; Gavrila et al., 2009; Low et al., 2004). Taken together, available findings on AMCI indicate a high prevalence rate, particularly in clinical populations, and a high dementia conversion rate, suggesting that sensitive and specific diagnostic methods could identify a group that may in future benefit from disease specific interventions.

1.2.2 AD Neuropathology in AMCI

Available evidence strongly suggests that AD neuropathology accumulates gradually for many years prior to the onset of the clinical syndrome and this provides an opportunity to identify, study and treat preclinical and prodromal stages. AD is neuropathologically characterised by numerous amyloid (neuritic) plaques and NFTs in the cerebral cortex, hippocampus, amygdala, nucleus basalis, locus coeruleus and hypothalamus (Markesbery et al., 2006). These changes are accompanied by substantial neuronal loss and subsequent cortical atrophy. NFTs and plaques also occur in normal ageing; however, findings from combined neuropathological and structural neuroimaging studies in health and AD indicate that neuropathological measures (NFT density and spread in entorhinal and hippocampal regions) correlate

inversely with hippocampal volume (r=-0.6) and cognitive performance (MMSE; -0.7) in AD but not in normal ageing (Jack et al., 2002). These neuropathological markers therefore suggest that AD is qualitatively different from normal ageing and not associated with hippocampal atrophy. Furthermore, the correlations indicate a gradual accumulation of AD neuropathology and this suggests that a prodromal AD stage will exist where the pathological load is lower and the clinical syndrome less pronounced.

Complaints of forgetfulness and the presence of episodic amnesia make AMCI clinically suggestive of prodromal AD. The notion that AMCI is in fact prodromal AD is supported by reports of inverse correlations between cognitive performance and NFT densities in medial temporal lobe (MTL) areas where AD neuropathology first appears (nucleus basalis of Meynert, entorhinal area). Inverse correlations between MTL NFT densities and episodic memory performance has been demonstrated in normal ageing, AMCI and AD (Guillozet et al., 2003). Furthermore, NFT densities in the ventromedial temporal lobe (entorhinal cortex, hippocampus area CA1, amygdala and subiculum) and inferior parietal lobe distinguished between normal ageing, AMCI and AD, and episodic memory (delayed word recall) correlated inversely with NFT density in entorhinal cortex (r=-0.44) and hippocampus (r=-0.50) (Markesbery et al., 2006; Mitchell et al., 2002). In nucleus basalis of Meynert neurones, NFT densities and pre-tangle tau accumulation similarly distinguished between normal ageing, AMCI and AD, and also correlated negatively with delayed word recall (Mesulam et al., 2004). There are therefore consistent reports of inverse correlations between NFT densities in the MTL and cognition that distinguish normal ageing from AMCI and AD, indicating that AMCI differs from AD quantitatively but not qualitatively in this area.

Neuropathological changes have also been demonstrated in cortical areas outside the hippocampal and basal forebrain areas in AMCI. Increased amyloid plaques are evident in the frontal, temporal, inferior parietal lobe, posterior cingulate gyrus, and amygdala, and increased NFTs are evident in temporal lobes (temporal pole, inferior temporal gyrus, fusiform gyrus), dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex, parietal cortex, amygdala and entorhinal cortex (Guillozet et al., 2003; Markesbery et al., 2006). Areas containing AD neuropathology in AMCI largely overlap with areas of cortical atrophy as demonstrated by volumetric studies. These areas include the, hippocampus, amygdala, parahippocampal gyrus (entorhinal area), fusiform gyrus, medial frontal areas, lateral temporal areas, posterior cingulate and precuneus (for a review see (Ries et al., 2008). A prospective cohort study of AMCI patients (n=34) who progressed to clinically diagnosed AD demonstrated neuropathological AD in 71% of cases (Jicha et al., 2006). Secondary pathologies thought to contribute to the diagnosis were also present (vascular lesion in 33%; Lewy bodies in 25%). Further evidence supporting the gradual emergence of AD neuropathology and the notion that AMCI is prodromal AD comes from neuroimaging studies.

A positron emission tomography (PET) study using a ligand that binds to amyloid and NFTs in vitro found that average binding in temporal, parietal, frontal and posterior cingulated areas distinguished between healthy ageing, AMCI and AD (Small et al., 2006). Furthermore, PET binding showed a similar amyloid and NFT distribution pattern in AMCI as seen in AD. Regional NFT involvement correlates well with behavioural impairment across the spectrum of healthy controls to AMCI to AD, whilst the amyloid load in AMCI is more similar to controls than AD (Petersen et al., 2006). This suggests that the ligand binding distribution pattern found in AMCI by

Small et al. is due to binding with NFTs rather than amyloid. Nevertheless, these findings reveal a consistent pattern of graded neuropathology proceeding from normal ageing to AMCI and AD, supporting the notion that AD neuropathology emerges gradually and that a prodromal and even preclinical stage may be identified.

In summary, the neuropathology in AMCI is qualitatively similar to that seen in AD and quantitatively somewhere between normal ageing and AD. Furthermore, the neuropathology affects areas crucial for memory processing, thereby explaining the high predictive value of episodic memory tests in dementia progression. In the next section, memory and other measures that predict progression to dementia are discussed.

1.2.3 AMCI Represents Prodromal AD

Prodrome is defined as "*an early sign of a developing condition"* or as "*the earliest phase of a developing condition or disease"* (Mosby, 2009). AMCI appears prodromal for AD because of its clinical and neuropathological characteristics, and due to the predictive value of neurocognitive, CSF and structural brain imaging biomarkers in AMCI.

The clinical phenotype of AMCI mirrors that of mild AD which presents with prominent episodic amnesia, measurable deficits in other domains and everyday activities, and progressive cognitive and functional decline (Morris, 2006). Furthermore, in well defined clinical samples, where AMCI is diagnosed using operationalised criteria and norm-based cognitive testing, more than 90% have AD confirmed at autopsy, with or without comorbid pathology (Storandt et al., 2006). It appears that ensuring high construct validity for AMCI as prodromal AD depends on specialist clinical settings that employ objective neurocognitive measures and

operationalised diagnostic criteria. However, even *pre-clinical AD* could potentially be identified based solely on a history of progressive episodic amnesia. A longitudinal study of individuals with informant corroborated complaints of progressively deteriorating memory, who were unimpaired on norm-based cognitive testing, found high conversion rates (91%) to neuropathologically confirmed AD and the mean duration of progression to AD was twice as long as in AMCI (Storandt et al., 2006). These findings indicate that sensitive neuropsychological measures and detailed symptomatic history can identify prodromal AD.

AD conversion rates in AMCI samples vary between clinic (10%-15% per year) and community (3.8% to 4.6% per year) based cohorts and the variation appears related to more advanced disease in clinical cohorts as evident from more pronounced everyday activity deficits (Farias et al., 2009). Impairment in everyday activities may be a motivating factor for seeking help resulting in referral. Although these impairments are subtle and not severe enough to indicate dementia, they nevertheless indicate a high risk of progression to dementia.

AMCI is characterised by episodic memory impairment which, if measured by delayed recall of spoken words or narrative, appears to be one of the most sensitive neuropsychological predictors of progression from normal ageing and AMCI to AD (Artero et al., 2003; Backman et al., 2001; Sarazin et al., 2007; Tierney et al., 1996; Tierney et al., 2005). Results from studies examining the predictive potential of delayed verbal recall measures compared to structural neuroimaging and cerebrospinal fluid (CSF) biomarkers for AD are summarised in **Table 1** (Decarli et al., 2007; Hansson et al., 2006; Korf et al., 2004). It is apparent that both neuropsychological measures and other special investigations can have satisfactory

sensitivity and specificity and that combination of these measures further improves diagnostic validity.

Study	**Measures**	**Se**	**Sp**	**Follow-up duration**	**n**	**Conversion rate**	**Sampling characteristic**
Artero et al. 2003	-Delayed verbal recall -Construction -Category fluency	73	99	2 year	127	30%	Symptomatic memory complaint
Tierney et al. 2005	-Delayed verbal recall -Animal fluency -Information	74	83	5 years	551	14%	Modified MMSE score
Sarazin et al. 2007	-Delayed verbal recall	80	90	3 years	251	24%	MCI
Hansson et al. 2006	-CSF total tau -CSF beta amyloid-42	95	83	4 to 6 years	137	42%	MCI
Korf et al. 2004	-Quantitative MTL atrophy	70*	68*	3 years	75	49%	MCI
DeCarli et al. 2007	-Qualitative MTL atrophy	51	69	3 years	187	34%	MCI

Table 1. Sensitivity and specificity of delayed verbal recall, structural imaging and CSF biomarkers for AD.

Se=sensitivity; Sp=specificity; n=number of AMCI enrolled at baseline. *=Sensitivity and specificity calculated for all types of dementia which included 8% vascular dementia cases.

Combining CSF markers with regional cerebral blood flow measures can also deliver highly predictive models (hazard ratio = 24), but this combines two highly specialist procedures which currently limits its clinical use (Hansson et al., 2009). Nevertheless, such specialist measures may become useful in future when disease specific treatments become available that arrest AD progression.

Some authors have argued that AMCI diagnosed in specialist memory clinics settings using sensitive episodic memory tests, is sensitive and specific enough to consider it prodromal AD (Dubois and Albert, 2004; Morris, 2006). This is supported by the neuropathological findings discussed above which indicate that the lesions are qualitatively similar and quantitatively somewhere between normal ageing and AD. Inclusion of the best progression predictors in diagnostic criteria for AMCI has further advanced the concept of AMCI as prodromal AD. These criteria are:

1. Memory complaints made by the patient or the family.
2. Progressive onset.
3. Normal or mildly impaired complex activities of daily living.
4. Amnestic syndrome of the "hippocampal type" defined by:
    a. Very poor free recall despite adequate (controlled) encoding;
    b. Decreased total recall because of insufficient effect of cueing or recognition;
    c. Numerous intrusions.
5. Persistence of memory changes at subsequent assessment.
6. Absence of fully developed dementia syndrome.
7. Exclusion of other disorders that may cause AMCI, with adequate tests, including neuroimaging and biomarkers (Dubois and Albert, 2004).

The combination of criteria 2 and 5 can overcome the concern raised by some critics of AMCI that memory impairment identified as deviations from norms (1.5 SD) is insufficient for it is not person specific (Werner and Korczyn, 2008). However, demonstrating the persistence of amnesia requires repeated measures and the ideal follow-up interval remains to be determined. Furthermore, this approach could attract criticism from the point of view that valuable treatment opportunities may be missed due to the delay. The efficacy and adverse effects of future treatments will determine the relevance of either viewpoint.

The criteria listed above are similar to what we adopted in our memory clinic and for this study to diagnose AMCI (§3.4). We required the increased diagnostic accuracy achieved by these criteria due to the relatively small sample size - more vulnerable to the adverse affects of heterogeneous aetiology –as required for fMRI studies as reported on in his thesis. The benefits of studying smaller samples with fMRI are offset by the potential confounds of heterogeneity introduced by unreliable diagnostic tools and poor sampling procedures. Accurate diagnosis of AMCI that is highly predictive of AD is therefore essential and we seem to have accomplished this as illustrated by the high AD conversion rate in our sample (§4.1).

Apart from the episodic memory tests mentioned, two other measures are also good predictors of conversion to AD from AMCI; (1) *CSF concentrations of tau and Aβ,* and (2) quantitative and qualitative *estimates of MTL atrophy* **(Table 1)**. Nevertheless, utilising neuropsychological measures to predict AD has several advantages over CSF and structural neuroimaging; they are affordable, portable and require relatively low levels of expertise to administer.

It should be noted, the measures of delayed verbal recall used for the work reported on in this thesis, and those included above (Table 1), are more sensitive than

standard measures such as the MMSE used in general psychiatric and neurological practice. The increased sensitivity of these measures is due to specific design elements. For example, the Free and Cued Selective Reminding Test used in the study by Sarazin et al. 2007 controls attentional and semantic processing during learning and is therefore robust against confounding effects of age-related changes in cognitive processing (Grober et al., 2009). The stimuli for encoding on this test are pictures of common objects presented in sets of four on a card. Subjects are asked to point and name an object following a cue during the learning phase. For example, the subject will point to and name "grapes" following the cue "fruit". This procedure ensures that all participants achieve a basic level of semantic elaboration and attentional focus that minimises possible confounding effects of impairments in these domains. The New Learning subscale from the Cambridge Cognitive Examination (CAMCOG), that we used to verify episodic memory impairment in our sample of AMCI, similarly controls for attention but not for semantic processing. It does however include both free recall and recognition and population norms are available (Huppert et al., 1995). The inclusion of recognition recall is relevant in our sample for it appears more sensitive at distinguishing AMCI from normal ageing (Anderson et al., 2008; Bennett et al., 2006; Westerberg et al., 2006). Combining two verbal learning tasks (Wechsler Memory Scale-III Logical Memory Test, California Verbal Learning Test-II) increases sensitivity (92%) and specificity (95%) and can accurately predict progression from AMCI to AD in 88% of cases (Rabin et al., 2009). Verbal learning tasks are therefore sensitive, specific and practical measures for identifying progressive AMCI.

Taken together, the findings from the studies discussed above indicate that accurate prediction of progression from AMCI to AD is possible and that tests of

delayed verbal recall are probably the most practical. Furthermore, the high degree of similarity between the neurocognitive measures and diagnostic criteria discussed above and our methods, suggest that our AMCI patients are prodromal for AD and this is supported by their high AD conversion rate.

Summary

The evidence presented in this section demonstrates that AMCI is neuropathologically qualitatively similar but less severe than AD. Furthermore, AMCI can be distinguished from normal ageing and AD in clinical populations with high inter-rater reliability when combinations of standardised and sensitive methods are used. Taken together these findings indicate that AMCI can be considered prodromal AD when specific methods are used. The prevalence of AMCI remains high when such methods are used rendering AMCI practical for studying prodromal AD. The similarities between the diagnostic methods with high predictive value described above and those we used to identify our sample suggest that we can confidently consider our sample of AMCI as prodromal for AD. Nevertheless, it does not necessarily indicate isolated AD neuropathology and anyone diagnosed with AMCI is likely to have comorbid cerebrovascular disease as is the case for clinically diagnosed AD and other neurodegenerative disorders. Some authors have argued that AD and AMCI are extreme presentations of normal cognitive ageing, undermining the construct validity of AD. Several flaws are apparent in this argument; nevertheless, a discussion of the relevant findings will inform the interpretation of our results and follows in the next section.

1.3 AMCI, AD and Ageing

There is considerable overlap between ageing, AMCI and mild AD. Normal ageing is associated with modest cognitive decline evident on everyday tasks and on neuropsychological testing. In addition, AD neuropathology (neuritic plaques and NFTs) is evident to a minor degree in the brains of older adults considered cognitively normal. These two findings have raised doubts over the status of AD as a disease and it has been proposed that AD is *accelerated ageing* (Anderton, 1997; Anderton, 2002). Conversely, it can be argued that cognitively normal older adults with AD neuropathology are in fact suffering from preclinical AD by virtue of the presence of diagnostic markers, and this introduces the notion that ageing is disease related. This is a key point because society treats ageing as a disease as illustrated by the substantial research efforts that have gone into treating ageing (Butler et al., 2008).

Ageing is associated with progressive loss of neocortical synapses and it has been suggested that even in the absence of concomitant AD neuropathology a critical limit of 60% synapses loss will be reached by the age of 130 years, thereby making dementia the inevitable outcome of ageing even in the absence of any distinct neuropathology (Terry and Katzman, 2001). However, this dementia is likely to be clinically distinct from AD that has a predilection for affecting certain areas that translates into specific symptoms (§1.1.3.2; 1.2.2)

These findings and views constitute the main controversies of the accelerated ageing debate. This section examines the relevant evidence on the accelerated ageing hypothesis by defining the terminology, comparing the causes of ageing and AD and by looking at the structural and neuropsychological similarities and differences. Age related cognitive changes that have bearing on our findings are also discussed.

1.3.1 Defining Normal, Ageing and Disease

Normal is defined as "*occurring naturally and not because of disease, inoculation, or any experimental treatment"* (Merriam, 2002). *Ageing* is the accumulation of changes to an individual over time due to environmental and genetic factors. *Disease* is defined as "an impairment of the normal state of the living animal or plant body or one of its parts that interrupts or modifies the performance of the vital functions, is typically manifested by distinguishing signs and symptoms, and is a response to environmental factors (as malnutrition, industrial hazards, or climate), to specific infective agents (as worms, bacteria, or viruses), to inherent defects of the organism (as genetic anomalies), or to combinations of these factors" (Merriam, 2002). This definition includes the term *normal*, which is defined in terms of naturally occurring events, and this introduces a contradiction because disease appears to affect all known living organisms and is therefore thoroughly natural. Both AD and healthy ageing can therefore be considered as *normal* because both appear naturally. Conversely, the current and historic attempts to find a cure for ageing demonstrate that society has long regarded ageing as a disease, similar to AD, and continues to do so. Neither society nor prevailing definitions therefore clearly distinguish ageing and AD. Debate about the difference between aging and disease has a long history and continues in the literature (for reviews see (Butler et al., 2008; Carnes and Olshansky, 2007). We next look at the aetiology of ageing and AD to see if they can be differentiated on this basis.

1.3.2 The Aetiology of Ageing and AD

Cellular ageing occurs when cellular division fails, which in humans appears predominantly related to the shortening of *telomeres* (Blackburn, 2001; Blasco, 2005;

Collins and Mitchell, 2002). Telomeres occur at the ends of chromosomal DNA chains and consist of repetitive DNA sequences that protect the chromosome from accidental enzymatic destruction and recombination. When cells without the enzyme that maintains the length of telomeres (telomerase) replicate, they gradually lose telomeric length and eventually stop dividing. Furthermore, the length of telomeres is maintained in cells that appear immortal. *Ageing of an organism* occurs when damaged tissue can no longer be replaced because cell division has failed. Ageing effects are not uniform in individuals of a species and are not inevitable for all living organisms as numerous species show negligible ageing effects. These and other findings suggesting contributions from other mechanisms of aging raise the possibility that ageing is a disease, apparently partly genetic.

Cellular ageing could affect brain function. There is comparatively little neuronal proliferation evident in the adult brain although it does occur, in particular in the hippocampus. Nevertheless, there is proliferation of neuroglia that support neurones directly and neurotransmission indirectly; neurones are dependent on neuroglia (astrocytes) for the energy essential for neurotransmission. Impaired neuroglial proliferation can affect their support of neurones and neurotransmission and can therefore influence cognition. There is also microglial (central nervous system immune cells) proliferation in the normal brain. Microglia defend the brain against infection and play a role clearing away amyloid and in supporting or destroying injured neurones (Lucin and Wyss-Coray, 2009). Ageing is associated with microglial degeneration and this may cause some of the age related structural changes seen in normal ageing and also in neurodegenerative disorders (Streit, 2006). Furthermore, recent evidence suggests that microglia lose the ability to replicate in old age indicating that the apparent telomeric mechanism of ageing discussed above

may be at work (Streit et al., 2008). Brain ageing may therefore also not be inevitable if neuronal and glial health could be maintained by continuous proliferation, and if brain tissue could be protected against exogenous and endogenous traumatic and toxic effects, including that caused by endogenous immune dysregulation.

A further difficulty encountered in distinguishing between AD and ageing is that the exact disease mechanism of AD is not clear. As mentioned in the introduction, several causative hypotheses exist for AD including genetic, environmental and infectious causes (§1.1.2). However, at present AD appears less like the result of a single mechanism and more like that of a complex interplay between genetic and environmental factors. AD could conceivably involve some of the mechanisms of ageing but appear different clinically due to genetic and environmental influences.

In the next section, we look at age-related cognitive decline in domains relevant to the work reported here, in order to inform the discussion of our findings.

1.3.3 Age-related Cognitive Decline

Age-related decline affects brain structure as well as several cognitive functions and, although there is overlap between ageing and AD, there are some distinct features apparent only in AD (for reviews see (Anderton, 1997; Anderton, 2002)).

1.3.3.1 Structural brain changes in AD and ageing

Table 2 summarises the known structural differences between normal ageing, AMCI and AD. Impairment on the majority of these measures progresses gradually from normal ageing towards AD supporting the accelerated ageing hypothesis. However,

age-related changes are absent on other measures where abnormalities are clearly present in AD therefore arguing against the accelerated ageing hypothesis. Findings that appear specific to AD include parietal lobe atrophy, neuronal loss in the nucleus basalis of Meynert, synapse loss in layers III and V in frontal cortex, and NFTs in the entorhinal, hippocampal C1 and thalamic areas (Anderton, 2002; Giannakopoulos et al., 2007; Giannakopoulos et al., 1997; Paskavitz et al., 1995). These areas are persistently implicated in AD indicating a qualitative difference from ageing. AD therefore appears to have regional specificity that distinguishes it from ageing. Supporting evidence comes from diffusion tensor imaging that reveals micro structural changes in normal ageing in frontal white matter, anterior cingulate and *genu* of the corpus callosum, and in AD and MCI in the posterior cingulate, parahippocampus, temporal white matter and *splenium* of corpus callosum (Chua et al., 2008).

		Ageing	AMCI	AD
Macro anatomical changes				
	Brain volume decrease	+	++	+++
	Increased ventricular volume	+	++	+++
	Hippocampal atrophy	+	++	+++
	Frontal atrophy	+	+	++
	Parietal atrophy	-	+	++
	White matter atrophy	+	++	++
Micro anatomical changes				
	Hippocampal neurone loss	+	++	+++
	Cortical neurone loss	+	++	+++
	Nucleus basalis neurone loss	-	++	+++
	Synapse loss	+	++	+++
	Synapse loss in layers III and V of frontal cortex	-	No data	++
	Amyloid plaques	+	++	+++
	Hirano bodies	+	No data	+++
	Granulovacuolar degeneration	+	No data	+++
	NFT in hippocampal C1 region	-	++	+++
	NFT in transentorhinal area	+	+	++
	NFT in entorhinal area	-	+	++
	NFT in thalamus	-	No data	+++
	Small infarcts	+	+	+
	Cerebral amyloid angiopathy	+	No data	+++

Table 2. Micro and macro anatomical similarities and differences between normal ageing, AMCI and AD.

Neuropathological findings in ageing, AMCI and AD reveal a gradual accumulation of abnormalities on many aspects; however, neuropathology affects distinct areas in AD and AMCI that are spared in normal ageing (shaded rows). These findings

suggest that neuropathology in AD and AMCI is qualitatively different from normal ageing and argues against the accelerated ageing hypothesis.

1.3.3.2 Neuropsychological findings in ageing

Advancing age is associated with a systematic decline in performance on a variety of cognitive tasks. Decline is not evident in all situations or on all cognitive domains and performance on some tasks may even improve with advancing age. There are currently two competing hypothesis explaining age-related cognitive decline. The first ascribes the widespread cognitive impairments to deficits in one or a few *general factors* (global interpretations), implying that the various impairments are related. The second hypothesis posits that impairments are *task or process specific* (analytical interpretations) caused by discreet structural or processing deficits (Van der Linden, 2002). We will look at each of these hypotheses in turn.

Global interpretations

Several general factors have been identified that may explain the decline of cognition associated with normal ageing. These include decreased *processing speed, reduced working memory capability, impaired inhibitory efficiency, and sensory processing deficits* (Park, 2000).

Processing speed refers to the speed with which elementary processing operations can be executed. Compelling evidence suggests that nearly all age-related variants in cognition can be explained by the variance in the speed at which individuals make rapid comparisons during perceptual speed tasks (Salthouse, 1996a; Salthouse, 1996b; Salthouse, 1996c). A decline in processing speed results in a decrease in the speed with which cognitive operations are executed. This leads to

behavioural deficits when the processing is to slow to allow completion of tasks (limited time mechanism) and when necessary working memory traces cannot be maintained long enough for later use (simultaneity mechanism). The decline in processing speed is linear and very gradual as indicated by a small intercept on scatter plots of age and processing speed (for a review of meta-analyses see (Verhaeghen and Cerella, 2002). The reaction times of older adults can therefore be expressed as a fixed ratio of younger adult's reaction times and this speed of processing deficit is called general slowing.

According to the *working memory hypothesis,* cognitive decline results from a reduction in the amount of cognitive resources available to temporarily store new information while simultaneously processing incoming or recently accessed information (Baddeley, 1986). *Working memory* refers to a cognitive system that stores and manipulates information during complex cognitive tasks such as learning, reasoning and language comprehension (§1.6.2). It is classically measured on dual-tasks where subjects are asked to both store and process information simultaneously. Much evidence suggests that age-related cognitive decline on several tasks is mediated by working memory deficits and there is some indication that the effects of processing speed and inhibitory attention mechanisms are mediated via working memory (Bopp and Verhaeghen, 2005; Salthouse et al., 1991; Van der Linden et al., 1994; Van der Linden et al., 1999). Dual-tasks differ from the divided attention task we employed by requiring storage of novel information during the task; this is discussed in detail in the methods section (§3.9.1.1).

According to the *inhibitory hypothesis*, irrelevant distracting information gains access to working memory in older adults due to deficits in inhibitory attentional mechanisms. The irrelevant information may come from external sources or from

automatic activation of semantic representations. It follows from this that task completion can be interrupted or delayed as processing resources are diverted to irrelevant information.

Sensory processing shows as strong age-based relationship with cognitive performance (Lindenberger and Baltes, 1994; Lindenberger and Baltes, 1997). A life-span study revealed compelling evidence that cognitive decline is in part mediated by decline in sensory function (Baltes et al., 1999). It has subsequently been shown that the decline is likely related to the integrity of brain structure and function and not due to the effects of impaired visual or auditory sensory acuity during testing (Lindenberger et al., 2001). Greater impairment in both near and far vision have been reported in AD compared to controls, and the degree of vision impairment correlates with the degree of cognitive impairment (Uhlmann et al., 1991). Similar findings have been reported in relation to hearing impairment and AD (Uhlmann et al., 1989). It is not certain at present if sensory impairments unmask and exacerbate the symptoms of dementia. It is also not clear if these sensory impairments are related to pathology in the sensory apparatus or visual processing.

It is most likely that these suggested general factors are interdependent as suggested by findings indicating that the contributions of slowed processing speed and inhibitory failure are indirect and mediated by working memory which itself appears to play a crucial role in age-related cognitive decline (Van der Linden et al., 1999). These findings have been contradicted by other studies and it has been suggested that the contributions to cognitive deficits in ageing is likely to be task dependent. The situation is further complicated by the composite nature of working memory. Baddeley's model of working memory is composed of a phonological loop, visuospatial sketchpad, central executive and episodic buffer (Baddeley, 1986;

Baddeley, 2000; Baddeley and Della, 1996). In turn the central executive appears to have distinguishable functions including the selection and manipulation of information in long-term memory, selecting and inhibiting information, attentional control, coordination of two or more concurrent activities, and updating working memory. It is therefore clear that working memory cannot be considered as a single factor influencing cognition and that an analytic approach may be more suitable to investigate cognitive decline in ageing.

I report on our comparison of divided attention performance in this thesis and divided attention is underpinned by processing speed, working memory and executive processing and therefore vulnerable to the effects of age-related decline in these processes. Divided attention performance does indeed decrease with age and these deficits are more pronounced during more complex dual-task conditions associated with complex task rules such as those require memory encoding or manipulation of information held in working memory (for a review see (Sarter and Turchi, 2002). Divided attention impairment has been demonstrated in healthy older adults on a driving simulation task using visual stimuli (Brouwer et al., 1991). A visual and auditory dual-task, requiring visual letter-stimulus matching and simple choice reaction to an infrequent auditory stimulus, revealed impairment in older adults compared to young adults for conditions where the probes followed a warning signal more than 75 ms. Nevertheless, on shorter trials (50 ms) the groups were comparable (Greenwood and Parasuraman, 1991). The authors further divided the older participants into two groups (60-69 years; 70-79 years) and found no differences in accuracy or speed between these two groups on simple choice reaction. These results indicate that age-related differences in divided attention are dependent on the type of task and the timing of stimuli. Furthermore, there appears to be no differences in the

age range 60 to 79 on the simple choice reaction aspect of the task suggesting that age-related differences are unlikely to be a source of significant variability in this age group on the choice reaction aspect of similar divided attention tasks. This finding is relevant to the interpretation of our results, on a similar divided attention task, in groups not closely matched on age.

Analytic interpretations

The *analytical approach* to cognitive ageing assumes that the cognitive processing involved in completing a specific task can be divided into the elements that constitute it. Age-related cognitive impairment may therefore be localised to specific brain areas and the frontal and medial temporal areas appear more vulnerable to ageing. Based on the approach, process specific deficits can be examined by reference to the attention related neural networks, each responsible for a different attentional function (Fan et al., 2005; Posner, 2004; Posner et al., 2000; Van der Linden, 2002; Wang and Fan, 2007). These functions include *alerting, orienting and executive control*.

Alerting achieves and maintains a heightened state of arousal in preparation for a task. It supports vigilance (maintained response readiness over long periods of time for infrequent targets), phasic alertness (periodic increased response readiness following a warning stimulus), and sustained attention (maintained response readiness over shorter periods of time to detect frequent targets) and is underpinned by a network which includes thalamic nuclei, frontal and parietal cortices. Alerting appears largely unaffected by ageing and older adults still benefit from warning stimuli. The only deficits reported are of earlier fatigue during demanding long (30 min) sustained attention tasks (Mouloua and Parasuraman, 1995).

Orienting focuses attention on one stimulus amongst many (selective attention) and relies on shifting attention from one stimulus to the next. It relies on a network that includes the parietal lobes, frontal eye fields, superior colliculi, and thalamus (pulvinar). Selective attention shows age-related impairment and this appears more pronounced when target stimuli are presented with highly similar distractor stimuli, and less pronounced when target stimuli are preceded by a cue, diminishing the need to process irrelevant stimuli (Hartley, 1993; Rogers, 2000).

Executive control is an amalgam of processes that monitor and resolve conflicts in planning, decision-making, it contributes to error detection, and it inhibits automatic execution of habitual actions. Contradictory findings have been reported in relation to the effects of ageing on executive control and this has been ascribed to methodological differences in assessing potential confounders such as slowed processing speed (Van der Linden, 2002). Nevertheless, deficits have been demonstrated which remain after controlling for the general effects of slowed processing speed.

1.3.3.3 Neuropsychological findings specific to AD and AMCI

In spite of the considerable overlap between ageing and AD, neuropsychological studies have demonstrated evidence of specific disease related cognitive impairments that cannot be accounted for by generalised effects of ageing.

Specific episodic memory impairment, demonstrated as impaired delayed recall of spoken words or narrative, appears to be one of the most sensitive neuropsychological predictors of progression from normal ageing and AMCI to AD and this has already been discussed (§1.2.3) (Artero et al., 2003; Backman et al., 2001; Sarazin et al., 2007; Tierney et al., 1996; Tierney et al., 2005).

Several reports found that recognition is less impaired that free recall in ageing compared to AMCI and AD (Anderson et al., 2008; Bennett et al., 2006; Westerberg et al., 2006). Furthermore, impairments in item, associative and semantic memory are evident in AMCI and AD compared to normal older adults and we will look at this in more detail in a later section (§1.4.4).

Normal ageing, AMCI and AD can be distinguished from one another on selective visual attention task performance (RT) after controlling for the potential confounding effects of general slowing in normal ageing by logarithmic transformation of behavioural measures, therefore indicating that the visual attention impairment in AMCI and AD is very likely disease related (Tales et al., 2005a). Patients with AD and AMCI also demonstrate significant deficits in the ability to disengage attention from an incorrectly cued location and the ability to use a visual cue to produce an alerting effect compared to controls and after controlling for the effects of general cognitive slowing (Tales et al., 2005b).

Summary

Taken together the findings from structural and neuropsychological studies indicate that in spite of the considerable quantitative overlap between ageing, AMCI and AD, qualitative and quantitative differences exist which distinguish normal ageing from AD. AD and AMCI therefore does not appear to be accelerated ageing. However, the effects of ageing on cognition continue to apply to AMCI and AD and this should be kept in mind when interpreting the result from studies.

1.4 Episodic Memory

Memory refers to the capacity to form mental representations of experiences (*memory encoding*) and to the ability to reactivate or reconstruct such representations (*memory retrieval*). Memory underpins important survival and social processes and amnesia is associated with declines in everyday activities. Memory appears particularly vulnerable to AD neuropathology and gradually progressive *amnesia* is typically the first symptom reported in AD and characteristic of both AMCI and AD. Amnesia usually presents in AD as complaints of forgetfulness, that appear related to neuropathology affecting the network of brain areas supporting *episodic memory*. Damage to this network makes it increasingly difficult to store new information whilst existing memory representations remain relatively intact. Episodic memory performance shows negative correlations with AD neuropathology in key areas in normal ageing, AMCI and AD (§1.2.2). The temporal gradient of memory loss accounts for the finding that amnesia is more pronounced for recently acquired memories than for distant memories. This finding is clinically useful because the ability to lay down new memories (*new learning*) can be measured and reflects the current integrity of episodic memory and is unconfounded by memory representations encoded prior to the onset of amnesia. Based on this disproportionate impairment in new learning in AD, tests of general intelligence such as the National Adult Reading Test (NART) can be used to assess premorbid intellectual functioning (O'Carroll et al., 1987). Good performance on the NART relies on recognition of written words and their correct pronunciation, both accessing memory acquired over the lifetime of the individual and therefore not dependent on recent or current encoding ability.

Memory deficits indicate high-risk, is essential for a diagnosis of AMCI and AD, predicts premorbid cognitive function and indicates pathological load. These

findings illustrate the utility of understanding the neuropsychology of episodic memory in prodromal AD. In this section, episodic memory is introduced in more detail and its associations with MTL and frontal lobe structures are discussed. Neuropsychological and functional neuroimaging findings in health and AMCI relevant to the interpretation of the results from our studies of verbal episodic encoding in AMCI are also presented and discussed.

1.4.1 Theoretical Background

Episodic memory is a type of long-term declarative memory where both factual knowledge of the world and the context (time, space, affect) in which it was acquired are amenable to conscious recollection (Baddeley, 2001). It involves the three processes of *encoding, consolidation and retrieval*. *Encoding* refers to the generation of new memory representations and *retrieval* to the recollection of these representations. The other type of long-term declarative memory is *semantic memory* where only factual knowledge is amenable to conscious recollection. Episodic memory was recently described by Endel Tulving, who first distinguished it from semantic memory in 1972, as "a recently evolved, late-developing, and early-deteriorating past-oriented memory system, more vulnerable than other memory systems to neuronal dysfunction, and probably unique to humans. It makes possible mental time travel through subjective time, from the present to the past, thus allowing one to re-experience, through autonoetic awareness, one's own previous experiences. Its operations require, but go beyond, the semantic memory system. Retrieving information from episodic memory (remembering or conscious recollection) is contingent on the establishment of a special mental set, dubbed episodic "retrieval mode." Episodic memory is subserved by a widely distributed network of cortical and

subcortical brain regions. This network overlaps with but also extends beyond the networks subserving other memory systems. The essence of episodic memory lies in the conjunction of three concepts—self, autonoetic awareness, and subjectively sensed time." (Tulving, 2002). *Autonoetic consciousness* refers to remembering episodic events and it is defined as the subjective feeling of re-experiencing or reliving the past and mentally travelling back in subjective time, and to therefore *remember* the time, place and affect associated with the encoding episode (Tulving, 1985).

The exact mechanisms that underpin episodic memory is not clear yet however it does appear to rely heavily on key structures that are vulnerable to AD and this probably explains the salience of amnesia in prodromal and mild AD. In the following section, I discuss the neural network that underpins episodic memory function.

1.4.2 Episodic Memory Network

Episodic memory depends on a widely distributed network of cortical and subcortical brain regions that overlap with and extend beyond the networks subserving other memory functions. Permanent representation of learned episodes seems to rely on distributing information into multiple neocortical areas and on the MTLs binding this information together (Squire and Zola, 1996). Normal MTL function during encoding, and for a period afterwards, appears essential for permanent declarative episodic memory formation. We will consider the contributions from the temporal and frontal lobes to episodic memory in more detail as they appear particularly important and altered activation in these areas have been demonstrated during episodic memory

processing in AD and high-risk states. The roles of the frontal and temporal lobes have been revealed by lesion and functional neuroimaging studies.

1.4.2.1 Lesion studies - the medial temporal lobes

The MTL is comprised of the hippocampal region (dentate gyrus, hippocampal cell fields C1-C3, subicular complex) and parahippocampal region (entorhinal cortex, Brodmann area (BA) 28,34; and perirhinal cortex BA 35) (Witter et al., 1989). During memory processing, information from sensory cortices pass to unimodal and polymodal association areas in frontal, temporal and parietal lobes and then through the parahippocampal region to the hippocampal region. The hippocampal region in turn projects back to the cortex via the parahippocampal region. The entorhinal cortex in the hippocampal region receives the majority of these projections from the parahippocampal region, via the dentate gyrus. This circuitry explains why episodic memory crucially depends on MTL structures as indicated by lesion studies. Medial temporal lesions results in dense amnesia for personal experiences whilst leaving semantic memory largely intact (Wheeler and McMillan, 2001). Selective lesions of MTL areas cause differential impairment on memory tasks and more extensive damage causes more substantial anterograde (formation of novel memory representations) and retrograde (recollection of pre-lesion memory representations) amnesia (Squire and Zola, 1996). MTL structures therefore appear crucial in episodic memory function and this has lead to the large number of studies that have focused exclusively on this area, to the exclusion of other areas.

1.4.2.2 Lesion studies - the frontal lobes

Lesion studies in humans also demonstrated impaired episodic memory following frontal cortex damage. Such impairments are evident on tasks that are more complex and that involve high levels of interference and disruption of the temporal order of memory (Incisa della Rocchetta and Milner, 1993; Janowsky et al., 1989). The influence of such interference and disruption is not routinely tested although similar distracting circumstances occur frequently in everyday life in crowded and noisy surroundings. Frontal lesions are thought to cause failure in the control processes of memory rather than of automatic storage processes and we will look at this in detail in a later sections on attention and executive control (§1.6.1-3).

Lesion studies in animals have revealed contributions from other structures including the fornix, mammillo-thalamic tract and anterior thalamic nuclei to normal episodic memory function (reviewed by (Aggleton and Pearce, 2001)).

1.4.2.3 The functional neuroanatomy of episodic memory

Functional neuroimaging has been widely used to investigate memory processes in cognitively normal individuals and in those with memory impairment. Verbal episodic encoding consistently activates left hemispheric prefrontal cortex (PFC) and temporal lobe structures, confirming the findings from lesion studies [for a review see (Cabeza and Nyberg, 2000)].

Left PFC activity during encoding relates to deep meaning-based *semantic* processing that optimises memory and involves the *executive processes* of *generating, maintaining, selecting and organising* semantically related information (Fletcher and Henson, 2001; Wagner et al., 1998). Indeed, disruption of left PFC function by transcranial magnetic stimulation results in impaired verbal encoding (Floel et al.,

2004), and disruption of semantic elaboration results in decreased left PFC activation and reduced memory performance (Fletcher et al., 1995; Grady et al., 1995). The extent of activation of the left frontal cortex and hippocampus during verbal encoding correlates positively with subsequent successful recognition (Morcom et al., 2003; Wagner et al., 1998).

The PFC can be divided by the inferior frontal sulcus into dorsolateral (DLPFC) and ventrolateral PFC (VLPFC) with the former loosely corresponding to Brodmann areas (BA) 9 and 46, and the latter to BA 44,45 and 47 (Fletcher and Henson, 2001). Activation in the left DLPFC reflects the working memory processes of reorganising information and subvocal rehearsal of working memory content whereas activation in VLPFC relates to semantic processing during verbal encoding (for reviews see (Desgranges et al., 1998; Fletcher and Henson, 2001).

Activation in the MTL appears predominantly on the left during word encoding and left sided lateralisation of activation for non-verbal stimuli appears related to the verbalisability of the stimuli (Desgranges et al., 1998; Golby et al., 2001). Not all verbal encoding tasks activate the MTL and it appears that novelty and associative encoding (encoding pairs of items) are more likely to recruit MTL areas. The effect of novelty and MTL activation is further illustrated by the finding that repeated exposure to initially novel face-name pairs results in *de*activation in the hippocampus (Rand-Giovannetti et al., 2006; Sperling et al., 2003a; Sperling et al., 2003b).

Activation in MTL and PFC predicts subsequent recall performance and demonstrate the interaction between these two key areas in episodic encoding (Kirchhoff et al., 2000; Sperling et al., 2003a; Staresina and Davachi, 2008; Wagner et al., 1998).

Recent studies have also revealed correlations between memory performance and parietal and cerebellar activation (Brassen et al., 2006; Fliessbach et al., 2007; Staresina and Davachi, 2006).

In summary, the PFC and MTL are key nodes in the network that underpins verbal episodic memory. This network also receives contributions from other cortical areas. Next, we look at the neuropsychology of normal memory that depends on this functional network.

1.4.3 The Neuropsychology of Episodic Memory

The design and choice of the memory paradigm that we used to study verbal episodic memory in AMCI is based on available findings from studies in health, AMCI and AD. What follows is an introduction to the neuropsychology of episodic memory that will inform the discussion that follows on the available findings in AMCI and on the design aspects of the tasks described in the methods section (§3.9.3).

Episodic memory can be examined on tasks that measure *retrieval success* following intentional or incidental *encoding* (learning phase) of sensory stimuli in any modality. *Free recall* is retrieval that is unsupported by external cues and the most demanding retrieval mode. A typical task would require a subject to name as many items as remembered from the set of items presented during the learning phase. The recall phase is termed *free* because no external cues related to the studied items are provided. Conversely, cues are presented during *recognition* tasks and retrieval is therefore supported and less demanding. For recognition tasks *cues* are presented and subjects have to decide whether or not they had been encountered during the learning phase. Typical cues are non-target semantic associates, such as a particular category

to which an item belongs, or previously learned items (*targets*). Recognition can be measured on forced-choice or yes/know tasks. During a *forced-choice recognition task,* a target and one or more non-target (*distractor*) items are presented simultaneously, and subjects are forced to choose one item as a target. For a *yes/not task,* a single probe is presented and the subject has to decide if it is a target or not. **Figure 3** illustrates the two types of recognition task and the difference between forced-choice and yes/no tasks.

Recognition may be more sensitive than free recall in distinguishing normal ageing from AD because it is more robust against age-related changes (Craik and McDowd, 1987; Parker et al., 2004). Recognition may therefore the preferred measure of episodic memory in smaller samples not closely matched on age. Recognition can be further divided into *familiarity,* which refers to a feeling of prior exposure to an item without recall of associated contextual information, and *recollection* referring to the retrieval of the item bound to contextual features such as the time, place or source of experience (for a recent review see (Yonelinas, 2002). Forced choice is a more sensitive measure of familiarity and, yes/know of recollection.

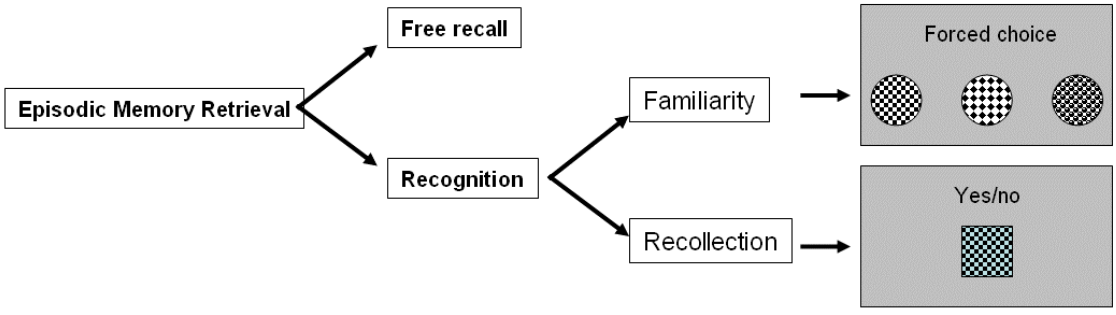


Figure 3. Measures of episodic memory retrieval.

The figure illustrates forced choice and yes/no recognition tests used to examine familiarity and recollection based recognition respectively. Recollection tests appear more sensitive at distinguishing normal ageing from AMCI.

Specific episodic memory tasks can distinguish between *item recognition* (recognition of an item as presented during learning on a given set of stimuli) and cross modal *associative memory* (encoding and recall of relations between items, such as for an object and its spatial location, or the pairing of two items). The verbal episodic memory task we employed consisted of an *implicit associative* learning task with a yes/know recognition phase. The associations were implicit because all the words in a list were semantically related but participants were not informed of this (§3.9.3-4). The ability to examine dissociable memory processes allows more sophisticated methodology to study specific brain areas and how they are affected by pathology. For instance, implicit associative learning allows examination of distinct memory processes such as automatic semantic elaboration that is strongly associated with the PFC, an area of interest in studies of AD. Also, the ability to examine dissociable memory processes may enable tracking of AD progression because distinct memory processes such as familiarity and recollection appear to depend on different cortical areas; recollection appears to rely on the hippocampus and prefrontal cortex and familiarity on the regions surrounding the hippocampus (Yonelinas, 2002). AD neuropathology proceeds from entorhinal cortex to hippocampus and then on to neocortical association cortices. Impairment on specific memory tasks may therefore indicate pathology in certain areas.

These findings illustrate the potential of specific memory studies to shed light on the component processes that constitute everyday memory processing. We next look at the findings from such studies in AMCI.

1.4.4 Neuropsychological Findings in AMCI

Episodic memory is characteristically impaired in AMCI and progress has been made to improve the sensitivity and specificity of tests that can distinguish normal ageing from AMCI and AD. Several reports indicate that recognition tests may be more sensitive than free recall at distinguishing AMCI from normal ageing and of the recognition tests, recollection rather than familiarity appears more sensitive (Anderson et al., 2008; Bennett et al., 2006; Westerberg et al., 2006). However, one recent study reported greater impairment in familiarity in AMCI when controlling for task difficulty (Wolk et al., 2008). These findings indicate that *yes/know recognition tasks are* sensitive in distinguishing between ageing, AMCI and AD.

Both item- and associative- memory appears affected in AMCI. An experiment examining famous person naming (person specific semantic memory), subsequent recognition (item recognition), and recall of the position where faces initially appeared (spatial associative episodic memory) revealed impairment in all three measures in AMCI (Dudas et al., 2005). A study of incidental and intentional item and associative encoding in AMCI demonstrated greater impairment on associative memory on two tasks (symbol-symbol pairs and object-location pairs) (Troyer et al., 2008). Taken together these findings indicate impairment in semantic memory, associative learning, free recall and recognition recall in AMCI and suggest that an associative learning task with a yes/no recognition phase may be a particularly sensitive measure to discriminate AMCI from normal ageing.

Although AMCI implies isolated episodic memory impairment, deficits in other areas including semantic memory have also been reported (De Jager et al., 2003; Dudas et al., 2005; Joubert et al., 2008). These results indicate that semantic memory for conceptual entities, which have distinctive and unique properties such as famous events and people, deteriorate before semantic memory for objects in AMCI. Nevertheless, the older reports on semantic memory precede the more recent *amnestic-single domain* distinction and may therefore refer to the more heterogeneous MCI group.

In summary, the findings discussed above illustrate the potential of specific memory paradigms to examine distinct memory processes that appear differentially affected in ageing, AMCI and AD. Combining such tasks with functional neuroimaging has the potential to reveal the functional neuroanatomy of memory processing in health, AMCI and AD and may be useful in diagnosis and treatment monitoring. In the next sections, I summarise functional neuroimaging findings of memory processing in health and AMCI.

1.4.6 Functional Anatomy of Episodic Memory Encoding in AMCI

We have seen that lesion and functional neuroimaging studies indicate that episodic memory relies on a network of brain areas. In this section, we look at the available findings from functional neuroimaging studies of episodic encoding in AMCI. Almost all functional neuroimaging studies of episodic memory in AMCI have used picture stimuli or picture-word pairs and only a few studies have employed visually presented words similar to our methods. This is at odds with the majority of clinical measures of delayed episodic memory, which use verbal stimuli (New York University Paragraph Recall Test, Logical Memory Test). Moreover, even when

picture stimuli are used, it usually involves naming the pictures as part of encoding (Boston Naming Test) (Fleisher et al., 2007). There appears to be a mismatch between clinical and research instruments and employing an experimental task involving auditory presented words or narrative would therefore more closely mirror clinical measures. It is at present not possible to adequately monitor compliance during verbal encoding tasks where stimuli are read to subjects because under such circumstances they could engage in cognitive activity unrelated to the task without the knowledge of the researcher. Monitoring compliance is especially important when studying clinical groups with cognitive impairment that can affect the ability to comply with task requirements. Researchers therefore often use tasks involving picture naming or overt reading of word lists during encoding which allows some compliance monitoring but does not exactly match clinical measures. Generalising results from picture encoding to verbal encoding is problematic due to the modality (visual or verbal) and memory phase (encoding or recognition) dependent lateralisation of activation in PFC and MTL (Johnson et al., 2003; Kelley et al., 1998; Otten and Rugg, 2001). What follows is therefore a discussion of available verbal encoding studies and selected picture-word and picture encoding studies that are of interest.

1.4.6.1 Verbal encoding studies

An encoding study using visually presented words demonstrated greater activation in AMCI subjects compared to controls in the left hippocampus, medial frontal gyrus (BA 6), anterior cingulate (BA 24) and post central gyrus (BA 3) specifically during encoding of words that were subsequently retrieved (Kircher et al., 2007). The authors suggest that greater activity in AMCI relates to compensatory mechanisms required in the face of failing resources because increased cognitive effort and more demanding

tasks lead to greater magnitude or extent of functional activation (Grady et al., 1994; Raichle et al., 1994). Results from this study indicate that matched encoding performance requires larger activation in relevant areas in AMCI.

1.4.6.2 Picture encoding studies

Using a picture encoding task, where novel scenes were contrasted with repeated scenes, greater *extent* of activation in the MTL regions correlated with better recognition performance but also paradoxically with greater functional impairment and faster cognitive decline in the subsequent 2.5 years in AMCI subjects (Dickerson et al., 2004). The authors recently conducted a further analysis which showed that greater *magnitude* of hippocampal activation at encoding correlated with greater degree and rate of subsequent cognitive decline (Miller et al., 2008). A further study by this group, using the same task, an expanded original participant group and a model-free independent component analysis of activation across the whole brain, revealed increased MTL activation in less impaired AMCI subjects and decreased activation in the more impaired AMCI subjects (Celone et al., 2006). Their findings could indicate that greater activation during a compensated state affords better recall but also heralds the onset of a period of fast decline, whereas decreased activation indicates more advanced impairment and relatively slower decline. Findings from these studies are unfortunately difficult to generalise to the AMCI population because the authors did not require objective evidence of episodic memory impairment for a diagnosis, instead they relied on subjective reports. Their patient group therefore included subjects that we would describe as suffering from *subjective cognitive impairment* and likely represents an even more heterogeneous group than MCI.

Finding a correlation between MTL activation and subsequent decline is of interest as a recent study that combined functional neuroimaging of encoding (picture-word pairs) and voxel-based morphometery reported a positive correlation between increased *posterior* MTL (hippocampus, parahippocampus, fusiform areas) activation and *anterior* MTL atrophy in AMCI subjects (Hamalainen et al., 2007). This correlation was absent in controls and AD patients and the authors suggest that increased posterior MTL activation is compensatory for anterior MTL atrophy in AMCI. The magnitude of compensatory activation could therefore correlate with disease progression and differentiate between AMCI, ageing and AD. These findings could also explain the result discussed above that indicate a correlation between MTL hyperactivation and subsequent rapid decline.

An incidental, deep picture encoding task (requiring participants to decide if a stimulus was man-made or natural) demonstrated impaired memory performance associated with decreased activation in AMCI in left hemispheric PFC (BA 47), entorhinal cortex (BA 34), superior temporal cortex, extrastriate visual cortex and anterior cingulate, in right hemispheric middle temporal gyrus, lentiform nucleus (putamen) and caudate, and in bilateral medial parietal (precuneus) cortex (Mandzia et al., 2007). In this study, recognition accuracy correlated with the extent of activation in bilateral parahippocampal gyri in controls but not in AMCI subjects. The result from this study reveals altered activation across a range of areas associated with sensory processing and higher processing of stimuli. The loss of correlation between activation and performance may indicate that parahippocampal areas were already maximally activated, and/or deployment of a compensatory encoding strategy that is less dependent on parahippocampal activation.

Altered metabolism in parahippocampal and bilateral medial parietal areas has also been reported in AMCI using the recently developed perfusion fMRI method. During picture encoding, AMCI demonstrated attenuated medial parietal (precuneus, cuneus and posterior cingulate) and absent right parahippocampal regional cerebral blood flow (CBF) increases compared to controls (Xu et al., 2007). The mean CBF in the medial parietal cluster correlated with MMSE score and more so with delayed verbal recall performance. Furthermore, when the groups were compared at rest, similar decreased medial parietal (precuneus and cuneus) perfusion persisted and again correlated with MMSE and delayed verbal recall performance. This appears to indicate a close association between activity in medial parietal areas, episodic memory and global cognition. The medial parietal area is comprised of the precuneus, posterior cingulate and retrosplenial cortex. This area appears vulnerable to AD neuropathology as evidenced by findings of volume loss, hypoperfusion and hypometabolism. Volumetric differences in this area are evident between normal and AMCI subjects and decreased volume in this area predicts dementia conversion. The area also suffers accelerated atrophy in progressive AMCI. Hypoperfusion and hypometabolism have been demonstrated in this area and these findings predict progression to AD from AMCI and also correlate with episodic memory performance (for a review see(Ries et al., 2008))(Xu et al., 2007). The medial parietal area receives cholinergic input from the BFCS via the medial cholinergic pathway and are the most distant area supplied via this pathway (Selden et al., 1998). Functional and structural changes could therefore be related to AD neuropathology in the BFCS affecting cholinergic innervation and therefore cholinergic regulation, first affecting the most distant areas innervated by the medial cholinergic pathway.

Impaired memory and decreased bilateral PFC and left cerebellar activation were demonstrated in AMCI on a face-name paired associates encoding task (Petrella et al., 2006). A more recent intentional picture naming encoding task revealed decreased left PFC activation and increased right MTL activation in AMCI compared to controls specifically for successfully encoded items (Trivedi et al., 2008). This indicates that matching the performance of controls requires different contributions from PFC and MTL structures, which may be compensatory.

The available experimental data does not allow a coherent description of functional activation changes associated with encoding processes in AMCI. This is due to the small number of studies, the varying encoding tasks and the lack of consistent application of operationalised diagnostic criteria for AMCI. Generalisable results from these few studies indicate that matching the encoding performance of controls is accompanied by greater MTL activation whilst altered PFC and medial parietal activation is often present but not clearly related to encoding success. Future studies need to clarify the roles of incidental/intentional and successful/failed encoding of verbally/visually presented material as the networks that underpin them may overlap but are not identical. At present there appears to be a lack of appropriate studies designed to reveal the neural correlates of impaired performance on standard clinical verbal episodic memory tests. The verbal episodic memory paradigm that we employed bares much closer resemblance to clinical measures and our data will therefore be informative of the neural correlations of clinically observed amnesia (§3.9.3). The following section deals with attention and the interaction between episodic memory and attention.

1.5 Attention

Attention is crucial for everyday functioning and the ability to direct attention to information sources relevant to survival helps to compensate for the limited capacity of human cognition. Attention underpins almost all other cognitive processing; consequently, attentional deficits can have widespread effects on behaviour. Attentional is impaired early in the course of AD but appears intact in AMCI. However, clinical observation in AD and the cholinergic hypothesis (§1.1.2) suggest that attentional deficits may be present in AMCI. We studied the neural correlates of selective and divided attention in AMCI, and in this section, attention is introduced and the available findings from behavioural and functional neuroimaging studies are discussed to inform the interpretation of the results.

1.5.1 Theoretical Background

Attention refers to the capacity to direct consciousness towards specific internal or external stimuli. It can be sustained (maintained over a period of time), selective (ignoring non-task relevant stimuli) and divided. *Divided attention* refers to the capacity to simultaneously attend to multiple stimuli that may come from one (within-modal) or more (across-modal) sensory inputs. Attention is typically studied using tasks during which subjects are required to monitor a series of stimuli and to react to the presentation of predetermined *target stimuli*. Pressing a button usually registers a participant's reaction and the time recorded is known as the *reaction time* (RT). Target stimuli can be presented at regular or varying frequency and in isolation or together with other *distractor stimuli*. *Sustained attention* is typically studied on tasks where target stimuli are presented with regular frequency and in isolation. *Selective attention* is studied on tasks where target stimuli are presented together with distractor

stimuli. A measure of *accuracy* is usually calculated and this can vary from the basic percentage of correct responses to more sophisticated measures that take into account incorrect responses on non-targets (false recognition). We will look into the details of measuring accuracy in the methods section (§3.9.1.3).

The increased cognitive effort required during divided attention results in a *dual-task decrement*, evident as slower RT and/or decreased accuracy (Posner, 1978). An example of such a divided attention task involves *serial visual search* of a series of images and responses to predetermined target images, and simultaneous *serial auditory search* of words or tones with responses to target stimuli.

Recent functional neuroimaging studies have identified three attention related neural networks, each responsible for a different attentional function (Fan et al., 2005; Posner, 2004; Posner et al., 2000; Wang and Fan, 2007). These functions include *alerting, orienting and executive control*. Understanding each of these functions and the network that underpins them will inform the discussion of our functional neuroimaging results and we will look at them in turn.

Alerting achieves and maintains a heightened state of arousal in preparation for a tasks, it is underpinned by a network which includes thalamic nuclei, frontal and parietal cortices. *Orienting* selectively focuses attention on single or multiple items among distracters, it relies on a network that includes the parietal lobes, frontal eye fields, superior colliculi, and thalamus (pulvinar). *Executive control* monitors and resolves conflicts in planning and decision-making, it contributes to error detection, and it inhibits automatic execution of habitual actions; it relies on a network that includes the PFC, anterior cingulate, supplementary motor area and basal ganglia. Varying contributions from these functions seem to underpin the attentional capacities referred to earlier (selective, divided, sustained) so whereas divided attention may

make heavy demands on all three capacities, selective attention will require less orienting and executive control. We next look at the findings from studies of attention in AMCI.

1.5.2 Neuropsychological findings of attention in AMCI

Although AD is characterised by episodic memory impairment, findings of impaired everyday activities in AD in excess of that expected for the degree of amnesia, as well as attentional impairment predicted by the cholinergic hypothesis of AD lead to a closer examination of attentional function in AD. Subsequent studies found attentional deficits in the early AD that typically followed the onset of episodic amnesia (Chun and Turk-Browne, 2007; Perry and Hodges, 1999). Divided attention appeared to be the first non-memory cognitive domain affected in AD. Furthermore, greater impairment on divided attention tasks could distinguished AD from ageing and the specificity of such tasks could be enhanced by increasing the similarity between target and distractor stimuli (Baddeley et al., 2001).

As attention appeared effected in very mild AD, studies were conducted to examine if attention was also impaired in MCI. In fact, attentional deficits were found in this more heterogeneous group and these deficits, together with other non-amnestic impairments, increased the risk of conversion to dementia (Levinoff et al., 2005; Nestor et al., 2004).

Only a few studies have examined attention in AMCI and these have found a range of deficits. A retrospective study found impaired forward digit span (a measure of sustained attention and phonological working memory) in 29% of AMCI patients who had converted to dementia (Bozoki et al., 2001). A study of visual selective attention found that increased interference from distractor stimuli resulted in longer RTs in AMCI compared to controls (Perry and Hodges, 2003). The task used in this

study required subjects to ignore a non-target stimulus that briefly appeared before a target. The results indicate a failure of attentional selection or *top-down* (task driven) control of attention. Top-down mechanisms are proposed to regulate attention according to the demands of predetermined goals (Sarter et al., 2005). Top-down attentional modulation is associated with activation in prefrontal and parietal nodes of the attentional networks and appears mediated by cholinergic neurotransmission. The modulation of attention is discussed in more detail in a later section (§1.6.3). A study of attention in normal ageing, AMCI and AD using a visual search task requiring selective visual attention and attentional shifting, revealed impaired visual attention (slower RT) in AMCI and AD which distinguished these conditions from normal ageing and from each other (Tales et al., 2005a). Furthermore, in this study the potential confounding effects of age related general cognitive slowing was controlled for by logarithmic transformation of behavioural measures and results indicate that the attention impairment is disease related. Impaired selective attention (on a task where subjects search for symbols on a map) and impaired attentional switching (on a task where subjects alternate between counting up or down) in AMCI have also been reported by another group which indicates the consistency of this finding (Silveri et al., 2007).

In summary, findings from the few behavioural studies conducted in AMCI reveal deficits on selecting, switching, dividing and sustaining attention. Furthermore, attentional deficits increasingly appear a consistent finding in AMCI when more sensitive neuropsychological measures are used. In the following section, the functional anatomy of divided attention is discussed before we look at findings from functional neuroimaging studies of attention in AMCI.

1.5.3 Functional anatomy of divided attention

A systematic review of functional neuroimaging findings of attention and other functions domains in control groups revealed that selective attention tasks typically activate frontal (BA 9) and parietal (BA 7, 40) cortex together with relevant sensory cortices whilst activation is suppressed in non-relevant sensory cortices (Cabeza and Nyberg, 2000). Divided attention tasks typically activate unilateral prefrontal cortex for both within-modal stimuli (visual) (Corbetta et al., 1991; D'Esposito et al., 1995) and for mixed modality stimuli (visual and somatosensory) (Johannsen et al., 1999; Johannsen et al., 1997). More complex *dual-tasks* (divided attention tasks that require additional working memory and semantic processing) typically activate bilateral prefrontal cortices (Iidaka et al., 2000a; Koechlin et al., 1999). A study employing a well-designed divided attention task requiring concurrent processing of auditory tones and visual patterns showed activation of the left prefrontal cortex when contrasted with activation during visual selective attention or auditory selective attention alone (Loose et al., 2003). **Figure 4** demonstrates the areas of activation during this task, which appears typical for divided attention conditions compared to selective attention conditions. The design of this task and the stimuli used are similar to the divided attention paradigm designed for our study.

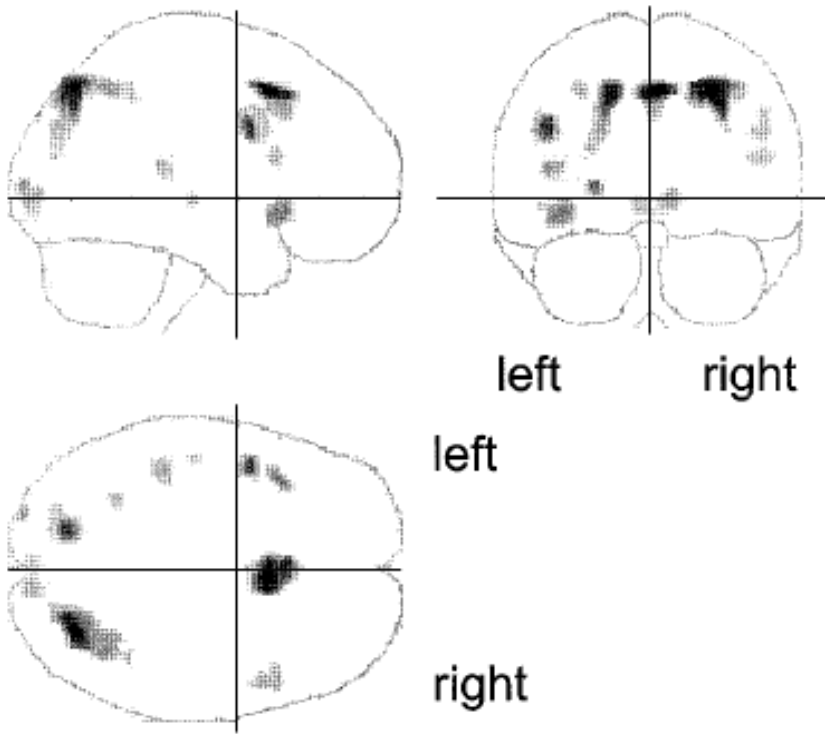


Figure 4. Functional activation during divided attention.

Activation during divided attention to auditory and visual stimuli is evident in bilateral anterior cingulate, prefrontal and occipital cortex, left superior temporal gyrus and precuneus and, right superior and inferior parietal cortex. (Loose et al., 2003). Reprinted with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc.

A more recent study comparing bimodal visual and auditory sustained attention, selective attention and divided attention also found left PFC activation only during divided attention (Johnson and Zatorre, 2006). Activation during divided attention appears not to be a simple summation of activation during selective attention; activation consistently decreases in relevant sensory cortices during divided attention relevant to selective attention and this has been ascribed to inter-sensory cortex inhibition or to limitations on processing control. The study by Johnson et al.

2006 revealed no differences in sensory cortex activation between divided attention and passive bimodal attention (attending to stimuli with no further reaction or processing required) indicating that sensory activation was not modulated by additional attentional task requirements. Selective attention to a sensory modality leads to increased activation in the relevant sensory cortex and decreased activation in the ignored modality's cortex, as would be expected. Combined sensory activation during divided attention was significantly less than the sum of activation during selective attention, supporting the idea of neural resource limitations. Furthermore, activation in DLPFC (BA 9) showed a negative correlation with task performance and sensory cortex activation. The authors suggested that poor performers were unable to sufficiently recruit sensory cortices during divided attention and that increased activation in DLPFC represents compensatory efforts and illustrates functional interactions between PFC and sensory cortices. A more recent study compared within-modal and across-modal divided attention in an effort to clarify if either is more resource demanding (Vohn et al., 2007). Auditory stimuli for the task consisted of serially presented single or paired musical notes and within-modal divided attention targets were note pairs increasing in tone. Visual stimuli were serially presented circles and squares, which increased (circles) or decreased (squares) in size during presentation; within-modal targets were consecutive shapes increasing in size. Across-modal targets were consecutive note pairs increasing in tones and consecutive visual squares decreasing in size. All divided attention conditions activated right PFC, inferior and superior parietal lobe and claustrum. Contrasting within-modal against across-modal conditions revealed increased activation during across-modal divided attention in bilateral DLPFC (BA 46), left DLPFC (BA 9), medial PFC (BA 8), and inferior parietal lobe (BA 40) and, in right anterior cingulate (BA 32). The authors

concluded that this reflected increased demand for coordination of cross-modal attentional resources, requiring more top-down control of attentional processing. Their were no significant behavioural differences during the different conditions and increased functional activation indicating greater effort during across-modal processing is therefore not supported by performance decrements; however, the task appears not to have been difficult enough to study this conclusively. From the few available studies, it appears that across-modal divided attention may be the more processing intensive attentional function and specifically associated with left DLPFC activation. Our divided attention task required across-modal divided attention therefore requiring greater cognitive effort making it more suitable as a probe for early attentional impairment. We look at findings from studies of attention in AMCI in the next section.

1.5.4 The functional anatomy of divided attention in AMCI.

Only a handful of functional studies of attention - none of divided attention - have been conducted in AMCI and we will therefore first look at the findings from studies of divided attention in AD. Even in AD, only a few studies have been conducted and this may be related to the difficulties in conducting functional imaging studies with participants who are cognitively impaired.

Functional neuroimaging studies of divided attention in AD

A PET study which correlated resting state cortical metabolism with RT during auditory and visual a selective attention tasks, as well as during a dual-task paradigm, found negative correlations between brain metabolism in the right PFC and parietal areas and RT only during the dual-task condition (Nestor et al., 1991). The task

required responses to the presentation of an auditory tone or when two serially presented visual stimuli were identical. Responses to the visual stimuli therefore required working memory, which renders this a dual-task rather than a divided attention task, and it would therefore be more reliant on PFC activation. The results from this study indicate an association between resting metabolism and attentional performance in AD.

A PET study using a divided attention task that required subjects to attend to and detect a change in frequency in a visual stimulus (a red and black reversing chequerboard) or tactile stimulus (vibration applied to the fingers of the right hand), demonstrated reduced activation in right cuneus and putamen, and left thalamus in AD (Johannsen et al., 1999). A further comparison of patients against controls across all attention tasks (divided attention, sustained attention) revealed reduced activation in AD in right PFC (BA 47), anterior pole (BA 10, 11) and occipital areas (BA 17). Increasing cognitive demand in the face of a limited resource leads to attenuated activation within that region and can therefore explain the attenuated activation during attention in AD (Goldberg et al., 1998; Nestor et al., 1991). That PFC is a key component of the attentional network and attention related PFC activation is related to modality independent executive control of attention whereas activation in occipital areas in the study above is likely specific to the visual modality. AD neuropathology have been demonstrated in the PFC in AD and AMCI (Guillozet et al., 2003; Kordower et al., 2001; Markesbery et al., 2006; Riley et al., 2002). The attenuated PFC activation may therefore be related to local neuropathology. Alternatively, distant pathology may influence PFC due to damaged association fibres or because of diminished cholinergic inputs into the PFC caused by neuropathology in the basal forebrain area.

Functional neuroimaging studies of attention in AMCI

The study of attention in high-risk AD states has received even less interest to date. Apart from our studies, only two other functional neuroimaging studies of attention in AMCI have been reported and none on divided attention. The first, an fMRI study limited to specific regions of interest (bilateral anterior cingulate, DLPFC and posterior parietal cortex), demonstrated greater PFC and posterior parietal cortex activation in AMCI patients when they matched the performance of control subjects on a task requiring visual sustained attention and working memory (Rosano et al., 2005). The task required participants to press a corresponding or opposing directional button following a probe presented by an arrow, each directional probe was preceded by a cue that indicated if responses should be congruent or incongruent with the directional. Increased activation whilst maintaining performance indicates that the AMCI group found the task more difficult. Interestingly, a parameterised increase in task difficulty was associated with greater activation of the posterior parietal cortex in AMCI patients, with decrements in speed and accuracy, whereas controls demonstrated greater PFC and anterior cingulate activity with a decrement only in speed. It is not clear if the failure to increase PFC activity in AMCI was due to a limitation in the reserve capacity for PFC activation or to deployment of an alternative but unsuccessful cognitive strategy. The second study examined the effects of reward on visuospatial attention. In AMCI, negative reinforcement (the prospect of losing money) enhanced attentional shifts and this correlated with posterior cingulated cortex activation, whereas positive reinforcement (the prospect of winning money) enhanced attentional shifts in controls and correlated with activation in orbitofrontal cortex (Bagurdes et al., 2008). The authors concluded that their results suggest a

reorganisation of the relationships between the limbic system and the spatial attention network in AMCI.

These limited findings indicate impaired sustained attentional processing in AMCI associated with PFC dysfunction and altered relationships between the nodes in the neural networks that underpin attention; however, due to the task characteristics these findings reflect altered processing in attention *and* executive processing as they were not separated. No functional studies of isolated sustained, selective or divided attention in AMCI had been published prior to our work and the functional status of these attentional conditions were therefore unknown in AMCI.

The PFC is a key constituent of the working memory network that controls attention and memory resources (Baddeley, 1986; Fletcher and Henson, 2001). Altered attentional and memory processing in AMCI may be related to impaired cholinergic modulation of cortical neuronal responsiveness and I discuss this in the next section where we also look at the role of the PFC in more detail.

Summary

In summary, behavioural findings in AD indicate divided attention as the first non-memory domain to show impairment following episodic memory deficits, most likely because it appears to be the most resource demanding attentional function. Divided attention could therefore serve as a potentially useful indicator of early regional cortical functional deficits in AMCI patients. Very limited functional neuroimaging findings are available for appraisal in AMCI but from these indicate that attentional impairment is associated with altered cortical activation. Based on the behavioural and functional neuroimaging findings of attention in AD and AMCI we hypothesised that AMCI will be associated with divided attention deficits and altered cortical

activation because increasing cognitive demand in the face of failing capacity leads to attenuated activation of participating brain areas. In the next section we discuss the relationships between attention, memory, the PFC and the BFCS as these relationships appear relevant to the results of the individual tasks as well as overall results of the work I report on here.

1.6 The Regulation of Memory and Attention: The Central Executive, Prefrontal Cortex, Basal Forebrain Cholinergic System and Brainstem Locus Coeruleus.

Memory and attention are controlled and optimised by the central executive that is closely related to PFC function. Close relationships, as well as significant overlap exists between the central executive, PFC, memory and attention. In addition, memory, attention and PFC activity are regulated via the BFCS and brainstem locus coeruleus. In this section, the PFC, BFCS and locus coeruleus are introduced and their relationships with memory and attention discussed.

1.6.1 Memory - Attention Interaction

The close interaction between memory and attention is complex and the distinction between these two cognitive modalities is increasingly less clear (Chun and Turk-Browne, 2007). Attention is optimised by previous experience on a task because task specific learning takes place - a memory effect. For example, practice on a dual-task or it subcomponents has considerable beneficial effects on behavioural performance during the dual-task. In turn, memory has a limited capacity and attention plays an important role in selecting specific stimuli for encoding. Consequently, focusing

attention on an item during encoding increases retrieval success. It follows that memory can be influenced by the demands made on attentional resources (ability to attend) and selection (consciously directing resources towards particular stimuli). When demands on attentional resources or selection reach critical levels, it can have a detrimental effect on memory performance. Disease processes affecting attentional resources can therefore impair memory by inhibiting the ability to attend or the mechanism of selection.

Impairment in divided attention or episodic memory is often reciprocated in the other. Divided attention deficits impair encoding of contextual information related to the episodic memory (*source monitoring*), which improves recall. In turn, memory deficits result impaired divided attention because the beneficial effects of practice, which reduces attentional load via automating parallel processing, are attenuated (Johnson, 1997; Sarter and Turchi, 2002; Schneider and Shiffrin, 1977). Encoding processes generally consume more cognitive resources than retrieval processes and memory processes overall make large demands on cognitive resources which makes it particularly vulnerable to the effects of attention deficits (Craik et al., 1996; Nyberg et al., 1997). The bidirectional relationship between divided attention and memory appears to explain the rapid decline from early deficits towards generalised cognitive impairment and dementia (Sarter and Turchi, 2002).

These findings indicate the close reciprocal relationship between attention and memory and that a deficit in one affects the other; furthermore, they illustrate why a thorough investigation of either would require close examination of both. Memory and attention are controlled and optimised by the central executive and we look at this next.

1.6.2 Central Executive Function

Working memory refers to a brain system that provides short-term storage of information and manipulation of this information that is required to complete complex cognitive tasks such as learning, reasoning and language comprehension (Baddeley, 1986). Working memory is proposed to consist of the *central executive*, the phonological loop, the visuospatial sketchpad and the episodic buffer (Baddeley and Della, 1996). In turn the central executive appears to have distinguishable functions including the selection and manipulation of information in long-term memory, selecting and inhibiting information, attentional control, coordination of two or more concurrent activities, and updating working memory. The role of the central executive is to optimise memory and control attentional processing. The phonological loop and visuospatial sketchpad are materials specific information stores, with limited capacity, concerned with the maintenance of verbal and visual spatial information respectively.

Memory optimisation relies on the executive functions of maintaining, listing, comparing and associating information in working memory. Executive control of attentional relates to switching, focusing or dividing attention. Memory optimisation and attentional control are required because the demands on memory and attention vary depending on the task. A dual-task requiring encoding of alternating visual and auditory stimuli can be demanding on attentional selection by requiring frequent fast switching between sensory modalities whilst having little or no effect on encoding selection or resources per se, as long as the stimuli are easily recognisable and memorable. Conversely, a dual-task requiring encoding of visual and auditory stimuli that are difficult to recognize, as for example faces with a large degree of similarity or distorted sound, could tax encoding resources whilst placing relatively little demand on attentional selection resources.

Central executive function is therefore essential for optimal memory and attention performance and executive deficits affect performance to varying degrees depending on the characteristics of the task. Central executive function is strongly associated with PFC activation and we look at the PFC in the next section.

1.6.3 The Prefrontal Cortex

Anatomy

The PFC corresponds to the lateral aspect of the frontal cortex and is subdivided into the dorsolateral prefrontal cortex (DLPFC) and ventrolateral prefrontal cortex (VLPFC)(Fletcher and Henson, 2001). The DLPFC loosely consists of the area superior to the inferior frontal gyrus and contains BA's 9 and 46 while the VLPFC consists of the lateral aspect of the inferior frontal gyrus and contains BA's 44, 45 and 47. The cortical area anterior to the PFC, known as the anterior frontal cortex, contains BA's 8 and 9.

Function

The DLPFC, VLPFC and anterior frontal cortex areas are involved in working memory functions; however, there appears to be a degree of functional specialisation. The DLPFC activates more often during tasks requiring manipulation, the VLPFC during tasks requiring maintenance of information, and the anterior frontal cortex during more complex processes requiring maintenance of goals and products of one task while performing another. Furthermore, there is some evidence of lateralisation depending on the type of material with left dominance for verbal material and right dominance for visuospatial material. (Fletcher and Henson, 2001).

The PFC in AD and AMCI

Episodic amnesia in AD and AMCI can readily be explained by AD pathology in MTL areas; however, attention deficits are more likely related to pathology affecting areas typically active during attention tasks including the PFC, cingulate and parietal cortices. The PFC typically activates during both verbal memory and attention tasks and PFC dysfunction could therefore contribute to impairments in both. PFC activation during memory and attention tasks appears related to the activity of the central executive as discussed above. Neocortical areas including the PFC and parietal cortex are already affected by neuropathology in very mild AD and neocortical synapse densities in these cortices are highly correlated with dementia severity in AD (Morris et al., 1991; Samuel et al., 1994). The attentional and memory deficits evident in AD and AMCI could therefore in part be due to interrupted connectivity between the PFC and other nodes in the networks supporting these cognitive functions. Alternatively, pathology affecting the BFCS and therefore cholinergic modulation could account for much of the cognitive impairments seen in the AD spectrum via direct effects on cortical areas and/or indirect effects mediated by PFC failure and concomitant executive failure. The BFCS provides the main cholinergic innervation to all neocortical areas and the hippocampi. It is affected in the very early stages by AD neuropathology and already shows evidence of tauopathy in AMCI (Mesulam et al., 2004; Mesulam and Geula, 1988). In this section, we have seen the important role of executive functioning in memory and attention processing and how this relates to the PFC. It is also apparent how the PFC and other cortical areas that constitute the networks that support executive functioning, memory and attention can be affected by local AD neuropathology or distant pathology affecting the BFCS and thereby

cholinergic modulation of cortical processing. These relationships are illustrated in **Figure 5**. We next look at the BFCS in more detail.

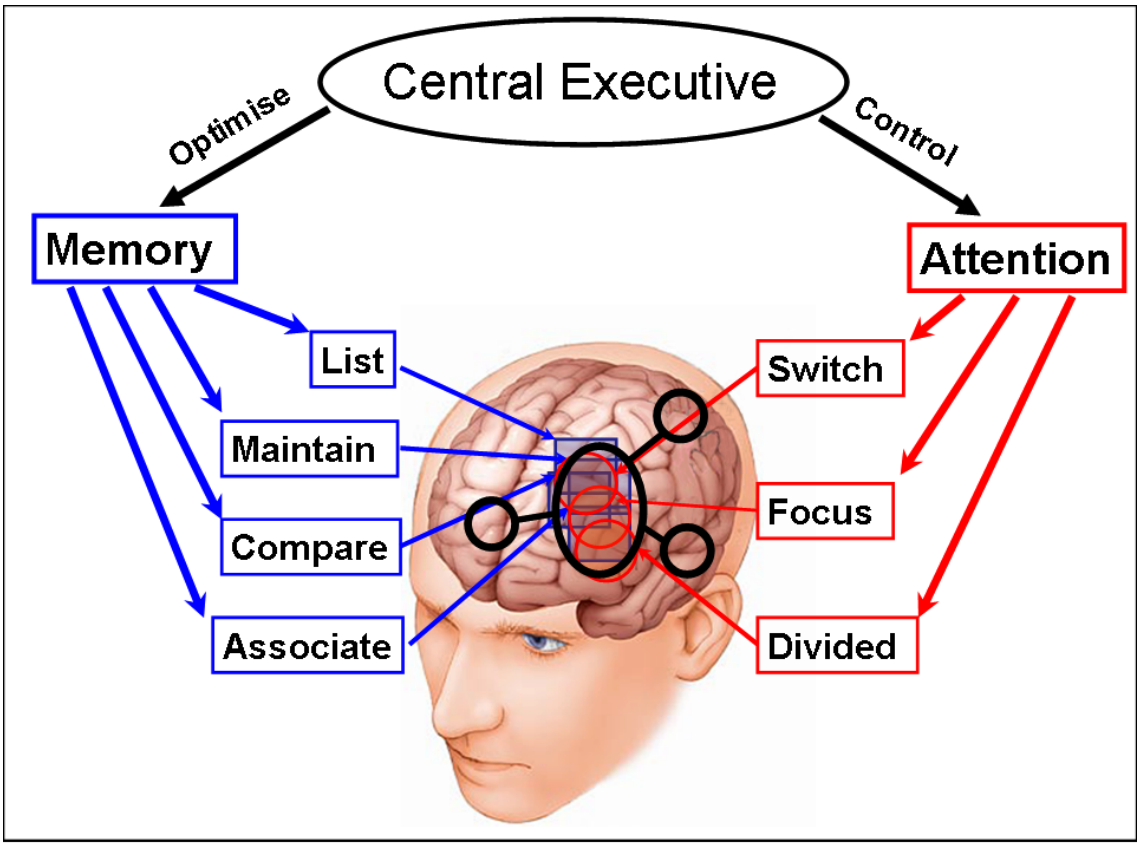


Figure 5. Executive control of memory and attention.

The figure illustrates the role of the central executive part of working memory in optimising memory and controlling attention. Memory tasks requiring manipulation in working memory (listing, maintaining, comparing or associating items to be encoded) activate the prefrontal cortex. Similarly, attention tasks requiring switching, focusing and dividing attention activate the prefrontal cortex. The prefrontal cortex appears to be a key node in the network that underpins executive functions. Other areas in the executive network include the anterior cingulate, premotor areas, limbic system, MTL and association cortices.

1.6.4 The Role of the BFCS in attention and memory

Anatomy

The basal forebrain cholinergic system (BFCS) is the major source of acetylcholine in the brain and provides its primary cholinergic input to the hippocampus and entorhinal cortex, but it also projects to all cortical areas and to the amygdala (for a review see (Auld et al., 2002). **Figure 6** illustrates the cholinergic pathways that originate from the Ch4 region of the nucleus basalis of Meynert in the human brain. The medial bundle supplies areas close to the midline including olfactory, cingulate, retrosplenial and medial occipital cortex. The lateral pathway supplies the rest of the cortex via two branches. The two pathways merge anterior in the orbitofrontal area and posterior in the occipital area. **Figure 7** illustrates the major cholinergic afferents from the BFCS to the neocortex, entorhinal cortex, amygdala and hippocampus.

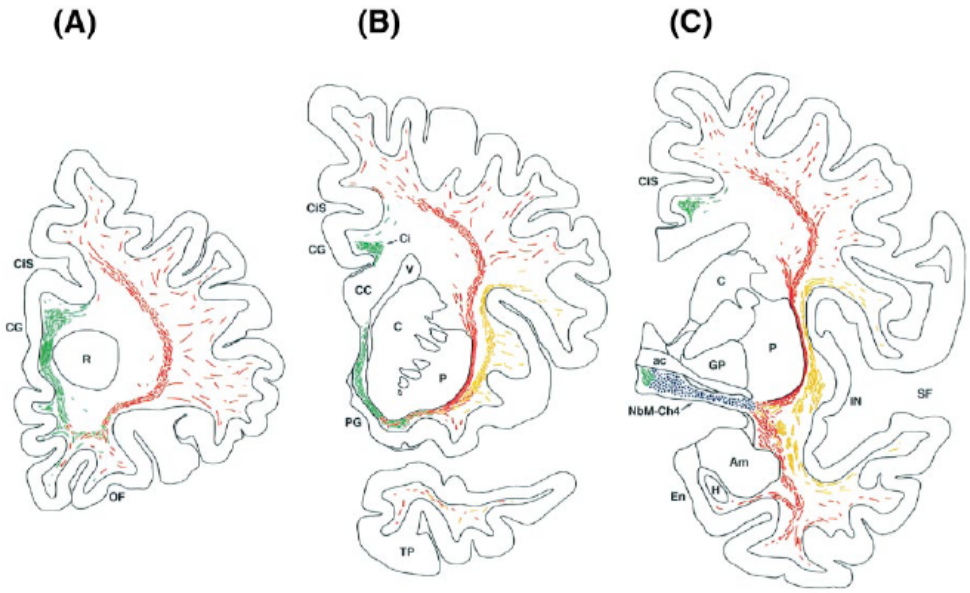


Figure 6. Cholinergic pathways in the brain.

This figure illustrates the medial (green) and lateral cholinergic pathways that originate from the Ch4 region of the nucleus basalis of Meynert in the human brain.

The medial bundle supplies areas close to the midline including olfactory, cingulate, restrosplenial and medial occipital cortex. The lateral pathway supplies the rest of the cortex via two branches (red and orange). The two pathways merge anterior in the orbitofrontal area and posterior in the occipital area. Sections proceed from rostral (A) to ventral (C). (Selden et al., 1998). Reproduced by permission of Oxford University Press.

Function

The function of Ach and therefore cholinergic neurotransmission in memory and attention processing is discussed in detail in §1.7.1.

The BFCS in AD and AMCI

From the comprehensive cholinergic innervation of the cerebrum discussed above, it follows that AD neuropathology affecting the basal forebrain nuclei could diminish cholinergic neurotransmission to all these areas in AMCI and AD. Research findings support this notion as cholinergic neurotransmission is prominently affected in AD where degeneration of cholinergic neurones of the basal forebrain nuclei leads to diminished cortical and hippocampal input (Francis et al., 1999; Perry et al., 1999; Sarter et al., 2003). The status of cholinergic neurotransmission in AMCI is less clear. Post mortem reports suggest reduced numbers of basal forebrain cholinergic neurones (Mufson et al., 2002), but upregulated cholinergic neurotransmission as indicated by elevated levels of cholinesterase acetyltransferase (responsible for acetylcholine synthesis) (DeKosky et al., 2002). Recent animal studies suggest that upregulated cholinergic neurotransmission may be caused by noradrenergic deficits whilst AMCI is associated with AD neuropathology in the locus coeruleus that is the source of

cerebral noradrenaline. We look at this in detail in the following section (§1.6.5). Findings from functional neuroimaging studies are more consistent. PET studies of in vivo activity of acetylcholinesterase (AChE), the enzyme that rapidly metabolises acetylcholine, have found a similar pattern of deficiency in AMCI as seen in AD (Rinne et al., 2003). Furthermore, decreased cortical AChE activity in AMCI predicted conversion to AD (Herholz et al., 2005). A recent PET study using a ligand that binds to the most prevalent cortical acetylcholine receptor (nicotinic α4β2 subtype) reported significantly decreased hippocampal, caudate, frontal cortex, temporal cortex, parietal cortex, anterior and posterior cingulated binding in AMCI and AD patients compared to controls (Sabri et al., 2008). Acetylcholine receptor binding in MTL positively correlated with MMSE scores, indicating that better global cognition associates with the availability of more cholinergic receptors. Interestingly, it appears that nicotinic acetylcholine receptor binding correlates more with attention than with episodic memory as significant correlations have been demonstrated between receptor binding (whole brain, frontal association cortex, parietal cortex) and measures of attention, with no correlations evident between receptor binding and episodic memory in any cortical area in mild AD (Kadir et al., 2006). In summary, these findings indicate that AMCI and AD are associated with reduced cholinergic innervation *and* reduced neuronal cholinergic receptors on the background of very early pathology in the BFCS, the source of Ach.

Animal models indicate that BFCS lesions can cause memory deficits *in the absence of hippocampal damage*, apparently mediated via impaired attentional modulation rather than specific memory impairment (Auld et al., 2002; Chiba et al., 1999). The cholinergic hypothesis of cognitive impairment in AD may therefore explain impairments in memory and non-memory functions such as attention

(Lawrence and Sahakian, 1995; Parasuraman et al., 1995; Parasuraman et al., 1992), which are regulated by the BFCS (for a review see (Sarter et al., 2005)).

Several lines of evidence indicate regulation of attentional processing via cholinergic input to sensory cortices where it improves the signal-to-noise ratio (signal-driven or bottom-up modulation), and to frontal and parietal cortex where it regulates processing in selective cortical areas (task driven or top-down modulation) (Sarter et al., 2005). BFCS pathology appears to be the earliest lesions in AD and it could affect the MTL via reduced cortical cholinergic innervation disrupting cognitive modulation, explaining the amnesia and impairment on other cognitive functions. **Figure 7** illustrates the cholinergic innervation of various brain areas. The entorhinal cortex and hippocampus receive inputs from the medial septal nucleus and diagonal band of Broca whilst the nucleus basalis of Meynert projects to the amygdala and neocortical association areas. This illustrates how neuropathology affecting any of the nuclei that comprise the BFCS can affect memory and attention either directly or indirectly.

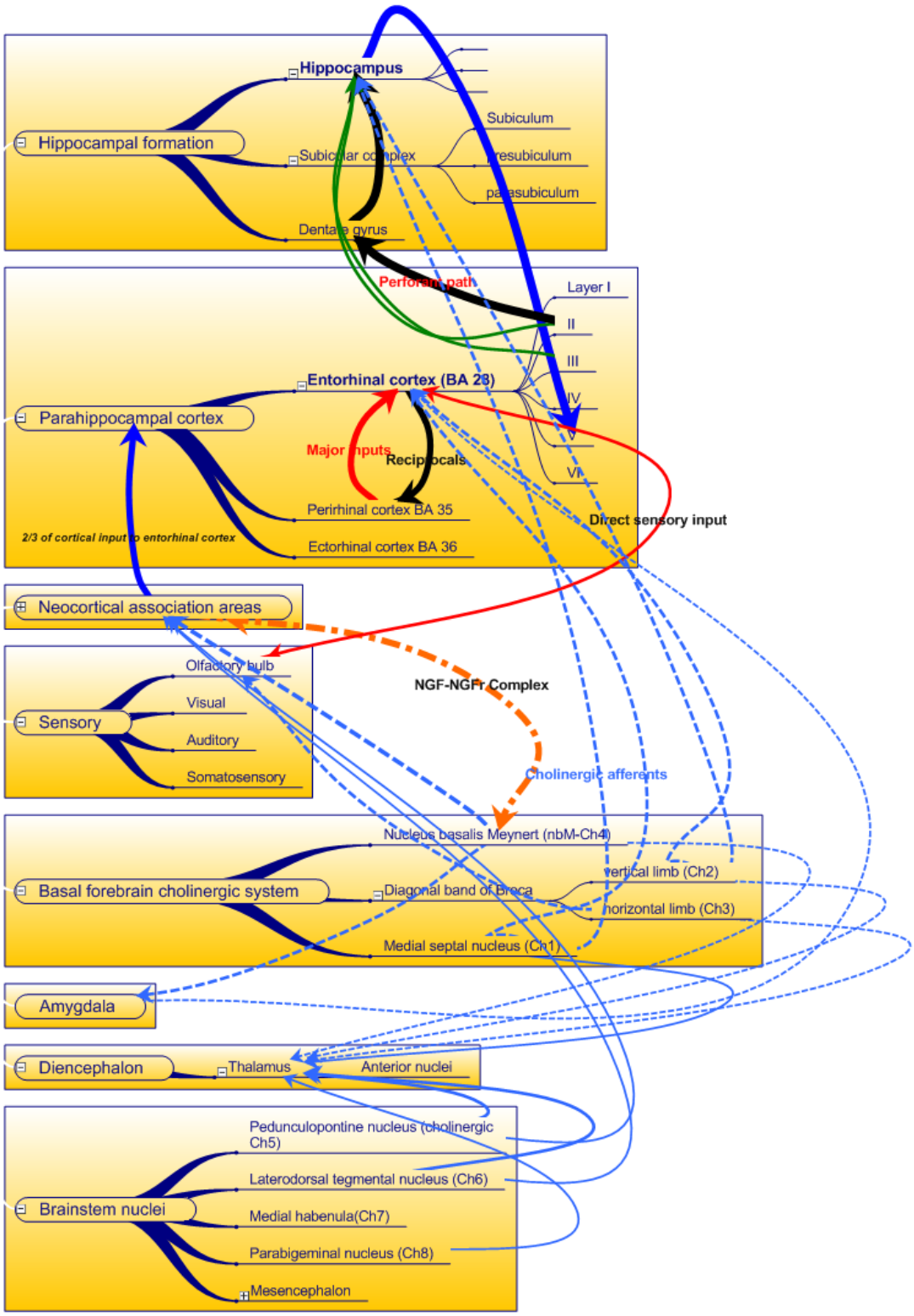


Figure 7. Cerebral cholinergic afferents.

The figure illustrates the major cholinergic afferents (dashed light blue lines) from the BFCS to the neocortex, entorhinal cortex, amygdala and hippocampus.

1.6.5 The Role of the Brainstem Locus Coeruleus in Attention and Memory

The locus coeruleus contributes substantially to the control of attention and memory. Its role in cognitive control, small size and vulnerability to AD neuropathology highlight it as one of the areas that may be key in the development of AD, similar to the BCFS discussed above (§1.6.4).

Anatomy and function

The locus coeruleus is located in the tegmentum in the brainstem and the sole source of noradrenaline to the neocortex, cerebellum, hippocampus and most of the thalamus. Noradrenaline from the locus coeruleus regulates attentional selection, arousal, and stress reactions related to environmental challenges (Aston-Jones, 2005; Foote et al., 1983).

Animal studies indicate that unpredictable but relevant stimuli activate the locus coeruleus more than irrelevant stimuli and this holds for multiple sensory modalities. The locus coeruleus also shows increased activation under other stressful situations including loud noise, punishment, pain, emotionally aversive images of snakes and angry faces (Raizada and Poldrack, 2008).

Lesions of the locus coeruleus, therefore *noradrenergic lesions*, affect learning in animal models and exacerbate the amnestic effects of cholinergic deficits induced by either muscarinic receptor blockade or BFCS lesions, on new learning and recall of pre-lesion episodic memory (Abe et al., 1997; Murchison et al., 2004; Ohno et al., 1993; Ohno et al., 1997). Furthermore, experimentally induced lesions of the locus coeruleus promote amyloid plaque deposition and neuronal loss in projection areas whilst sparing non-projection areas (Heneka et al., 2002; Heneka et al., 2006). These

lesions also reduce cerebral glucose metabolism and aggravates memory deficits in a transgenic animal model of AD. These findings indicate that noradrenergic lesions result in cognitive and metabolic changes characteristic of AD.

Activation in the locus coeruleus, and in the right PFC, appears to correlate with sudden unpredictable increases in task difficulty that constitutes a challenge to attentional resources. This was demonstrated by an fMRI study of attentional resource allocation during challenging attentional conditions (Raizada and Poldrack, 2008). The task for this study employed high intensity visual (flashed white disc) and auditory stimuli (bursts of noise) with unpredictable onset. A period of stimulation would start with either visual or auditory, or visual and auditory stimulation with the latter condition being the most challenging. Increased activation in the locus coeruleus and right PFC occurred during the most challenging attentional condition. These findings revealed a close correlation between activation in these two areas, indicating a high level of functional connectivity that is supported by structural and functional connectivity studies in animals. Furthermore, they demonstrated close correlation between activation in these areas and task difficulty; however, PFC activation increased gradually whilst the brainstem only activated during the most challenging conditions. Activation in the locus coeruleus was also correlated with activation in the visual, auditory and parietal cortices. The locus coeruleus is the main source of noradrenaline to the cortex and this pattern of correlation mirrors that of the known widespread projections from the locus coeruleus. The authors suggested their findings indicate that meeting attentional demands on challenging tasks are mediated by a frontal-brainstem network wherein the brainstem signals the onset of a challenging attentional condition and the right PFC allocates cognitive resources.

The locus coeruleus in AD and AMCI

AD neuropathology in the locus coeruleus has long been implicated in the pathogenesis of AD and several studies have revealed prominent neuronal loss (reaching 70% in the rostral nucleus) and significant reduction of cortical and limbic NA levels in AD (Grudzien et al., 2007; Heneka, 2009). The reduction of noradrenaline concentration is highly correlated with disease progression, memory deficits and cognitive impairment. Findings indicate that more extensive neuronal loss in the locus coeruleus correlates better with disease progression compared to cholinergic cell loss in the in the nucleus basalis of Meynert (Forstl et al., 1994; Zarowet al., 2003). Meta-analysis of locus coeruleus neuronal loss (311 brains from 24 autopsy studies) indicates that AD patients invariably had substantially reduced cell counts (effect sizes = 2.28; 95% confidence interval = 2.06–2.51) (Lyness et al.2003). Altered activation in the locus coeruleus in AMCI may be caused by NFTs that occur therein in AMCI and AD; the number of NFTs correlate inversely with cognitive performance as measured by the MMSE (Grudzien et al., 2007).

The locus coeruleus contain a small number of neurones, approximately 20 000 in total, therefore minor pathological lesions can have widespread effects on cognition. Serotonin release in the hippocampus, and Ach release in the hippocampus and nucleus basalis of Meynert is regulated (inhibited) by locus coeruleus derived NA (Siniscalchi et al., 1994), and serotonin and Ach are upregulated in MCI compared to both age-matched controls and AD patients (DeKosky et al., 2002; Truchot et al., 2008). Animal studies reveal that chemical ablation of the locus coeruleus can upregulate Ach and serotonin metabolism similar to that reported in MCI (Jackisch et al., 2008). These findings suggest that AD neuropathology in the locus coeruleus impairs noradrenergic outflow to the cortex and hippocampus in AMCI, resulting in

unopposed serotonin and Ach release. Furthermore, findings that indicate synaptically linked noradrenergic input from the locus coeruleus to the nucleus basalis of Meynert suggest direct modulation of Ach release from the latter by the former (Jones and Yang, 1985; Smiley et al., 1999). This has lead to the suggestion that degeneration of the locus coeruleus would have to precede degeneration of the nucleus basalis of Meynert (and raphe nucleus) in order to give rise to the observed upregulation of Ach metabolism in AMCI, followed by the reduction that marks advanced AD (Heneka, 2009).

The locus coeruleus in ageing

Locus coeruleus noradrenaline signalling may also play a role in ageing. Performance on certain attentional tasks can be restored in aged rats to levels seen in young animals by electrical stimulation of the locus coeruleus. Furthermore, memory is enhanced by pharmacological manipulation to increase locus coeruleus activity, intraventricular transplantation of locus coeruleus neurones, and by direct intraventricular infusion of noradrenaline ((Heneka, 2009) and references therein). The beneficial effects of increased noradrenergic neurotransmission by these manipulations suggest that early AD neuropathology in the locus coeruleus in AMCI can exacerbate the cognitive deficits caused by BFCS pathology. However, it remains to be established if similar locus coeruleus stimulation will be beneficial in AMCI and AD where neuropathology affects connectivity.

Taken together, these findings indicate that AD neuropathology is likely to be present in the locus coeruleus in AMCI and that it can have widespread cortical effects that can contribute to attention and memory deficits.

Summary

This section has highlighted the interactions between memory, attention, the central executive, PFC, BFCS and locus coeruleus. Attention is regulated in sensory cortices by the central executive via the PFC and BFCS, and both these areas are regulated by the locus coeruleus. Memory is regulated in the MTL by the central executive via the PFC, BFCS and locus coeruleus. In addition, memory and attention closely interact. It is also apparent how neuropathology affecting the BFCS and locus coeruleus very early in the course of AD may account for the widespread cognitive deficits associated with AD. In the following section would take a closer look at acetylcholine and acetylcholinesterase inhibitor treatment.

1.7 Acetylcholine and Acetylcholinesterase Inhibitor Treatment

Acetylcholine (Ach) is a neurotransmitter in both the central and peripheral nervous systems where it functions as a neuromodulator. The cortical effects of the *cholinergic system*, comprised of the cholinergic neurons and Ach, are predominantly excitatory. *Cholinergic function* plays a role in arousal and reward mechanisms and in the regulation of sensory attention and sustained attention. AD neuropathology affects cholinergic neurotransmission by damaging cholinergic neurons in the BFCS and cholinergic axons. In this section, we look at the role of acetylcholine in memory and attention before we look at acetylcholine esterase and acetylcholinesterase inhibitors. I will discuss the treatment effects of acetylcholinesterase inhibitors on attention in health and on cognitive and behavioural impairments in AD and AMCI

1.7.1 The Role of Acetylcholine in Memory and Attention

Memory

Acetylcholine (Ach) appears critical for the modulation of memory consolidation. Animal studies in the 1950's and 1960's showed the beneficial effects of increased Ach following acetylcholinesterase inhibitor (ACEI) exposure and the detrimental effects of the cholinergic receptor antagonists atropine and scopolamine (which could be reversed by ACEI administration) on maze learning in rats (for a reviews see (Blokland, 1995; Power et al., 2003). Increased Ach could potentially influence memory directly or via effects on attention. Studies where cholinergic enhancement followed the learning phase were able to dissociate its effects on attentional processing at the time of learning from its effects on memory consolidation and the effects on memory consolidation appear independent of any attentional enhancement. Infusion of a cholinergic drug directly into isolated brain areas revealed its strategic role in memory consolidation in the amygdala, hippocampus and anterior cingulate. Long term disruption of Ach production by local infusion of the high affinity choline uptake inhibitor AF64A in frontoparietal cortex resulted in impaired episodic memory ((Power et al., 2003) and references therein). It appears from these findings that cholinergic inputs into the amygdala, hippocampus, anterior cingulate and frontoparietal cortex all contribute to normal episodic memory function. Findings from functional neuroimaging reveal considerable overlap with these areas in spite of being dependent on task design and choice of stimuli. Left prefrontal and MTL activation consistently appear during memory processing with less reliable activation evident in cingulate, parietal, occipital and other areas (for a review see (Cabeza and Nyberg, 2000). Together, these studies demonstrate the key areas where cholinergic modulation of episodic memory takes place and again highlight the role of the BFCS,

which is the main source of cholinergic innervation to all these areas, in memory function.

Attention

Ach regulates attention by promoting attentional selection and stimulus discrimination. It is released diffusely throughout the neocortex during periods of increased attentional demand. It regulates processing in higher cortical areas (prefrontal) through *bottom-up* or *stimulus-driven* effects from sensory cortices and, in early sensory cortices (e.g. primary visual cortex) through *top-down* or *task-driven* effects from higher cortical areas which amplify signal and reduces noise in sensory cortices (for a review see (Knudsen, 2007) (McGaughy and Sarter, 1998; Murphy and Sillito, 1991; Sato et al., 1987). Information about the world enters the nervous system and is processed in sensory cortices where salience filters allow infrequent or important stimuli to pass through to higher cognitive processing, this is know as *bottom-up* or *signal driven* modulation of processing. Signals related to sensory information, memories, affective state and movement compete for entry into working memory. Goal orientated representations active in working memory can direct *top-down* biased signals to regulate the sensitivity of representations that are being processed in working memory, i.e. *task driven* modulation.

Ach therefore appears crucial in memory consolidation and in top-down and bottom-up regulation of attention. The role of cholinergic neurotransmission in attention and memory therefore indicates potentially beneficial effects of increased synaptic Ach following ACEI treatment in disease states associated with cholinergic deficits. In the

next section, we look at the enzyme acetylcholinesterase (AChE), which inactivates Ach.

1.7.2 Acetylcholinesterase

Ach released from the axon terminal in response to neuronal depolarisation is rapidly hydrolysed to choline and acetate by cholinesterase enzymes. AChE is the major cholinesterase enzyme and is found throughout the brain in neurones and axons and its action is specific to Ach. Butyrylcholinesterase also metabolises Ach and other neuropeptides, it is mainly found in glial and endothelium cells and occurs in high concentrations in the hippocampus, thalamus and amygdala (Lane et al., 2006). Ach is mainly regulated by AChE with additional regulation from butyrylcholinesterase. It is for this reason that AChE became a target of drug development with the potential of increasing Ach through inhibition of AChE. We next look at acetylcholinesterase inhibitors (ACEI) and in particular at rivastigmine.

1.7.3 Acetylcholinesterase Inhibitors and Rivastigmine

There are three main ACEI drugs in general clinical use (rivastigmine, donepezil, galantamine) and their introduction followed initial trials with tacrine which was discontinued due to more pronounced side effects. Rivastigmine (Exelon – Novartis Pharmaceuticals) is a non-competitive inhibitor of AChE (Spencer and Noble, 1998). It readily crosses the blood-brain barrier and inhibits AChE and butyrylcholinesterase for about 10 hours following a single dose. It is available as oral preparations taken twice daily and the daily dose range is between 3mg and 12 mg. The recommended dosing regime starts with 1.5mg twice daily and increases in steps of 1.5mg twice

daily at intervals of at least 2 weeks. Common side effects include asthenia, anorexia, dizziness, drowsiness, nausea and vomiting. Side effects are dose related and commonly occur at the start of therapy or when the dose in increased.

1.7.4 ACEI Treatment Effects on Verbal Episodic Memory in Health, AMCI and AD.

Delayed verbal episodic memory as measured by delayed recall of word lists does not appear to benefit from ACEI treatment in healthy subjects. A double blind placebo controlled treatment trial of donepezil for 30 days, in 30 young healthy adult males, revealed improvement on verbal working memory (immediate recall) but not on delayed recall after 30 minutes (Gron et al., 2005). A double blind placebo controlled trial of 14 days donepezil treatment in healthy elderly subjects (n=26), which used a sensitive computerised cognitive test battery, failed to demonstrated treatment effects on verbal episodic memory and found *impaired* working memory and executive functioning (Beglinger et al., 2005). The same group also reported absent treatment effects on episodic memory and similar impairments on executive functioning and working memory in healthy young adults following seven days of donepezil treatment (Beglinger et al., 2004).

Findings from ACEI treatment trials in AMCI have also failed to demonstrate clear improvements in verbal episodic memory. A double blind placebo controlled study of ACEI treatment effects (donepezil, 28 days) in AMCI patients (n=270) failed to demonstrate improvements on delayed verbal episodic memory on the intention-to-treat, last observation carried forward analysis (Salloway et al., 2004). A large double blind placebo controlled trial (n=769) of donepezil and vitamin E treatment for AMCI failed to demonstrate improved episodic memory at study endpoint (36 months) as

measured by a compound score which included immediate (working memory) and delayed verbal recall; however, slight improvements were evident at 18 months (Petersen et al., 2005). It is not clear from this report if the improved memory score at 18 months was due to effects on tests assessing immediate recall, a measure of working memory, or on delayed recall, a measure of episodic memory. A meta-analysis of three unpublished and two published (Petersen et al., 2005; Salloway et al., 2004) randomised controlled ACEI treatment trials conducted in AMCI-multiple domain, between 1999 and 2004, demonstrated no significant effects on verbal episodic memory (Raschetti et al., 2007). A Cochrane review of two published and one unpublished double blind placebo controlled trials of donepezil found no significant effect on verbal episodic memory (Birks and Flicker, 2006).

Meta-analysis of rivastigmine and of rivastigmine, donepezil and galantamine combined, have not demonstrated any significant treatment effects on verbal episodic memory although modest effects were evident on global cognitive performance (Birks, 2006; Birks et al., 2000; Birks et al., 2009).

Taken together these findings in health, AMCI and AD fail to indicate significant ACEI treatment effects on verbal episodic memory. Treatment of cholinergic neurone loss by increasing the availability of synaptic Ach has been modelled on the replacement of dopamine in Parkinson's disease and assumes that cortical cholinergic inputs have predominantly tonic activity (Sarter and Turchi, 2002). However, it is unlikely that the complex neuronal systems mediating episodic memory function operate on the basis of tonic activity. Increased synaptic Ach is therefore unlikely to replace the functions of presynaptic neurones and may in fact impair the ability of the cholinergic system to regulate cortical information processing because of dissociation between presynaptic activity and postsynaptic receptors (Bowers et al., 1964; Sarter,

1994). In fact, increased Ach in the synaptic cleft may impair the ability of presynaptic neurones to encode information because of excessive autoreceptor stimulation by Ach (Becker and Giacobini, 1988a; Becker and Giacobini, 1988b; Sarter and Turchi, 2002). The findings in AD can also in part be related to the lack of sensitivity of the commonly used outcome measure, the Alzheimer's Disease Assessment Scale (Benge et al., 2009; Rosen et al., 1984). It is as yet not clear why visual episodic memory appears to respond to ACEI treatment but not verbal episodic memory and it may be related to differential processing of verbal and visual stimuli as far as hippocampal involvement in long term storage is concerned (Gron et al., 2005).

1.7.5 ACEI Treatment Effects on Attention in Health, AMCI and AD.

The effects of ACEI on attentional processing are mediated via task-driven effects in prefrontal and parietal cortices and via signal-driven effects in sensory cortices, as described above. Only a few behavioural studies have examined the effects of prolonged ACEI treatment on attention in healthy subjects in a manner resembling clinical treatment. Healthy airline pilots treated with donepezil for 30 days demonstrated superior sustained (visual) attention during flight simulator exercises compared to treatment with placebo (Mumenthaler et al., 2003; Yesavage et al., 2002). A similar study in healthy young subjects found improvements in verbal working memory and visual episodic memory, but not in attention (Gron et al., 2005). The results from these studies do not appear consistent but the reports related to the flight simulator exercises lacks sufficient detail to be appraised alongside the later study by Gron et al. 2005. Nevertheless, the findings from single dose and prolonged ACEI treatment in healthy controls demonstrate beneficial effects on aspects of cognitive processing.

Functional neuroimaging studies of attention and working memory have demonstrated ACEI effects on behaviour and activation. Given the small number of relevant studies, we will look at the findings from behavioural and functional studies in AMCI and AD together in this section.

Improved visual working memory (faster reaction times) together with decreased activation in associated frontal cortical regions and increased activation in extrastriate visual cortex were reported following single dose physostigmine administration in healthy adults (Furey et al., 2000; Furey et al., 1997). The authors suggested their findings indicate that improved perceptual processing in primary sensory cortices leads to simplified working memory demands which in turn required less PFC participation. This conclusion is supported by findings from recent animal studies demonstrating that visual cortex stimulation causes increased local acetylcholine release via a pathway that proceeds from visual cortex to PFC to the basal forebrain and back to visual cortex. Increased sensory cortex responsiveness due to physostigmine could consequently require less effort from the PFC to enhance sensory cortex excitability (Rasmusson et al., 2007). Physostigmine administration also resulted in improved reaction time associated with decreased primary visual cortex and increased extrastriate cortex activation during visuospatial working memory and selective visual attention tasks with additionally improved accuracy on the later (Bentley et al., 2004). These results suggest that the improvements in sensory processing during working memory following ACEI treatment is the results of improved signal-noise ratios in primary sensory cortices that increases activation in secondary sensory areas and reduces the requirements of top-down regulation from the PFC.

The effects of ACEI treatment on attentional processing have not being studied in AMCI; however, the effects on working memory have been studied and we will look at the results from these studies. Ten weeks of donepezil treatment improved accuracy on a verbal working memory task (n-back, letter stimuli) and this was associated with increased left PFC and occipital activation, and decreased left temporal activation (Saykin et al., 2004). Increased left PFC activation correlated positively with accuracy improvements. Five days of galantamine treatment improved speed and accuracy on a visual working memory task (n-back, letter stimuli) and this was associated with increased activation in right precuneus and right PFC (BA 9, 47) (Goekoop et al., 2004).

The effects of ACEI on attention have been studied in AD and treatment appears to improve attention. Treatment with the ACEI tacrine, which has been withdrawn due to adverse effects, improved accuracy and reaction time during attention test in AD (Riekkinen et al., 1997; Sahakian et al., 1993). Not all AD patients responded to treatment and responders were less impaired on world list recall, category fluency, letter fluency and MMSE; furthermore, non-responders demonstrated severe frontal dysfunction evident as decreased resting glucose metabolism (Riekkinen et al., 1997). The authors interpreted their results as indicating that frontal dysfunction due to decreased cholinergic neurotransmission resulting from the BFCS pathology blocks the therapeutic effects of ACEI. This makes sense because local cholinergic innervation is required for ACEI treatment to take effect on cholinergic neurotransmission. A study examining the effects of one year of treatment with the newer ACEIs rivastigmine (n=29) or donepezil (n=47) found improved sustained attention as measure by the trail making test part A which requires participants to connect a sequence of numbers presented on an A4 sized paper

(Borkowska et al., 2005). Part A of the trail making test therefore measures sustained attention and attentional engagement, disengagement, orientation and re-engagement, and time and accuracy are recorded as performance measures. The participants in the study did not show any benefits of ACEI treatment on the MMSE or trail making test part B. Part B has the same format as part of A but subjects are required to alternate between two cognitive sets: sequential numbers and sequential letters of the alphabet. The authors concluded that although the treatment improved performance during a sustained attention task it did not prevent deterioration in working memory and executive functions. A controlled study of donepezil treatment in AD found improved accuracy during a selective attention task (Foldi et al., 2005). The task required participants to identify and mark target stimuli from amongst distractors on a sheet of paper. Accuracy during the task was measured using signal-detection theory similar to what we employed for behavioural data analysis and this is discuss in more detail in the methods section (§3.9.1.3). The use of signal-detection theory to analyse behavioural performance makes it possible to calculate a measure of response bias. The study by Foldi et al. found that patients who were treated had a more conservative bias in association with improved accuracy. This study had a small sample size (n=17) and the authors concluded that using higher order behavioural measures such as signal detection theory makes it possible to study the effects of interventions on small samples. The results also suggest that increased cholinergic availability directly affects attention and that measures of selective attention appear sensitive to detect treatment effects. A study which combined behavioural and PET measures to examine the effects of 12 weeks of donepezil treatment found that the degree of cortical AChE inhibition correlated positively with performance on a working memory task, requiring sustained attention and conflict resolution, but not with any measures of

episodic memory (Bohnen et al., 2005b). The effects of donepezil on cortical AChE inhibition at standard clinical dosing regimes were modest overall (22%) and most robust in the anterior cingulated cortex followed by the DLPFC, posterior cingulated, parietal and lateral temporal cortices. It was also demonstrated in this AD group (n=15) by comparison to controls (n=12) that mean cortical AChE activity, combined for all the regions of interest mentioned above and for the temporal areas separately, was significantly associated with measures of attention and working memory but not with tests of episodic memory (Bohnen et al., 2005a). These findings are a further indication of the relationship between cholinergic function, working memory and attention.

Taken together these findings indicate improvements in working memory and attention following ACEI treatment in health, AMCI and AD that correlate with regional cortical metabolism. Improved working memory and sustained attention following ACEI treatment may therefore also benefit performance during divided attention tasks that make heavy demands on these.

1.7.6 The Current Status of ACEI Treatment in AD and MCI

Cochrane reviews of treatment efficacy have been completed for rivastigmine, donepezil and galantamine and for all three combined in AD (Birks, 2006; Birks and Melzer, 2000; Loy and Schneider, 2004). All three ACEI show efficacy on cognition, behaviour and everyday activities but these effects are small and do not appear to persist beyond 12 months of treatment. It appears that the effects on separate cognitive domains such as memory and attention have not reached significance as only improvement in global measures such as the MMSE and Alzheimer's Disease Assessment Scale have been reported.

A recent review of published and unpublished data concluded that ACEI treatment does not have any significant effects on cognition or behaviour in AMCI-multiple domain (Raschetti et al., 2007). It did however highlight the variable application of available diagnostic criteria for AMCI and the lack of more advanced cognitive measures that can examine both cognitive speed and accuracy. It is therefore difficult to generalise these results to patients with AMCI and further studies will be required to determine if these treatments are of benefit in AMCI. Current national clinical guidelines based on available findings do not recommend ACEI treatment in AMCI (NICE, 2006 (Amended 2007, 2009)).

Summary

Acetylcholine plays a pivotal neuromodulatory role in cortical processing of memory and attention and cholinergic deficits in AD have detrimental effects on these cognitive processes. Cholinergic enhancement in health and AD with ACEIs benefits attention and working memory but not episodic memory. It appears from available findings that ACEI treatment effects are modest at best and only reliably evident in AD and not in AMCI. However, the cognitive impairment in AMCI is mild and only modest improvements may be expected from treatment that only partially addresses cortical processing deficits. The effects of ACEI treatment on attention had not been studied in AMCI and it therefore appeared appropriate to do so with sensitive behavioural measures and functional neuroimaging.

2. Rationale and Aims of the Thesis

Prevention is considered the gold standard in health promotion, followed by curative and then symptomatic treatment. Developing effective prevention and treatment methods requires a detailed understanding of a disease or disorder. The work reported in this thesis was undertaken with this purpose in mind.

2.1 Overall Aims

The overall aims of this study were to:

1. Investigate if attentional processing is impaired and/or altered in prodromal AD.
2. Determine the neural correlates of the established episodic amnesia in prodromal AD.
3. Determine the functional and behavioural effects of current drug treatments in prodromal AD.

AMCI is considered a prodromal stage for AD and detailed characterisation of the neuropsychological and functional brain changes evident in AMCI could aid in early diagnosis, treatment development and treatment response monitoring.

Early diagnosis is required if early intervention is to be achieved. AD is associated with several modifiable risk factors and reliable early identification of AD can allow targeted prescription of risk-modifying treatments that may be costly and associated with side effects. Understanding AD disease mechanisms and their effects on brain function is essential for the *development of targeted treatments*. Functional neuroimaging increasingly appears to be a valuable research method that allows investigation of brain function in vivo. *Measuring treatment effects* using

neuroimaging allows greater understanding of disease processes and can aid in treatment development by indicating cortical areas displaying treatment response.

This work sets out to answer the following questions:

1. Is divided attention impaired in AMCI as predicted, and if so, what are the neural correlates?
2. Is there evidence of altered visual and auditory attentional processing in AMCI that could contribute to divided attention deficits and amnesia?
3. Is the amnesia in AMCI related to failing executive control of encoding processes and if so, what are the neural correlates?
4. What are the effects of ACEI treatment on attention in AMCI?

I set out the rationale and specific hypothesis for each of the experiments below in turn.

2.2 Divided Attention

Divided attention deficits had been predicted in the prodromal stages of AD because attentional deficits were found in very mild AD and typically followed the onset of episodic amnesia (Chun and Turk-Browne, 2007; Perry and Hodges, 1999). Divided attention appears to be the first non-memory cognitive domain affected in AD. It makes heavy demands on the distributed cortical network that support attention; furthermore, key areas in this network are affected by AD neuropathology in AMCI and divided attention is therefore likely to be affected in prodromal AD. Attentional deficits had been demonstrated in the more heterogeneous MCI group and these were associated with increased dementia conversion risk (Levinoff et al., 2005; Nestor et al., 2004). The few studies of attention in AMCI have found behavioural deficits on a task requiring selective attention and a task requiring working memory that controls

the allocation of attentional resources (Bozoki et al., 2001; Perry and Hodges, 2003). These results appear to indicate a failure of attentional selection or *top-down* (task driven) control of attention. Top-down attentional control is associated with activation in the PFC and parietal areas, key constituents of the attentional network, and appears mediated by cholinergic neurotransmission. AD neuropathology affect processing in PFC and parietal areas directly (via local neuropathology) and indirectly (via decreased cholinergic neurotransmission due to basal forebrain neuropathology) early in the course of AD. These findings in addition to the cholinergic hypothesis of AD form the rationale for expecting divided attention deficits associated with altered cortical activation, and ACEI treatment response in AMCI.

Attentional deficits have been associated with reduced activation in right PFC, anterior pole and occipital areas in AD and this could be explained by the finding that increasing cognitive demand, in the face of limited resources, commonly leads to attenuated activation within that region (Goldberg et al., 1998; Nestor et al., 1991). The PFC is a key component of the attentional network and AD neuropathology has been demonstrated in the PFC in AD and AMCI (Guillozet et al., 2003; Kordower et al., 2001; Markesbery et al., 2006; Riley et al., 2002). Divided attention is strongly associated with activation in the PFC, which already demonstrates AD neuropathology in AMCI, and is therefore likely to be affected in AMCI. Functional neuroimaging could reveal evidence of functional abnormalities even in the absence of behavioural deficits, as demonstrated by the studies on memory processing in other high-risk AD groups (Bookheimer et al., 2000; Johnson et al., 2006b; Smith et al., 1999). We therefore examined divided attention in AMCI for evidence of the predicted functional and behavioural deficits.

Hypotheses:

1. Behavioural deficits will be evident during divided attention in AMCI.
2. Altered cortical activation will be evident in attention processing areas during divided attention in AMCI.

2.3 Visual and Auditory Selective Attention

Basic sensory attention processing had not been investigated in prodromal AD in spite of the fact that deficits in basic attention processing may affect higher order processing during divided attention and encoding. Altered cortical activation had been reported in advanced AD; PET imaging studies of visual attention of AD have found reduced activation in occipital visual areas (Johannsen et al., 1999). However, primary sensory areas are spared from AD neuropathology until the more advanced stages. The status of basic sensory attention processing therefore remained to be determined in prodromal AD and we therefore decided to examine basic visual and auditory attention processing.

Hypotheses:

1. Based on the relatively late appearance of AD neuropathology in sensory cortices, we hypothesised that functional brain activation would be unchanged compared to controls in AMCI.

2.4 Verbal Episodic Memory

The functional correlates of the characteristic episodic memory impairment in AMCI remains to be established and most of the studies that had been conducted examined visual memory. However, verbal memory tests are used routinely in clinical practice and modern life is heavily dependent on language. Furthermore, functional neuroimaging studies of memory in AMCI have almost exclusively focussed on the

MTL areas in spite of the fact that memory is processed by a network of areas. It is therefore appropriate to investigate verbal memory processing in prodromal AD using whole brain fMRI. The MTLs are essential for episodic encoding and the PFC also makes important contributions to memory processing (§1.4.2.1-2). MTL lesions cause dense amnesia for events and their context and PFC lesions cause amnesia for context; furthermore, the MTLs and left PFC consistently activation during verbal episodic encoding (§1.4.2.3). Left PFC activity relates to deep meaning-based (semantic) processing which optimises memory and disruption of semantic elaboration results in decreased left PFC activation and reduced memory performance. Additionally, the extent of activation of the left PFC and hippocampus during verbal encoding correlates positively with subsequent successful recognition. Moreover, AD neuropathological has been found in the PFC and MTL in AMCI (§1.2.2). Taken together, the role of the PFC in episodic memory and the early appearance of AD neuropathology that can affect PFC function suggest that episodic amnesia in prodromal AD may in part be due to PFC dysfunction.

We therefore set out to study verbal episodic encoding and retrieval in AMCI by comparison to controls. In order to study functional activation related to verbal encoding and the possible contributions of executive failure to impaired encoding in AMCI, we employed whole brain fMRI and a verbal encoding task sensitive to semantic elaboration (§3.9.3.1). The episodic memory paradigm requires semantic elaboration and can therefore examine PFC function as described above. The combination of sensitive measures of encoding with fMRI allows correlation analyses between brain activation and behaviour that could provide additional support for any observed associations.

Hypotheses:

1. Based on the established roles of the MTL and PFC in episodic memory encoding and the consistent activation in these areas during functional neuroimaging we hypothesised that PFC activation would correlate with semantic processing as revealed by recognition performance.
2. Recognition would be impaired in AMCI and associated with altered activation in PFC and/or medial temporal areas.

2.5 ACEI treatment Effects on Attention and Episodic Memory in AMCI.

Impaired cholinergic neurotransmission due to BFCS pathology and the strong associations between attention, memory and cholinergic function, provided the rationale for treating AD with interventions that prolong the effects of Ach in synapses. The gradual accumulation and NFTs in the BFCS, which correlates with memory function, provided the rationale for similar treatment in AMCI (§1.1.2). Attention and memory are powerfully regulated by Ach; nevertheless, the effects of ACEI treatment on attention and verbal episodic memory had not been studied conclusively in AMCI although ACEIs had been available for the treatment of AD for some time.

Studies of ACEI treatment in health and AMCI indicate beneficial effects on cortical activation and on working memory, which controls attention (§1.7.5). Improved visual and visuospatial working memory (faster reaction times) associated with decreased PFC and primary visual cortex activation and increased extrastriate visual cortex activation have been found in healthy adults following ACEI treatment (Bentley et al., 2004; Furey et al., 2000; Furey et al., 1997). Studies of ACEI

treatment on working memory in AMCI have found improved accuracy and speed, associated with increased PFC and occipital activation and decreased temporal and precuneus activation (Goekoop et al., 2004; Saykin et al., 2004). Furthermore, ACEI treatment has been found to improve sustained and selective attention in AD (Borkowska et al., 2005; Foldi et al., 2005). Taken together these findings suggest that ACEI treatment may improve divided attention in AMCI as it depends on working memory and on the attentional processes that underpin sustained and selective attention. These behavioural and associated functional neuroimaging findings provided the rationale for studying the effects of rivastigmine on basic sensory attention and divided attention in AMCI with fMRI.

Verbal episodic memory, as measured by delayed verbal recall, is not significantly improved by ACEI treatment in healthy adults (Gron et al., 2005). Furthermore, ACEI treatment does not appear to benefit verbal episodic memory in AMCI. Five randomised controlled ACEI treatment trials were conducted in AMCI-multiple domain between 1999 and 2004, of which two were published (Petersen et al., 2005; Salloway et al., 2004), and meta-analysis of these five trials demonstrated no significant effects on verbal episodic memory (Raschetti et al., 2007).

The premise of the follow-up study was that the impaired cholinergic neurotransmission in AMCI, which leads to attentional deficits and altered cortical activation, would respond to ACEI treatment and result in normalisation of attentional processing.

Hypotheses:

1. Rivastigmine treatment would improve speed or accuracy during divided attention in AMCI.

2. Rivastigmine treatment would decrease PFC activation and/or increased secondary sensory cortex activation during divided attention.
3. Rivastigmine treatment would decrease primary sensory cortex activation during selective visual and auditory attention.
4. Verbal episodic memory would not improve following rivastigmine treatment.

3. Methods

In this chapter, we take a detailed look at the methodology used for this study.

- Section 3.1 considers ethical issues related to early diagnosis and how this applies to AMCI, and particular issues related to the study I report on here.
- Section 3.2 describes the study design.
- Section 3.3 describes recruitment methods, Section 3.4 the AMCI sample, and Section 3.5 the control sample.
- Sections 3.6 and 3.7 describes the clinical neuropsychological tests and behavioural measures.
- Section 3.8 deals with fMRI methods and analysis.
- Section 3.9 provides detailed descriptions of the cognitive paradigms.
- Section 3.10 describes the additional methods and post hoc analyses.

3.1 Ethical Considerations

Early diagnosis is associated with ethical dilemmas for patients and healthcare in terms of emotional response and interventions. The response to early diagnosis depends mainly on the perceived seriousness of the disorder and on the effectiveness and availability of treatment. Early diagnosis of a curable tumour is therefore less likely to have adverse psychological effects compared to the diagnosis of a late emerging outosomal dominant disorder, like Huntington's disease in a patient who already had offspring. Patients are faced with the prospect of knowing the likely cause of their death if the disease is terminal, like AD. Patients given this advanced warning may react by changing their lives to reflect neglected values such as relationships and desired experiences and this can often lead to a renewed appreciation of the world.

For other people it may be socially stigmatising and detrimental to their self-image. It can also have serious consequences for employment and financial matters such as investments and insurance. The impact on patients will also depend on the life stage in which they find themselves. Old age and retirement are associated with lower health expectations and poorer outcomes but younger patients usually have more dependents, and higher health and personal expectations.

Advances in healthcare are at present moving steadily in the direction of early diagnosis and intervention on the back of advances in genetics. This may in time lead to changing public perceptions of what normal means. As I indicated in Section 1.3, for humans being ill appears to be the norm, whilst what is considered a disease or disorder depends on cultural as well as scientific values.

The main concerns from critics of AMCI and AD centre on the ethics of making a diagnosis of AD or prodromal AD when it is occasionally incorrect and at present invariably incurable and poorly responsive to treatment. The ethics concerning a diagnosis of MCI, the more heterogeneous condition, has been considered in some detail and I will summarise the main issues that also apply to AMCI (Werner and Korczyn, 2008). Revealing a diagnosis relieves anxiety about symptoms by putting a label on them and allows patients to be involved in decision-making regarding their future. Concerns about disclosing a diagnosis include precipitating fear, distress and depression, and reducing hope and positive thinking. However, available findings do not support this in AD and it has not been studied conclusively in MCI. The situation in MCI is more complex due to uncertainty concerning the prognosis, as not all cases will progress. The authors stress that this point is particularly valid in sites where there are fewer experts knowledgeable about the complexities of the diagnosis of MCI. This point is not unique to MCI and pooling

expertise and using combinations of measures that improve sensitivity and specificity, as implemented in our memory clinic, are reliable methods for increasing diagnostic accuracy that reduce the ethical dilemmas associated with inaccurate diagnosis. The emergence of AMCI as a more specific prodromal stage for AD and the available evidence supporting its validity should resolve some of the dilemmas associated with MCI. The level of stigma associated with MCI or AMCI is not certain yet and it will likely be influenced by the effectiveness and availability of treatment, and by the possibility of restrictions imposed on certain high-risk activities such as driving.

In our memory clinic we deal with some of these ethical dilemmas by asking patients and their families if they want to know the outcome of the assessment. Patients unaware of their diagnosis can be managed as such in clinical settings. However, it is difficult to recruit such patients into research studies with potential adverse effects that must be explained so that informed consent can be obtained. It is rare in our clinic sample that patients prefer not to know their diagnosis and all the potential participants we approached knew their diagnosis.

Research on patients with cognitive impairment requires additional consideration and care in terms of obtaining informed consent. The AMCI group in particular could have trouble in retaining information on which to base a decision. Approval for the study was obtained from the West Essex Local Research Ethics Committee and all participants provided written informed consent. In obtaining consent, we allowed sufficient time for participants to digest and discuss the proposed studies with relatives and took care to simplify and repeat information.

We thought that the extended travel involved in attending the scanning facility as well as noise from the scanner would be the most taxing aspects. Reports from participants confirmed that the procedures were noise but travel was not mentioned as

a problem. During the scanning sessions participants were familiarised with the environment before functional scanning commenced, they also had constant access to an emergency call button.

The move towards earlier diagnosis assisted by advances in biotechnology is gathering pace. This will present us with new ethical dilemmas related to diagnostic disclosure, treatment and research participation, with advances in ethics more likely to follow technical advances. We are therefore left with the general principals of ethics to guide us.

3.2 Study Design

A controlled observational trial design was used to examine divided attention, verbal episodic memory encoding and recognition, and selective visual and auditory attention in AMCI. For this purpose we recruited a AMCI sample (**AllAMCI**) and follow them to also identify the subgroup that converted to AD after 2 years (**CoAMCI**)(also see §4.1). A controlled, repeated measures design was used to examine the treatment effects of rivastigmine on cognition in AMCI and involved comparing treated (**RivAMCI**) and untreated (**NxAMCI**) at baseline and after 12 weeks on repeated measures. The total sample of participants was therefore stratified into four AMCI patient groups and one Control group. For the repeated measures design, we used different stimuli to control for learning effects whilst the rest of the methodology remained unchanged. The first participant was recruited and scanned in March 2003 and data collection was completed in October 2008.

3.3 Participant Recruitment

We recruited participants diagnosed with AMCI from known patients and new referrals attending the Derwent Memory Clinic at St Margaret's Hospital, Essex, and controls from patient's relatives, friends and staff. All participants were studied at baseline and at 12 weeks follow-up. The first AMCI group recruited (**RivAMCI**) received rivastigmine after baseline for 12 weeks until follow-up. Because inclusion of an untreated AMCI group would likely improve the analysis of treatment effects, we later recruited the untreated comparison AMCI group (**NxAMCI**) when additional funding became available. The NxAMCI group (n=10) was scanned using the same parameters but they did not receive treatment.

3.4 AMCI Patients

We recruited right-handed patients diagnosed with AMCI from the Derwent Memory Clinic at Princess Alexandra and St Margaret's Hospitals in North Essex. The clinic was established in 1993 and takes referrals from general practitioners and specialists in the area, for patients with cognitive impairment. Consultant psychiatrists and trainees, under close supervision of senior clinicians, carry out assessments after training and instruction on the specific procedures that include taking a detailed history, conducting a thorough physical examination, and use of special investigations. Particular emphasis is placed on examination of the neurological and cardiovascular systems that are vulnerable to many disorders that can present with cognitive impairment.

Around 260 assessments are carried out annually and are comprised of both follow-up and initial assessments. The clinical population is aetiologically varied and in 2008, out of the 111 new patients, we diagnosed: 5 with vascular dementia, 2 with

Lewy body dementia, 24 with AD, 7 with frontotemporal dementia, 10 with mixed AD-vascular dementia, 3 with Parkinson's disease dementia, 27 with AMCI (single and multiple domain), 7 with subjective cognitive impairment, 7 were unclear, 2 with unspecified dementia, 5 with depressive disorder, 2 with anxiety disorder and 10 with other disorders. As part of the assessment, the psychiatrist completes a standard battery of behavioural scales. The scales have been selected to elucidate and measure dementia symptoms. An assistant neuropsychologist, supervised by a clinical neuropsychologist, carries out a comprehensive neuropsychological test battery on all referrals and follow-up patients. Additional neuropsychological testing is undertaken when results from the standard batteries are equivocal. Assessments are discussed in a weekly multidisciplinary meeting that includes psychiatrists and neuropsychologists, and consensus diagnoses are recorded. Clinicians have access to routine structural imaging results (magnetic resonance imaging, computed tomography) during diagnostic meetings and metabolic imaging (dopamine transporter PET, glucose PET) is requested when indicated. Patients receive annual follow-up until a stable diagnosis of dementia is returned for more than a year. Assessment data are entered on a central database from where potential research participants can be identified.

Participants in this study were diagnosed with AMCI using specific operational criteria (Petersen et al., 2001) which included: (1) memory complaint corroborated by an informant, (2) abnormal memory function documented by impaired recall on the New Learning subscale of the CAMCOG (Roth et al., 1986), (3) preserved general cognitive function based on a Clinical Dementia Rating Score ≤ 0.5 (Morris, 1993) and CAMCOG total score within 1 SD of population mean for age (≥78 for age <79 years; ≥75 for age>80 years), (4) intact activities of daily living, (5) and not meeting National Institute of Neurological and Communicative Disorders and

Stroke/Alzheimer's Disease and Related Disorders Association criteria for AD (McKhann et al., 1984). The New Learning subscale score, which contributes to the memory subscale of the CAMCOG, was used as a measure of delayed recall. It is comprised of free recall (6 marks) followed by recognition (6 marks) of 6 pictured items (shoe, typewriter, scales, suitcase, barometer and lamp) encoded during a picture naming task, and delayed free recall of a 5 item address verbally encoded and written by subjects, to make a total score of 17. Episodic memory was considered impaired if there was a clear discrepancy between memory performance on the New Learning subscale and premorbid IQ as measured by the National Adult Reading Test (Nelson, 1982). We used a cut-off score ≤12 (1.5 SD below population mean) for subjects with average IQ and a cut-off score ≤ 14 for above average IQ based on published population data (Huppert et al., 1995). In addition to applying the criteria, patients were screened for any other identifiable medical or psychiatric cause of cognitive impairment. All participants received face-to-face feedback on their assessment from one of the memory clinic specialists.

Potential participants were identified from existing AMCI patients on the database and from new referrals. Their case notes were systematically reviewed for diagnostic accuracy and for the presence of exclusion criteria relevant to fMRI. Patients presenting with significant vascular risk factors attracting modified Hachinski score ≥ 4 were excluded as they were at increased risk of having significant comorbid cerebrovascular disease (Hachinski et al., 1975). FMRI exclusion criteria included general exclusion criteria for MRI and additionally being predominantly left handed and having facial tattoos or extensive metal dental implants. Being left handed is avoided in fMRI especially when motor responses are required due to differences in hemispheric dominances which can influence inter-subject comparisons. Handedness

was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Patients were also excluded if they suffered claustrophobia, were unable to lie supine for an hour, or had visual or hearing impairment that could not be corrected with aids. Visual impairment was corrected prior to scanning by using lens prescription information and fMRI compatible glasses. Hearing impairment was corrected prior to commencing scanning by adjusting the respective channel volume of the stereo fMRI compatible headphones.

3.5 Controls

Right-handed healthy control subjects, with no evidence of cognitive impairment were also recruited from relatives, friends of patients, and staff. Control subjects completed a neuropsychological test battery consisting of the CAMCOG and MMSE, in order to exclude cognitive impairment. Controls had to be on a stable dose of any medication they were prescribed for 6 months and were excluded if they were on any psychotropic drugs.

3.6 Neuropsychological Measures

The standard neuropsychological test battery used for assessments in our memory clinic includes the following measures: CAMCOG, MMSE, Wechsler Memory Scale - III Logical Memory Test (Wechsler, 1997), Halstead Trail Making test parts A and B (Davies, 1968), NART, Verbal Fluency and Category Fluency (Lezak, 1995). The CAMCOG, and specifically the New Learning subscale, are relevant to this thesis and will be discuss in more detail.

The CAMCOG is a self-contained part of the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) (Roth et al., 1986). It is a standardised

instrument used to assess the level of cognitive impairment and therefore the extent of dementia. It assesses orientation, language, memory, praxis, attention, abstract thinking, perception and calculation. The CAMCOG consists of 67 items and includes all the items from the MMSE. It is divided into 8 subscales: orientation, language (comprehension and expression), memory (remote, recent and new learning), attention, praxis, calculation, abstraction and perception. The *orientation* subscale is comprised of 10 items taken from the MMSE. The *language* subscale assesses comprehension through verbal and nonverbal responses to spoken and written questions. Language expression is assessed through tests of naming, repetition, fluency and definitions. The *memory* subscale assesses remote memory (famous events and people), recent memory (news items, prime minister, etc.), and *new learning* (the recall and recognition of non-verbal and pictorial information learned intentionally as well as incidentally). New learning is comprised of free recall followed by recognition recall of 6 pictured items encoded incidentally during a picture naming task, and delayed free recall of a 5 item address verbally encoded and written by subjects. *Attention* is assessed by subtracting sevens serially from 100, and by counting backwards from 20. Carrying out instructions, copying, drawing, and writing assesses *praxis*. Performing addition and subtraction involving money is used for assessment on the *calculation* subscale. Questions on the similarities between items an apple and a banana, a shirt and a dress, a chair and a table, and a plant and an animal are used for the *abstraction* subscale. For the perception subscale, famous people and familiar objects viewed from unusual angles have to be identified from photos shown. The total score is 107 and the score for each subscale is as follows:

- Orientation - 10
- Language comprehension - 9

- Language expression - 21
- Remote memory - 6
- Recent memory - 4
- New learning - 17
- Attention/calculation - 9
- Praxis - 12
- Abstract thinking - 8
- Perception - 9

Population norms are available for total and subcategory scores. We used these norms to calculate cut-off scores for the New Learning subscale to verify episodic memory impairment. Episodic memory was considered impaired if there was a clear discrepancy between memory performance on the New Learning subscale and premorbid IQ as estimated by the National Adult Reading Test (Nelson, 1982). We used a cut-off score ≤12 (1.5 SD below population mean) for subjects with average IQ and a cut-off score ≤ 14 for above average IQ based on published population data (Huppert et al., 1995). The New Learning task controls for the effects of attention and includes both free recall and recognition recall, with the latter appearing more sensitive at distinguishing AMCI from normal ageing (Anderson et al., 2008; Bennett et al., 2006; Westerberg et al., 2006). Episodic memory measures can be confounded by rehearsal of studied items in working memory. Rehearsals is curbed on the New Learning task by requiring completion of unrelated tasks during a time delay between encoding and recall phases.

It is apparent from the description of the CAMCOG and New Learning subscale that these measures are similar to those discussed in Section 1.2.3 that have

high sensitivity and specificity in identifying individuals with progressive AMCI. This increases the validity of considering our AMCI sample as prodromal for AD.

3.7 Behavioural Measures

Our standard memory clinic assessment involves completion of behavioural measures included to aid in the identification of comorbid disorders and to quantify impairment. These include measures of everyday functioning: Instrumental Activities of Daily Living (Lawton, 1969), Clinical Dementia Rating (CDR)(Morris, 1993). We also use the Geriatric Depression Scale to screen for depression, which due to associated cognitive impairment requires exclusion for a more valid diagnosis of AMCI.

The Instrumental Activities of Daily Living scale is used to ascertain if there are any impairments in everyday activities, which if present would indicate dementia rather than AMCI. The activity areas covered included telephone use, shopping, food preparation, housekeeping, washing laundry, transportation, responsibility for own medications and ability to handle finances. Impairment in any of these areas can be due to cognitive and/or physical impairment and this distinction is considered when functional impairment is evaluated.

The CDR measures functioning in six domains: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. It yields a global score and a summated score (sum of box score) and is used to measure dementia severity and change in everyday activities in response to treatment in MCI (Petersen et al., 2005). A diagnosis of AMCI is compatible with a global score of 0.5 if this results from a 0.5 score in the memory domain. A 0.5 score in the memory domain indicates “consistent slight forgetfulness; partial recollection of events; benign forgetfulness”. A score of more than 0.5 is more indicative of dementia

as it implies impaired everyday activities whilst a score of 0 indicates the absence of persistent memory complaints that is therefore incompatible with AMCI. We diagnosed AMCI when the score was equal to 0.5. Another way to use the CDR is to add up the scores from the domain boxes and this *sum of box score* is predictive of progression to dementia. The likelihood of dementia increases by a factor of 2.3 for every point increase on the CDR sum of box score (Lynch et al., 2006). The sum of boxes score appears to be a more sensitive indicator of severity than the global score as the range of possible scores is wider. We used the CDR global score to aid in diagnosis and the sum of boxes score to investigate the difference between the treated and untreated AMCI groups, which I discuss later.

3.7.1 Statistical Methods for Behavioural Data Analyses

Analysis of variance (ANOVA) was used to compare behavioural data between groups on normally distributed data with homogeneous variances. Data violating normality assumptions were compared using the Mann-Whitney analysis with Kruskal-Wallis Exact tests. Repeated measures factorial analyses were conducted to examine for interaction effects on repeated measures. All data were analysed in SPSS 12 for Microsoft Windows. The coefficient of determination, $\mathbf{R^2}$ is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect. R^2 indicates how much of the variability in one measure is accounted for by the measure it correlates with; R^2 multiplied by 100 gives the percentage of variability e.g. R^2=0.30 indicates 30% variability explained by the correlate.

3.8 Functional Magnetic Resonance Imaging

Functional neuroimaging increasingly appears to be a valuable research method that allows investigation of brain function in vivo. It can identify brain areas demonstrating increased or decreased activity at rest or during specific task conditions. To this end, groups can be compared on activation maps and altered activation patterns in clinical populations may indicate disease related activation failure and/or compensatory activation in other areas (for a review in AD and ageing see (Han et al., 2009). For example, studies of subjects at high-risk of AD by virtue of a positive family history have found alterations in cortical activation whilst matching the performance of controls (Bookheimer et al., 2000; Johnson et al., 2006b; Smith et al., 1999). These findings may indicate the consequence of local neuropathology or distant neuropathology resulting in reduced neural input to the areas, and/or compensatory recruitment of extra neurones, or increased firing rates of neurones required to match the performance of controls. Correlation between the magnitude of activation and behavioural measures can be examined and compared to results in controls. Loss of normal correlation associated with behavioural impairment indicates deficits in the area or network that supports the behaviour. The effects of drug treatment and other interventions on brain function can also be studied using fMRI (for a review see (Dickerson, 2007). Treatment effects may be indicated by the restoration of normal activation patterns and correlations. It may also be evident from factorial analysis of data from repeated measures in treated and control groups by the presence of Group x Time interactions. Interaction effects indicate that activation varied significantly over time between the groups as a consequence of the intervention under investigation. Measuring treatment effects using neuroimaging allows greater understanding of disease processes and can aid in treatment development by

indicating cortical areas displaying treatment response. We next look at the theoretical background, technical aspects and statistical analysis of fMRI in more detail.

3.8.1 Biophysics

FMRI is a whole brain functional imaging modality that employs the difference in magnetic properties between oxygenated and deoxygenated haemoglobin to estimate changes in blood oxygenation and by further inference neuronal activity. Neuronal activity results in an initial decrease in oxyhaemoglobin due to increase oxygen use, followed by a relative over supply of oxyhaemoglobin, peaking between 4 and 8 seconds after the onset of activity, due to local vasodilatation via neurovascular coupling (Villringer and Dirnagl, 1995). The signal detected is known as the blood oxygenation level dependent (BOLD) signal and appears to be generated by the combined post synaptic activity of large populations of neurones thus, fMRI allows examination of cognition at a system level (Logothetis, 2002; Logothetis et al., 2001; Rugg et al., 2002). FMRI, at the commonly used 1.5 Tesla field strength, has a finer temporal (typically a few seconds) and spatial resolution (typically 3mm x 3mm x 6mm) than positron emission tomography (PET) and single photon emission tomography. This makes it particularly useful in investigating higher cognitive processes such as memory and attention on tasks with durations ranging from a few seconds to minutes. Cognition is examined by comparing the cognitive function of interest, such as verbal episodic encoding, to a reference or control condition; activation during the control condition is subtracted from activation during the experimental condition. By matching the experimental condition and the control condition as closely as possible, apart from on the cognitive function of interest,

activation only evident during the experimental condition can be inferred as specific to that function. This type of design is typically implemented in a block-designed paradigm where blocks of the experimental condition are alternated with blocks of the control condition.

3.8.2 Imaging Sessions

Participants were transported, accompanied by the researcher, to the Institute of Psychiatry by private taxi on the day of scanning. All task procedures were revised in a dedicated room prior to scanning. Patients were positioned in the scanner after site-specific consent was obtained and fMRI safety checks performed by the radiographers.

Participants held a three-button response box in their left hand during scanning sessions; they responded with the index, middle and ring fingers of the right hand. The button box was designed specifically for the study and the buttons were laid out so that they were easily accessible and provided tactile feedback when a response was made.

Before fMRI could commence, a localiser and a high-resolution structural scan were obtained on which any imaging artefacts would be apparent. Participants with poor alignment evident from these scans were repositioned. For follow-up scans, participants were carefully positioned so that head alignment fell within 1° of previous alignment parameters in order to optimise transformation of functional data to structural templates.

Auditory and visual stimulus perception was tested before functional scanning was commenced. The instructions for each task were repeated immediately prior to scanning. Participants were able to indicated distress or discomfort by pressing an

emergency button or by speaking to the research team via a microphone mounted on the head coil.

Functional and structural scans were acquired in a sequence dictated by task design. The sequence was as follows: High resolution structural scan, visual-auditory attention task, encoding task, spoiled gradient structural scan, divided attention task, recognition task, and a T2 fast spin-echo structural scan. The first high-resolution structural scan is used during the functional data analysis as the subject-specific template and the latter two structural scans to examine for evidence of structural brain pathology.

3.8.3 Image Acquisition

Gradient echo echo-planar imaging (EPI) data were acquired on a neuro-optimised GE Signa 1.5 Tesla system (General Electric, Milwaukee WI, USA) at the Institute of Psychiatry, King's College London. Image quality was ensured by a semi-automated quality control procedure. A quadrature birdcage head coil was used for radio frequency transmission and reception. Foam padding was placed around the subject's head in the coil to minimize head movement. T2*-weighted whole-brain volumes were acquired during the paradigms at each of 16 near-axial non-contiguous planes parallel to the intercommissural (anterior commisure-to-posterior commisure) line (matrix = 64 x 64). Imaging parameters for each of the paradigms appear in **Table 3**. The encoding paradigm had a shorter echo time (TE) and included a pass delay of 2.5 s after presentation of each stimulus in order to sample BOLD signal after overt speech production in order to minimise movement artefact. These EPI data sets provided almost complete brain coverage. A high-resolution gradient echo image of the whole brain was also acquired for co-registration of functional images (43 slices;

slice thickness=3 mm; gap =0.3 mm; TR = 3 seconds; flip angle = 90°; matrix = 128 x128).

	Encode	**Recognition**	**Visual-Auditory Attention**	**Divided Attention**
Number of slices	16	16	16	16
Slice thickness	7 mm	7 mm	7 mm	7 mm
Gap	0.7 mm	0.7 mm	0.7 mm	0.7 mm
TR	1500 ms	2000 ms	2000 ms	2000 ms
TE	40	40	40	40
Flip angle	90 °	70 °	70 °	70 °
Nr of images per location	112	280	144	140
Total scan time	07:28	09:28	04:56	04:48
Total number of images	1808	4496	2320	2256
Pass delay	2500 ms			

Table 3. Imaging parameters for fMRI paradigms.

TR= repetition time, TE= echo time.

3.8.4 Data Analysis

Data analysis was undertaken using the XBAM software package written and maintained by the Institute of Psychiatry at King's College London, UK (http://brainmap.it). It was conceived by Ed Bullmore and Michael Brammer in 1995 with the aim of writing "an fMRI analysis package with the minimum possible

number of assumptions" because testing for activation at thousands of different points in the brain required rigorous methods. The standard methods at the time involved either correlational analysis between the experimental design and the time series at each voxel (volumetric pixels), or t-statistics using means and variance. Both these approaches assume normal theory approximations, which have not being established in large samples of fMRI data. These types of analyses are typical of the more commonly used fMRI analysis software packages such as SPM (http://www.fil.ion.ucl.ac.uk/spm/), which are more sensitive than non-parametric approaches such as XBAM but also more prone to false positives. SPM is probably the most widely used functional neuroimaging analysis package and the default analysis settings are usually appropriate for analysing functional data from healthy control groups. XBAM analysis differs significantly from SPM analysis by not assuming normality of activation data and consequently implements permutation testing to construct the null distribution to make inference about the probability of activation under the null hypothesis. A recent large fMRI study (81 subjects) investigated the assumption of normality in fMRI analysis and found substantial departure in 22% of voxels; this was improved to 9% using the standardized statistics adopted by XBAM (Thirion et al., 2007). Based on their findings the authors recommended the use of cluster level statistics, robust and permutation-based testing rather than normal theory based inference, strategies already adopted by XBAM. Median statistics are used in XBAM rather than means in order to control for outlier effects. XBAM analysis adopts a two-level approach to data analysis in which the response sizes computed from the model fit for each individual are standardised with respect to their variances before embarking on the second, multi-subject, phase of analysis. This permits mixed-effects analyses of group level fMRI data by taking into

account both intra and inter subject variances. Mixed-effects analysis allows generalisation of findings from the participant population to the sampled population (Brammer et al., 1997). Wavelet-based time series permutation is used to control for the noise in fMRI data (Bullmore et al., 2001; Bullmore et al., 1999). XBAM uses 3-dimentional cluster-level statistics based on cluster mass (the sum of all statistical values from all the voxels in the cluster) for activation thresholding and the typical statistical threshold is therefore usually described as the probability value where less than one false cluster will be evident per volume. We next look in more detail at the implementation of the analysis at individual and group levels.

3.8.4.1 Individual analysis

To minimise motion related artefact, the data were first realigned by calculating a 3-dimensional volume template consisting of the average intensity at each voxel over the whole experiment and then realigning 3-dimensional image volume at each time point to this template by computing the combination of rotations (around the x, y, and z axes) and translations (in x, y and z) that maximised the correlation between the image intensities of the volume in question and the template (Bullmore et al., 1999). The data were then smoothed using a Gaussian filter (full width at half maximum of 5 mm) to improve signal to noise characteristics of the images. This resulting value for each voxel is the weighted average of all the neighbouring voxels with the average more weighted towards the central voxel. Responses to the experimental paradigms were then detected by time-series analysis using Gamma variate functions (peak responses at 4 and 8 sec) to model the BOLD response. The analysis was implemented as follows. First, each experimental condition was convolved separately with the 4 and 8 second Poisson functions to yield two models of the expected

haemodynamic response to that condition. The weighted sum of these two convolutions that gave the best fit to the time series at each voxel was then computed. This weighted sum effectively allows voxel-wise variability in time to peak haemodynamic response. In order to constrain the possible range of fits physiologically plausible BOLD responses, a constrained fitting procedure was used (Friman et al., 2003). Following this fitting operation, a goodness of fit statistic was computed at each voxel. This was the ratio of the sum of squares of deviations from the mean intensity value due to the model (fitted time series) divided by the sum of squares due to the residuals (original time series minus model time series). This statistic is called the SSQratio. The percentage BOLD signal change at each voxel was also calculated. This was: ((fitmax – fitmin)/mean signal intensity) * 100, where fitmax and fitmin were the maximum and minimum values of the fitted response for the time series in question.

In order to sample the distribution of SSQratio under the null hypothesis that observed values of SSQratio were not determined by experimental design (with minimal assumptions), the time series at each voxel was permuted using a wavelet-based resampling method (Breakspear et al., 2003; Bullmore et al., 2001). This process was repeated 20 times at each voxel and the data combined over all voxels, resulting in 20 permuted parametric maps of SSQratio at each plane for each subject. The same permutation strategy was applied at each voxel to preserve spatial correlational structure in the data during randomisation. Combining the randomised data over all voxels yields the distribution of SSQratio under the null hypothesis. A test that any given voxel is activated at any required type I error can then be carried out by obtaining the appropriate critical value of SSQratio from the null distribution. For example, SSQratio values in the observed data lying above the 99^{th} percentile of

the null distribution have a probability under the null hypothesis of <=0.01. This permutation method gives very good type I error control with minimal distributional assumptions (Breakspear et al., 2003; Bullmore et al., 2001).

3.8.4.2 Group mapping

In order to extend inference to the group level, the observed and randomised SSQratio maps were transformed into the standard space. This involved a two stage process requiring first a rigid body transformation of the fMRI data into a high-resolution gradient echo image of the same subject followed by an affine transformation on to a structural template (Brammer et al., 1997). By applying the two spatial transformations computed above for each subject to the statistic maps obtained by analysing the observed and wavelet-randomised data, a generic brain activation map could be produced for each experimental condition. The median observed SSQratio over all subjects at each voxel (median values were used to minimise outlier effects) can then be tested at each intracerebral voxel in standard space against a critical value of the permutation distribution for median SSQratio ascertained from the spatially transformed wavelet-permuted data (Brammer et al., 1997). The standard space used for analyses in this study was that of Talairach (Talairach, 1988). In order to increase sensitivity and reduce the multiple comparison problem encountered in fMRI, hypothesis testing was carried out at the cluster level using a method shown to give excellent cluster-wise type I error control in functional fMRI analysis (Bullmore et al., 1999). When applied to fMRI data, this method estimates the probability of occurrence of clusters under the null hypothesis using the distribution of median SSQratio computed from spatially transformed data obtained from wavelet permutation of the time series at each voxel. Image-wise expectation of the number

of false positive clusters under the null hypothesis is set for each analysis at <1. Consequently, correction for multiple comparisons was not required, as thresholds were set on an image-wide basis, not a voxel-wise basis.

3.8.4.3 Group differences

For group comparisons, analysis of variance was carried out on the SSQratio maps in standard space by first computing the difference in median SSQratio between groups at each voxel. Subsequent inference of the probability of this difference under the null hypothesis was made by reference to the null distribution obtained by repeated random permutation of group membership and recomputation of the difference in median SSQratios between the two groups obtained from the resampling process. As with the generic brain activation maps, cluster-level maps were then obtained with the cluster-wise probability equivalent to less than one false positive cluster per image.

3.8.4.4 Correlation analysis

Correlational analysis can be performed between the BOLD effect data for each individual and behavioural data. To implement this, the Pearson product-moment correlation coefficient (r) between the observed behavioural and BOLD effect data is first calculated, followed by calculation of the null distribution of correlation coefficients by permuting the BOLD data at each voxel many times (a minimum of 50) and combining the data over all voxels. Thresholded voxel and cluster level maps where r is significant can then be computed at any desired level of expected voxel or cluster-level type I error.

Correlations between activation data and behavioural data can also be examined by extracting the peak SSQ value for each participant from the coordinates of the most activated voxel in a cluster of difference. Spearman's correlation coefficients are then calculated due to the relatively small sample sizes and non-normally distributed data. Values deviating more then 2 standard deviations from the mean are excluded.

3.8.4.5 Interaction analysis

Interaction effects are tested for in factorial design studies where two (or more) groups (i.e. controls and patients) perform a single task at two time points (baseline and follow-up). The analysis is implemented by constructing a *design matrix* coding for the non-repeated group factor, the repeated condition factor and the interaction, which is the product of the first two factors. The interaction column tests for regions where the effect of one factor is modulated by the status of the other. If there is no interaction between group and condition, supporting the null-hypothesis, then plotting mean data for the groups and conditions will reveal parallel lines between the two time points. Conversely, if an interaction is present then the lines will deviate. Interpreting interactions therefore requires plots of activation data to be examined along with interaction maps.

3.4.8.6 Local permutation

For group comparisons of functional data we used an advanced method compared to our earliest reports. This method became available during the course of this study. It was designed to be more sensitive in brain areas where there is relatively smaller

signal change. It entailed a different method of random permutation, which is the process of generating a null distribution by repeated random sampling of the observed data at all, time points. This *local permutation* involved calculating a voxel-specific critical threshold as opposed to a critical threshold calculated from all voxels in the volume studied (*global permutation*). With *global permutation*, statistics from each voxel from the observed data is compared against a single threshold of significance used across the brain, which is taken from the null distribution of F-statistics calculated from all voxels over all randomisations. Therefore, voxel statistics from areas with large signal change enter into the global distribution and increase the significance threshold across the brain, decreasing detection in areas with smaller signal change. The magnitude of differences at thousands of spatially removed voxels therefore determines the significance at any one voxel. At a voxel threshold of e.g. 0.05 the value of the test statistic at that percentile (95^{th}) of the null distribution is calculated and any voxel with a test statistic larger than the critical value is identified as significant. Global permutation therefore assumes uniform voxel statistic magnitudes across the brain, an assumption that has not been established and is unlikely to hold in disease states such as AD. With *local permutation* a voxel-specific threshold is calculated and significance at each voxel is assessed against its own null distribution. This voxel-specific threshold is therefore not contaminated by statistics from other brain areas. Cluster level significance testing was still done against a null distribution of cluster mass generated from supra threshold voxel statistics across the brain as a local test of cluster mass is not available yet. However, the cluster level null distribution now included statistics from voxels that would otherwise not have contributed due to their comparatively smaller task related signal change.

3.4.8.7 UNIX analysis

Anonymised data were transferred to the UNIX computer system at the Institute of Psychiatry at King's College London. Each experiment was assigned a unique identifier. Corrupted data files were removed and raw experimental data were archived. A specific data file containing information about the experimental conditions was created for each experiment; these are known as *infiles* for block-designed experiments and as *newstarts* for event related designs. **Table 4** shows the infile data for the encoding task, and how it corresponds to the experimental condition and stimuli for the task. Experiments were processed in batches using server clusters. Most of the analyses were carried out remotely via the Internet, using Secure Shell Host and Hummingbird Exceed software. Each analysis step produces a log file and each of these were examined to ensure that the analysis ran as planned.

Infile data	Condition	Stimulus
1	Experimental	flag
1	Experimental	cross
1	Experimental	pole
1	Experimental	symbol
1	Experimental	stars
1	Experimental	stripes
1	Experimental	march
1	Experimental	England
0	Control	wait
0	Control	wait
0	Control	wait
0	Control	wait
1	Experimental	cabin
1	Experimental	scout
1	Experimental	summer
1	Experimental	pack
1	Experimental	lake
1	Experimental	trail
1	Experimental	canvas
1	Experimental	holiday

Table 4. Infile data for experimental analysis.

The data in the Infile data column informs the analysis procedure by indicating if the volume acquired represents the experimental or control condition. In this example from the encoding paradigm, it can be seen that the experimental condition is coded with the digit 1, and this corresponds to the presentation of different English nouns. The repeated presentation of the word “wait” comprises the control condition that is coded with the digit 0.

3.4.8.8 Identifying areas of activation

Brain areas where task related activation or group differences occurred were identified by their coordinates and cluster size using the Talairach atlas (Talairach, 1988). Clusters containing less than 10 voxels were ignored. Brain areas containing a cluster and surrounding areas up to 10 mm away were included in identified areas. Clusters were followed in all three spatial dimensions in order to identify all relevant brain areas. **Figure 8** shows the Talairach z-coordinates for axial images from the Talairach template used in XBAM; it has 25 slices with a 5.5mm gap between slices.

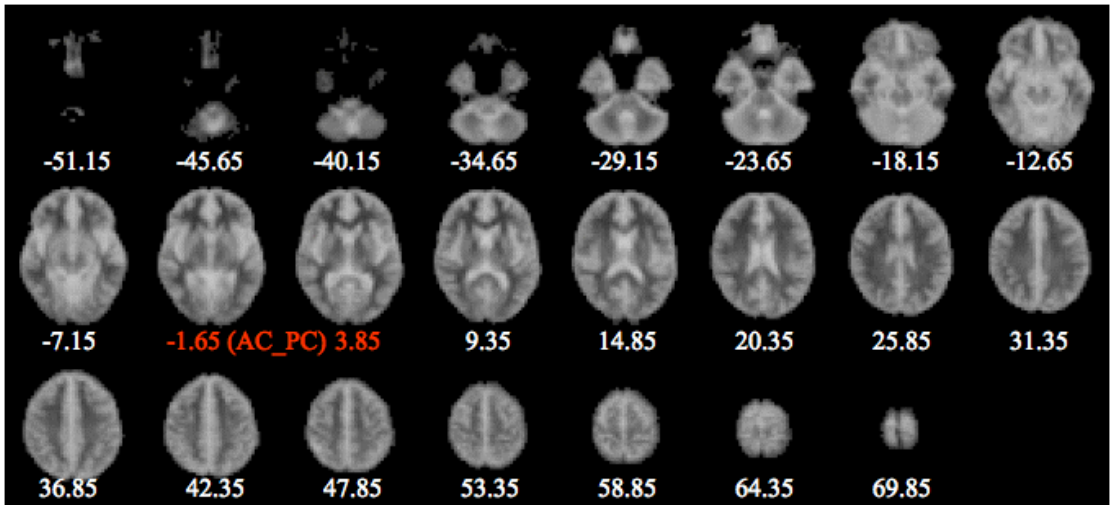


Figure 8. Talairach template.

The map shows axial brain slices with corresponding Talairach atlas z-coordinates. Slices with a negative z-value are located below the line that extends from the anterior commisure to the posterior commisure (AC_PC) that serves as an anatomical landmark. Axial slices proceed from left to right, from the most caudal in the top left corner to the most rostral in the right bottom corner of the map. The left hemisphere appears on the right and vice-versa.

3.8.5 Stimuli Presentation

Auditory stimuli were presented via MRI compatible air-conducting headphones and visual stimuli were back-projected with a LCD projector (Proxima Desktop Projector 5500) onto a screen 2.5m from the subject's head and were visible to the subject via a prism mounted on the head coil. The tasks were programmed in Microsoft Visual Basic Professional 6.0, presented on a PC running MS Windows NT and triggered by the scanner during image acquisition where applicable.

3.9 Overview of cognitive paradigms

All of the cognitive paradigms that were used in this study were designed specifically for use with fMRI. The encoding, recognition and visual-auditory paradigms had been designed at the Institute of Psychiatry and used in prior studies. We designed the divided attention paradigm and piloted it on controls and patients. I discuss the cognitive paradigms in detail below.

3.9.1 Divided Attention Paradigm

We designed a divided attention paradigm to examine the associated behaviour and brain activation. The rationale for examining divided attention has already been discussed (§2.2) and what follows below are details of the theoretical background, experimental design, behavioural data analysis and pilot data.

3.9.1.1 Theoretical background

Processing two streams of information simultaneously or completing two cognitive tasks simultaneously has a cost in terms of accuracy and\or speed known as the *dual-task decrement*. This appears related to limitations in executive control of attentional resources, attentional resources per se, and interference effects (Sarter and Turchi, 2002). The dual-task decrement results in decreased speed and/or accuracy during divided attention but this can vary between subjects so that differential impairment on either speed or accuracy can occur depending on automatic or conscious cognitive strategies favouring one or the other. Interpreting such data presents a difficulty and usually requires calculation of a behavioural index that combines speed and accuracy. Adequate performance, well above chance level, is also necessary in order to make inferences about task related functional activation. FMRI paradigms are therefore often designed to ensure behavioural equivalence that then allows interpretation of functional activation data with fewer assumptions. Extensive practice on a divided attention task may reduce dual-task cost in terms of response accuracy which then allows reaction time (RT) to be used as a valid measure of residual divided attention cost (Sarter and Turchi, 2002)). A carefully designed divided attention task together with sufficient practice on the task therefore allows comparisons between groups on RT. Moreover, as divided attention appears to be the first non-memory cognitive domain to be affected in mild AD, abnormal brain function may be evident on fMRI in the absence of behavioural differences.

We designed a divided attention task using relatively naturalistic stimuli (spoken numbers and visually presented letters and numbers). Using familiar letter and number stimuli reduces cognitive processing related to novelty detection and this was desirable because the aim was to minimise non-attentional processing during the

task so that results specifically related to attentional functioning. Processing the stimuli on the task already involved some automatic semantic processing related to targets identification and the aim was to limit any other non-relevant processing.

A *blocked designed* paradigm was used because this type of design has excellent BOLD signal detection properties. Optimal detection power was required due to the similarity between the experimental and control conditions and because AMCI patients may have reduced functional activation similar to that demonstrated in AD. Block duration is a further consideration and blocks should be long enough for the BOLD signal to return to baseline (>10 s) in areas not activated by a condition, as this ensures maximal variability in the data which in turn increases detection power. Block length during this task was 26 s.

In order to generate an fMRI contrast specific for the divided attention condition (DA), it was contrasted it with a control attention condition (CA). An ideal fMRI paradigm has a contrasting condition that matches the experimental condition on all sensory and processing aspects apart from those of interest. If this can be achieved then activation only evident during the experimental condition will likely be specific to the cognitive processing of the experimental condition. This *subtraction logic* hypothesis underpins most fMRI paradigms. By this logic, subtracting control condition activation from experimental condition activation leaves only activation particular to the experimental condition.

3.9.1.2 Experimental design

The final task consisted of 10 blocks: 5 DA blocks alternating with 5 CA blocks. Each block comprised 16 pairs of stimuli with an inter-stimulus interval of 1.75 s and ran for 26 s giving an overall duration of 280s for the task. Subjects practised the task

outside the scanner until they were comfortable with the instructions and able to complete it with high accuracy. The stimuli used for practice were different from those used during the experiment. Subjects were reminded of instructions immediately prior to commencing scanning. Instructions were identical for both conditions. Subjects were instructed to press the button with their right index finger whenever they saw the target letter "q" or heard the target number "8". The auditory-digit component consisted of the numbers 0 to 9 read aloud in a pseudo-random order by an unfamiliar male voice. The visual-letter component comprised of discrete lower case letters (a, b, I, l, m, q, r, s, u, w, z) presented in a pseudo-random order. Letters were coloured yellow and appeared in a large familiar font (Times New Roman) on a blue background. During the DA-blocks, subjects were simultaneously presented with visual (letters) and auditory (spoken digits) stimuli **(Figure 9)**. Each block contained three auditory and three visual targets. The CA blocks were designed to match the auditory, visual and motor aspects of the DA blocks. Subjects were presented with matching identical auditory and visual stimuli (e.g. an auditory "eight" and visual "8"), in a repeating sequence, with a target cue every third stimulus (008008008…). **Table 5** contains a sample of the first 48 stimulus pairs, reaction time, accuracy and time stamp from a response file. The designs characteristics as explained above are evident from the table.

Subjects were not warned of a switch from the DA to the CA blocks and block length differed from that used during training sessions in order to minimize habituation that could reduce cortical activation. RT and accuracy of each response were recorded for all stimuli.

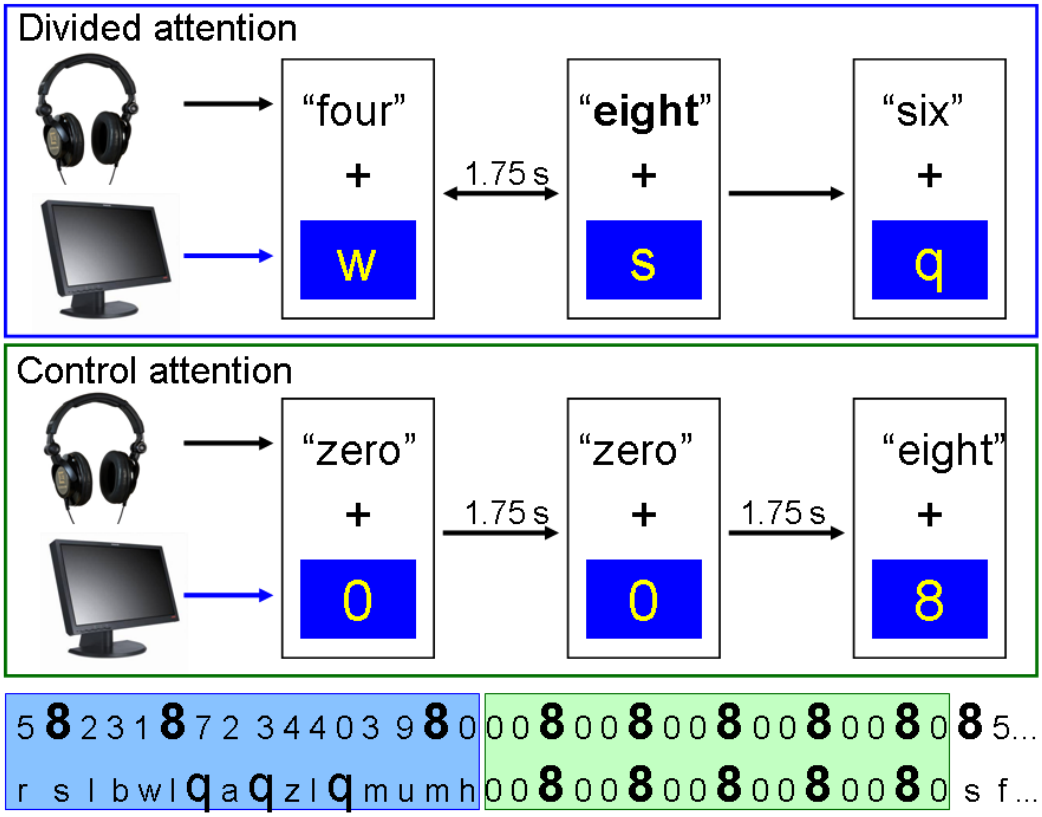


Figure 9. Divided attention task.

During the DA condition (top panel) pairs of stimuli were presented every 1.75 s and comprised of numbers between zero and nine, and discreet lower case letters. Targets were the number "eight" and the letter "q". The CA condition (centre panel) comprised of a repeating sequence of semantically identical verbal and visual stimuli. Blocks of 16 stimuli pairs (bottom panel) alternated so that five blocks of each condition were presented.

Stimuli	Letter	Number	RT	Correct=1	Time from start
1	r	5	0	0	0
2	s	8	0.729	1	1.752
3	l	2	0	0	3.504
4	b	3	0	0	5.256
5	w	1	0	0	7.008
6	l	8	0	0	8.76
7	q	7	1.171	1	10.512
8	a	2	0	0	12.264
9	q	3	0.58	1	14.016
10	z	4	0	0	15.768
11	i	4	0	0	17.52
12	q	0	0.544	1	19.272
13	m	3	0	0	21.024
14	u	9	0	0	22.776
15	m	8	0	0	24.528
16	h	0	0	0	26.28
17	0	0	0	0	28.032
18	0	0	0	0	29.784
19	8	8	0.487	1	31.536
20	0	0	0	0	33.288
21	0	0	0	0	35.04
22	8	8	0.484	1	36.792
23	0	0	0	0	38.544
24	0	0	0	0	40.296
25	8	8	0.305	1	42.048
26	0	0	0	0	43.8
27	0	0	0	0	45.552
28	8	8	0.436	1	47.304
29	0	0	0	0	49.056
30	0	0	0	0	50.808
31	8	8	0.327	1	52.56
32	0	0	0	0	54.312
33	s	8	0	1	56.064
34	f	5	0.25	0	57.816
35	r	0	0	0	59.568
36	j	9	0	0	61.32
37	w	1	0	0	63.072
38	z	5	0	0	64.824
39	m	2	0	0	66.576
40	q	6	0.644	1	68.328
41	z	4	0.005	1	70.08
42	i	3	0	0	71.832
43	q	5	0.653	1	73.584
44	m	0	0	0	75.336
45	v	8	0	0	77.088
46	k	4	0	0	78.84
47	o	8	0	0	80.592
48	q	9	0.573	1	82.344

Table 5. Divided attention response file.

The table contains a sample result of the first 48 stimuli pairs, reaction time, accuracy and time stamp. Blocks are colour coded with DA light grey and DA dark grey in the Letter and Number columns. In the Stimuli column, the shaded boxes indicate instances where the subject did not respond to targets. In the RT column the shaded

boxes indicate examples of instances where the RT-rule (stimulus nr. 34) and button rule (stimulus nr. 41) would be applied (see below).

3.9.1.3 Behavioural analysis

Behavioural measures collected for group comparisons of divided attention processing included: visual stimuli RT (Vis-RT), auditory stimuli RT (Aud-RT), and discrimination index (PrDA). Behavioural measures were calculated and collated in Microsoft® Office Excel 2003. Each response file was imported into a worksheet and the formulas were then copied from a master sheet. Response data were then inspected and cleaned according to standard rules. We devised rules to deal with RT longer than 1.75 s (*long RT rule*) and for instances where subjects accidentally kept the response button depressed after making a response therefore resulting in a erroneous response recorded for the next stimuli pair (*button rule*). For the *long RT rule* we inspected data for instances where a *false alarm* response to a non-target was made following an apparently missed target, and the RT was < 0.3 s. We considered such a response as a late response to a target because valid responses to stimuli in under 0.3 s are not ordinarily possible. The adjusted RT was calculated as the sum of the RT on the non-target and the inter-stimulus duration (1.75 s). Making this adjustment improves the accuracy measure, whilst a time penalty for a slow response enters analysis, which would otherwise have been lost if not adjusted. For the *button rule,* we inspected data for occurrences of very fast RTs (<1.0 s) following responses to targets. In these cases the incorrect responses were removed because they would otherwise be calculated as incorrect responses. Examples of instances where both rules would be applied are shown in **Table 5**.

For DA-RT and CA-RT, we calculated means from RTs on correct responses, excluding RTs on *false alarms*. The mean for each block was first calculated and then the mean for the five blocks. To measure recognition accuracy we used the corrected recognition rate (Pr) calculated from the Hit Rate (HR) and False Alarm Rate (FAR) as follows:

Pr = Hit Rate (targets correctly identified / total targets) minus False Alarm Rate (false alarms / non-targets).

It is evident from the calculation that Pr can vary between 1 and 0, with 1 indicating an ideal performance (Snodgrass and Corwin, 1988). By including the FAR in the calculation an adjustment is made for subjects who have a liberal response bias and make more errors on non-targets. Using Pr is an advance over the traditional use of percentage correct responses (= targets correctly identified/total targets) where false recognition is not taken into account.

3.9.1.4 Piloting the divided attention task

We piloted an initial version on 12 healthy volunteers who were able to complete the task satisfactorily. Five patients with mild AD and two with AMCI then completed the task. Some of the patients performed poorly on accuracy and reported that this was due to the fast rate of stimulus presentation. In response, we increased the inter-stimulus interval from 1.25 s to 1.75 s in order to ensure high accuracy on the task. Due to the longer inter-stimulus interval, the number of stimuli per block had to be reduced from 24 to 16 in order to maintain overall block and task length. The task was tested in the scanner environment on two runs to ensure compatibility. Pilot behavioural data are reported in the Results section (§4.4).

3.9.2 Visual and Auditory Selective Attention Paradigm

We compared the CoAMCI and Controls at baseline on auditory and visual attention processing in order to establish if there were any generalised non-specific effects of AMCI on fMRI signal. We compared RivAMCI and NxAMCI over time to ascertain if there were any generalised effects of ACEI treatment on visual and auditory attention processing which could affect the interpretation of results on the divided attention and memory tasks. We used an existing paradigm to study basic auditory and visual attention. The attentional conditions are considered *selective* rather than sustained because the scanner environment introduces highly salient sensory distraction in the form of scanner noise (see §1.5.1 for definitions of attention).

3.9.2.1 Experimental design

This paradigm had a blocked design that comprised of three alternating conditions. The control condition consisted of crosshair fixation (FIX), the visual condition of a circular black and white checkerboard pattern that filled up the entire screen a (VIS), and the auditory condition of lists of English nouns (AUD). Conditions were presented in alternating epochs. During the VIS component, the checkerboard squares were reversed at three distinct frequencies (2, 4 and 8 Hz) for fixed epochs of 16 seconds. The order of reversal frequencies was randomised within each set of three consecutive stimulation/fixation cycles. The auditory component consisted of a male voice reading a list of nouns presented at three randomised word rates (30, 60 and 90 words per minute) for fixed epochs of 24 seconds. The visual and auditory stimuli were presented asynchronously from each other over the 288 seconds duration of the entire task and overlapped (VISAUD) for 136 seconds. For functional analysis, we modelled four conditions: VIS, AUD, FIX and VISAUD.

3.9.3 Verbal Episodic Encoding Paradigm

We used an existing verbal encoding paradigm that we adapted by reducing the number of stimuli. The paradigm was designed to measure the effects of semantic elaboration related to PFC function, which we hypothesised may be impaired in AMCI. I describe the task in detail below by reference to its theoretical underpinnings, experimental design and behavioural data analysis.

3.9.3.1 Theoretical background

We set out to study functional activation related to verbal encoding and the possible contributions executive failure makes to impaired encoding in AMCI. We therefore employed fMRI and a verbal episodic memory encoding task similar to the Dees-Roediger-McDermott memory paradigm (Deese, 1959; Roediger and McDermott, 1995). In this paradigm, false recognition (incorrect recognition of a novel item as a studied item) can be manipulated by including recognition probes that are either unrelated (novel) or semantically related (lures) to studied items. It is therefore sensitive to the effects of spontaneously adopted, unsupported encoding strategies that reflect everyday memory function and reflect semantic processing. In healthy subjects, semantic elaboration at encoding predictably increases the misidentification of semantically related items (called lures) as studied items (targets). False recognition of lures can be quantified and AD patients show reduced false recognition of lures due to failure to generate and encode semantically related information (Balota et al., 1999; Budson et al., 2000; Budson et al., 2002; Budson et al., 2003). This

paradigm can therefore be used to study the possible contribution of executive failure during attempted encoding in individuals with AMCI.

3.9.3.2 Experimental design

We adapted an existing blocked-designed encoding task by reducing the number of stimuli, which reduced the overall duration of the task and made it practical for our purposes. The adapted task consisted of alternating epochs of experimental encoding (ENCODE) and control (CONTROL) conditions. The ENCODE blocks required subjects to read aloud and memorise commonly occurring English nouns, visually presented in 9 lists **(Table 6)**. Audible speech was used in order to monitor compliance during the task. Each list contained 8 words semantically highly related to a critical non-presented word taken from a known list (Stadler et al., 1999). For example, the items hiking, pack, trail, scout, cabin, summer, lake, canvas and holiday made up the list related to the critical non-presented word camp. Each word in the task was presented for 2.5 sec followed by a fixation cross presented for 1.5 sec and thus a new word was presented every 4 sec. ENCODE blocks were alternated with CONTROL blocks which consisted of 4 consecutive presentations of the word 'wait', which also had to be read aloud **(Figure 10)**. CONTROL blocks matched ENCODE blocks for attention to a visual stimulus, reading and vocalisation, but not on the requirement to encode words or on the variety of vocalisation associated with the ENCODE stimuli. Participants were trained on the tasks prior to entering the scanner using a set of training stimuli, and reminded of the instructions immediately prior to commencing. They were instructed to read all the words out loud, including the word "wait", and to memorise as many words as possible because recognition would be

tested during a subsequent task. They were aware of task duration and of the number of words and lists.

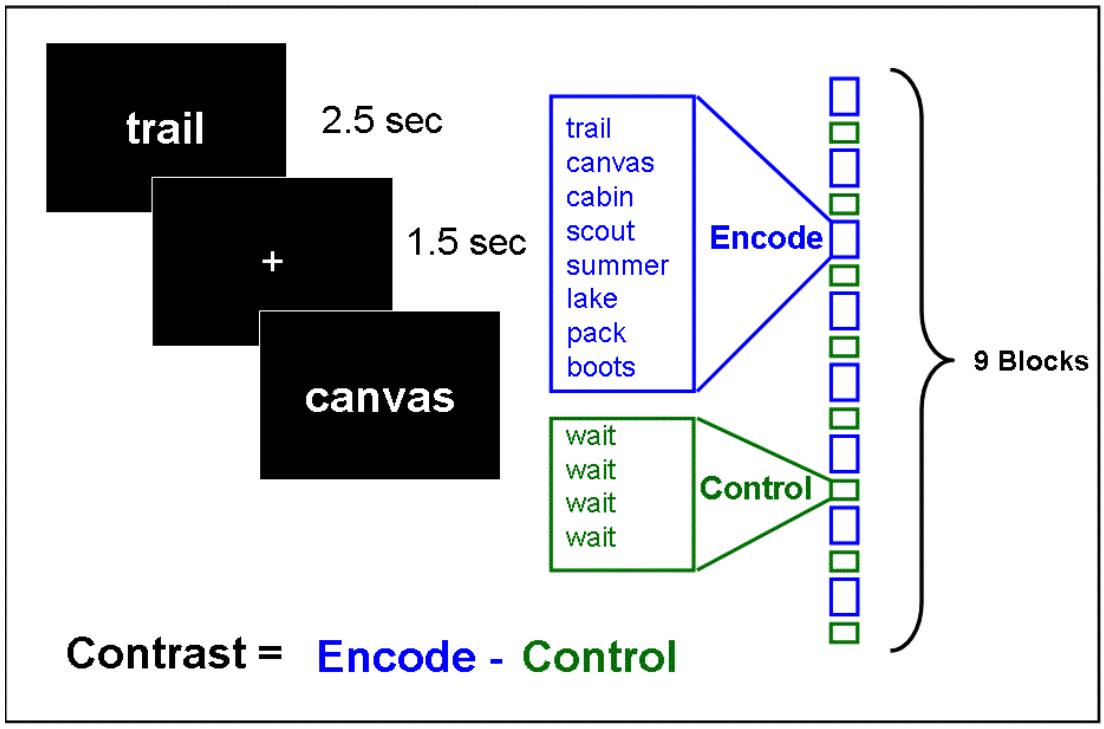


Figure 10. Verbal encoding task.

Commonly occurring English nouns were presented sequentially in lists of eight during ENCODE blocks which alternated with CONTROL blocks during which the word "wait" was presented four times. Each word was presented for 2.5 seconds followed by a pass delay of 1.5 seconds during which time a white cross was presented. Nine blocks of each condition were presented.

List 1	**List 2**	**List 3**	**List 4**	**List 5**
flag	cabin	sour	pin	bed
cross	scout	bitter	sewing	yawn
pole	summer	candy	haystack	night
symbol	pack	good	thimble	snore
stars	lake	cake	cotton	snooze
stripes	trail	nice	thorn	blanket
march	canvas	tooth	point	awake
England	holiday	honey	injection	dream
wait	wait	wait	wait	wait
wait	wait	wait	wait	wait
wait	wait	wait	wait	wait
wait	wait	wait	wait	wait
List 6	**List 7**	**List 8**	**List 9**	
daft	hive	mad	rubber	
fool	bumble	fear	bounce	
dense	hornet	temper	nylon	
genius	hum	tension	tyre	
slow	sting	punch	ball	
loser	insect	jealous	band	
clever	keeper	hate	foam	
dim	wax	drunk	glue	
wait	wait	wait	wait	
wait	wait	wait	wait	
wait	wait	wait	wait	
wait	wait	wait	wait	

Table 6. Word lists presented at baseline for the encoding paradigm.

Nine lists comprised of eight discreet words and four repetitions of the word “wait” were presented during encoding. Words from lists 1 and 9 were not included as recognition probes in order to control for recency and primacy.

3.9.3.3 Behavioural analysis

Overt speech was used during this task in order to monitor compliance with task instructions. Speech was monitored continuously during the task for correct identification of words and overt vocalisation. Encoding success was measured on the recognition paradigm, which I describe next.

3.9.4 Verbal Episodic Memory Recognition Paradigm

We employed a recognition task to accompany the encoding task in order to measure behavioural performance during recognition and associated functional brain activation. The task was adapted from an existing task by reducing the number of stimuli in accordance with the shortened encoding task.

3.9.4.1 Theoretical background

We have seen in the introductory section that episodic memory can be tested by tasks measuring *free recall* or *cued recall*. Recognition is a type of cued recall that is supported by presentation of probe stimuli that are either targets or distractors. During a *yes/not* recognition task, a single probe is presented and the subject has to decide if it is a target or not. Recognition tests appear more sensitive at distinguishing AMCI from normal ageing (Anderson et al., 2008; Bennett et al., 2006; Westerberg et al., 2006) and may also be more sensitive than free recall in distinguishing normal ageing from AD because it is less affected by age-related changes (Craik and McDowd, 1987; Parker et al., 2004). These findings suggest that a recognition task would be preferred to a free recall task for our purpose of examining recognition in AMCI, which falls between ageing and AD. Furthermore, both item and associative memory

appears affected in AMCI (Dudas et al., 2005). Taken together these findings suggest that an associative learning task with a yes/no recognition phase may be a particularly sensitive measure to discriminate AMCI from normal ageing.

Recognition can be further divided into *familiarity* which refers to a feeling of prior exposure to an item without recall of associated contextual information, and *recollection* referring to the retrieval of the item bound to contextual features such as the time, place or source of experience (Yonelinas, 2002).

The recognition task we used was *yes/no implicit associative item recognition* task, which followed an intentional encoding phase. The associations are implicit as all the words in a list are semantically related but subjects are not explicitly made aware of this, which enables examination of distinct memory processes such as automatic semantic elaboration.

In summary, these findings suggest the potential of specific recognition tasks to examine distinct memory processes that appear differentially affected in ageing and AMCI. Combining such tasks with functional neuroimaging has the potential to reveal the functional neuroanatomy of recognition processing in controls and AMCI.

3.9.4.2 Experimental design

Recognition was tested after a delay of 12 minutes during which an unrelated attention task and a structural scan were performed. This delay was included to curb rehearsal of studied items because recognition following rehearsal would reflect working memory capacity rather than episodic encoding success. Probe words were presented individually on a screen and subjects used a button to indicate their choice from three possible responses: “Remember” (familiarity with both recognition of the probe and recollection of study episode), “Know” (familiarity in the absence of

recollection of the study episode) or “New” (unfamiliar). The position of choices presented on the screen was similar to the position of the buttons on the button box so that “Know appeared to the bottom right, “Remember” to the top middle, and “New” to the bottom right. The probe list consisted of 17 *target* words, 16 *novel* words and 14 *lures*. The first and last lists, as well as the first and last words in each of the remaining lists, were excluded from the recognition task to control for the effects of recency and primacy. Targets were typically words in position 2, 5 and 7 in the remaining lists. The lures consisted of the non-presented critical word highly semantically associated with words in each list plus one more non-presented word from the list associated with the lure **(Figure 11)**. Novel words were taken from non-presented word lists. “Remember and “Know” responses were added together as familiarity responses during behavioural analysis. “Remember" refers to recollection of a studied item which is accompanied by conscious retrieval of aspects of the individual’s experience at the time of study, which may include what they thought about or what preceded it, so in a sense being able to relive the moment of study. Knowing implies only recollection of the studied item (Tulving, 1985). This distinction was included as there is mounting evidence for distinct neural networks involved in remembering and knowing with PFC activity predominantly involved during remembering (for a review see (Knowlton, 1998). The difference between “Remember” and “Know” was explained to participants, they practiced the task outside the scanner, and were reminded of instructions immediately prior to scanning.

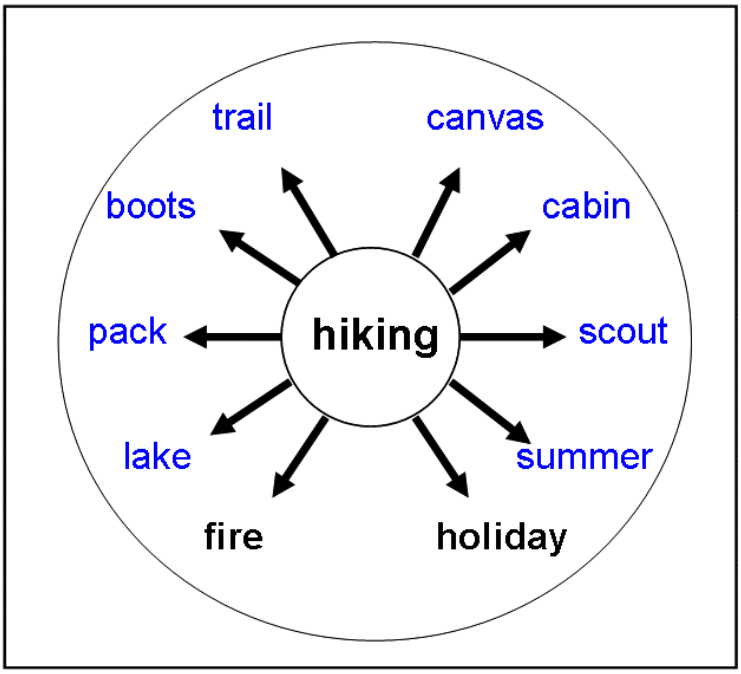


Figure 11. Recognition task.

The figure illustrates the relationship between the critically non-presented highly semantically associated word "hiking" and words that were studied during encoding (blue), and words used as lures (black).

3.9.4.3 Behavioural analysis

For behavioural analysis we calculated corrected recognition rates (Pr) exactly as described in the section above on divided attention. We also calculated a response bias measure (Br) after Feeman and Snodgrass who found normal response bias for patients with isolated amnestic disorders (Korsakoff's, MTL pathology) but more liberal response bias for patients with AD (Snodgrass and Corwin, 1988).

Br = false alarm rate / (1- Pr)

Subjects might have high levels of familiarity for both old and new probe items if stimuli were not encoded distinctively or when semantic memory representations have been inappropriately activated by prior presentation of target and distractor items.

Stimuli that were not encoded distinctively at presentation could adequately match stored representations of similarly poorly encoded target items or very familiar items in semantic memory, leading to a general increase in recognition responses to both old and new items. A difference in response bias between AMCI and controls could therefore indicate impaired semantic processing.

We also examined whether the groups were comparable on false recognition of lures by calculating the false recognition rate for lures:

Lure Pr = lure HR - FAR

Recognition responses can be classified into four categories **(Figure 12)**. Targets can be correctly identified as previously studied or old (*hits*) or fail to be recognised (*misses*). Whereas novel or distractor items can be incorrectly recognised as old (*false alarm*) or correctly rejected as not studied previously (*correct rejection*). The *two-high-threshold* model of recognition memory can be used for interpreting the cognitive processes underlying these four behavioural responses (Snodgrass and Corwin, 1988). This model assumes that recognition involves selection between discrete memory states rather than a continuum of memory strengths as signal detection models of memory assumes (Snodgrass and Corwin, 1988). Recognition of an item is therefore based on the memory signal trace meeting a specific threshold level. This proposes the existence of distinct memory thresholds for the recognition of old items as old and for the recognition of new items as novel. These two thresholds separate three possible memory states that can be experienced by individuals: recognition of items as old/familiar, recognition of item as novel/unfamiliar and an intermediate state of uncertainty. Items are correctly recognised as old or familiar when the strength of the memory trace crosses the "high" threshold level. Conversely, if an item fails to activate a memory trace over the "low" threshold it is not recognised

and therefore deemed as new. If the level of memory trace associated with an item is too strong to be novel or too weak to be familiar and therefore ambiguous, then it falls into the uncertain state. Items in the uncertain state are classified as old or new based on the individual's particular response biases in that situation (e.g. "I must have seen that before" or "If I had not seen that I would remember"). The HR represents the number of times that an individual correctly recognised a target item *plus* the correct guesses from the uncertain recognition state. The FAR originates from the uncertain recognition state and occurs when a novel item is not recognised as new and a target not recognised as such. Both the Pr and Br are obtained from the HR and FAR which indicates that these measures take into account all the behavioural responses.

		Source of Stimuli	
		Target	Distractor
Subject Responses	"Old"	Hit	False Alarm
	"New"	Miss	Correct Rejection

Figure 12. Recognition response classifications.

Targets can be correctly identified as previously studied or old (*hits*) or fail to be recognised (*misses*). Novel or distractor items can be incorrectly recognised as old (*false alarm*) or correctly rejected as not studied previously (*correct rejection*).

On a recognition task with equal numbers of targets and novel distractor items, a Br value of 0.5 represents the neutral or chance value indicating that the individual is equally likely to say that an unrecognised item is old or new. A value < 0.5 represents a *conservative bias* or a tendency to say that an item is new and a value > 0.5

represents a liberal bias or a tendency to say that unrecognised items are old when uncertain.

The functional analysis of this task was event related and required that events of interest be defined and that the timing of each event of interest was required for each experimental run.

Based on the two-high-threshold model of recognition we set up the functional analyses of the recognition data to examine activation during five events:

1. Remember and Know (Old) responses to targets (*Hits*)
2. New responses to targets (*Misses*)
3. Remember and Know (Old) responses to lures (*False alarm on a lure*)
4. New responses to lures (*Correct rejection of a lure*)
5. New responses to novel and lure distractors (*Correct rejection of any distractor*)

Examining these conditions in conjunction with the semantic processing manipulation could enable us to draw conclusions about the nature of recognition failure in AMCI. **Table 7** shows the recognition task response data from a sample file for the task at baseline. Probes were presented in the order in which they appear in the table. The timing of presentations was used to create the *newstarts* file mentioned in the fMRI methods section and identifies the conditions of interest during the analysis process.

Stimuli	Word	Response	Time	Stimuli	Word	Response	Time
1	salt	know=wrong	0.001	1	attack	new=correct	346.002
2	scout	rem=correct	8.002	2	bumble	know=correct	364.002
1	scissors	know=wrong	16.002	1	wing	new=correct	372.002
3	leaf	know=wrong	26.002	3	chess	rem=wrong	382.002
3	oak	rem=wrong	44.002	3	sound	rem=wrong	398.002
2	canvas	know=correct	52.002	1	gentle	rem=wrong	406.002
3	write	new=correct	66.002	3	scribble	rem=wrong	418.002
1	idiot	know=wrong	76.002	2	keeper	rem=correct	428.002
2	awake	rem=correct	94.002	3	cage	rem=wrong	444.002
1	sleep	know=wrong	102.002	2	yawn	new=wrong	452.002
2	sewing	know=correct	116.002	3	tiger	know=wrong	464.002
2	fool	new=wrong	132.002	1	sugar	know=wrong	474.002
1	bee	know=wrong	144.002	2	cotton	know=correct	484.002
3	piano	know=wrong	152.002	1	needle	know=wrong	500.002
2	sting	know=correct	166.002	1	silk	rem=wrong	512.002
2	nice	rem=correct	182.002	2	point	new=wrong	520.002
3	branch	new=correct	192.002	1	camp	know=wrong	538.002
1	furious	new=correct	200.002	3	gym	new=correct	546.002
2	hate	rem=correct	214.002	1	attack	new=correct	346.002
3	circus	know=wrong	226.002	2	bumble	know=correct	364.002
2	slow	rem=correct	236.002	1	wing	new=correct	372.002
1	buzz	know=wrong	252.002	3	chess	rem=wrong	382.002
3	lion	new=correct	264.002	3	sound	rem=wrong	398.002
2	cake	rem=correct	276.002	1	gentle	rem=wrong	406.002
3	tree	rem=wrong	286.002	3	scribble	rem=wrong	418.002
3	fit	new=correct	302.002	2	keeper	rem=correct	428.002
2	clever	rem=correct	310.002	3	cage	rem=wrong	444.002
3	dice	rem=wrong	328.002	2	yawn	new=wrong	452.002
2	fear	rem=correct	336.002	3	tiger	know=wrong	464.002
1	attack	new=correct	346.002	1	sugar	know=wrong	474.002
2	bumble	know=correct	364.002	2	cotton	know=correct	484.002
1	wing	new=correct	372.002	1	needle	know=wrong	500.002
3	chess	rem=wrong	382.002	1	silk	rem=wrong	512.002
3	sound	rem=wrong	398.002	2	point	new=wrong	520.002
1	gentle	rem=wrong	406.002	1	camp	know=wrong	538.002
3	scribble	rem=wrong	418.002	3	gym	new=correct	546.002
2	keeper	rem=correct	428.002				

Table 7. Recognition task response data at baseline.

The table shows the type of recognition probe (1=lure; 2=target; 3=novel), probes, response, accuracy, and time of presentation from the start of the task for the recognition task at baseline. The probe list consisted of 17 *target* words, 16 *novel* words and 14 *lures*.

3.9.5 Post Hoc Analyses

3.9.5.1 Stratification of AMCI groups to examine for potential selection bias

After it became apparent that the RivAMCI and NxAMCI groups differed significantly on cortical activation at baseline on the divided attention task (see results section), we decided to explore this further in order to see if this was related to any identifiable systematic error or bias introduced due to the later recruitment of the NxAMCI group. We first investigated if the groups were different as far as the severity of cognitive impairment was concerned. Based on population data available for the CAMCOG, we devised a procedure designed to calculate a predicted maximum total score against which each subjects actual score could be measured (Huppert et al., 1995). We could therefore judge the relative level of global cognitive impairment for subjects at the time of their participation in the study. Similar methods have been used in studies where the sum of the boxes rather than the global CDR score is used as a measure of impairment; an increase in CDR sum of boxes is associated with increased risk of being diagnosed with dementia (Lynch et al., 2006). AMCI patients have also been stratified according to their level of functional impairment using the CDR sum of boxes for fMRI studies (Celone et al., 2006). The CAMCOG predicted maximum schedule is shown in **Table 8**. In order to stratify participants into more and less impaired groups, we calculated the percentage difference between predicted and actual scores.

Gender	Male	+2
	Female	-2
Age	<65	+14
	65-70	+9
	71-75	+5
	76-80	-0
	81-85	-5
	>85	-10
Education	<12	-4
	12-13	-2
	14	-0
	15-16	+2
	>17	+4
Social Class	Unskilled	-4
	Skilled manual	-0
	Skilled non-manual	+2

Table 8. Predicted total CAMCOG score calculation

Variables derived from the above table are used to calculate the individual predicted CAMCOG total by adding or subtracting from a starting total of **85**. A maximum predicted score of 107 is possible.

4. Results

4.1 Participants Demographics

We recruited 22 right-handed AMCI patients (14 women, 8 men, mean age 69 years, range 44 to 81 years) and 11 right-handed healthy control subjects (Controls) who were comparable on age (6 women, 4 men, mean age 68 years, range 50 to 84; $F=0.03$; $p<0.9$) and educational attainment (AMCI mean 11.3, SD 2.0, range 9 to 15, Controls mean 10.0; SD 1.3; range 9 to 12; $F=3.1$; $p<0.9$). Sixty-three potential AMCI participants were screened and 60 screened positive. Participants failed screening due to being left-handed and suffering significant neurological disorders since being diagnosed with AMCI; meningitis and transient ischaemic attack.

AMCI patients were recruited to two groups: The **RivAMCI** group received rivastigmine treatment after baseline and the **NxAMCI** group did not receive drug treatment. Patients were recruited in two phases as indicated in **Figure 13**. The first participant was recruited and scanned in March of 2003 and data collection was completed in October 2008. Control and RivAMCI groups participated from March 2003 onwards and the majority completed within nine months. The untreated NxAMCI group participated between November 2004 and October 2007. We replaced one participant from the RivAMCI group when it became clear they suffered diffuse Lewy body disease and this accounts for the recruitment of RivAMCI patient in July of 2007. At two-year follow-up, four patients in the RivAMCI group remained stable, four had progressed to mild probable AD, two to mild dementia of probable mixed AD and vascular aetiology. In the NxAMCI group, six patients converted to AD, three remained stable and one was lost to follow–up. All Controls remained cognitively normal at 2-year follow-up.

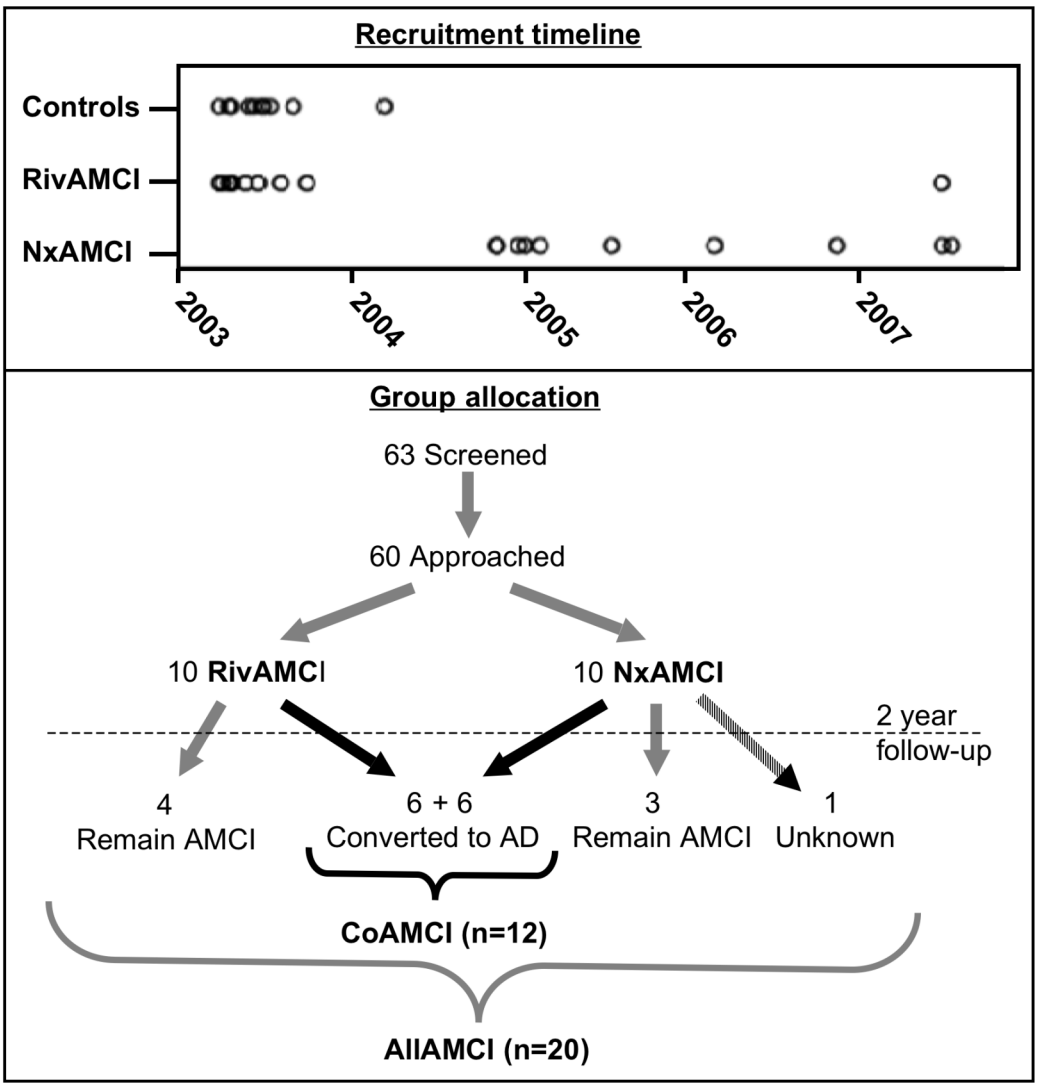


Figure 13. Recruitment and group allocation of AMCI participants.

The Control and RivAMCI groups participated from March 2003 and the NxAMCI group from November 2004 onwards (upper panel). For the later analysis on the RivAMCI group one patient was replaced as it became obvious that he was suffering diffuse Lewy body disease, accounting for the recruitment of a replacement patient in July of 2007. AMCI participants were recruited to the RivAMCI and NxAMCI groups and, for functional and behavioural analysis, these two groups were combined as the AllAMCI group whilst those AMCI that converted to AD after 2 years of follow-up were pooled as the CoAMCI group that represent prodromal AD (lower panel).

For behavioural and functional analysis, data were pooled from the RivAMCI and NxAMCI samples into the **AllAMCI** group, and from AMCI patients in the RivAMCI and NxAMCI groups that subsequently converted to AD into the **CoAMCI** group. Findings from the AllAMCI group can therefore be used to make inferences about clinical samples of AMCI whereas findings from the CoAMCI group can be used to make inferences about prodromal AD at the stage of AMCI. AllAMCI and CoAMCI groups were compared to Controls at baseline for the behavioural and functional analyses of attention and memory. To study the effects of rivastigmine in AMCI, participants were grouped into treated (RivAMCI) and untreated groups (NxAMCI) and compared over time.

4.2 Neurocognitive Results

Results for Controls, AllAMCI and CoAMCI at baseline are summarised in **Table 9**. Controls and AllAMCI were comparable on age and education although the mean age for the AMCI converter group (CoAMCI) was older. AllAMCI and CoAMCI groups scored lower on the CAMCOG and MMSE compared to Controls. Controls performed better than AllAMCI and CoAMCI groups on the New Learning subscale but the groups were comparable on attention.

A significant correlation was evident between age and CAMCOG total for Controls and CoAMCI ($r= -0.35$; $p<0.05$; $R^2=0.12$) but not on new learning.

				Control vs AllAMCI			Control vs CoAMCI		
	Control n=10	**AllAMCI n =20**	**CoAMCI n=12**	P	F	R^2	P	F	R^2
Age	68.0 (13.5)	68.8 (10.3)	74.5 (6.1)	ns			ns		
Years in education	10.0 (1.3)	11.3 (2.0)	11.1 (2.2)	ns			ns		
NART	115 (5.2)	112 (9.3)	113 (8.8)	ns			ns		
MMSE	28.8 (1.2)	26.4 (1.9)	26 (2.0)	<0.01	10	0.3	0.001	15	0.5
CAMCOG (max=107)	100.1 (2.5)	89.1 (5.1)	86.6 (4.6)	0.001	25	0.5	<0.001	63	0.6
Attention (max=9)	8.8 (0.4)	8.0 (0.9)	8.5 (0.7)	ns			ns		
New Learn (max=17)	14.4 (1.3)	9.1 (3.8)	8.0 (3.1)	0.001	18	0.4	<0.001	38	0.6

Table 9. Neurocognitive data and group comparisons for Controls and AMCI groups.

Controls were comparable to AMCI patients on age, years in education, premorbid intelligence (NART) and attention score, and they performed better that patients on global cognitive measures (MMSE, CAMCOG) and new learning. Data are presented for Controls, all AMCI participants (AllAMCI) and AMCI dementia converters (CoAMCI).

Data are mean (standard deviation), ns=not significant, F=ANOVA test statistic. The coefficient of determination, R^2 is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect.

4.3 Overview of Scanning Sessions

We conducted 63 scanning sessions on 32 participants. One participant completed only one session as structural imaging revealed evidence of a cortical infarct and he was therefore excluded from further participation. Seventeen of the 252 experimental runs had to be restarted or abandoned due to technical failure or poor compliance with task instructions. Behavioural performance was monitored throughout experimental runs and experiments were repeated where necessary. Four runs of the recognition task had to be abandoned and consequently no useful functional data were collected. On one run a participant refused to complete the task due to discomfort in the scanner and on three other runs technical problems resulted in loss of functional data. Behavioural data were unusable on five runs of the recognition task: three due to compliance issues (holding the button box upside down, continuously pressing a button); data from the remaining two runs could not be used because the encoding task had to be repeated, with a different set of stimuli, that therefore interfered with the encoding – recognition sequence required for valid use of the task. During recognition tasks, distractor items that are presented are automatically encoded and subjects are inclined to make false recognition responses to such items if the task is repeated. It is therefore not possible to collect reliable data by repeating a recognition task. A disproportionate number of technical and compliance problems occurred

during the recognition task (9 out of 15) and this is likely due to the task being presented last, when participants were tired.

4.4 Divided Attention Task Pilot Data

During the development of the divided attention task, we collected data from 14 controls, 5 mild AD patients and 2 AMCI patients. Task parameters were adjusted based on these data to ensure high accuracy in all participants. As mentioned in the Methods (§3.9.1), RT is considered a reliable and useful performance measure in divided attention experiments when tasks are well practiced and completed with very high accuracy levels. Controls had high accuracy on both DA (PrDA=0.97) and CA (PrCA=0.97) conditions at the initial interstimulus interval of 1.25 s. We also collected data on patients with mild AD who would already suffer some impairment in divided attention. Their performance was impaired on DA (PrDA=0.59) and CA (PrCA=0.86) conditions. By increasing the interstimulus interval to 1.75 s, we ensured high accuracy in the patient group (PrDA=0.84; PrCA=0.98). This level of accuracy in the mild AD group suggested that patients with AMCI with no obvious attentional impairment should be able to perform as well as controls. This was confirmed in the AMCI patients (PrDA=0.98; PrCA=1.0) and by the behavioural results reported on below.

4.5 Divided Attention at Baseline

We compared Controls with AllAMCI and CoAMCI at baseline to determine if AMCI is associated with impaired divided attention and related functional activation.

4.5.1 Behavioural Results

Results at baseline for comparisons on divided attention are summarised in **Table 10**. Controls and AMCI had high accuracy on the dA and cA conditions. Responses were faster for visual targets than for auditory targets and Controls and AMCI were faster and more accurate on the cA condition. Between-group comparisons revealed comparable auditory RT and high accuracy (Pr); however, AllAMCI and CoAMCI subjects had significantly slower visual RT. VisRT did not correlate with age and all groups demonstrated longer RT to auditory targets.

				Control vs AllAMCI			**Control vs CoAMCI**		
	Control n=10	**AllAMCI n=20**	**CoAMCI n=12**	P	F	R^2	P	F	R^2
dA- condition									
Corrected recognition rate (Pr)	0.94 (0.16)	0.92 (0.07)	0.89 (0.08)	ns			ns		
Visual RT	0.60 (0.03)	0.67 (0.07)	0.69 (0.09)	0.01	7	0.3	0.01	9	0.3
Auditory RT	0.73 (0.14)	0.76 (0.09)	0.77 (0.09)	ns			ns		
cA-condition									
Corrected recognition rate (Pr)	0.99 (0.02)	0.99 (0.03)	0.98 (0.03)	ns			ns		
RT	0.46 (0.07)	0.51 (0.08)	0.51 (0.08)	ns			ns		

Table 10. Divided attention task behavioural results and group comparisons at baseline.

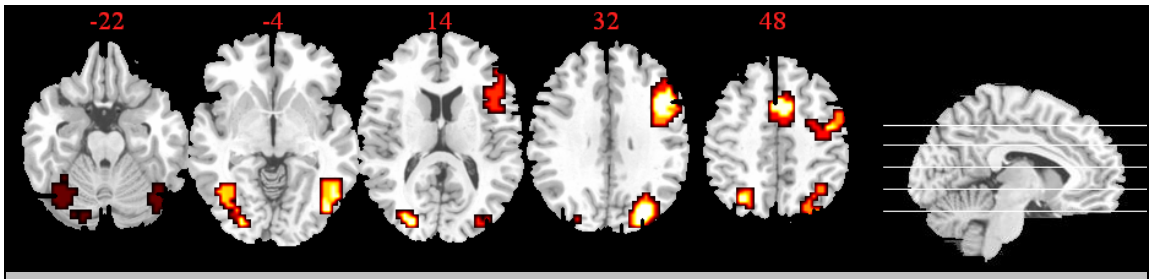
All participants had high target recognition accuracy during divided attention as indicated by corrected recognition rates (Pr). AMCI patients were slower than controls on visual target response speed (Visual RT) but comparable on auditory

target response speed (Auditory RT). Data are presented for Controls, all AMCI (AllAMCI) and AMCI dementia converters (CoAMCI). Data are mean (standard deviation), ns=not significant; F=ANOVA test statistic. The coefficient of determination, R^2 is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect.

4.5.2 Functional Results

4.5.2.1 Group activation

Combining Controls and AllAMCI revealed activation in ***bilateral*** medial parietal areas (precuneus), fusiform gyrus (BA 37), extrastriate visual cortex (BA 19) and cerebellum, and in ***left*** hemispheric DLPFC (BA 9, 46), VLPFC (BA 44, 45, 47), premotor cortex (BA 4, 6), insula and anterior cingulate **(Table 11)**.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
All subjects						
Prefrontal cortex	L	4, 6, 9, **44**, 45, 46, insula	-40	4	31	173
Medial frontal	L	**32**	-4	11	42	51
Medial parietal	L	**19**, 31	-25	-70	26	100
Medial parietal	R	19, **31**	25	-60	31	22
Cerebellum	L	**Cerebellum**, 19, 37	-40	-56	-18	76
Cerebellum	R	**Cerebellum**, 19, 37	43	-63	-18	104

Table 11. Functional activation during divided attention for Controls and AllAMCI combined.

The table and brain activation maps show the areas where activation increased during the divided attention condition compared to the control attention condition across all participants. Activation was evident in PFC, parietal and occipitotemporal areas (see text for details).

Table legend: The table contains the cerebral region and Talairach coordinates of the voxel of maximal activation in each cluster and the cortical or Brodmann areas

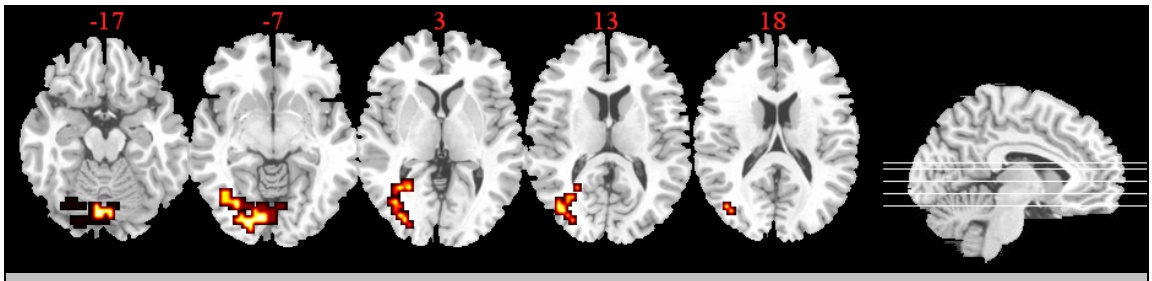
activated in the cluster; the area of maximal activation appears in **bold**. Areas of activation are indicated on the brain map. Results are reported at a cluster-wise probability of less than one false positive cluster per volume, unless otherwise specified. Axial slices proceed from left to right, from the most caudal to the most rostral. Lines on the saggital image indicate the level of axial slice with the lowest slice corresponding to the far left image. Z-coordinates appear in red above axial slices. The left hemisphere appears on the right and vice-versa. L= Left hemisphere; R = Right hemisphere; BA = approximate Brodmann's or cortical area.

4.5.2.2 Group differences

Controls vs AllAMCI: AllAMCI had decreased activation in ***right*** hemispheric extrastriate visual cortex (BA 18, 19), hippocampus and occipitotemporal areas (BA 37, 39) **(Table 12)**. This was confirmed after controlling for the possible effects of slower reaction times on the BOLD signal in AMCI using analysis of covariance (ANCOVA) with visual RT as covariant. Slower RT could therefore not account for decreased activation in AMCI participants. There were no areas of greater activation in AMCI.

Controls vs CoAMCI: CoAMCI had decreased activation in ***right*** cerebellum, inferior temporal lobe (BA 20, 36, 37), auditory cortex (BA 22, 42), visual cortex (BA 18, 19), hippocampus, insula, medial parietal (BA 31) and lateral parietal areas (BA 40), and in ***left*** fusiform gyrus (BA 37), auditory association cortex (BA 21, 22) and lateral parietal lobe (BA 40) **(Table 13)**.

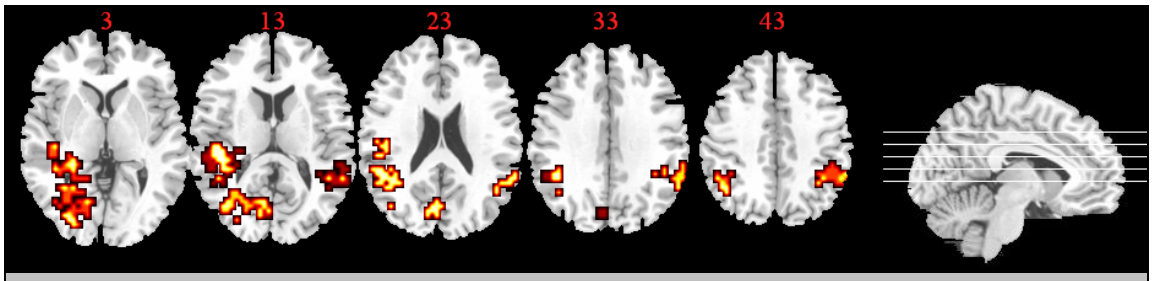
CoAMCI activated more in ***bilateral*** thalamic, brainstem and medial parietal areas (BA 29, 30).



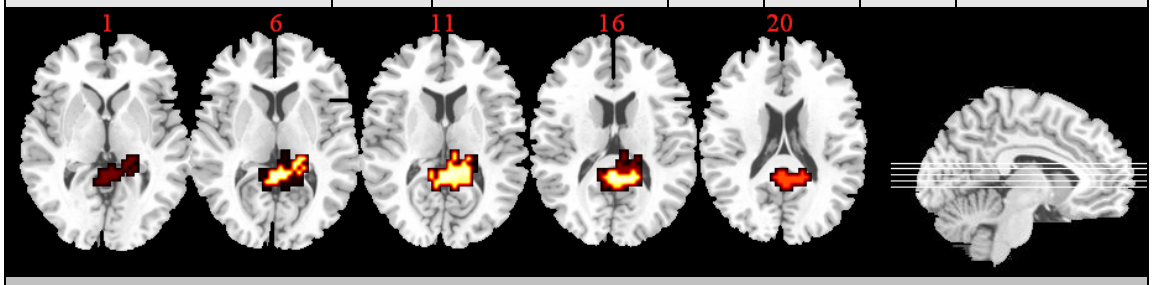
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls >AllAMCI						
Occipital	R	Cerebellum, hippocampus, **18**, 19, 37, 39	22	-81	-13	100

Table 12.Functional activation differences on divided attention between Controls and AllAMCI.

The table and brain activation maps show the areas where Controls had greater activation compared to all AMCI participants on divided attention. Differences were apparent in a large cluster in occipitotemporal areas (see text for details). For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls >CoAMCI						
Occipital	R	Cerebellum, hippocampus, insula, **18**, 19, 20, 22, 37, 42, 40	22	-81	-13	127
Occipitotemporal	R	18, 19, 31, 35, **36**, 37	29	-33	-7	190
Temporoparietal	L	21, 22, 37, **40**	-50	-44	26	68



CoAMCI > Controls						
Midbrain-thalamic	L	**Thalamus**, brainstem, 29, 30	-10	-37	4	75

Table 13. Functional activation differences on divided attention between Controls and CoAMCI.

The table and brain activation maps show the areas where Controls had greater activation compared to CoAMCI (top) and vice versa (bottom). Controls had greater activation in three clusters covering occipitotemporal and temporoparietal areas whereas CoAMCI had greater activation in a posterior midline cluster (see text for details). For table legend, see Table 11.

4.5.2.3 Functional-behavioural correlations

A negative correlation of medium effect size was evident between VisRT and activation in ***right*** occipitotemporal cortex (fusiform gyrus; BA 36) where CoAMCI had attenuated activation **(Table 14; Figure 14)**. A positive correlation of medium effect size was evident between VisRT and activation in the ***midbrain-thalamic*** area where CoAMCI had increased activation **(Figure 15)**.

Positive correlations with medium and small effect sizes were evident between activation in all three cortical areas where CoAMCI had attenuated activation, and CAMCOG total and New Learning subscale scores.

	Controls + CoAMCI	**R^2**
VisRT vs Right Occipital	ns	
VisRT vs Right Occipitotemporal	-0.45 (<0.05)	0.20
VisRT vs Left Temporoparietal	ns	
VisRT vs Midbrain-thalamic	0.54 (<0.05)	0.29
CAMCOG vs Right Occipital	0.41 (<0.05)	0.17
CAMCOG vs Right Occipitotemporal	0.50 (<0.01)	0.25
CAMCOG vs Left Temporoparietal	0.53 (<0.01)	0.28
CAMCOG vs Midbrain-thalamic	ns	
New Learn vs Right Occipital	0.44 (<0.05)	0.19
New Learn vs Right Occipitotemporal	0.49 (<0.01)	0.24
New Learn vs Left Temporoparietal	0.42 (<0.05)	0.18
New Learn vs Midbrain-thalamic	ns	

Table 14. Correlations between activation and behavioural measures for Controls and CoAMCI on divided attention.

The table shows correlations between behavioural measures and cortical areas where Controls and CoAMCI differed significantly.

Data are: Spearman's correlation coefficient (p). The coefficient of determination, R^2 is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect.

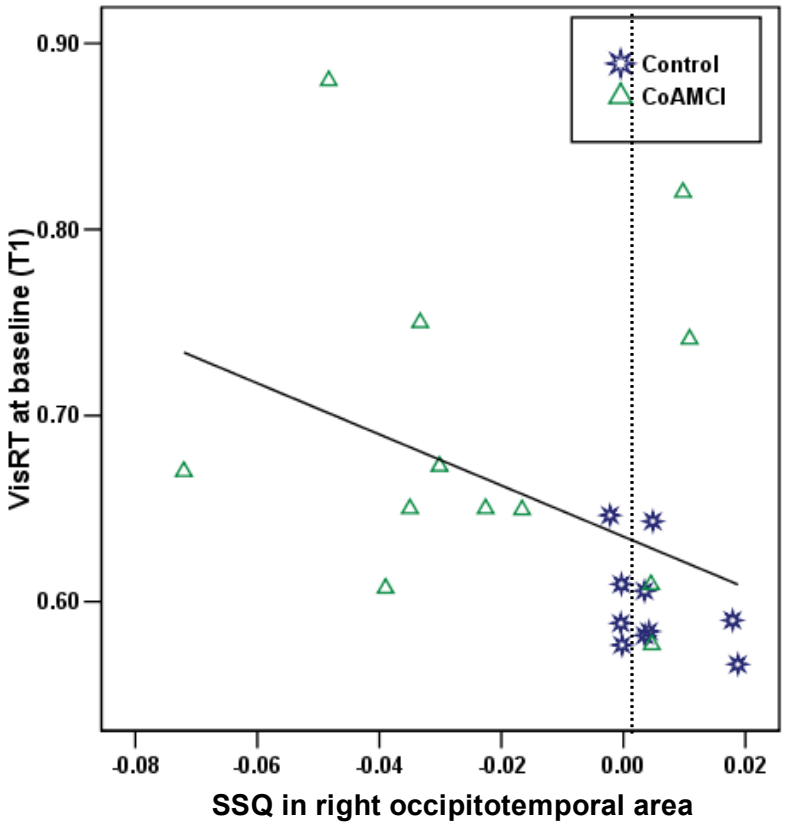


Figure 14. Functional-behavioural correlation of visual processing speed and cortical activation in the right occipitotemporal area in Controls and CoAMCI.

A significant correlation (r= -0.5 ($p<0.05$) was evident between visual processing speed (VisRT) and the magnitude of activation (SSQ) in the right occipitotemporal area where CoAMCI had attenuated activation and associated slower visual target response times during divided attention.

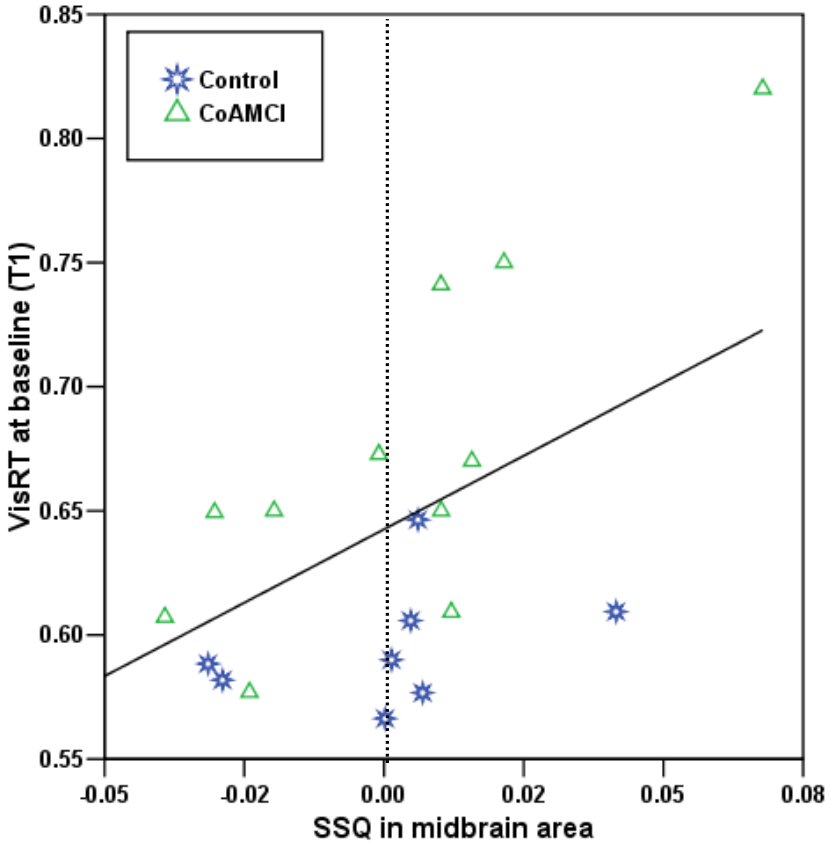


Figure 15. Functional-behavioural correlation of visual processing speed and cortical activation in the midbrain area in Controls and CoAMCI.

A significant correlation ($r= 0.5$; $p<0.05$) was evident between visual processing speed (VisRT) and the magnitude of activation (SSQ) in the midbrain-thalamic area where CoAMCI had greater activation and associated slower visual target response times during divided attention.

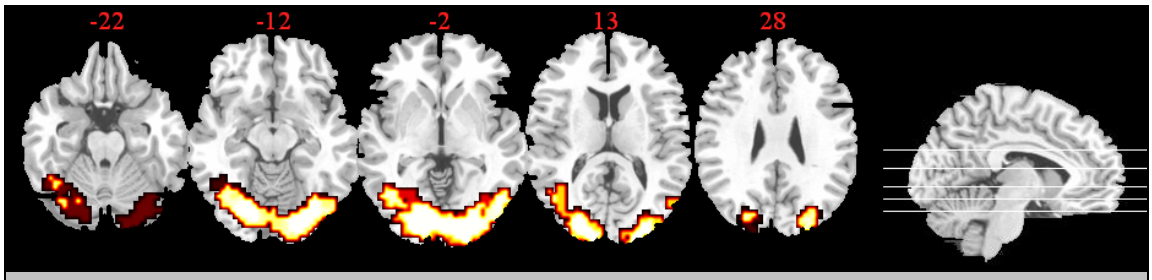
4.6 Visual and Auditory Selective Attention at Baseline

We compared AMCI subjects with Controls at baseline on selective visual and auditory attention in order to determine if basic sensory attention processing is altered in AMCI.

4.6.1 Functional Results Visual Selective Attention

4.6.1.1 Group activation for visual selective attention

Combining Controls and AllAMCI revealed activation in ***bilateral*** cerebellum, primary visual cortex (BA 17), extrastriate visual cortex (BA 18, 19) and fusiform gyrus (BA 37) **(Table 15)**.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
All subjects						
Cerebellum	L+R	Cerebellum, 17,**18**, 19, 37	18	-78	-13	870

Table 15. Functional activation on visual selective attention for Controls and AllAMCI combined.

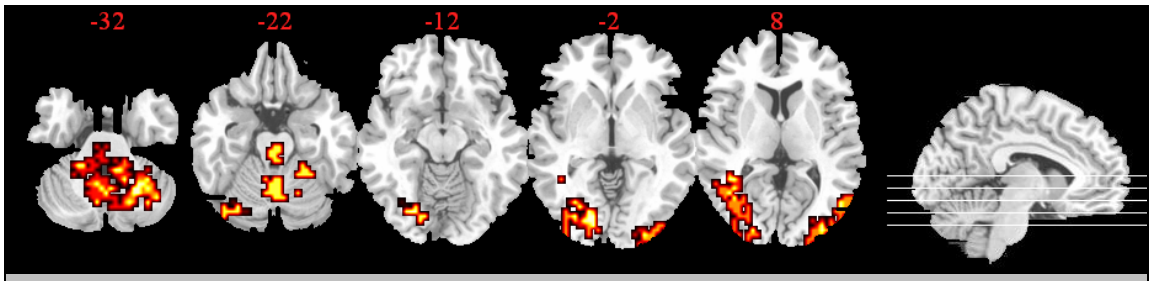
The table and brain activation maps show areas of increased activation during selective visual attention to a highly salient reversing chequerboard stimulus across all participants. A large cluster of activation was evident in bilateral occipitotemporal visual areas (see text for details). For table legend, see Table 11.

4.6.1.2 Group differences for visual selective attention

Controls vs AllAMCI: AllAMCI had decreased activation in ***bilateral*** cerebellar cortex, extrastriate visual cortex (BA 18, 19), occipitotemporal areas (BA 39) and brainstem, and in ***left*** hemispheric primary visual cortex (BA 17) **(Table 16)**.

Controlling for the possible effects of age on the BOLD signal in the AMCI participants (ANCOVA) revealed similar results with additional decreased activation in AllAMCI in the hippocampus. Generalised effects of ageing on cortical activation could therefore not account for these differences.

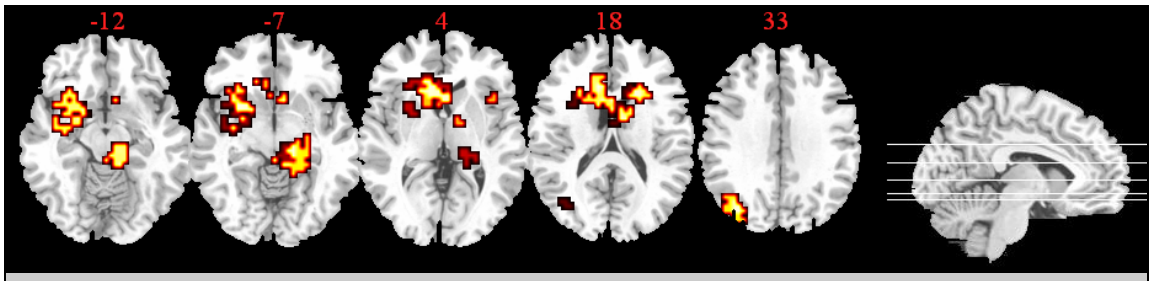
Controls vs CoAMCI: CoAMCI demonstrated greater activation in ***left*** MTL (hippocampus, parahippocampus (entorhinal (BA 28) and perirhinal (BA 35) areas)) and brainstem, and in ***right*** insula, medial frontal, prefrontal (BA 46, 47), anterior temporal (BA 38), hippocampal, extrastriate visual (BA 19) and lateral parietal (BA 39) areas, and in ***bilateral*** thalamus, caudate and anterior cingulate (BA 24, 25) **(Table 17)**. There were no areas of greater activation in Controls at the set threshold; however, running the analysis at a less conservative threshold (expected number of false clusters per volume < 2, conventional analysis <1) revealed increased activation in a cluster in ***right*** occipitotemporal areas (BA 19, 37).



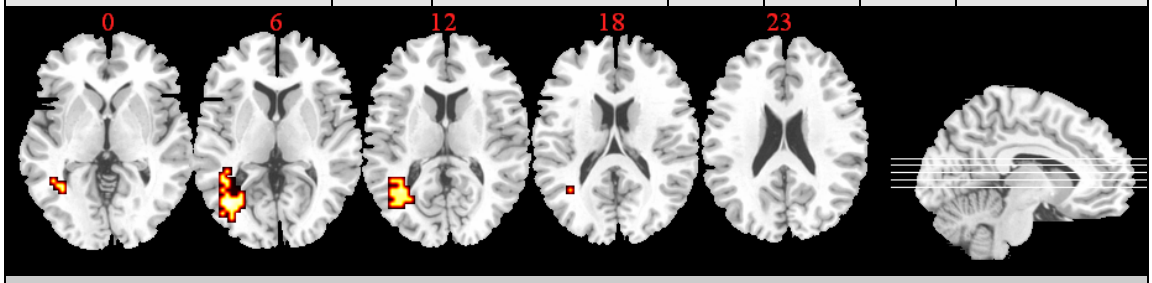
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls > AllAMCI						
Cerebellum	R	**Cerebellum**, 18, 19, brainstem	7	-48	-35	156
Cerebellum	R	Cerebellum, **18**, 19, 37	21	-78	-13	110
Occipital cortex	L	Cerebellum, 17, **18**, 19, 37, brainstem	-32	-85	-7	70

Table 16. Functional activation differences on *visual* selective attention between Controls and AllAMCI.

The table and brain activation maps show areas of increased activation in Controls compared to all AMCI participants during selective visual attention. Thee clusters of increased activation were evident in occipitotemporal visual areas and in the brainstem (see text for details). For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
CoAMCI > Controls						
Medial temporal	L	Hippocampus, **brainstem**, 28, 35	-14	-22	-13	54
Medial frontal	L+R	Thalamus, caudate, **24**, 25	18	26	-7	238
	R	Insula, hippocampus, 32, 38, 46, 47				
Occipitoparietal	R	19, **39**	36	-70	26	52



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls > CoAMCI						
Occipitotemporal	R	**19**, 37	40	-63	-2	48

Table 17. Functional activation differences on *visual* selective attention between Controls and CoAMCI.

The table shows the areas where CoAMCI had greater activation than Controls during the visual attention condition (top), and where Controls had greater activation than

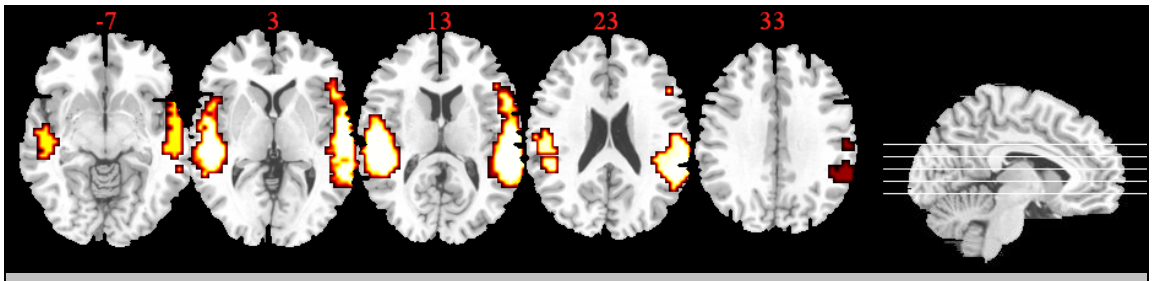
CoAMCI (bottom). The increased activation in occipitotemporal areas seen in Controls was the only cluster of difference evident at a less conservative statistical threshold (see text for details).

For table legend, see Table 11.

4.6.2 Functional Results Auditory Selective Attention

4.6.2.1 Group activation for auditory selective attention

Combining Controls and AllAMCI revealed activation in ***bilateral*** symmetrical clusters including primary auditory cortex (BA 41, 42), auditory association cortex (BA 21, 22), occipitotemporal areas (BA 37), temporal pole (BA 38), inferior parietal lobe (BA 40), VLPFC (BA 44, 45, 47) and insula **(Table 18)**.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
All subjects						
Temporal lobe	R	**21**, 22, 37, 38, 40, 41, 42, 44, 47, insula	58	-19	-2	283
Temporal lobe	L	21, **22**, 37, 38, 40, 41, 42, 44, 45, 47, insula	-54	-19	4	423

Table 18. Functional activation on *auditory* selective attention for Controls and AllAMCI combined.

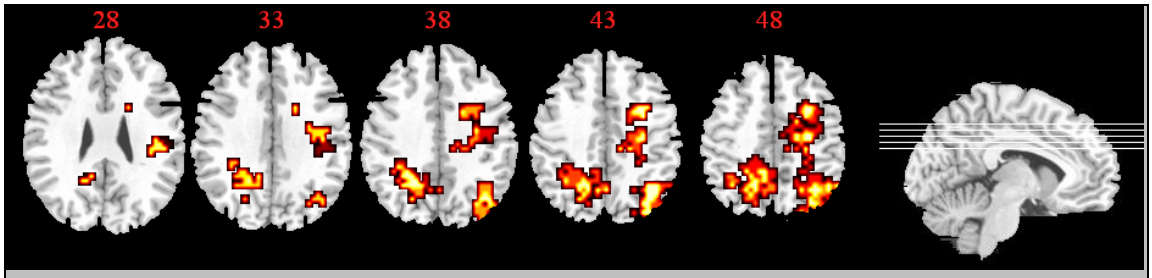
The table and brain activation maps show areas of increased activation during selective auditory attention to English nouns across all participants. Bilateral large clusters of activation were evident in lateral temporal auditory areas (see text for details). For table legend, see Table 11.

4.6.2.2 Group differences for auditory selective attention

Controls vs AllAMCI: AllAMCI had decreased activation in two clusters on the ***left*** including DLPFC (BA 9), premotor (BA 6), anterior cingulated (BA 24, 32), posterior cingulate (BA 7, 31), and lateral parietal areas (BA 39, 40). A cluster on the ***right*** included medial parietal (BA 7, 30, 31) and lateral parietal areas (BA 40) **(Table 19)**.

Further analyses controlling for the possible effects of age on the BOLD signal in the AMCI participants (analysis of covariance (ANCOVA)) revealed similar results thus, it could not be accounted for by the effects of ageing on cortical activation.

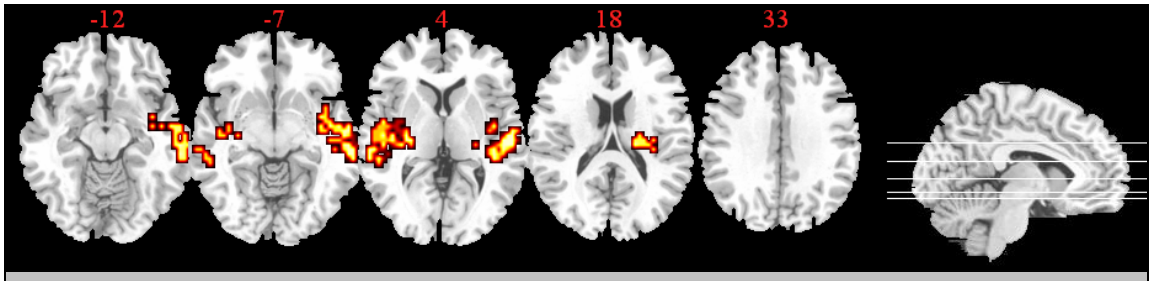
Controls vs CoAMCI: CoAMCI had greater activation in ***bilateral*** auditory association cortices (BA 21, 22) and hippocampi, and ***left*** putamen, thalamus and insula **(Table 20)**. There were no areas of greater activation in Controls.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls > AllAMCI						
Medial parietal	R	7, 30, **31**, 40	25	-44	26	131
Medial parietal	L	**7**, 19, 31, 39, 40	-25	-55	48	176
Frontal	L	6, **9**, 24, 32	-29	0	42	148

Table 19. Functional activation differences on *auditory* selective attention between Controls and AllAMCI.

The table and brain activation maps show areas of increased activation in Controls compared to all AMCI participants during selective auditory attention. Clusters of increased activation were evident in bilateral parietal cortex and in frontal areas (see text for details). For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
CoAMCI > Controls						
Temporal	L	Hippocampus, insula, thalamus, putamen, 20, **21**, 22	-54	-15	-7	129
Temporal	R	Hippocampus, 21, **22**	54	-4	-2	79

Table 20. Functional activation differences on *auditory* selective attention between Controls and CoAMCI.

The table shows the areas where CoAMCI had greater activation than Controls during selective auditory attention. Increased activation was evident in bilateral MTL areas (see text for details). For table legend, see Table 11.

4.7 Verbal Episodic Encoding at Baseline

We compared Controls with AllAMCI and CoAMCI at baseline to determine if the episodic amnesia in AMCI is associated with altered cortical activation during verbal episodic memory encoding.

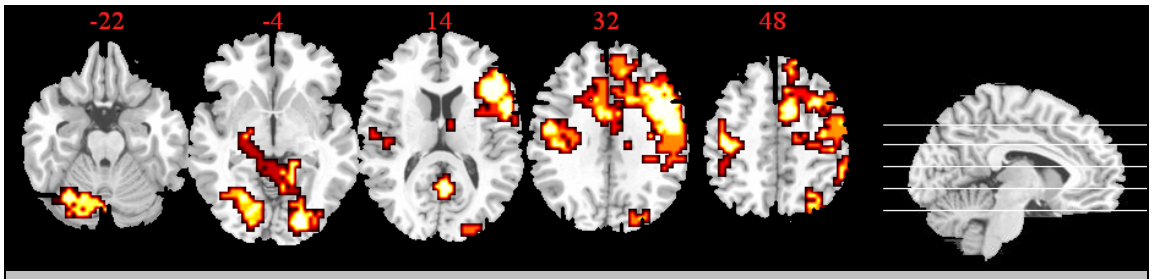
4.7.1 Behavioural Results

Encoding performance is deduced from recognition results success and these are presented in the following section on recognition (§4.8.1; Table 25).

4.7.2 Functional Results

4.7.2.1 Group activation

Combining Controls and AllAMCI revealed activation in ***bilateral*** cerebellum, primary and extrastriate visual cortex (BA 17, 18, 19), in ***right*** hippocampus, amygdala, entorhinal (BA 28, 34), anterior cingulate (BA 24, 32), frontal pole (BA 9), posterior cingulate (BA 30, 31), fusiform (BA 35, 36), insula, premotor ((BA 4, 6), primary auditory (BA 41, 42) and VLPFC areas (BA 40) **(Table 21)**. Activation was also evident in ***left*** insula, frontal pole (BA 8, 10), VLPFC (45, 47), DLPFC (BA 9, 46), anterior cingulate (BA 24, 32, 33) and posterior cingulate (BA 23).



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
All subjects						
Cerebellum	R	**Cerebellum**, 17, 18,19	18	-74	-13	173
Cerebellum	L	Cerebellum, 17, **18**	-14	-81	-13	67
Brainstem	R	Hippocampus, amygdala, **brainstem**, 19, 28, 30, 31, 34, 35, 36	0	-48	4	108
Occipital cortex	L	18, **19**	-22	-78	31	42
Precentral	R	Insula, **4**, 6, 40, 41, 42, 44	40	-22	42	125
Insula	L	**Insula**, 6, 8, 9, 10, 23, 24, 32, 33, 45, 46, 47	-36	26	15	850
	R	9, 24, 32				

Table 21. Functional activation during verbal episodic encoding for Controls and AllAMCI combined.

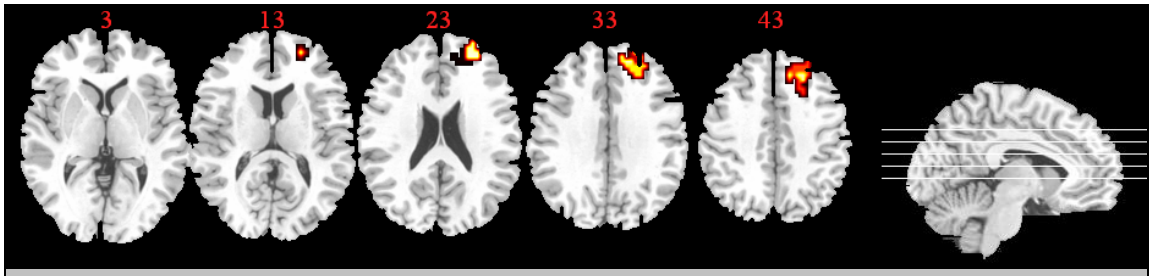
The table and brain activation maps show the areas where activation increased during overt reading and encoding of words lists compared to repeatedly reading a control word, across all participants. Several clusters of activation were evident in occipital,

MTL, lateral temporal, PFC and cingulate areas (see text for details). For table legend, see Table 11.

4.7.2.2 Group differences

Controls vs AllAMCI: AllAMCI had decreased activation in ***left*** frontal pole cortex (BA 9, 10) **(Table 22)**. Including age as covariant revealed similar results.

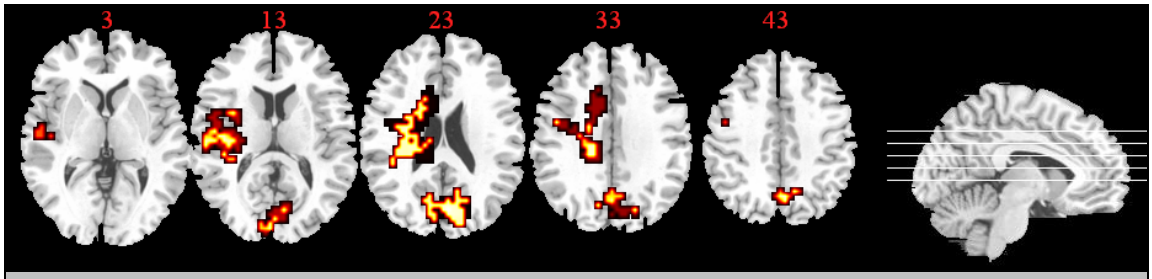
Controls vs CoAMCI: Greater activation was evident in CoAMCI in ***right*** insula, primary auditory cortex (BA 41, 42), auditory association cortex (BA 21, 22), anterior cingulate (BA 24, 32), thalamus and putamen, and in ***bilateral*** posterior cingulate (BA 31, 32), medial parietal (BA 7) and extrastriate visual cortex (BA 18) **(Table 23)**.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls>AllAMCI						
Prefrontal cortex	L	**9**, 10	-18	41	31	66

Table 22. Functional activation differences during verbal episodic encoding between Controls and AllAMCI.

The table and brain activation maps show areas of increased activation in Controls compared to all AMCI participants during verbal encoding. A single cluster of increased activation was evident in PFC in Controls (see text for details). For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
CoAMCI > Controls						
Temporal lobe	R	Insula, putamen, caudate, thalamus, 21, **22**, 41, 42	51	-7	9	162
Medial parietal	L+R	**7**, 18, 23, 31	0	-70	15	125

Table 23. Functional activation differences during verbal encoding between Controls and CoAMCI.

The table and brain activation maps show areas of increased activation in CoAMCI compared to Controls during verbal encoding. Clusters of increased activation were evident in medial parietal and lateral temporal areas (see text for details). For table legend, see Table 11.

4.7.2.3 Functional –behavioural correlations

Activation in the areas of difference showed significant correlations with recognition measures **(Table 24)**. Negative correlations were evident between Pr and activation in right temporal and posterior cingulate areas **(Figures 16 and 17)**. Negative correlations were also evident between activation in the posterior cingulate and Pr for lures. Neuropsychological measures also correlated significantly with activation: CAMCOG score showed inverse correlation with activation in temporal and cingulate

areas so that better performers had less activation. New Learning subscale scores also showed a negative correlation with posterior cingulate activation. Large effect sizes were evident for the correlations between activation and neuropsychological measures.

	Controls + CoAMCI	**R^2**
Pr vs Right Temporal	-0.40 (<0.05)	0.16
Pr vs Bilateral Posterior Cingulate	-0.41 (<0.05)	0.17
FAR vs Right Temporal	ns	
FAR vs Bilateral Posterior Cingulate	ns	
Pr lures vs Right Temporal	ns	
Pr lures vs Bilateral Posterior Cingulate	-0.36 (<0.01)	0.13
CAMCOG vs Right Temporal	-0.42 (<0.05)	0.18
CAMCOG vs Bilateral Posterior Cingulate	-0.55 (<0.01)	0.30
New Learn vs Right Temporal	ns	
New Learn vs Bilateral Posterior Cingulate	-0.58 (<0.01)	0.34

Table 24. Correlations between activation and behavioural measures for Controls and CoAMCI on verbal episodic encoding.

The table shows correlations between behavioural measures and cortical areas where Controls and CoAMCI differed significantly (see text for details).

Data are: Spearman's correlation coefficient (p). The coefficient of determination, R^2 is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect.

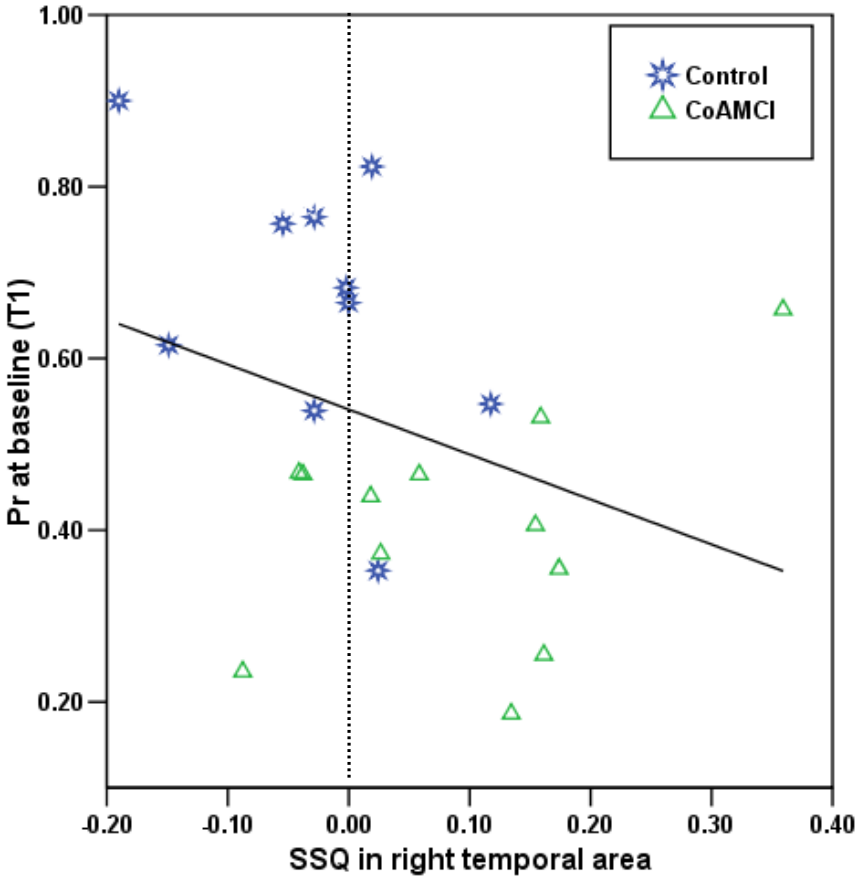


Figure 16. Functional-behavioural correlation of encoding performance and cortical activation in the right temporal area in Controls and CoAMCI.

A significant negative correlation ($r = -0.40$ ($p<0.05$)) was evident between encoding success as measured by corrected recognition rates (Pr) and the magnitude of activation (SSQ) in the right temporal area where CoAMCI had greater activation and associated impaired verbal episodic memory.

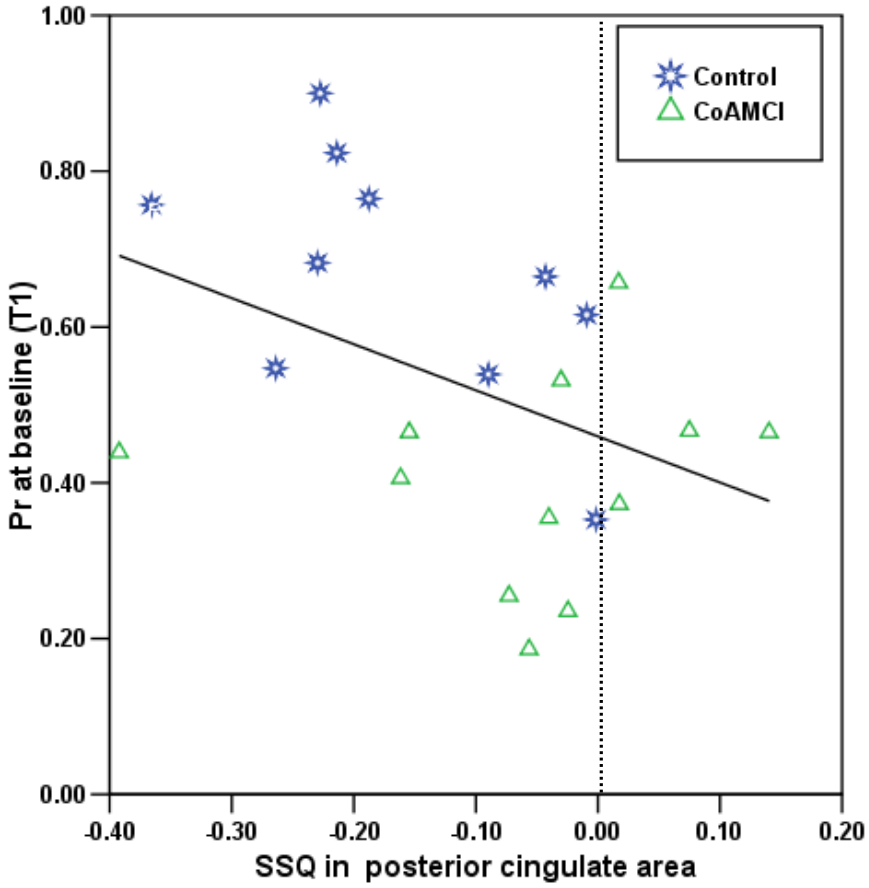


Figure 17. Functional-behavioural correlation of encoding performance and cortical activation in the posterior cingulate area in Controls and CoAMCI.

A significant negative correlation (r= 0.41 ($p<0.05$)) was evident between encoding success as measured by corrected recognition rates (Pr) and the magnitude of activation (SSQ) in bilateral posterior cingulate areas where CoAMCI had greater activation and associated impaired verbal episodic memory.

4.8 Recognition at Baseline

We compared AMCI patients to Controls on verbal episodic memory performance to determine if the amnesia in AMCI is in part due to semantic elaboration failure.

4.8.1 Behavioural Results

Results are summarised in **Table 25**. AMCI were impaired on corrected recognition (Pr) and this was predominantly due to higher *false alarm* rates (FAR) **(Figure 18)**. As a proportion of *false alarms*, AMCI subjects displayed significantly reduced false recognition for lures (Pr lures). In the AMCI groups the ranges of values for Pr and FAR were wide and groups were compared using non-parametric tests. The differences in response bias (chance bias = 0.5; liberal bias >0.5; conservative bias<0.5) between Controls and AMCI groups did not reach statistical significance.

Significant correlations were evident on divided attention and recognition measures that Controls and CoAMCI differed on: VisRT during divided attention correlated with Pr ($r= -0.45$; $p<0.02$; $R^2= 0.20$), FAR ($r= 0.63$; $p<0.001$; $R^2= 0.40$) and Pr lures ($r= -4.7$; $p<0.02$; $R^2= 0.22$). Age did not correlate significantly with any of the measures that differed between CoAMCI and Controls.

				Control vs AllAMCI			Controls vs CoAMCI		
	Control	**AllAMCI**	**CoAMCI**	P	F	R^2	P	F	R^2
Recognition (Pr)	0.66 (0.2)	0.46 (0.2)	0.40 (0.1)	0.003	*	0.3	0.001		0.1
Hit rate (Hr)	0.76 (0.2)	0.71 (0.2)	0.67 (0.2)	ns			ns		
False alarm rate (FAR)	0.10 (0.1)	0.25 (0.1)	0.26 (0.1)	0.001	*	0.1	0.003		0.01
Bias rate (Br)	0.35 (0.3)	0.51 (0.3)	0.47 (0.2)	ns			ns		
Pr Lures	0.21 (0.2)	0.01 (0.2)	-0.01 (0.2)	0.01	7	0.1	0.02	7	0.3

Table 25. Behavioural results for the recognition task for Controls, AllAMCI and CoAMCI.

The table shows behavioural recognition data for Controls, AllAMCI and CoAMCI. Controls performed better on corrected recognition (Pr) and *false alarm* rates (FAR), had higher *false recognition for lures* rates (Pr Lures) and the groups were comparable on hit rates (Hr).

Data are mean (standard deviation), ns=not significant; F=ANOVA test statistic, *=MannWhitney test. The coefficient of determination, R^2 is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect.

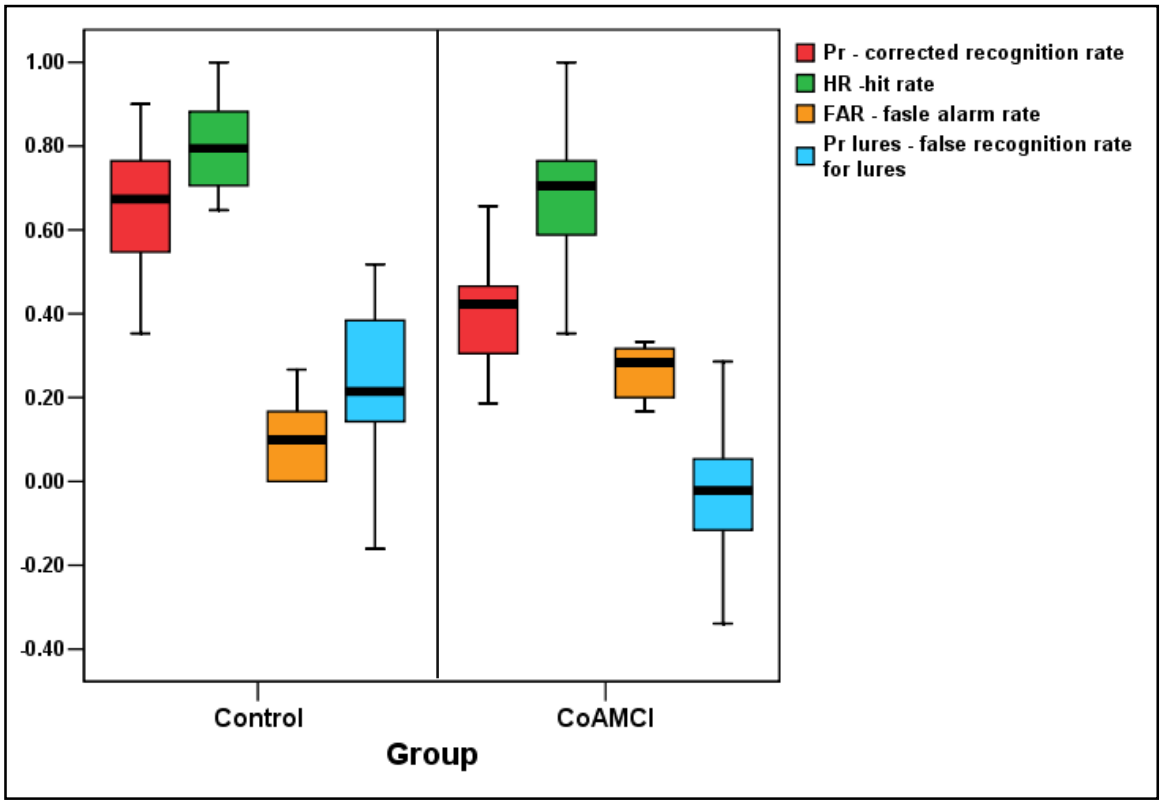


Figure 18. Recognition task performances for Control and CoAMCI groups.

The boxplot depicts behavioural results for the encoding task for the Control group (left) and CoAMCI group (right). Although the groups had comparable *hit* rates, the CoAMCI group had a lower corrected recognition rate that was mediated by a higher *false alarm* rate. The CoAMCI group had a significantly lower false recognition rate for lures. On the boxplot the central black lines indicate median values; upper and lower edges of boxes represent the interquartile range.

4.8.2 Functional Results

Behavioural data from 9 Controls, 16 AMCI and 11 CoAMCI were sufficient to allow functional analysis and comparisons of *hits, correct rejection* and *false recognition of lures*.

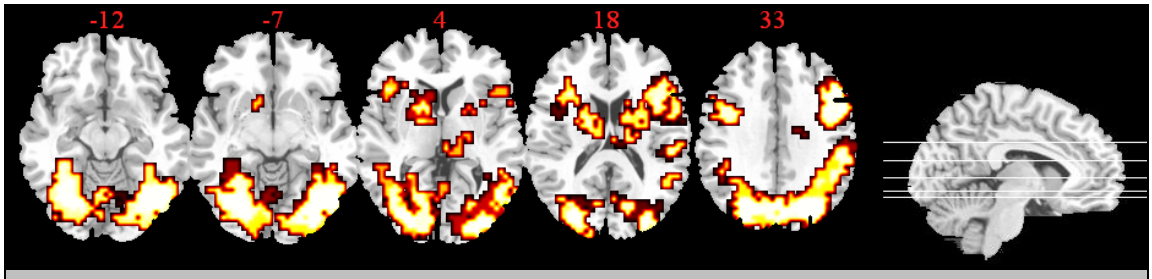
4.8.2.1 Group activation

Results for *hits* (targets correctly identified) are reported for all subjects with available data (9 Controls + 16 AMCI) as the groups did not differ on behavioural measures. Functional results on *correct rejection* of any distractor (lure and non-lures) and *false recognition of lures* are reported for Controls because the patient and control groups differed significantly on these measures.

Hits *(all subjects):* Activation was evident in ***bilateral*** cerebellum, visual cortex (BA 17, 18, 19), fusiform (BA 36, 37), caudate, lentiform nucleus (putamen, globus pallidus), thalamus, DLPFC (BA 9, 46), VLPFC (BA 44, 45, 47), premotor (BA 4, 6) auditory association cortex (BA 21, 22), insula, temporoparietal areas (BA 39, 40), anterior cingulate (BA 24, 32), posterior cingulate (BA 7, 31), and brainstem **(Table 26)**. Activation in the ***left*** hemisphere was evident in primary auditory cortex (BA 41, 42) and somatosensory areas (BA 1, 2, 3).

Correct rejection of any distractor *(in Controls):* Activation was evident in ***bilateral*** cerebellum, visual cortex (BA 17, 18, 19), fusiform (BA 36, 37), caudate, lentiform nucleus (putamen, globus pallidus), DLPFC (BA 9, 46), VLPFC (BA 44, 45,), premotor (BA 4, 6), insula, temporoparietal areas (BA 39, 40), anterior cingulate (BA 24, 32), and posterior cingulate (BA 31) **(Table 27)**. Activation on the ***left*** was evident in primary auditory (BA 41, 42), auditory association (BA 22), and on the ***right*** in the thalamus.

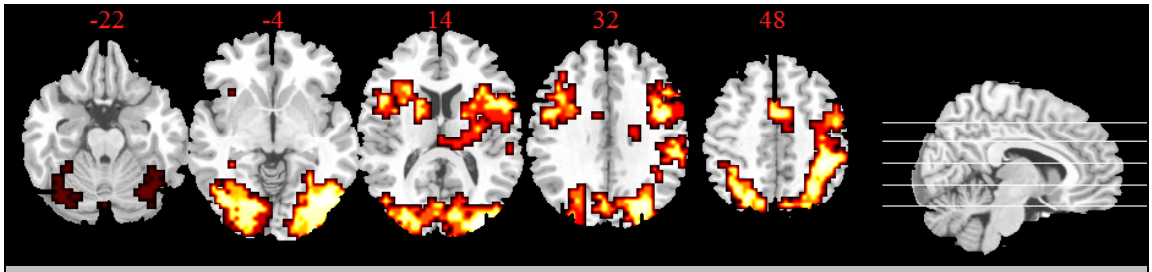
False recognition of lures *(in Controls):* Activation was evident in a cluster in left PFC (Talairach coordinates: x= -36; y=33; z=9; size = 56 voxels) than included VLPFC (BA 45, 47), DLPFC (BA 46) and anterior polar cortex (BA 10), and in a cluster in right PFC (Talairach coordinates: x= 43; y=7; z=20; size = 132 voxels) that include VLPFC (BA 44, 45, 47), anterior polar cortex (BA 10) and insula.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Correct recognition of targets in all subjects						
Striatum	R	**Striatum**	22	0	15	98
Temporal	R	**Insula**, caudate, lentiform nucleus, thalamus, 44, 45, 46, 47	43	22	-2	48
Temporal	R	4, 6, **9**, 44, 45, 46, 47	40	0	37	115
Occipital	L+R	Cerebellum, 7, 17, 18, **19**, 21, 22, 31, 32, 36, 37, 39, 40	-22	-67	26	2649
	L	1, 2, 3, 41, 42				
Frontal	L	Insula, thalamus, brainstem, 4, 6, **24**, 31, 32, 44, 45, 46, 47	-4	-4	48	181

Table 26. Functional activation during correct recognition of targets (*hits*) for Controls and AllAMCI combined.

Combining all participants revealed activation in PFC, temporal, striatal, occipital, parietal and brainstem areas during correct recognition of targets (see text for details). For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Correct rejection of any distractor in Controls						
Prefrontal	R	4, 6, 9, **44**, 45, 46, insula, caudate, thalamus, lentiform nucleus	43	8	20	338
Occipital	L	22, 41, 42	33	-67	-18	2517
	R	4, 6, 9, 44, 45, 46, insula, caudate, lentiform nucleus				
	L+R	**Cerebellum**, 7, 17, 18, 19, 24, 31, 32, 36, 37, 39, 40,				

Table 27. Functional activation during *correct rejection of any distractor* in Controls.

Controls showed activation in two large clusters during *correct rejection of any distractor* in predominantly bilateral PFC, occipitotemporal, medial and lateral parietal, striatum, insula, thalamus, and cerebellar areas (see text for details). For table legend, see Table 11.

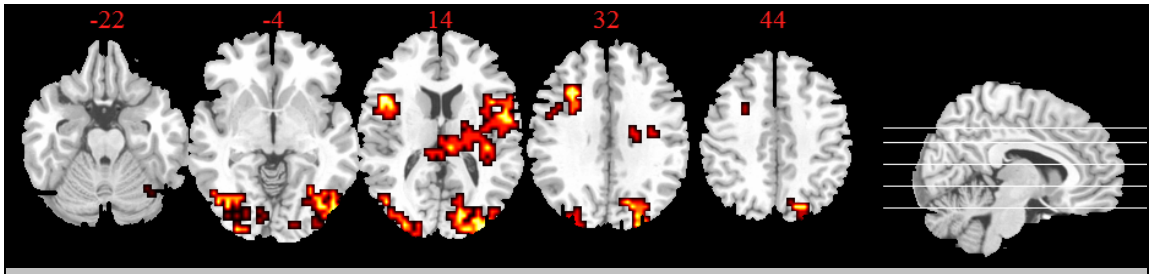
4.8.2.2 Group differences

We compared 9 Controls and 11 CoAMCI on three conditions.

Hits**:** *Controls vs. CoAMCI:* CoAMCI had decreased activation in ***bilateral*** cerebellum,

visual (BA 17, 18, 19), fusiform (BA 37), insula, VLPFC (BA 44, 45, 47), posterior cingulate (BA 31) areas, in ***right*** DLPFC (BA 9, 46), and in ***left*** PFC (BA 6), auditory association (BA 22), primary auditory (BA 41, 42), putamen and caudate areas **(Table 28)**.

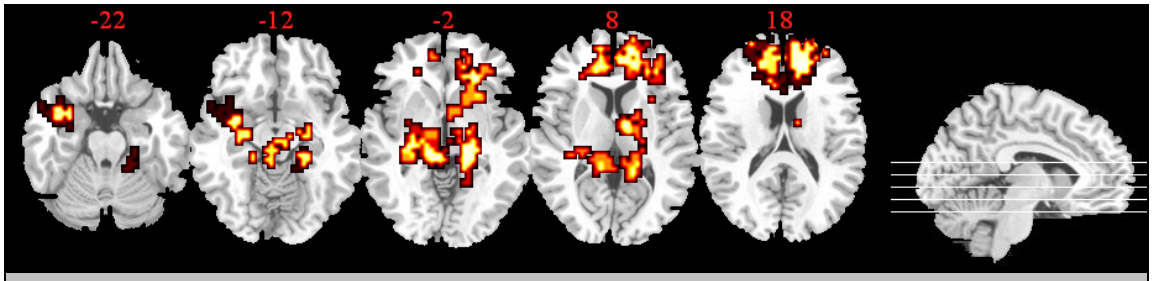
CoAMCI had increased activation in ***bilateral*** anterior frontal cortex (BA 9, 10), medial temporal (BA 28, 34, amygdala, hippocampus, NBM), brainstem, fusiform (BA 36) in ***right*** auditory association (BA 21), perirhinal (BA 35), temporal pole (BA 38), and in ***left*** DLPFC (BA 46), putamen and caudate areas **(Table 29)**.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls>CoAMCI						
Occipital	R	Cerebellum, 17, **18**, 19, 37	40	-82	-2	118
Frontal	L	Putamen, caudate, **insula**, 6, 22, 41, 44, 45, 47,	-51	11	4	138
Frontal	R	Insula, 9, 44, **45**, 46, 47	50	19	4	58
Temporal	L	Cerebellum, 17, 18, 19, 31, **37**	-36	-59	-13	216

Table 28. Functional activation differences during *hits* between Controls and CoAMCI: Controls > CoAMCI

CoAMCI had decreased activation in cerebellar, occipitotemporal visual, PFC, cingulate, temporal auditory, putamen and caudate areas compared to controls during cortical processing of *hits* (see text for details). For table legend, see Table 11.



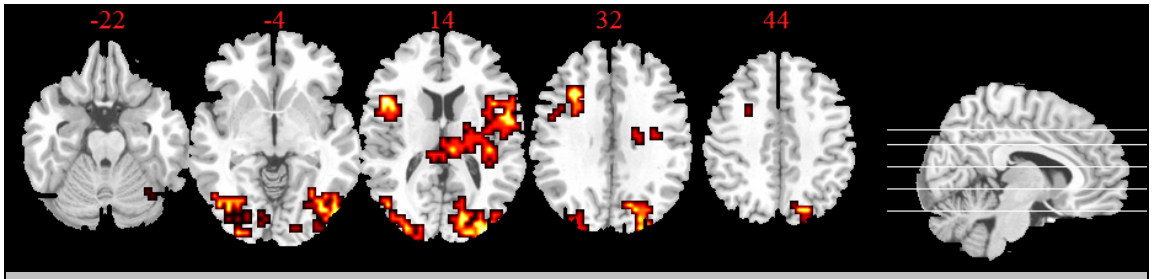
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
CoAMCI>Controls						
Medial temporal	R	**Hippocampus**, amygdala, 21, 27, 28, 30, 34, 35, 36, 38	33	-26	-7	171
Posterior medial parietal	L	Putamen, caudate, brainstem, amygdala, hippocampus, nucleus basalis, 25, 27, 28, **30**, 34, 35, 36	-11	-41	-2	116
Frontal pole	L+R	**10**, 11	0	59	20	467

Table 29. Functional activation differences during *hits* between Controls and CoAMCI: CoAMCI > Controls

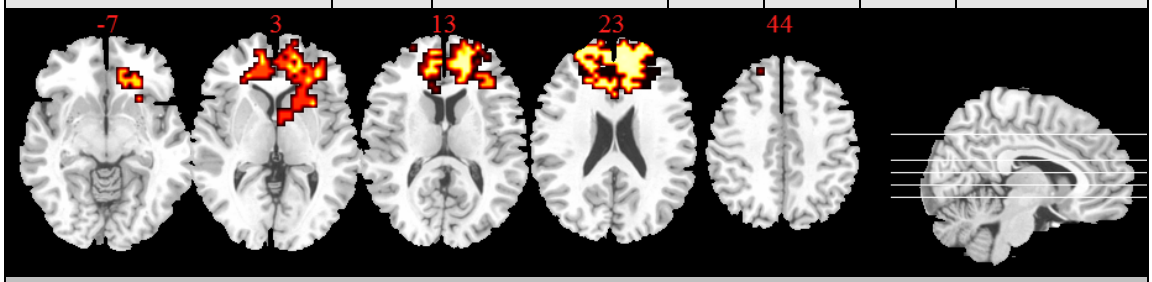
CoAMCI had increased activation in anterior frontal, medial temporal, brainstem, temporal pole, and PFC areas (see text for details). For table legend, see Table 11.

Correct rejection of any distractor**:** *Controls vs CoAMCI:* CoAMCI had decreased activation in ***bilateral*** visual (BA 17, 18, 19), fusiform (BA 37), posterior cingulate (BA 31), VLPFC (BA 44, 45) and insula, and ***left*** thalamus, striatum, and caudate, and ***right*** DLPFC (BA 9, 46, 47) **(Table 30)**.

CoAMCI had greater activation in ***bilateral*** frontal pole (BA 10), anterior cingulate (BA 32), and frontal cortex (BA 9, 47), and in ***left*** frontal pole (BA 11), anterior cingulate (BA 25), caudate, putamen and insula, and in ***right*** anterior cingulate, (BA 25).



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls>CoAMCI						
Occipital	R	17, **18**, 19, 37, 31	38	-81	-2	118
Frontal	L	**Insula**, thalamus, striatum, caudate, 44, 45	-51	11	4	138
Frontal	R	Insula, 9, 44, **45**, 46, 47	50	19	4	58
Temporal	L	Cerebellum, 17, 18, 19, 31, **37**	-36	-60	-13	216



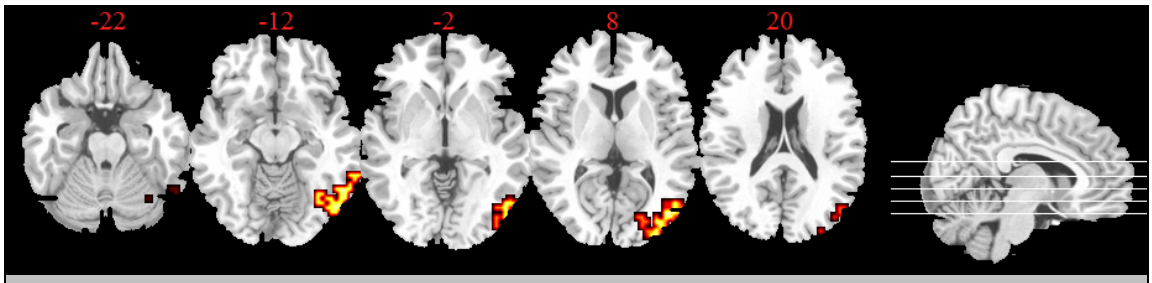
CoAMCI>Controls						
Frontal pole	L+R	9, **10**, 32, 47	0	59	20	467
	L	10, 25, caudate, putamen, insula				
	R	25				

Table 30. Functional activation differences during *correct rejection of any distractor* between Controls and CoAMCI.

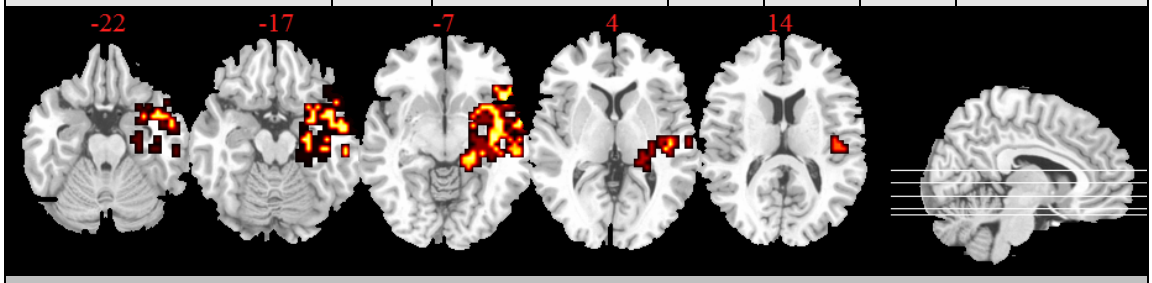
CoAMCI had decreased activation in occipitotemporal visual, medial parietal, PFC, insula, thalamus and striatum during *correct rejection of any distractor* (top). CoAMCI had greater activation in a large anterior cluster in frontal polar, anterior cingulate, caudate and insular areas (bottom). For table legend, see Table 11.

False recognition of lures: *Controls vs CoAMCI:* CoAMCI had decreased activation in ***left*** cerebellum, extrastriate visual (BA 18, 19), fusiform (BA 37), and inferior parietal (BA 39) areas **(Table 31)**.

CoAMCI had greater activation in left VLPFC (BA 47), hippocampus, parahippocampus (BA 28, 35), fusiform gyrus (BA 36), temporal pole (BA 38), insula and amygdala.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
Controls>CoAMCI						
Cerebellum	L	18, 19, **37**, 39, cerebellum	-47	-59	-18	134



CoAMCI>Controls						
Frontotemporal	L	Insula, amygdala, hippocampus, 20, 21, 28, 34, 35, 36, 38, **47**	-43	22	-13	204

Table 31. Functional activation differences during *false recognition of lures* between Controls and CoAMCI.

CoAMCI had decreased activation in a cluster in left cerebellar, occipitotemporal and lateral parietal areas during false recognition of lures (top). CoAMCI had greater activation in left PFC, MTL, temporal pole and insula (bottom). The CoAMCI > Controls result is at a cluster p-level where 1.02 false cluster can be expected instead of <1. For table legend, see Table 11.

4.9 Rivastigmine Treatment Effects on Divided Attention in AMCI

We compared RivAMCI and NxAMCI on repeated functional and behavioural measures on the divided attention task to determine the treatment effects of rivastigmine.

4.9.1 Rivastigmine Dosage and Tolerability

The average daily dose of rivastigmine during the study period was 5.09 mg (Range 3 to 9 mg). Side effects were common; five patients reported nausea and vomiting, three reported nausea only and one suffered significant weight loss. One year after initiating treatment six patients remained on rivastigmine, three on another cholinesterase inhibitor and one was not taking treatment due to intolerable adverse effects.

4.9.2 Neurocognitive and Behavioural Results

Baseline (T1) and follow-up (T2) neurocognitive and divided attention behavioural results for RivAMCI and NxAMCI are summarised in **Table 32**. Groups were comparable on age, education and premorbid intelligence but the NxAMCI group had superior performance on MMSE and Attention subscale scores.

Group comparisons revealed significantly faster VisRT and AudRT on the divided attention task in RivAMCI at follow-up. Entering MMSE as a covariant into the analysis revealed similar results on VisRT ($p<0.05$; $F=3.7$; $R^2=0.3$) and AudRT ($p<0.02$; $F=5.2$; $R^2=0.4$). The groups were comparable on other measures. A trend towards improvement over time in accuracy and speed was evident in RivAMCI whilst a trend towards deteriorating performance was evident in NxAMCI. Results are reported as significant at $p<0.05$.

PrDA Main effect of time: There were no time effects evident between the groups as all performed at ceiling at baseline (T1) and follow-up (T2).

PrDA interaction (Group x Time): No interaction effects were evident indicating that rivastigmine treatment did not affect accuracy.

VisRT Main effect of time: There were no significant effects of time on VisRT.

VisRT Group x Time interaction: There were no significant interaction effects on VisRT although a trend towards improved performance in RivAMCI appeared **(Figure 19)**.

AudRT Main effect of time: There were no significant time effects on AudRT.

AudRT interaction (Group x Time): A significant interaction effect ($F(1, 18)=6.2$) was evident indicating faster RT in RivAMCI following treatment with slower RT in NxAMCI **(Figure 20)**.

			RivAMCI vs NxAMCI		
	RivAMCI	**NxAMCI**	P	F	R^2
Age	70.7 (9.2)	66.9 (11.5)	ns		
Years in education	10.7 (2.4)	11.9 (1.5)	ns		
NART	111.1 (9.1)	111.8 (10.0)	ns		
MMSE	24.9 (1.9)	27.8 (1.6)	0.001	*	0.5
CAMCOG	87.3 (5.1)	90.9 (7.1)	ns		
Attention	8.0 (0.9)	9.0 (0.00)	0.003	*	-0.5
New Learning	8.8 (3.3)	9.4 (4.4)	ns		
T1 Pr	0.90 (0.1)	0.93 (0.1)	ns		
T2 Pr	0.92 (0.1)	0.88 (0.1)	ns		
T1 VisRT	0.65 (0.1)	0.70 (0.1)	ns		
T2 VisRT	0.59 (0.1)	0.72 (0.2)	0.043	4.8	0.2
T1 AudRT	0.74 (0.1)	0.78 (0.1)	ns		
T2 AudRT	0.69 (0.1)	0.86 (0.2)	0.007	9.2	0.3

Table 32. Demographic, neuropsychological and divided attention task behavioural data at baseline (T1) and follow-up (T2) for RivAMCI and NxAMCI groups.

The groups were comparable on age, education and premorbid intelligence but the NxAMCI group had superior performance on MMSE and Attention subscale scores. RivAMCI were significantly faster on auditory and visual RT at follow-up. A trend towards improvement over time in accuracy and speed is evident in RivAMCI and a

trend towards deteriorating performance in NxAMCI. Results are reported as significant at $p<0.05$.

Pr=corrected recognition; VisRT=visual target reaction time; AudRT=auditory target reaction time; ns=not significant; F=ANOVA test statistic; *= Mann-Whitney test. The coefficient of determination, R^2 is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect.

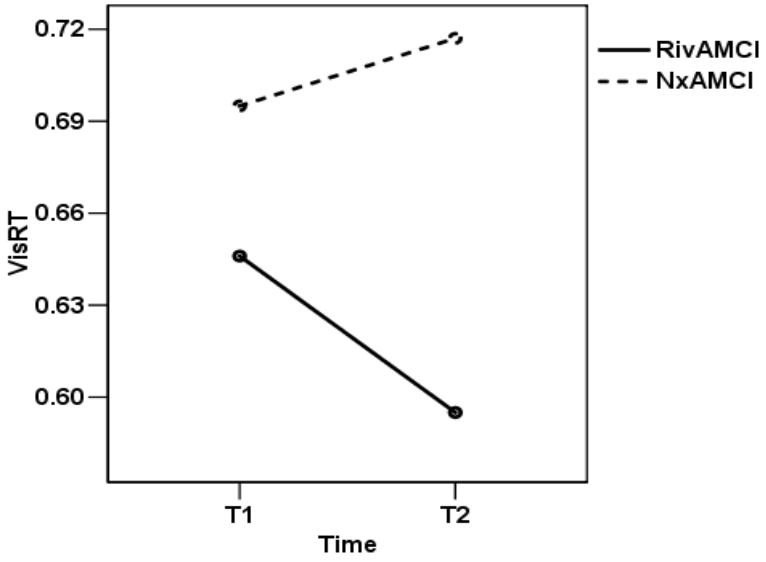


Figure 19. Interaction (Group x Time) effects on visual reaction time (VisRT) in RivAMCI and NxAMCI groups.

VisRT is faster in RivAMCI following rivastigmine treatment and slower in untreated NxAMCI over time. Means for VisRTs are displayed on the vertical axis and time on the horizontal axis.

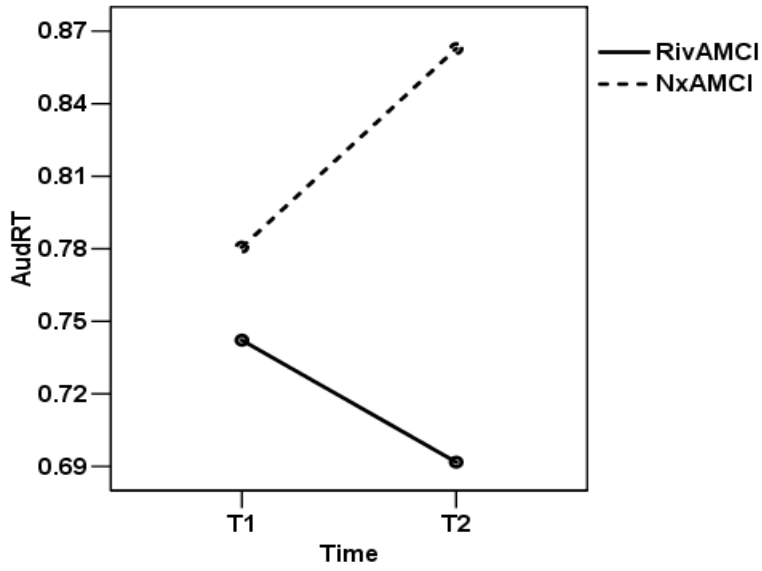


Figure 20. Interaction (Group x Time) effects on auditory reaction time (AudRT) in RivAMCI and NxAMCI groups.

AudRT is faster in RivAMCI following rivastigmine treatment and slower in untreated NxAMCI over time. Means for AudRT are displayed on the vertical axis and time on the horizontal axis.

4.9.3 Functional Results

Group comparisons at T1: RivAMCI had greater activation at T1 in ***bilateral*** cerebellum and visual cortex (BA 17, 18), and in ***left*** fusiform gyrus (BA 37) on the divided attention task **(Table 33)**. There were no areas of greater activation in NxAMCI.

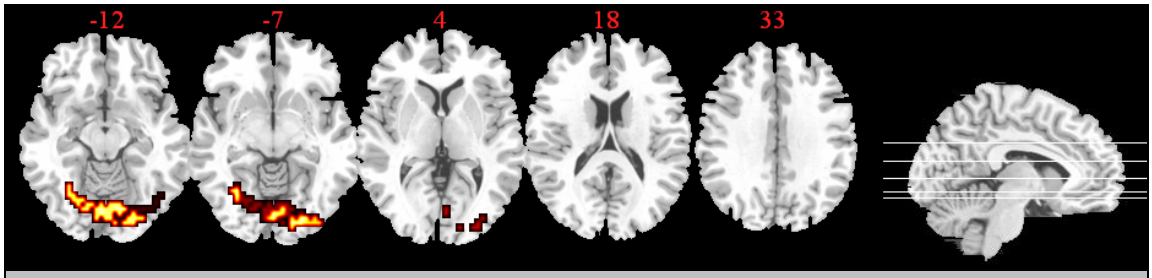
Main effect of time: Significant time effects were evident in ***left*** temporal auditory association cortex (BA 21, 22), inferior temporal lobe (BA 20), anterior temporal pole (BA 38) and hippocampus where both groups reduced activation over time **(Table 34)**. Both groups increased activation over time in ***bilateral*** posterior cingulate (BA 23, 29, 31) and hippocampal areas, in ***right*** somatosensory (BA 1), temporal (BA 22),

insula, fusiform (BA 37), extrastriate visual (BA 19), caudate and parietal (BA 40) areas, and in ***left*** brainstem, thalamus, parahippocampus (BA 28) and cerebellum.

Main effect of group: None.

Interaction effects (Group x Time): Significant interaction effects were evident in ***left*** primary and auditory association cortex (BA 21, 22, 42), fusiform (BA 37), extrastriate visual (BA 19), insula, premotor (BA 4, 6), DLPFC (BA 9, 46), striatum and lateral parietal areas (BA 39) **(Table 35; Figures 21, 22)**. In these areas RivAMCI decreased activation and NxAMCI increased activation over time.

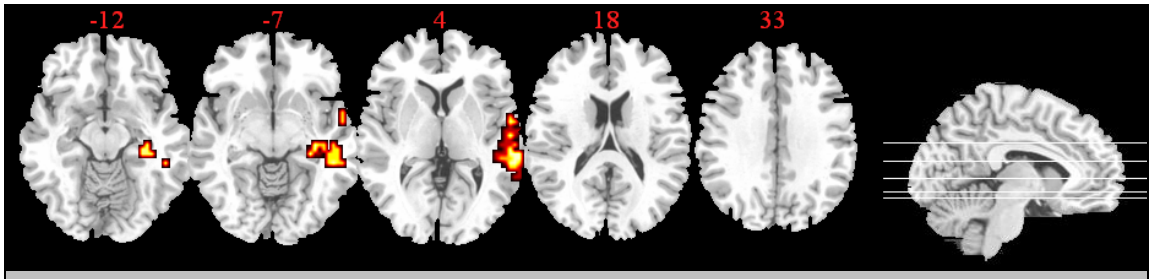
No linear correlations were evident between activation change over time and change in reaction times in RivAMCI. This may indicate a binary effect or lack of power to demonstrate linear effect



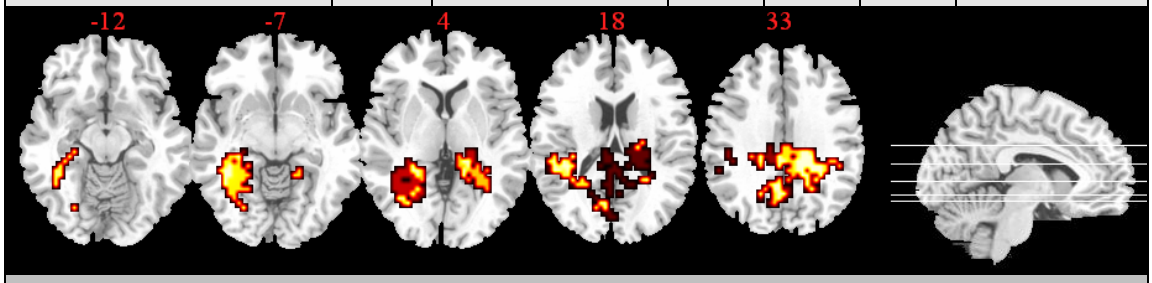
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
RivAMCI > NxAMCI						
Cerebellum	L+R	**Cerebellum**, 17, 18, 19, 37	-15	-70	-29	90

Table 33. Group differences at baseline on divided attention between RivAMCI and NxAMCI groups.

The table and brain activation maps show the cerebellar and occipitotemporal areas where RivAMCI had greater activation during divided attention at baseline compared to NxAMCI (see text for details). For table legend, see Table 11.



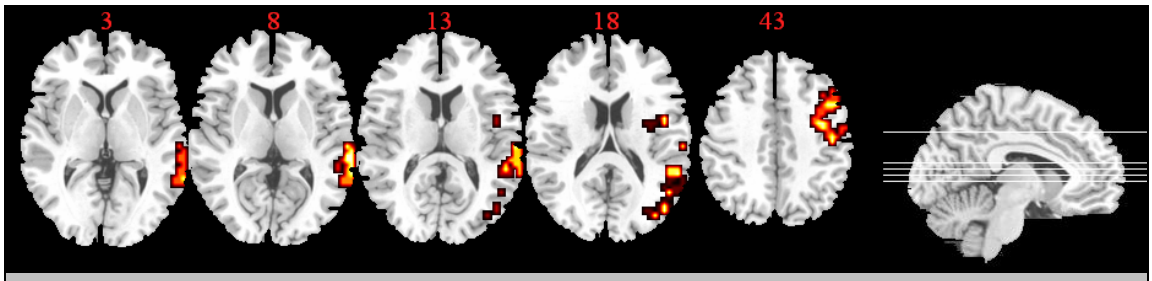
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
T1 > T2						
Temporal	L	20, 21, 22, 38, hippocampus	-47	-26	-13	86



T2 > T1						
Temporal	R	1, 19, 20, caudate, insula, hippocampus, 22, 23, 29, **37**, 40	40	-41	-13	159
Parietal	L	Brainstem, cerebellum, hippocampus, thalamus, 23, 28, 29, **31**	25	-26	37	352

Table 34. Main effects of time on the divided attention task for the pooled AllAMCI sample (RivAMCI + NxAMCI).

The table and brain activation maps show areas of increased activation in the combined AMCI groups at baseline in temporal areas (top), and at follow-up (bottom) in temporal, parietal and midbrain areas. See text for details. For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
RivAMCI decrease vs NxAMCI increase						
Occipital	L	18,19, 21, 22, 37, **39**, 42	-25	-70	15	70
Prefrontal	L	4, 6, **9**, 46, insula, striatum	-36	-4	37	73

Table 35. Interaction effects (Group x Time) for RivAMCI and NxAMCI groups on the divided attention task.

The table and brain activation maps show occipitotemporal and frontotemporal clusters where RivAMCI decreased activation following rivastigmine treatment compared to increased activation in NxAMCI (see text for details). For table legend, see Table 11.

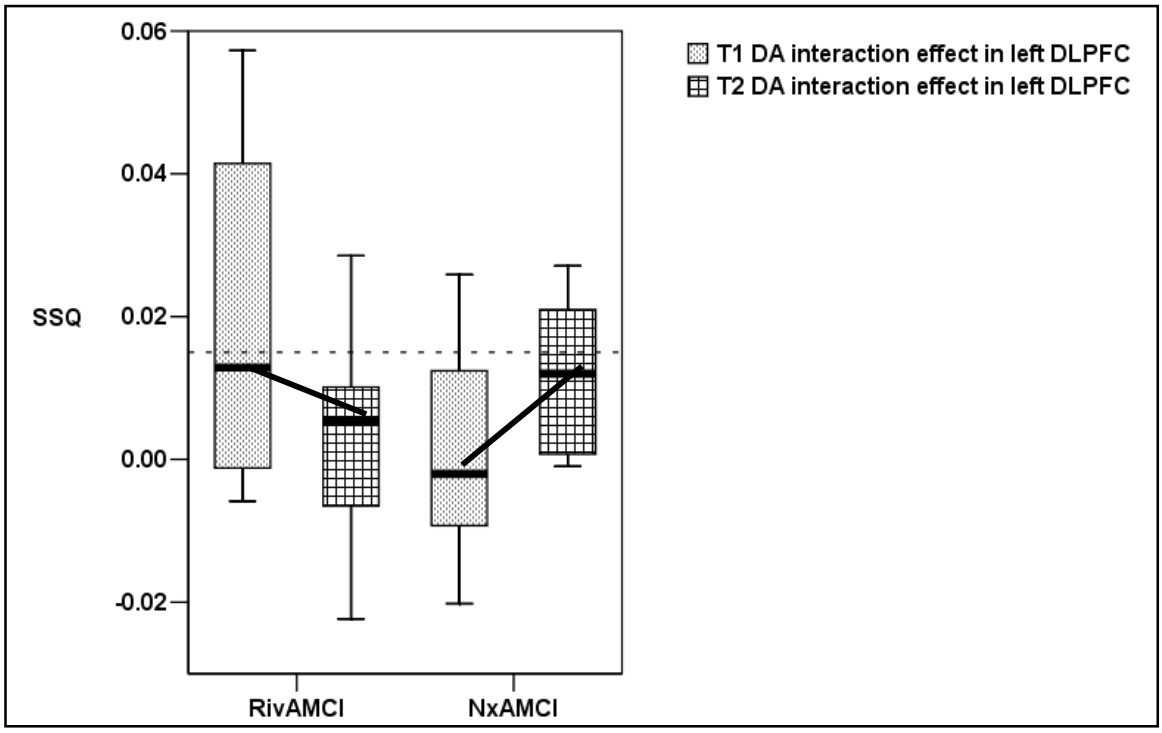


Figure 21. Interaction (Group x Time) effects on divided attention between RivAMCI and NxAMCI in left DLPFC.

The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI decrease and NxAMCI increase activation over time in the left DLPFC.

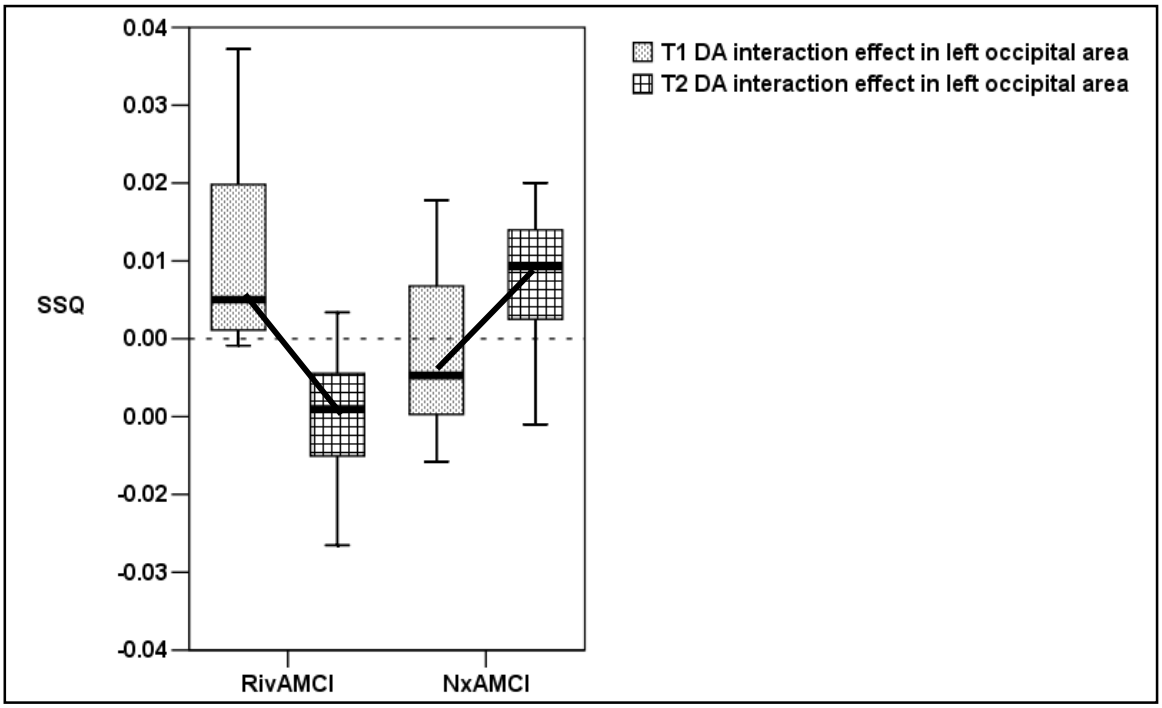


Figure 22. Interaction (Group x Time) effects on divided attention between RivAMCI and NxAMCI in left occipital area.

The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI decrease and NxAMCI increase activation over time in the left occipital area.

4.9.4 Post-hoc Analyses

We conducted post hoc analyses to determine if the group differences in functional activation evident at baseline were due to sampling bias as the groups were recruited at different times, albeit with identical sampling procedures. The groups did not differ on the severity of cognitive impairment as measured by the deviation of individual scores from predicted CAMCOG totals. The ten less severely impaired AMCI group included six NxAMCI and the more impaired group four **(Table 36)**. Eight Controls were in the group of ten least impaired participants. Insufficient data was available to calculate a predicted CAMCOG score for one control.

Subject	% Predicted CAMCOG	Rank
Control	127	1
Control	123	2
Control	122	3
Control	118	4
NxAMCI	118	5
Control	114	6
Control	112	7
Control	110	8
Control	110	9
RivAMCI	108	10
RivAMCI	108	11
RivAMCI	106	12
Control	106	13
RivAMCI	106	14
NxAMCI	106	15
NxAMCI	106	16
NxAMCI	105	17
NxAMCI	105	18
NxAMCI	103	19
RivAMCI	101	20
NxAMCI	101	21
RivAMCI	100	22
NxAMCI	99	23
RivAMCI	98	24
NxAMCI	96	25
RivAMCI	94	26
RivAMCI	93	27
RivAMCI	91	28
NxAMCI	83	29

Table 36. Participant stratification based on predicted CAMCOG total.

The table shows percentage of total predicted CAMCOG scores obtained by participants. Group A (RivAMCI) was recruited at the same time as control subjects and recruitment of group B (NxAMCI) followed that. In the rank column, the ten least impaired AMCI appear in the light grey cells and the 10 more impaired AMCI in darker grey cells.

4.10 Rivastigmine Treatment Effects on Visual and Auditory Selective Attention

We examined the effects of rivastigmine on functional activation during basic visual and auditory attention processing by comparing RivAMCI and NxAMCI on repeated functional measures.

4.10.1 Functional Results

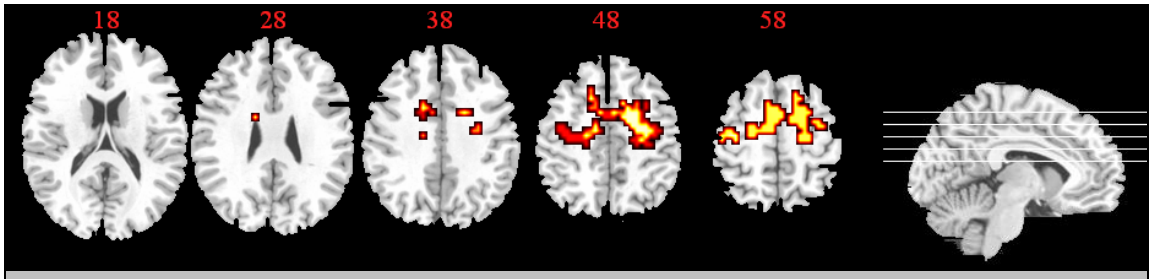
4.10.1.1 Visual selective attention

Main effect of time: Both groups decreased activation over time in ***bilateral*** premotor (BA 4, 6) and anterior cingulate cortex (BA 24, 32), and in ***right*** caudate **(Table 37)**. Both groups increased activation over time in ***left*** amygdala, hippocampus and parahippocampus (BA 28), fusiform, (BA 36), anterior temporal pole (BA 38), auditory (BA 21, 41, 42), lateral parietal (BA 39, 40), caudate, putamen and extrastriate visual cortex.

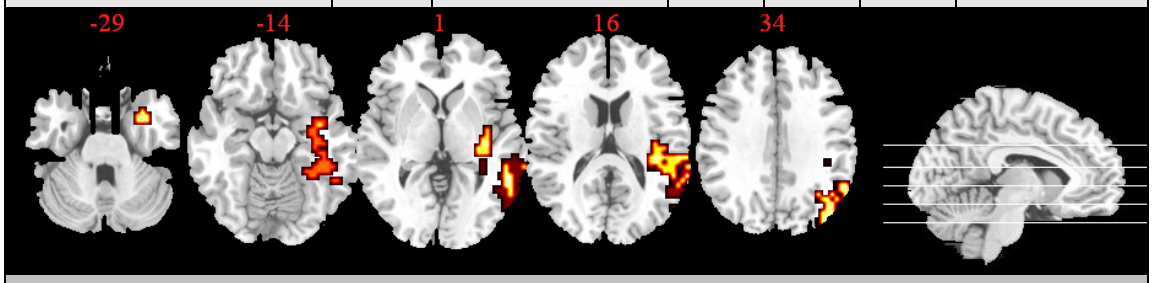
Main effect of group: RivAMCI had greater activation at both time points in ***bilateral*** visual cortex (BA 17, 18, 19) and posterior cingulate (BA 31), and in ***right*** cerebellum **(Table 38)**. NxAMCI had greater activation at both time points in ***bilateral*** caudate, thalamus, striatum and insula, in ***right*** superior temporal cortex (BA 22) and PFC (BA 6, 44), and ***left*** PFC (BA 45).

Interaction effects (Group x Time): Significant interaction effects were evident in ***right*** lateral temporoparietal areas (BA 21, 22, 37, 39, 40, insula) where RivAMCI decreased and NxAMCI maintained activation over time **(Table 39; Figure 15)**.

Further interaction effects were evident in PFC areas where RivAMCI increased activation and NxAMCI decreased activation **(Figure 16)**. These differences illustrate the treatment effects of rivastigmine on visual attention processing.



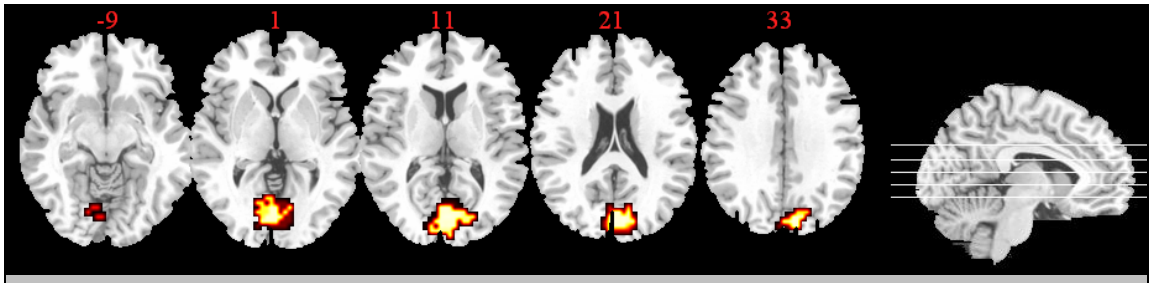
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
T1>T2						
Premotor	L+R	4, **6**, 24	-22	-11	42	201
	L	8				
	R	Striatum, 1, 32				



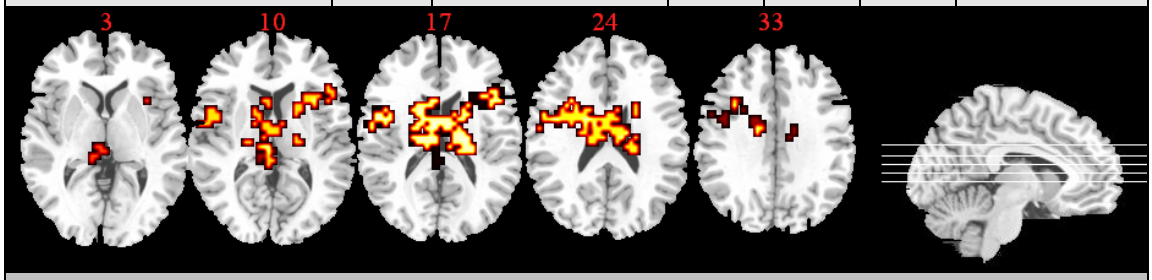
T2>T1						
Temporal	L	Hippocampus, thalamus, striatum, 21, 22, 28, 35, **37**, 38, 39, 41	-51	-52	-2	287

Table 37. Main effects of time on *visual* selective attention in RivAMCI and NxAMCI groups.

Both groups demonstrated larger activation in lateral and medial frontal areas at baseline (top) and larger activation in temporoparietal areas at follow-up (bottom). For table legend, see Table 11.



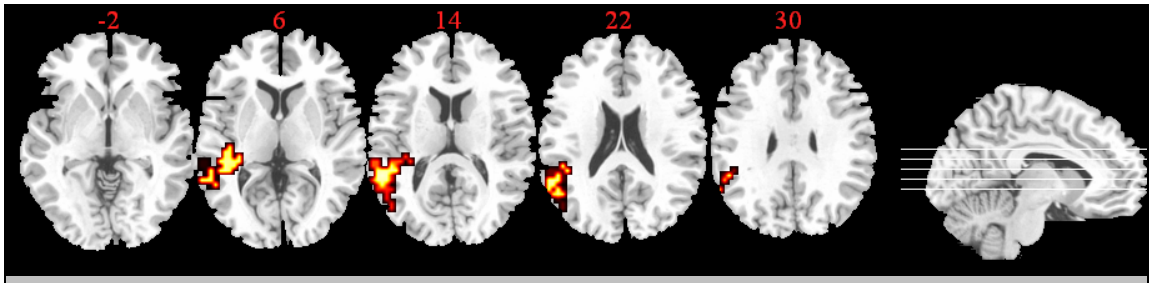
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
RivAMCI>NxAMCI						
Occipital	L+R	17, **18**, 19, 31	-4	-78	-15	171
	R	Cerebellum				



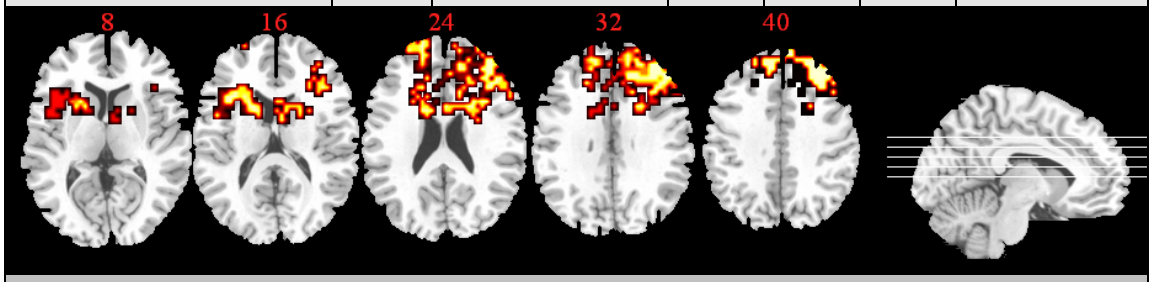
NxAMCI>RivAMCI						
Prefrontal	L+R	Caudate, striatum, thalamus, insula	54	4	9	263
	L	45				
	R	**6**, 22, 44				

Table 38. Main effects of group on *visual* selective attention in RivAMCI and NxAMCI groups.

RivAMCI had larger activation in posterior medial areas compared to NxAMCI over time (top) and NxAMCI had larger activation in PFC and temporal areas (bottom). For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
RivAMCI decrease vs NxAMCI maintain						
Temporal	R	Insula, **21**, 22, 37, 39, 40	54	-41	9	91



RivAMCI maintain vs NxAMCI decrease						
Prefrontal	L+R	Caudate, **9**, 10, 24, 32, 45	-7	56	31	415
	L	46				
	R	Insula, 44, 47				

Table 39. Interaction effects (Group x Time) for RivAMCI and NxAMCI groups on the *visual* selective attention condition.

RivAMCI decreasing activation over time in right lateral temporoparietal areas (top) and NxAMCI decreased activation in frontal areas (bottom) compared to RivAMCI, revealing the neural correlates of rivastigmine treatment in RivAMCI. For table legend, see Table 11.

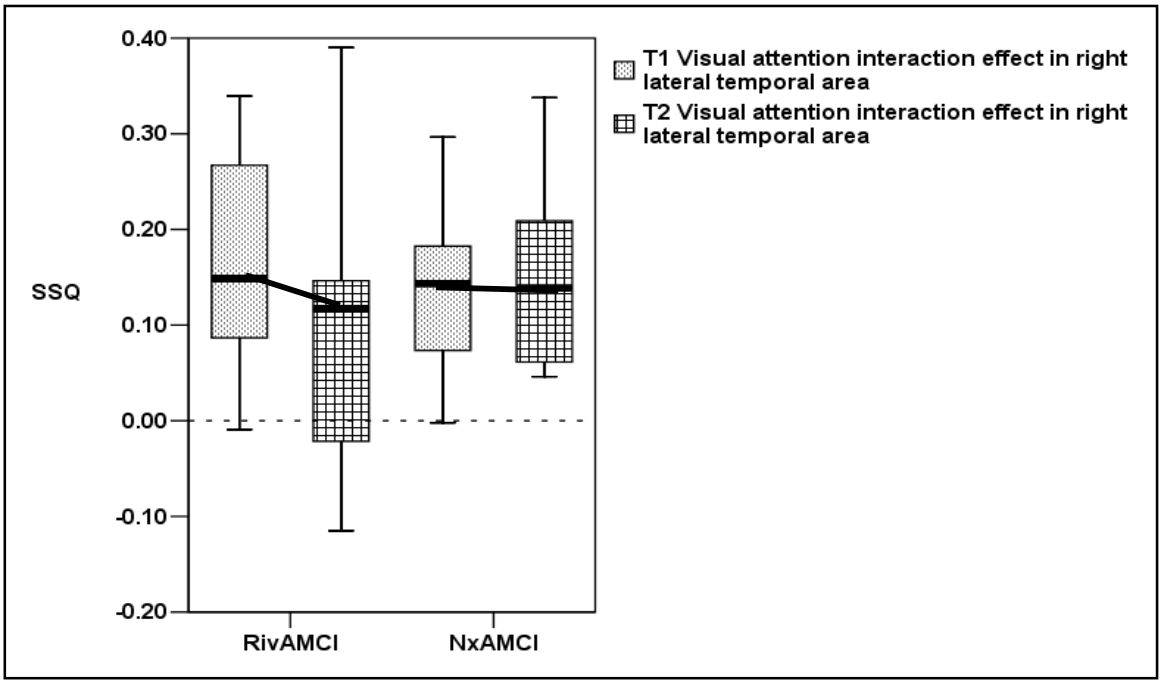


Figure 23. Interaction (Group x Time) effects on *visual* selective attention between RivAMCI and NxAMCI in right lateral temporal area.

The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI decrease and NxAMCI maintained activation over time in the right lateral temporal area.

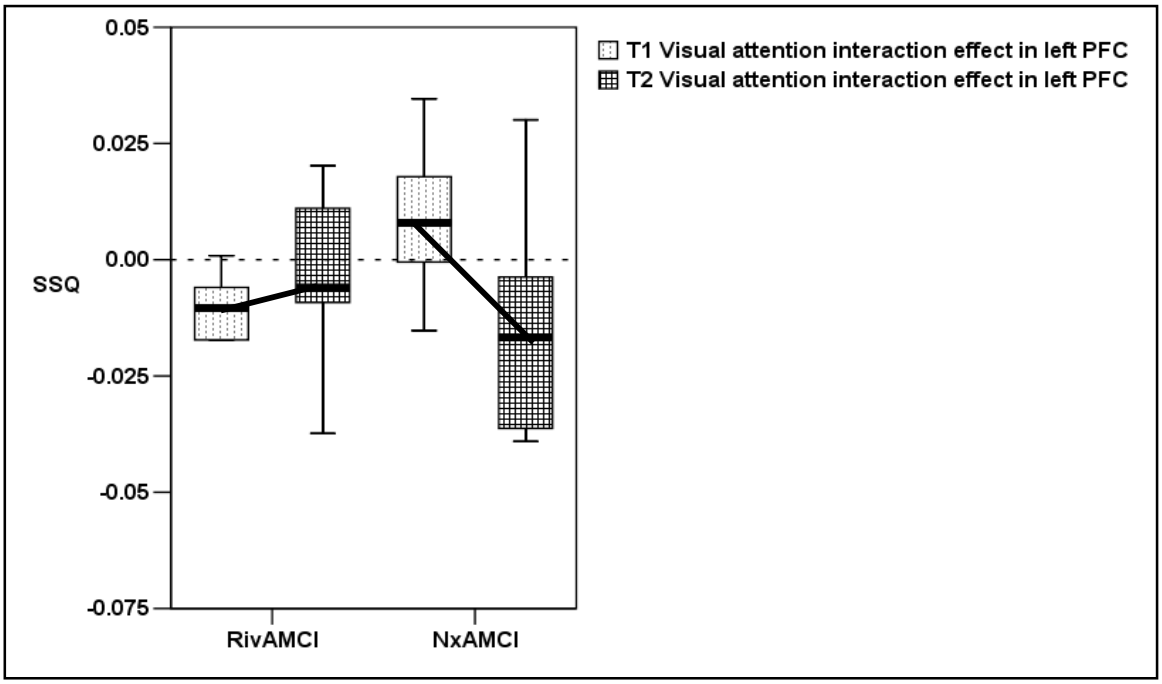


Figure 24. Interaction (Group x Time) effects on *visual* selective attention between RivAMCI and NxAMCI in left PFC.

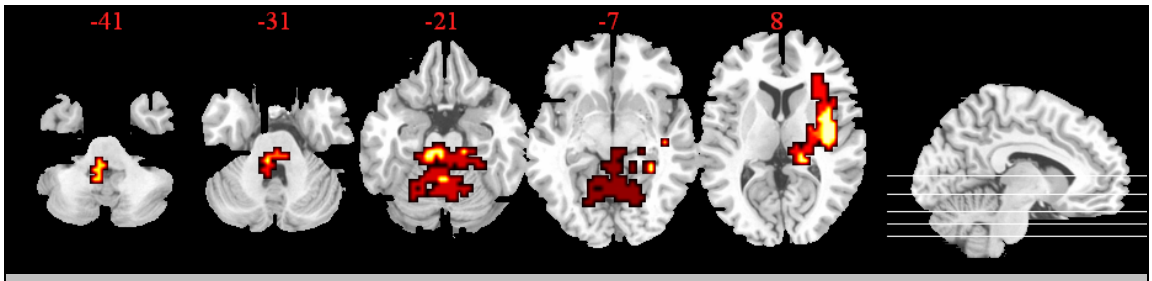
The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI increased and NxAMCI decreased activation over time in the left PFC.

4.10.1.2 Auditory selective attention

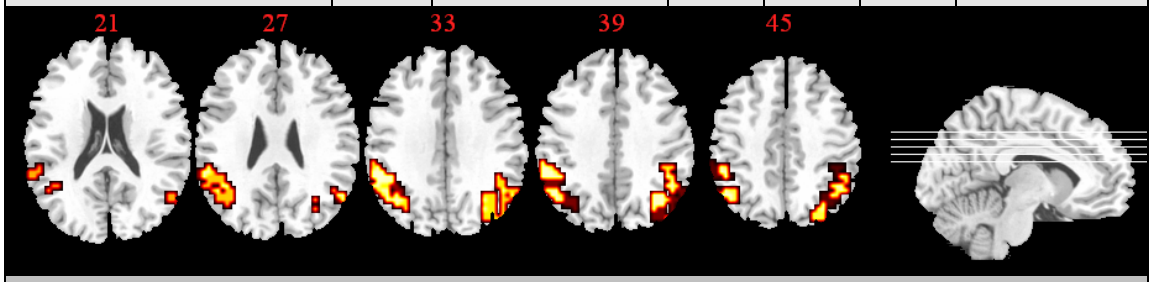
Main effect of time: Both groups activated more at baseline a cluster in ***bilateral*** cerebellum, brainstem, in ***left*** parahippocampal (BA 27, 30) and fusiform areas, and in a cluster in PFC (BA 45), putamen, thalamus and insula **(Table 40)**. Both groups activated more in ***bilateral*** occipitoparietal (BA 19, 39, 40) areas at follow-up.
Main effect of group: RivAMCI had greater activation across time in ***left*** temporal (BA 21, 22, 42), parietal (BA 39, 40), visual (BA 19), anterior cingulate (BA 24), motor and premotor (BA 1, 2, 3, 4, 6) areas **(Table 41)**. NxAMCI had greater activation across time in ***bilateral*** medial parietal (BA 7, 23, 29, 30, 31), ***left*** visual

(BA 17, 18) and cerebellar areas, and in ***right*** lateral parietal (BA 39), medial temporal (hippocampus) and insula.

Interaction effects (Group x Time): Significant interaction effects were evident in a cluster in ***right*** cerebellum, visual and fusiform areas ((BA 19, 36, 37) **(Figure 17)**, a cluster in ***bilateral*** medial occipitoparietal (BA 17, 31) **(Figure 18)** and ***right*** visual areas (BA 18, 19) **(Figure 19)**, and a cluster in left temporoparietal (BA 21, 22, 40, 41, 42) and somatosensory areas (BA 1, 2, 3) **(Table 42)**. In these areas RivAMCI demonstrated relatively decreased activation compared to NxAMCI. Further interaction effects were evident in brainstem, medial occipitotemporal (BA 19, 34, amygdala, hippocampus), posterior cingulate (BA 29, 30), thalamus and striatum where RivAMCI had increased activation over time and NxAMCI decreased **(Figure 20)**.



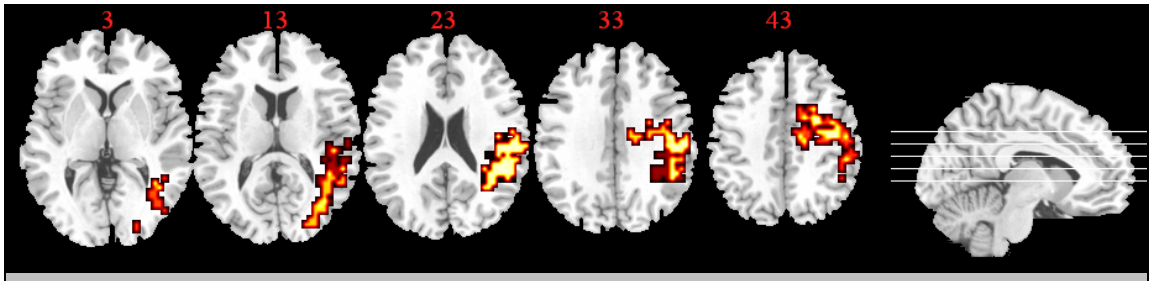
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
T1>T2						
Cerebellum	L+R	**Cerebellum**, brainstem, 27, 30,	-4	-52	-18	189
	L	35, 36, 37				
Frontotemporal	L	Insula, thalamus, putamen, **45**	-29	26	9	152



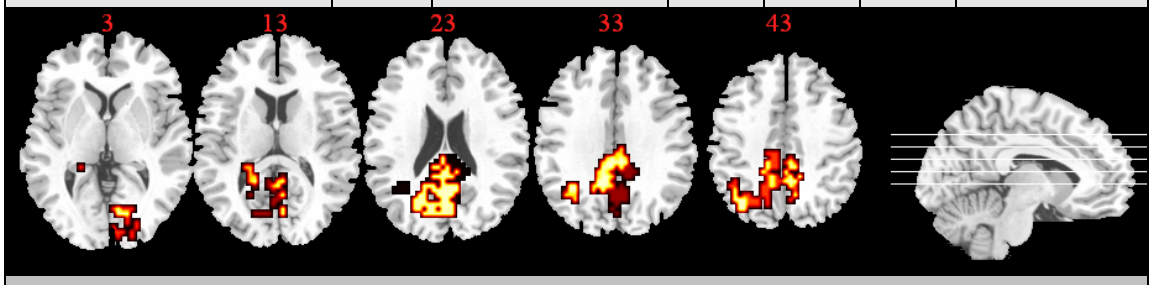
T2>T1						
Parietal	R	19, 21, 39, **40**	54	-41	26	140
	L	19, **39**, 40	-33	-70	26	130

Table 40. Main effects of time on *auditory* selective attention in RivAMCI and NxAMCI groups.

The groups had relatively larger activation in left frontotemporal and bilateral occipitoparietal and brainstem areas at baseline (top), and in bilateral occipitoparietal areas at follow-up (bottom). For table legend, see Table 11.



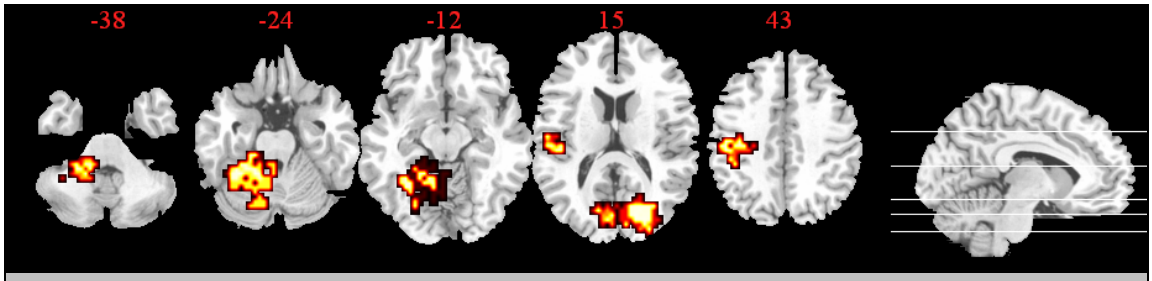
Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
RivAMCI > NxAMCI						
Temporal	L	1, 2, 3, 4, 19, 21, **22**, 24, 37, 39, 40, 42	-43	-37	15	309



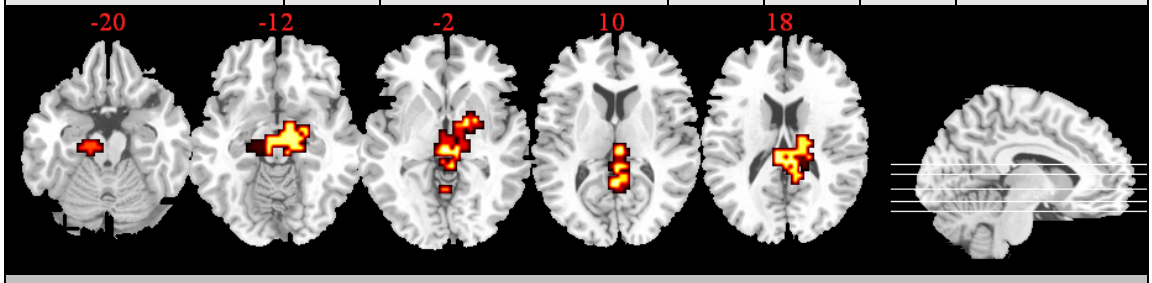
NxAMCI > RivAMCI						
Medial parietal	L+R	7, **23**, 30, 31	4	-59	20	377
	L	Cerebellum, 17, 18				
	R	Hippocampus, insula, 39				

Table 41. Main effects of group on *auditory* selective attention in RivAMCI and NxAMCI groups.

RivAMCI had greater activation in lateral occipitotemporal areas across time (top) and NxAMCI in medial occipitoparietal areas (bottom). For table legend, see Table 11.



Cerebral Region	L/R	Brodmann's or cortical area	Talairach coordinates x	y	z	Cluster size (voxels)
RivAMCI decrease vs NxAMCI increase						
Cerebellum	R	**Cerebellum**, 19, 36, 37	29	-41	-29	192
Occipital	L	17, **18**, 19, 31	-7	-70	2	121
Temporal	R	1, 2, 3, **21**, 22, 40, 41, 42	54	-15	-2	138



RivAMCI increase vs NxAMCI decrease						
Occipital	L+R	**Brainstem**, 19, 34	-4	-41	4	179
	L	Thalamus, striatum, amygdala, hippocampus, 29, 30				

Table 42. Interaction effects (Group x Time) for RivAMCI and NxAMCI groups on the *auditory* selective attention condition.

RivAMCI decreased activation over time in cerebellar, occipital and lateral temporal areas (top) and increased activation in brainstem areas (bottom) compared to NxAMCI and this reveals the neural correlates of rivastigmine treatment. For table legend, see Table 11.

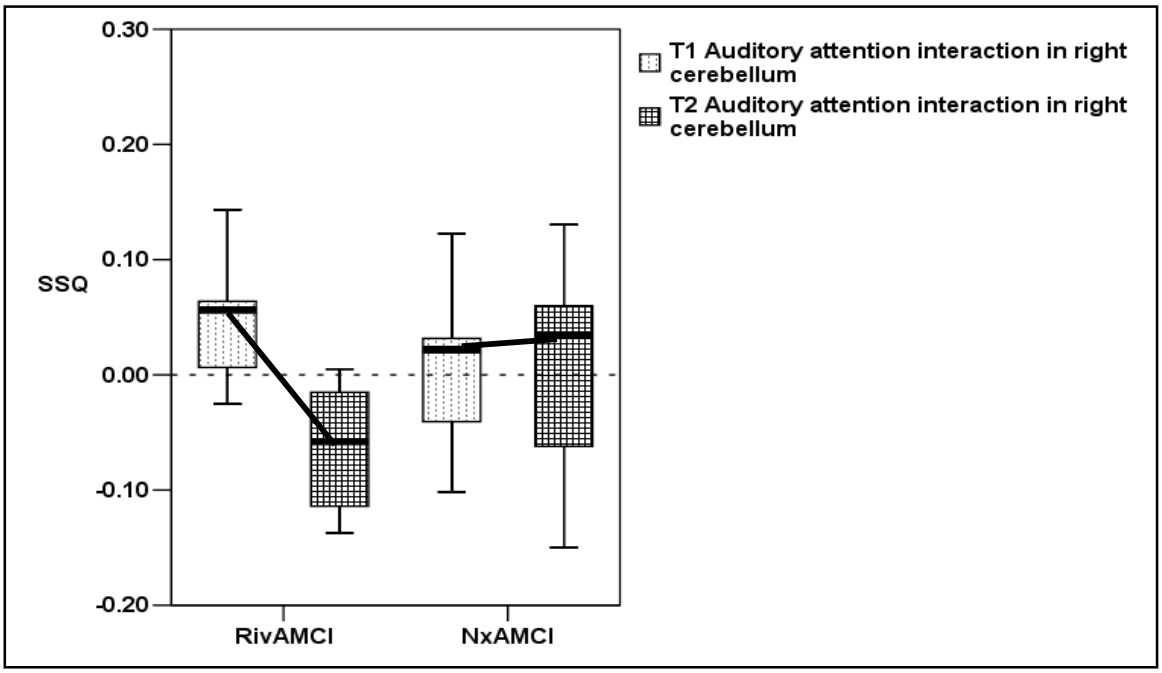


Figure 25. Interaction (Group x Time) effects on *auditory* selective attention between RivAMCI and NxAMCI in right cerebellum.

The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI decreased and NxAMCI increased activation over time in the right cerebellum.

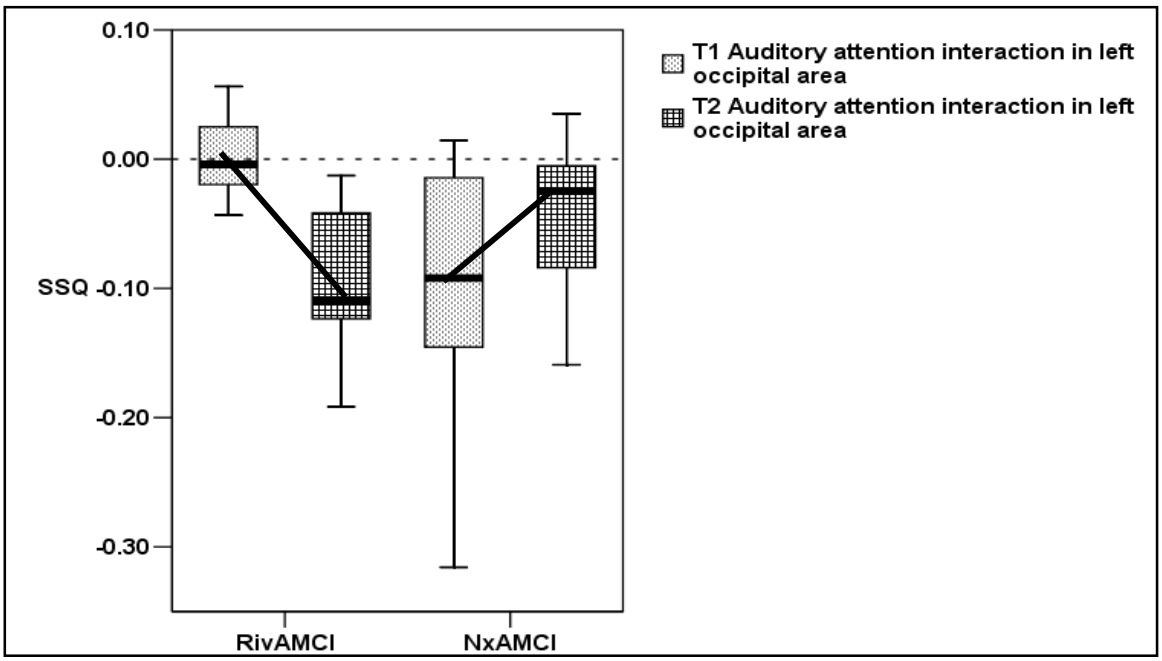


Figure 26. Interaction (Group x Time) effects on *auditory* selective attention between RivAMCI and NxAMCI in left occipital areas.

The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI decreased and NxAMCI increased activation over time in the left occipital areas.

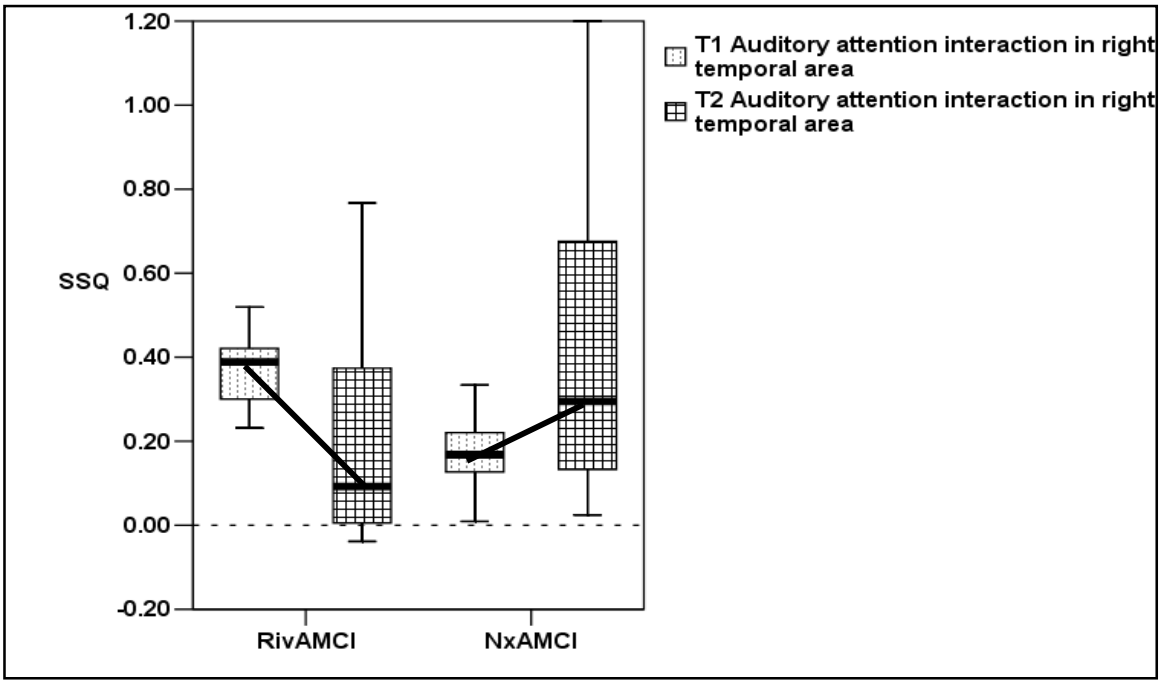


Figure 27. Interaction (Group x Time) effects on *auditory* selective attention between RivAMCI and NxAMCI in right temporal areas.

The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI decreased and NxAMCI increased activation over time in right temporal areas.

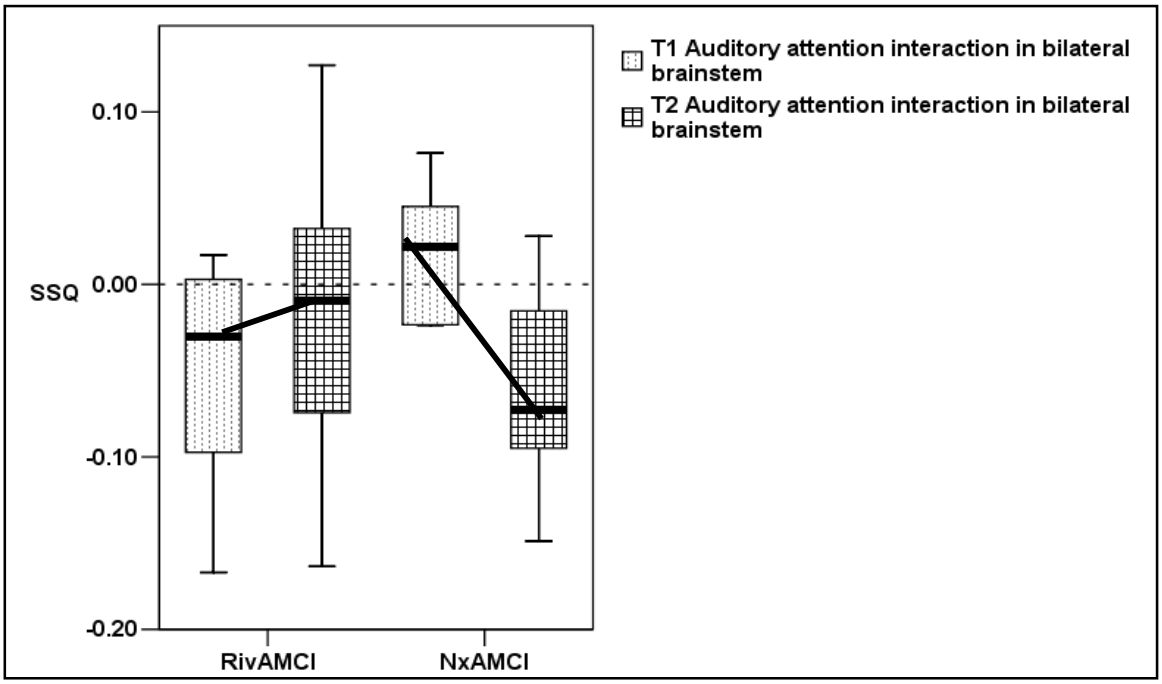


Figure 28. Interaction (Group x Time) effects on *auditory* selective attention between RivAMCI and NxAMCI in brainstem areas.

The boxplot shows baseline (T1) and follow-up (T2) data for the groups. RivAMCI increased and NxAMCI decreased activation over time in the brainstem.

4.11 Rivastigmine Treatment Effects on Verbal Encoding and Recognition

4.11.1 Behavioural Results

There were no significant differences between the RivAMCI and NxAMCI groups on any of the recognition measures at any time point, and the differences between these groups and Controls evident at baseline remained **(Table 43)**. All participants showed decreased Pr and increased FAR at follow-up. A main effect of time was evident for FAR in RivAMCI and NxAMCI, revealing overall increased FAR at follow-up ($F(1,17)=8.4$; $p<0.01$); however, the consequential decrease in Pr was only near-

significant ($F(1,17)=3.5$; $p=0.77$). No main time effects were evident on Br or Pr for Lures. No interactions (group x time) were evident on FAR, Pr, Br or Pr for Lures.

4.11.2 Functional Results

Longitudinal functional data from the encoding task were not analysed for the RivAMCI and NxAMCI rivastigmine treatment comparison because treatment had no apparent effect on recognition performance and evidence from treatment studies in control and AMCI groups failed to demonstrate beneficial effects on verbal memory (§1.7.4). In addition, insufficient data were available for meaningful analysis of the recognition task due to the technical and participant factors discussed before (§4.3).

				RivAMCI vs NxAMCI			RivAMCI vs Control			Control vs NxAMCI		
	Control	**RivAMCI**	**NxAMCI**	P	F	R^2	P	F	R^2	P	F	R^2
Corrected recognition rates (Pr)												
T1	0.66 (0.2)	0.48 (0.1)	0.43 (0.2)	ns			0.01	7	0.3	0.007	9	0.6
T2	0.61 (0.1)	0.38 (0.2)	0.36 (0.2)	ns			0.01	8	0.4	0.002	14	0.7
Hit rates (Hr)												
T1	0.76 (0.2)	0.71 (0.2)	0.71 (0.2)	ns			ns	-		ns		
T2	0.75 (0.2)	0.66 (0.2)	0.72 (0.2)	ns			ns	-		ns		
False alarm rates (FAR)												
T1	0.10 (0.1)	0.22 (0.1)	0.28 (0.1)	ns			0.01	8	0.4	0.003	12	0.6
T2	0.14 (0.1)	0.28 (0.2)	0.36 (0.1)	ns			0.04	5	0.3	0.001	21	0.7
Bias rates (Br)												
T1	0.35 (0.3)	0.47 (0.2)	0.55 (0.3)	ns			ns			ns		
T2	0.41 (0.4)	0.46 (0.2)	0.61 (0.2)	ns			ns			ns		
Lure recognition rates (Pr lures)												
T1	0.21 (0.2)	0.05 (0.2)	-0.03 (0.2)	ns			0.05	*	0.2	0.01	*	0.5
T2	0.13 (0.2)	-0.07 (0.2)	-0.21 (0.2)	ns			0.05	*	0.2	0.001	*	0.6

Table 43. Behavioural results for Controls, RivAMCI and NxAMCI at baseline (T1) and follow-up (T2) on the recognition task.

There RivAMCI and NxAMCI groups were comparable on all recognition measures across time and differences with Controls evident at baseline remained. Pr decreased and FAR increased across all participants at follow-up.

Data are mean (standard deviation), ns=not significant, F=ANOVA test statistic; *=Mann-Whitney test. The coefficient of determination, R^2 is reported as a measure of effect size: ±0.01 = a small effect, ±0.10 a medium effect, ±0.25 a large effect.

5. Discussion

We examined episodic memory and attention in prodromal AD and the effects of ACEI treatment on deficits in these cognitive domains. We therefore used fMRI to examine the neural correlates of attention and memory processing in AMCI that represent prodromal AD. The longitudinal demographic and neurocognitive findings from our patient group lend strong support to the validity of AMCI diagnosed in specialist memory clinics as prodromal AD (§5.1-3). Continued follow-up of AMCI patients enabled us to identify those who progressed to AD, the CoAMCI group, and to compare them with Controls in allowing us to examine attention and memory in prodromal AD. Comparisons between Controls and the pooled AllAMCI group (RivAMCI + NxAMCI), that was overall less impaired than the CoAMCI group, provide some insight into altered behaviour and activation earlier on in the progression of AD, although continued follow-up will be required to determine if all our AMCI subjects progress to AD.

CoAMCI demonstrated altered cortical activation during selective visual and auditory attention suggesting that basic attentional processing is affected by AD neuropathology in the prodromal stages (§5.7). Behavioural and functional findings on the divided attention task indicate its sensitivity to differences in relatively small groups and therefore its appropriateness for fMRI studies of attention in AMCI (§5.5). Altered functional activation and impaired behavioural performance during divided attention indicate that attentional deficits occur early in the course of AD (§5.6). Behavioural findings from the verbal episodic memory task indicate that executive failure contributes significantly to the episodic amnesia in AMCI and functional findings suggest that this is associated with failed cognitive regulation and compensatory activation (§5.8-9). The recognition task revealed altered activation

areas including the posterior cingulate that accords with findings from other studies and supports the suggestion that altered posterior medial parietal (cingulate) activation appears early and consistently in AMCI (§5.6.2.2).

The longitudinal study on the behavioural and functional effects of ACEI treatment on attention in AMCI revealed that rivastigmine treatment improved visual and auditory response processing during divided attention (§5.10). This was associated with decreased activation in primary and secondary visual and auditory cortices, and in the PFC and lateral parietal areas of the executive-attention network suggesting improved cortical processing efficiency.

Rivastigmine treatment was associated with decreased activation in non-relevant auditory and language processing areas during the selective visual attention condition and in visual processing areas during selective auditory attention, suggesting improved down regulation of activation in areas not relevant to a given condition following rivastigmine (§5.11). Rivastigmine had no apparent behavioural effects on recognition (§5.12). All these findings are discussed in more detail below.

5.1 Participant Demographics

Controls and AMCI groups did not differ significantly on age and education although the mean ages of the AllAMCI and CoAMCI groups were older (§4.1; Table 9). The non-significance of the age difference may be due to the relatively small sample sizes reducing statistical detection power. However, statistical analysis of other functional and behavioural variables will suffer from the same lack of statistical power, consequently observed significant differences will have large effect sizes, and would therefore remain significant in larger samples. Nonetheless, differences in age should be taken into account and where possible controlled for by matching samples because

age cannot be covaried for as it is systematically related to the defining characteristic (episodic memory) of the AMCI group and doing so would remove real variance and corrupt the grouping variable itself (Miller and Chapman, 2001). The potential effects of age differences between CoAMCI and Controls were examined by correlation analyses with behavioural and neurocognitive measures on which the groups differed significantly (CAMCOG and New Learning subscale §4.2; VisRT on divided attention §4.5.1; Pr, FAR and Pr lures on recognition §4.8.1). The only significant correlation was an inverse correlation between age and total CAMCOG scores in CoAMCI and Controls, indicating that 12% of the difference between the groups could be attributed to differences in age. The age difference between these two groups therefore accounts for less than two of the 15-point difference on mean total CAMCOG scores. The absence of any significant correlations between age and any of the experimental behavioural measures that differed between Controls and CoAMCI suggests that age is unlikely to account for related observed functional differences.

Numerically different age-means are evident in several referenced studies comparing AD and AMCI to controls (Celone et al., 2006; Chetelat et al., 2003; Laine et al., 2008; Okonkwo et al., 2008; Tales et al., 2005a; Tales et al., 2008; Trivedi et al., 2008). This is likely due to the increasing incidence of comorbid medical conditions in older adults that preclude participation as controls and because of the increased incidence of AMCI with age increasing the likelihood of recruiting older AMCI patients. Age is not a reliable predictor of progression from AMCI to AD, despite being a predictor of incident AMCI and AD, and increasing age therefore does not appear to correlate with the time it takes to progress to dementia, and therefore decreasing cognitive functioning, in AMCI samples (Artero et al., 2003; Fleisher et

al., 2007). The effects of age are therefore more relevant to comparisons of different aged controls whilst not appearing relevant to comparisons between AMCI groups.

We were able to identify and excluded participants who progressed to dementia other than AD, thereby improving the homogeneity of the AMCI patient group. This is reflected by the high AD conversion rate that makes our results generalisable to prodromal AD populations but no to MCI samples that have more heterogeneous aetiologies.

We report findings for the AllAMCI and CoAMCI groups as we consider the former group to present prodromal AD despite the fact that not all members had progressed to AD after 2 years. All participants were recruited using the same methods and this, together with the findings that (1) the AllAMCI group's mean scores on behavioural measures fall between that of Controls and CoAMCI (Tables 9, 10, 25) and (2) that AllAMCI differ from Controls on less voxels than do CoAMCI on divided attention (Tables 12-13), visual attention (Tables 16-17) and encoding (Tables 23-24), leads to the conclusion that the non-converters are less impaired but will progress in future.

Taken together these findings indicate that age differences are frequently encountered in studies of AMCI and that it is unlikely to account for the observed differences from Controls in our results. Furthermore, we can identify AMCI with a high rate of progression over a relatively short follow-up period. AMCI that did not progress are still likely to progress and findings from behavioural and functional measures suggest they are intermediate between Controls and converters. Continued follow-up of non-converters will enable further clarification of the sensitivity and specificity of our measures.

5.2 Neurocognitive Findings

Lower CAMCOG, MMSE and New Learning subscale scores were anticipated in AMCI and indicate episodic memory impairment. The absence of impairment on the attention subscale of the CAMCOG illustrates the low sensitivity of this measure. The attention subscale score is derived from the serial-seven subtraction and counting backwards from 20 tasks, and therefore tests working memory and executive functioning. Demonstrating slower RT on divided attention in AMCI suggests that more sensitive measures of attention are useful in evaluating attention in clinical settings.

5.3 Diagnostic Stability and Disease Progression

More than half of the AMCI patients had progressed to AD after two years. This high conversion rate is similar to that demonstrated by studies using sensitive and specific cognitive, CSF or structural neuroimaging indicators of disease progression (Artero et al., 2003; Hansson et al., 2006; Korf et al., 2004; Sarazin et al., 2007). Furthermore, it confirms the construct validity of AMCI as prodromal AD and illustrates the high sensitivity and specificity of rigorously applied operationalised diagnostic criteria in expert memory clinic settings.

5.4 Overview of Scanning Sessions

Our results are characterised by high follow-up and technical success rates (§4.3). We conducted 63 scanning sessions on 32 participants and we were unable to use data from only three sessions; two sessions were excluded as the participant was subsequently diagnosed with Lewy body dementia and one session was excluded form a participant who had evidence of a cortical infarct on structural imaging.

Seventeen experimental runs had to be restarted or abandoned due to technical failure or poor compliance. Eleven of these were on the recognition task; four runs had to be abandoned without collection of useable functional data, behavioural data from five runs could not be used due to compliance issues, and data from two runs were corrupted by the necessity to repeat the encoding task using different stimuli as the initial run failed. This relatively high failure rate adversely affected the functional analysis of the recognition task because not enough events of the conditions of interest were available to allow adequate statistical power. Most of the failures occurred on the recognition task, which was presented last, and it may therefore be related to participant fatigue. It therefore appears that in its present form the task is suitable for behavioural studies but not ideal for functional studies in AMCI.

5.5 Piloting the Divided Attention Task

We designed a divided attention paradigm suitable for fMRI that proved useful in demonstrating behavioural and functional changes in AMCI. We used data from the pilot study (§4.4) to adjust task parameters to ensure high response accuracy across AMCI and Controls that renders RT a useful outcome measure (§3.9.1), and the high accuracy scores in Controls and AMCI confirmed this. Furthermore, the difference demonstrated on RT between Controls and AMCI groups illustrates the utility of the task to measure aspects of attentional processing and indicates that it may be useful in identifying AMCI and in monitoring treatment responses in clinical practice (§4.5.1). The task generated functional activation in areas associated with divided attention and the cognitive contrast (experimental condition - control condition) therefore appears suitable for evaluation of the neural correlates of divided attention (§4.5.2.1). Comparing Controls and AMCI on the task at baseline revealed functional brain

activation differences indicating the paradigm is also suitable for group comparisons (§4.5.2.2). Furthermore, the behavioural and functional results from the rivastigmine treatment study reveal the potential of the task in studying the neural and behavioural correlates of treatment interventions (§4.9). The short training period required and the short task duration make it practical for clinical and research use.

5.6 Divided Attention in AMCI at Baseline

We compared AMCI to Controls on divided attention at baseline to investigate if prodromal AD is associated with attentional impairment as predicted by the cholinergic hypothesis and the presence of attentional deficits in very mild AD. We found that the CoAMCI group that converted to dementia had slower visual target processing during divided attention and this was associated with altered activation in relevant sensory and attention processing areas. Activation in visual processing areas correlated positively with visual processing speed in other words superior performance required increased activation. CoAMCI had attenuated activation in these visual areas suggesting impaired cognitive regulation. This is supported by the inverse correlation between activation in midbrain areas - which activate during very high attentional demand - and visual target processing response speed, suggesting that processing speed decrements were more pronounced in AMCIs that found the task more demanding. These results suggest disconnection between midbrain areas that drive attention and cortical areas that process stimuli in prodromal AD.

5.6.1 Behavioural Findings

General task related findings

All subjects found the dA condition more demanding than the cA condition as demonstrated by increased RT and lower accuracy on the former (§4.5.1; Table 10). These findings indicate the expected dual task decrement in performance during divided attention and are in line with published findings (Klingberg, 1998; Loose et al., 2003; Posner, 1978).

The groups did not differ on accuracy during the dA or cA tasks, indicating that the task design allowed the high accuracy levels required to utilise RT as an outcome measure as described in the methods section (§3.9.1.1).

AMCI groups and Controls took longer to respond to auditory compared to visual stimuli during the dA condition and this is likely due to the physical characteristics of the stimuli. All the information necessary for identification is presented instantly for visually presented letter stimuli but auditory-presented number stimuli require longer evaluation for identification. This difference in appraisal time is therefore the likely cause of RT differences between visual and auditory targets.

Behavioural deficits on divided attention in CoAMCI

We demonstrated slower visual RT during the dA condition in CoAMCI indicating impaired target response speed during divided attention in prodromal AD. We initially demonstrated this in the RivAMCI group and this was the first report of impaired divided attention in AMCI (Dannhauser et al., 2005). One prior study had demonstrated impaired visual selective attention in AMCI, evident as longer RTs on a task designed to examine the effects of interference from distractor stimuli (Perry and Hodges, 2003). Their task required subjects to ignore a non-target stimulus that briefly appeared before a target and is therefore similar in some aspects to the divided attention condition where non-target distractor stimuli appear before *and*

simultaneous with targets. These findings of slower visual target processing may therefore be related. A later study that controlled for the effects of ageing also revealed impaired visual attention (slower RT) in AMCI and AD, which distinguished these conditions from normal ageing (Tales et al., 2005a). Their visual search task required selective visual attention and attentional shifting and these processes are also required for the dA condition. A more recent study found impaired visual selective attention and attentional shifting in AMCI (Silveri et al., 2007). Impaired visual divided attention and visual-auditory divided attention have subsequently been reported in AMCI (Laine et al., 2008; Okonkwo et al., 2008). Our initial results have therefore being replicated and taken together these findings indicate impaired divided attention in AMCI whilst our findings in CoAMCI indicate impaired divided attention in confirmed prodromal AD.

Neuropsychological testing at baseline revealed that CoAMCI had isolated episodic amnesia and did not differ from Controls on measures of attention. Taken together, these findings indicate that visual attention and divided attention deficits are evident in AMCI when sensitive measures are used that record RT. Moreover, available findings suggest that deficits are restricted to the visual domain as all the tasks were either visual or had a visual component. These visual attention deficits may be related to failing executive *top-down* (task driven) control of attentional selection that depends on cholinergic neurotransmission (§1.6.3). Some support for this notion comes from our functional results that reveal decreased activation during selective visual attention in occipital visual processing areas that are regulated by PFC and parietal nodes of the attentional networks (§4.6.1.2; Tables 16, 17).

Divided attention was also impaired in the AllAMCI group that included converters and non-converters suggesting that divided attention may, similar to

delayed recall, predict AD. It remains to be seen if divided attention is as good a predictor of progression to AD in AMCI and longer follow-up is required to establish if all our AMCI patients will progress to AD.

Taken together our results indicate that episodic amnesia in progressive AMCI does not occur in isolation, as it appears accompanied by impaired divided attention.

The presence of divided attention deficits in AMCI is an important finding because it can partly explain the episodic amnesia. The interaction between memory and attention is complex and memory processing in everyday life requires intact divided attention for such functions as source monitoring that improves episodic recall (§1.6.1). Memory has a limited capacity and it can be influenced by the demands made on attentional selection. Disease processes affecting attentional resources can therefore impair memory by inhibiting attentional selection. This is further supported by the strong correlations between visual target response speed decrements and recognition deficits in the CoAMCI groups (§4.8.1).

Encoding processes generally make large demands on cognitive resources, which make them particularly vulnerable to the effects of attention deficits (Craik et al., 1996; Nyberg et al., 1997). The bidirectional relationship between divided attention and memory appears to explain the rapid decline from early deficits towards generalised cognitive impairment and dementia (Sarter and Turchi, 2002). Studies of cortical cholinergic function in mild AD have found that it correlates with attention but not with episodic memory and this suggests a more central role for attentional impairment in prodromal AD (Kadir et al., 2006). The exact contribution of divided attention decrements to episodic memory deficits in AMCI remains to be established.

Sustained attention in AMCI

We found comparable performance on sustained attention (cA condition), suggesting it remains intact in CoAMCI. This is supported by recent reports of impaired visual divided attention but comparable sustained and selective attention in AMCI (Okonkwo et al., 2008). However, the cA condition arguable also required an element of selection due to the noisy scanner environment and this should be taken into account when interpreting results. Nevertheless, at present there are no reports of impaired sustained attention in AMCI suggesting considerable resilience in the network underpinning it; alternatively current measures are insensitive to deficits. Divided attention depends on sustained and selective attention and it is useful to confirm that these are unimpaired prior to making inferences about findings on divided attention. Okonkwo et al. used a different measure of visual divided attention that determined the processing time required to perform at 75% accuracy on the task by varying the presentation time of targets. Their results indicate that AMCI require longer visual processing time, similar to our results.

Taken together, these findings from behavioural studies indicate impairment in visual target processing during divided attention in AMCI; however, auditory processing has not been studied sufficiently to determine its status in AMCI. AMCI could be associated with isolated visual processing deficits or visual attention may be more vulnerable to the effects of AD neuropathology and therefore show impairment earlier. Future studies can clarify this by using auditory divided attention task or across modal tasks that do not include visual stimuli.

Disease mechanisms

BFCS lesions

It has been proposed that disruption of two neural systems may underlie the attentional deficits in AD and these therefore also apply to AMCI (Perry and Hodges, 1999). The first is the BFCS that provides the main cholinergic input to the neocortical areas involved in attention, especially the prefrontal and parietal cortex and the thalamus (Mesulam and Geula, 1988). The BFCS includes the nucleus basalis of Meynert, which is one of the areas prominently affected by neuropathology in AD (Arnold et al., 1991). Cholinergic neurotransmission is prominently affected in AD where degeneration of cholinergic neurones of the basal forebrain nuclei diminish cortical and hippocampal input (Francis et al., 1999; Perry et al., 1999; Sarter et al., 2003). The presence of neurofibrillary tangles and pre-tangle cytopathology have been reported in autopsied MCI patients, with pre-mortem measures of cognitive impairment correlating with the percentage of neurones affected (Mesulam et al., 2004). This suggest that cholinergic neurotransmission is affected in AMCI and findings from studies of in vivo cholinergic activity support this, indicating a similar pattern of deficiency as seen in AD and correlation with cognitive impairment (Rinne et al., 2003) (Sabri et al., 2008). Decreased cortical AChE activity in AMCI also appears to predict conversion to AD (Herholz et al., 2005). Several lines of evidence indicate that attentional processing is mediated via cholinergic input to sensory cortices (signal-driven or bottom-up modulation), and to frontal and parietal cortex (task driven or top-down modulation) (Sarter et al., 2005). Considering the role of cholinergic innervation from the BFCS in attentional processing and the early presence of AD neuropathology in this area, impaired divided attention in AMCI is likely related to impaired attentional modulation in sensory and frontoparietal cortices

due to interrupted cholinergic innervation from the BFCS. The correlations between activation in visual processing areas and visual processing speed evident from our results support the suggestion of impaired modulation in sensory cortices.

Cortico-cortical pathway lesions

The second neural system relevant to attentional dysfunction in AMCI is comprised of the cortico-cortical pathways, such as the longitudinal fasciculi that connect frontal and parietal cortices. Neocortical synapse density in frontal and parietal cortices are highly correlated with dementia severity (Samuel et al., 1994) and AD neuropathology is present early in the neocortex in very mild AD (Morris et al., 1991). Autopsy studies in MCI found early neuropathological changes of AD (Price and Morris, 1999). A longitudinal study which followed MCI patients (up to 9.5 years) reported that 100% progressed to AD and 84% had neuropathological changes of AD at autopsy (Morris et al., 2001). Furthermore, PET studies of amyloid reveal widespread cortical deposition in AMCI and specific increases in prefrontal, parietal, lateral temporal and medial parietal areas (Jack et al., 2008; Kemppainen et al., 2007; Lowe et al., 2009). Although no neuropathological study of AMCI appears to have been conducted, the findings from mild AD and MCI, and amyloid PET studies strongly suggest that AD neuropathology will be present in the cortex in AMCI. This in turn suggests that functional connectivity between cortical areas may be impaired in AMCI and this has recently been demonstrated in AMCI, supporting view that AD neuropathology is present in the cortex in AMCI (Sorg et al., 2007; Sorg et al., 2009). Several lines of evidence therefore suggest that cortico-cortical pathways may be affected in AMCI and therefore that decreased processing speed in AMCI during

divided attention may be secondary to altered functional connectivity between cortical attention network nodes such as the PFC and parietal cortex.

Sensory impairment

Greater visual impairment has been reported in AD compared to controls, and vision impairment correlates positively with cognitive impairment (Uhlmann et al., 1991). Similar findings have been reported in relation to hearing impairment and AD (Uhlmann et al., 1989). It is not known if these sensory impairments are related to pathology in the sensory apparatus or visual processing; however, impaired performance on visual targets during divided attention in CoAMCI is unlikely to be related to sensory visual impairment as this was controlled for by optimising the visual acuity for all participants. Nevertheless, a comparison of visual acuity between AMCI and controls has not been done yet and AMCI may be associated with greater visual impairment.

Ageing

Divided attention is underpinned by processing speed, working memory and executive processing and therefore vulnerable to the effects of age-related decline in these processes (§1.3.3.2). Divided attention performance does decrease with age; however, these deficits are more pronounced on more complex dual tasks with complex task rules that require working memory encoding or item manipulation (Sarter and Turchi, 2002). A visual and auditory dual-task requiring visual letter-stimulus matching and simple choice reaction to an infrequent auditory stimulus revealed no difference in performance between two elderly age groups (60-69 years; 70-79 years) suggesting that age-related differences are unlikely to be a source of

significant variability in this age group during similar divided attention tasks (Greenwood and Parasuraman, 1991). Furthermore, no significant correlations were evident between age means and behavioural measures that CoAMCI and Controls differed on (VisRT, Pr, FAR, Pr lures; §5.1), indicating that generalised ageing effects are unlikely to be responsible for the observed differences.

The effects of ageing on alerting, which achieves and maintains a heightened state of arousal in preparation for a task, have only been demonstrated on long (30min) sustained attention tasks (Mouloua and Parasuraman, 1995). Although the divided attention task required sustained attention, the duration was relatively short and it is therefore unlikely that ageing effects on alerting impacted on performance.

Orienting, which focuses attention on one stimulus amongst many (selective attention) and relies on shifting attention from one stimulus to the next shows age-related impairment and this appears more pronounced when target stimuli are presented with highly similar distractor stimuli, and less pronounced when target stimuli are preceded by a cue, diminishing the need to process irrelevant stimuli (Hartley, 1993; Rogers, 2000). Stimuli were presented in pairs in different sensory modalities after a visual cue (cross-hair) during the dA condition and there was no interference from simultaneous similar distractor stimuli. The effects of ageing on selecting stimuli for processing are therefore likely limited.

Although the effects of ageing on executive control remains controversial, deficits have been demonstrated which remain after controlling for the general effects of slowed processing speed. We have seen above that ageing effects are more pronounced on more complex dual-tasks but that differences were not evident in one study that compared older groups spanning the age range of our groups. As mentioned

above, age did not correlate with any of the measures that differed between CoAMCI and Controls and all of these relied on executive processing.

Ageing affects many aspects of cognition but it is unlikely that the slightly younger age of controls can explain the observed differences because (1) they are also evident and more pronounced in AD, (2) age did not correlate with visual target processing speed, and (3) age did not correlate with episodic memory measures on which CoAMCI were impaired, thereby also discounting the possible deleterious effects of age related memory deficits on attentional processing. Furthermore, our results of impaired divided attention are supported by findings from a study comparing AMCI (mean age 70 years; n=51) and older controls (mean age 68 years; n=58) where sample sizes were larger and groups were matched more closely on age (Okonkwo et al., 2008). The divided attention deficits seen in the AMCI group therefore appears to be due to underlying AD neuropathology and if ageing did contribute, then it was not pronounced and unlikely to explain the observed differences.

Summary

In summary, we demonstrated slower visual RT during the dA condition in CoAMCI indicating slower visual attention processing during divided attention in prodromal AD. It therefore appears that episodic amnesia does not occur in isolation, even in AMCI, but is accompanied by attentional impairment. Attention and memory are closely related and deficits in these domains evident in AMCI also appear related. This may be due to memory-attention interactions or shared upstream disease mechanisms affecting both. AD neuropathology can affect attention in AMCI by disrupting regulation in sensory cortices via lesions in the BFCS and/or locus

coeruleus. Support for these causative mechanisms comes from the correlations between visual areas activation and visual processing speed.

Findings from larger age-matched studies together with the absence of significant correlations between age and behavioural measures indicate that generalised ageing effects are unlikely to be responsible for the observed differences.

Our results also support the current view that sustained attention is unimpaired in AMCI. Below we look at the neural correlates of divided attention and functional brain changes in AMCI associated with impaired divided attention.

5.6.2 Functional Findings

Activation was evident in cortical areas associated with sensory, attentional and executive processing. The CoAMCI group demonstrated attenuated activation in visual (extrastriate, fusiform, inferior temporal lobe), auditory (lateral temporal, insula) and attentional processing (cerebellum, lateral parietal) areas and this was associated with slower visual target processing. CoAMCI demonstrated greater activation in the thalamus. Slower visual target processing correlated with attenuation of activation in visual processing areas (fusiform) and with greater activation in midbrain-thalamic areas associated with demanding attentional tasks. These findings are discussed in more detail below.

5.6.2.1 Task related activation

The dA condition required (1) appraisal of simultaneously presented auditory stimuli (numbers read by a male voice) and visual stimuli (lower case letters presented on a screen) followed by, (2) a motor response upon target detection or, (3) response

inhibition when presented with distractor stimuli. In terms of attention, it required sustained attention over the duration of the condition and, for divided attention, a repeating sequence of attentional orientation, response selection, behavioural response execution or inhibition, and attentional disengagement. Attentional disengagement and orientation, the key elements of divided attention, are not required during the cA condition where the visual and auditory targets provided identical semantic information (numbers). The subtraction analysis (dA – cA) therefore provides information about neural activity specifically required for attentional switching during dA, whilst closely matching other attentional requirements and sensory stimuli.

Combining activation data for all Controls and AllAMCI during dA revealed activation in areas associated with processing visual stimuli (extrastriate, fusiform), attention (PFC, anterior cingulate, parietal, cerebellum) and letter stimuli (insula) and we look at these in turn (§4.5.2.1; Table 11).

Visual processing areas

Activation in bilateral extrastriate and fusiform areas most likely reflects processing of visually presented letter stimuli necessary only during the divided attention condition. Processing different visual stimuli, and different attributes of identical stimuli, activate discreet regions of extrastriate visual cortex (Corbetta et al., 1991; Heinze et al., 1994; Mangun et al., 1997). Furthermore, functional and behavioural studies in healthy controls indicate the presence of separate neural substrates for letter and digit processing (Polk and Farah, 1998). The additional activation in visual areas during divided attention is therefore likely related to letter processing.

The absence of additional activation in auditory cortex is in line with findings of activity decreases in sensory cortices during divided attention compared to selective

attention (Corbetta et al., 1991; Johnson and Zatorre, 2006; Loose et al., 2003), and the absence of additional parietal activation appears to indicate that it is already sufficiently or maximally engaged by the cA condition.

PFC, anterior cingulate and medial parietal areas

Task related activation during divided attention occurred in areas reliably associated with dividing attention (PFC) and sustained sensory attention (anterior cingulate, medial parietal) (Johnson and Zatorre, 2006; Loose et al., 2003; Vohn et al., 2007). The left PFC is associated with executive tasks including attentional orientation, divided attention, response inhibition, cognitive set-shifting, memory encoding and retrieval, working memory and organisation of information, and activation here during divided attention likely reflects executive processing (Cabeza and Nyberg, 2000; Floel et al., 2004; Johnson et al., 2003; Nyberg et al., 1996). The combination of PFC, anterior cingulate and parietal activation, as seen in our groups, reliably activate specifically during tasks requiring response inhibition e.g. the divided attention task.

Our findings are consistent with the result of a similar fMRI divided attention study, employing visual and auditory stimuli, in healthy young subjects (Loose et al., 2003). The auditory targets for their divided attention task were successive presentation of identical frequency tones and this makes greater demands on working memory than our task. Other studies requiring older participants to monitor visual and somatosensory modalities have reported right PFC and parietal activation (Johannsen et al., 1997). Differences in the laterality of PFC activation between studies may be related to the different sensory modalities of stimuli as these determine the attentional network recruited for each individual task (Corbetta et al., 1991; Johannsen et al.,

1999; Johannsen et al., 1997). For example, in the study by Johannsen et al. (1999) study participants were told to expect targets but none were presented and activation due to target responses were therefore absent whilst response inhibition processing was active, furthermore the absence of behavioural measures makes it difficult to establish if participants were complying with task instructions. Bilateral prefrontal and parietal activation has been reported on more complex dual tasks requiring working memory and semantic processing (Iidaka et al., 2000b; Koechlin et al., 1999). A study comparing bimodal visual and auditory sustained attention, selective attention and divided attention also found left PFC activation only during divided attention (Johnson and Zatorre, 2006). A more recent study contrasting within-modal against across-modal conditions revealed greater activation during across-modal divided attention in bilateral DLPFC (BA 46), left DLPFC (BA 9), medial PFC (BA 8), and inferior parietal lobe (BA 40) and, in right anterior cingulate (BA 32), suggesting that the increased demand for coordination of cross-modal attentional resources required more top-down control of attentional processing (Vohn et al., 2007). From the few available studies, it appears that across-modal divided attention may be the more processing intensive attentional function and specifically associated with left DLPFC activation, similar to our task and findings.

Activation in the anterior cingulate has been found during divided attention and when visual attention is maintained at a single spatial location (Vandenberghe et al., 2000; Vandenberghe et al., 1997), and both these conditions occur during our dA condition.

Medial parietal activation in the left hemisphere has been demonstrated in studies requiring silent reading similar to that required on our divided attention task (Cabeza and Nyberg, 2000).

Insula and cerebellum

Activation in the insula may be related to processing of the visually presented letter stimuli or to working memory maintenance of the target letter as this area frequently activates during tasks requiring reading and working memory maintenance of letters or words (Cabeza and Nyberg, 2000).

Cerebellar activation has been associated with attentional orientation such as is required during divided attention (Cabeza and Nyberg, 2000).

Summary

In summary, our findings are in line with those from studies of divided attention whilst activation in other areas area likely related to specific characteristics of the task. Taken together, our findings indicate that the divided attention task is a useful paradigm for examining divided attention.

5.6.2.2 Group differences

Reduced activation in CoAMCI

The CoAMCI group demonstrated less activation in areas associated with visual object processing (extrastriate, fusiform, inferior temporal lobe), auditory speech processing (primary auditory and auditory association, insula) and attentional processing (cerebellum, lateral parietal), and these activation changes were associated with slower visual target processing (Table 13). In AD, impaired performance of a divided attention task that required monitoring of visual (chequerboard) and somatosensory (vibro-tactile) stimuli was associated with both increased activation (primary visual cortex, putamen, thalamus) and decreased (pons and cerebellar

peduncle) (Johannsen et al., 1997). The methodology and patient group of this study makes it unsuitable for detailed comparison with our findings; however, it illustrates that divided attention deficits associate with functional changes in AD. Consequently, decreased activation in visual areas could be caused by local or distant pathology that explains the decrements in processing speed in AD. Indeed, better performance during divided attention correlates with greater activation in sensory cortices in healthy controls, supporting this view (Johnson and Zatorre, 2006).

Functional-behavioural correlations

Slower responses to visual targets in AMCI correlated with decreased activation in visual processing areas (fusiform), indicating that cortical processing deficits in these areas contribute significantly to the behavioural deficits seen (Table 14). Indeed, better performance during divided attention correlated with greater activation in sensory cortices in healthy controls (Johnson and Zatorre, 2006) further supporting this conclusion.

Inter-sensory cortex inhibition or failed cognitive regulation

AD neuropathology could decrease activation in visual areas via altered inter-sensory cortex inhibition or failed cognitive regulation. Activation during divided attention appears not to be a simple summation of activation during selective attention and it consistently decreases in relevant sensory cortices during divided attention compared to selective attention. This has been ascribed to inter-sensory cortex inhibition or to limitations on processing control (§1.5.3). If decreased visual area activation was due to inter-sensory cortex inhibition then one might expect greater activation in the other sensory cortex i.e. auditory cortex during dA. However, activation was also decreased

in auditory cortex suggesting rather failed cognitive regulation. These findings imply that activation in both sensory areas decreased more in the CoAMCI group during the dA condition, or that it was already decreased during selective attention and only revealed by the dA condition.

We did not study selective visual and auditory attention using the stimuli from the divided attention task but we can consider the findings from the visual-auditory task to look for evidence of impaired selective attention in the CoAMCI group (§4.6.1.2; Table 17). Findings on *visual attention* indicate greater activation in a small area of visual cortex (BA 19) and in several other apparently task non-relevant areas in CoAMCI; however, this task involved letter stimuli and processing in the ventral visual stream is therefore not comparable with the reversing chequerboard thereby limiting the conclusions that can be drawn from a comparison. However, greater activation in many task non-relevant areas in CoAMCI strongly suggests impaired cortical regulation. The auditory conditions of the two tasks are very similar as both consisted of common English nouns. The CoAMCI group had greater activation than Controls in bilateral auditory association cortices during *auditory attention*; consequently, decreased activation during the dA condition indicates more pronounced decreases in these areas (Table 20). Greater auditory cortex activation in CoAMCI during auditory attention indicates failed attentional control per se, and decreased activation in both visual and auditory areas during divided attention is further evidence of failed attentional control; furthermore, the functional findings on the dA condition are supported by behavioural findings. Taken together, these findings indicate deficits in attentional resource allocation that result in behavioural deficits in AMCI.

Auditory processing during divided attention

Decreased activation in auditory association cortex during the dA condition may, similarly to that in visual cortex, indicate deficits and auditory processing; however, although CoAMCI responded slower to auditory targets the groups did not differ significantly. This suggests that auditory processing is more robust against the effects of AD neuropathology or that our methods were not sensitive enough.

Decreased lateral parietal activation in AMCI

CoAMCI also had decreased activation in cerebellar and lateral parietal areas that underpin attentional orientation. The dA condition makes high demands on attentional orientation because attention needs to be shifted between two stimuli every 1.75s. Lateral parietal areas, along with the PFC, exercises top-down control of attention by modulation of sensory cortex responsiveness. Consequently, decreased parietal activation may be expected to correlate with decreased sensory cortex activation and behavioural impairment in AMCI, but this was not evident. Nonetheless, lateral parietal activation did correlate with CAMCOG and New Learning subscale scores and this may indicate that parietal activation correlates more with global cognition and episodic memory than divided attention in AMCI.

Disease mechanisms

It is striking that CoAMCI had decreased activation in many of the key areas required for processing this task and not only in visual, auditory or general attention areas. This suggests that AD neuropathology has widespread cortical effects in AMCI. Of the two leading hypotheses of behavioural impairment in AD discussed above, damage to BFCS neurones could cause such widespread functional alteration as the BFCS

provides the main source of Ach that is released throughout the cortex during high attention demand. Functional connective deficits due to synaptic loss in cortico-cortical pathways can also contribute and impaired functional connectivity between parietal and medial temporal areas have been demonstrated in AMCI and AD (Sorg et al., 2007; Sorg et al., 2009).

Comparison with published findings

Our first report of divided attention in AMCI detailed the results of the comparison between Controls and RivAMCI at baseline that revealed decreased left PFC activation associated with slower RT in AMCI (Dannhauser et al., 2005). These functional and behavioural result have been replicated in AMCI using a similar divided attention task comprising visual and auditory stimuli (Laine et al., 2008). Unfortunately, the authors limited their analysis to PFC regions only and further comparisons with our findings are not possible.

The results reported here are different to our initial report and this is likely due to sample characteristics and fMRI analyses differences. The CoAMCI group reported on here comprised patients who progressed to AD whereas not all the members of the RivAMCI group of the initial report had progressed and they are therefore arguably less impaired or heterogeneous. Deterioration everyday activities, which precipitate the clinical dementia syndrome, are strongly correlated with executive impairment but not amnesia and executive functioning is strongly associated with PFC activity (Bisiacchi et al., 2008; Royall et al., 2004; Royall et al., 2005). Disparate PFC activation between these two patient groups and Controls may therefore be related to dissimilar disease severity and explain the absence of activation differences between CoAMCI and Controls in PFC areas. Resting state PFC activation appears altered in

AMCI and this maybe more or less pronounced in CoAMCI and during task performance (Qi et al., 2010). Some support for the influence of disease severity appears evident from our finding that Controls differed from AllAMCI in 100 voxels but from CoAMCI in 469 voxels (Tables 12, 13). The other possible explanation for the disparate results is related to the advanced fMRI analysis technique implemented for the results reported in this thesis but not for our first report on divided attention in AMCI mentioned above. This method is more sensitive in areas with smaller signal change and this could explain the increased number and extent of observed differences (§3.4.8.6). Analyses with this technique revealed functional-behavioural correlations that accord with theoretical and experimental data whereas such correlations were not found on the initial analysis. The newer technique therefore appears superior but it is not widely used yet and our results await replication.

Greater activation in CoAMCI

CoAMCI had greater activation in a medial posterior area that included the thalamus, brainstem and medial parietal cortex (posterior cingulate).

Thalamus

CoAMCI demonstrated greater activation in the thalamus; furthermore, this correlated negatively with visual target processing speed. Thalamic activity has an established relationship with attention and animal studies reveal that thalamic stimulation increases alertness whilst lesions impair aspects of attention (Newman and Burk, 2005). In humans, thalamic and brainstem activation associates with the onset of attention demanding choice reaction tasks, similar to the transition between the cA and dA conditions (Kinomura et al., 1996; Raizada and Poldrack, 2008). Thalamic activation appears related to attentional orientation and it plays a pivotal role in

sensory processing as information from the senses enter the cortex via the thalamus (for a review see (Newman, 1995)). It also has extensive reciprocal cortical connections with unimodal and polimodal association cortices and appears to mediate decisional processing by linking sensory stimuli with actions (Newman and Mair, 2007). Thalamic activation is therefore associated with non-specific attentional arousal that increases during periods of increased attentional demand. Consequently, greater activation in CoAMCI may indicate compensatory efforts to overcome deficits in functional connectivity with relevant sensory areas. The inability to overcome these deficits could therefore result in increased thalamic and decreased visual area activation, similar to our results. Alternatively, increased thalamic activation could be related to decision-making processes particular to the dA condition. Irrespective of the exact attentional process that is supported by the thalamus during the divided attention task, it appears altered in AMCI and correlates with behavioural impairment suggesting it is affected by AD neuropathology.

Medial parietal

CoAMCI demonstrated greater activation in the ventral posterior cingulate that forms part of the medial parietal area, which includes the precuneus and retrosplenial cortex. The medial parietal areas (BA 23, 29, 30, 31) form part of the default network that is further comprised of medial prefrontal (BA 9, 10, 24, 32), MTL (hippocampus, BA 28, 34, 35), lateral parietal (BA 39, 40) and lateral temporal areas (BA 21). This network is active at rest and its activity appears related to inwardly directed cognitive processes including memory and planning. The magnitude of *deactivation* in the default network correlates with task difficulty in healthy controls suggesting that more difficult tasks require more outwardly directed cognitive processes that in turn

reduces default activity due to cognitive resource limitations (for a review see (Buckner et al., 2008; Raichle et al., 2001). The degree of deactivation in medial parietal areas has been correlated with the severity of impairment in AMCI : less impaired AMCI deactivate more and show slower cognitive decline (Celone et al., 2006; Miller et al., 2008). These findings have been considered as evidence of compensatory efforts or loss of cognitive regulation. In AD, failure to deactivate regions of the default network correlates with poorer cognitive performance and has similarly been interpreted as loss of functional regulation (Lustig et al., 2003). Evidence of *failed* compensatory activation or, viewed differently, failed deactivation has been found in medial DLPFC areas (BA 9) in poorer performing healthy controls during divided attention (Johnson and Zatorre, 2006). The authors suggested that poor performers were unable to sufficiently recruit sensory cortices during divided attention and that greater activation in DLPFC represents compensatory efforts and illustrates functional interactions between PFC and sensory cortices. However, their findings also fit a failed deactivation interpretation. According to this view, greater activation evident in the medial parietal area in CoAMCI may be related to a failure of deactivation similar to that seen in the studies mentioned above. This is further supported by the fact that this failure of deactivation is evident in the CoAMCI group that progressed to AD but not in the AllAMCI group that included non-converters and was therefore arguably less impaired. Furthermore, this apparent failure of medial parietal deactivation is associated with impaired performance on the divided attention task in the CoAMCI group. Viewed this way, our findings support the suggestion that failure to deactivate medial parietal and other default network areas, during demanding task conditions, predicts conversion to dementia in AMCI.

Recent work relates the default network to so-called cortical hubs that represent cortical areas with a high degree of activation correlation across the entire brain (Buckner et al., 2009). Cortical hubs and default network areas overlap most strikingly in the medial and lateral parietal, and medial prefrontal areas and this in turn shows considerable overlap with areas of amyloid deposition (Buckner et al., 2005; Jack et al., 2008; Kemppainen et al., 2007; Lowe et al., 2009). These hubs are thought to be areas that integrate diverse information sources. They thereby reduce the need for segregated, specialised pathways, and minimise wiring and metabolism costs by providing a limited number of long-distance connections which integrate local networks (Bassett and Bullmore, 2006).

The medial parietal area appears particularly vulnerable to AD related structural and functional changes. Volumetric differences in this area have been demonstrated on comparisons of normal and AMCI subjects, decreased volume here predicts dementia conversion and the area suffers accelerated atrophy in progressive AMCI. Functional studies reveal medial parietal hypoperfusion and hypometabolism which predicts conversion and correlates with episodic memory performance (for a review see(Ries et al., 2008))(Xu et al., 2007). The medial parietal areas receive cholinergic input from the BFCS via the medial cholinergic pathways and are the most distant areas supplied via these pathways (Selden et al., 1998). Functional and structural changes could therefore be related to AD neuropathology in the BFCS affecting cholinergic innervation and therefore cholinergic regulation, first affecting the most distant medial parietal areas innervated by the medial cholinergic pathway (§1.6.4; Fig 1.6). This notion is supported by recent findings from diffusion tensor imaging studies that demonstrated microstructural pathology in the posterior cingulate

that distinguished AMCI from non-AMCI subjects with sensitivity of 80% and specificity of 60% (Chua et al., 2009).

Although functional and structural neuroimaging studies consistently reveal abnormalities in the medial parietal area, the direction is inconsistent as resting studies show decreased activity and studies using activation tasks show a correlation between failed deactivation and performance. This could however be explained by available findings: functional isolation of default network areas due to lesions affecting neurovascular coupling and connectivity and with other areas could leave them firing at a relatively stable rates that would appear reduced in resting studies and increased during tasks where it is deactivated in controls. Further support for this notion comes from a recent resting state functional study in AMCI that found decreased activation in the default network (bilateral medial parietal, right inferior parietal lobule, and left fusiform areas) increased activation in left PFC, inferior parietal and middle temporal areas (Qi et al., 2010).

The explanatory models discussed above for increased activation in AMCI differ and it is not implied that one mechanism will be responsible for the functional changes reported, indeed it is more likely that a variety of mechanisms contribute to the observed findings.

Functional-behavioural correlations

Better performance during divided attention has shown positive correlations with greater activation in sensory cortices in healthy controls (Johnson and Zatorre, 2006). Our results revealed similar correlations between activation in sensory areas and visual processing speed. Furthermore, visual processing speed during divided attention showed an inverse correlation with activation in the posterior medial area of

the thalamus. The thalamus is associated with alerting and attentional orienting and this correlation may indicate increased thalamic effort in more impaired CoAMCI. These correlations link behavioural and functional findings and provide strong support for the notion that the behavioural deficits are caused by abnormal brain metabolism in these areas.

Summary

Our results revealed altered activation during divided attention in task related sensory and attention processing areas and this correlated with impaired performance. Furthermore, abnormal default network activation was evident only in AMCI that progressed to AD and it therefore appears to predict disease progression in our sample, commensurate with findings from similar studies. It therefore appears that divide attention is impaired in AMCI and associated with activation changes related to local and/or distant AD neuropathology. Furthermore, altered activation correlated with neuropsychological measures of overall cognition and episodic memory and this indicates that memory and attention deficits share a common mechanism in AMCI. The most likely mechanism would involve the BFCS based on the crucial role it plays across a variety of cognitive processes including memory and attention, and the presence of AD neuropathology in the area very early on (§1.1.2; §1.6.4). Crucially, mounting evidence now indicates that progressive AMCI is associated with attentional deficits that should be considered part of the clinical syndrome and assessed more accurately as it predicts progression.

5.7 Visual and Auditory Selective Attention in AMCI at Baseline

We examined selective visual and auditory attention in AMCI at baseline by comparison to controls in order to determine if basic sensory attention processing is altered in prodromal AD.

5.7.1 Functional Findings - Visual Selective Attention

5.7.1.1 Task related activation

Visual stimulation was produced by a reversing circular chequerboard image and this generated the expected activation in bilateral visual processing areas (§4.6.1.1; Table 15). Cerebellar activation was also evident and is likely related to attentional processing and in particular attentional orientation (Cabeza and Nyberg, 2000). Activation in the cerebellum suggests that processing the visual stimuli required additional attentional resources compared to the control condition (attending to a small white cross presented on a uniform light grey background) and this is expected as the reversing chequerboard stimuli were highly salient and would therefore engage attention more.

5.7.1.2 Group differences

Control vs AllAMCI

Controls had greater activation in primary and visual association areas compared to AllAMCI (Table 16). Decreased visual cortex activation was also evident in this group during selective auditory attention that was accompanied by low level visual stimulation and therefore implies decreased visual cortex activation also during lower salience visual stimuli (§4.6.2.2.). These findings indicate altered basic visual sensory

processing in AMCI; however, primary sensory cortices are relatively spared by NFTs and show only modest accumulations even in advanced AD; NFT numbers increase gradually from primary sensory cortex, to primary (sensory) association cortex and non-primary (higher) association cortex (Arnold et al., 1991). NFT density in the higher order association cortices (ventromedial temporal lobe, inferior parietal lobe) distinguishes between normal, AMCI and AD subjects and episodic memory (delayed word recall) shows significant negative correlation with NFT counts in higher order association areas (entorhinal cortex, hippocampus) (Markesbery et al., 2006; Mitchell et al., 2002). These findings suggest that basic visual and auditory attention processing should be intact in prodromal AD if it only depends on local neuronal function. On the other hand, attentional modulation depends on long cortico-cortical interneurones between sensory and association cortices that appear particularly sensitive to NFT formation in AD. Therefore, the altered activation is more likely the effect of distant pathology.

Selective attention to a sensory modality leads to greater activation in the relevant sensory cortex and decreased activation in the cortex of the ignored sensory modalities. Decreased activation in AMCI may therefore be related to impaired cholinergic innervation due to AD neuropathology affecting the BFCS or due to lesions along the paths of the cholinergic bundles in deep white matter, or due to lesions in PFC which relays signals from visual cortex to the basal forebrain (Rasmusson et al., 2007; Selden et al., 1998).

Cholinergic modulation of sensory cortex (auditory) via stimulation of the basal forebrain has been demonstrated in animals (Metherate and Ashe, 1991) and somatosensory cortex responsiveness can be increased by repeated pairing of basal

forebrain stimulation and sensory stimulation. Furthermore, these modulatory effects of basal forebrain stimulation are inhibited by atropine, which blocks cholinergic stimulation of muscarinic receptors, and by chemical (muscimol) inactivation of PFC. (Rasmusson and Dykes, 1988; Rasmusson et al., 2007; Tremblay et al., 1990). Regardless of the specific mechanism, this apparent impairment in basic sensory attentional processing may underlie the increasing number of studies reporting attentional impairment in AMCI; however, this will need to be clarified by employing basic sensory attention processing paradigms that incorporate behavioural measures.

Taken together these findings suggest that decreased primary and secondary visual cortex activation may be due to distant lesions affecting functional connectivity thereby impairing attentional regulation.

Controls vs CoAMCI

Comparing Controls to CoAMCI did not reveal similar decreased visual cortex activation in the latter group as seen in AllAMCI and this may be related to comparatively less detection power on the smaller CoAMCI group comparison. A slightly less conservative statistical threshold did reveal the expected decreased activation in primary and secondary visual areas and adding some support to notion that distant pathology impairs modulation of cortical processing in early sensory areas (Table 17).

CoAMCI had greater activation in visual object processing areas (medial temporal, fusiform, occipital, caudate), attentional control processing areas (brainstem, PFC, lateral parietal, anterior cingulate, lateral geniculate nucleus of the thalamus), and in the anterior temporal pole that is associated with language processing. Greater activation in CoAMCI in such widespread visual and attentional

processing areas strongly suggests failed top-down regulation of attentional and sensory resources, resulting in compensatory hyperactivation and failed down-regulation of non-relevant areas. There are two alternative explanations for these findings: compensatory hyperactivation and poor task compliance. Although greater activation could be accounted for by compensatory activation, none was required as the task only required selective attention to the highly salient reversing circular chequerboard. Nonetheless, increased activation in the lateral parietal, fusiform, occipital and thalamic areas have been observed in young controls as a consequence of increased task load across visual attention and visual working memory tasks and this indicates the neural substrate of increased attentional processing demands (Tomasi et al., 2007). Increased activation in these areas in CoAMCI may indicate that they found the task more demanding due to failing resources. Greater activation in executive attentional control processing areas (lateral PFC, anterior cingulate, brainstem) could reflect failed compensatory efforts of top-down attentional modulation mechanisms to down-regulate activation in sensory and attentional processing networks that was triggered by the highly salient stimuli. We have already seen that the PFC and lateral parietal areas are affected by AD neuropathology resulting in altered functional connectivity (§1.1.3) and this could cause the failure of top-down attentional modulation. Impaired top-down regulation of attention can in turn alter activation in the visual attention areas mentioned above.

Activation in the anterior temporal pole is associated with word processing that occurs during the verbal condition and continued activation during the visual condition suggest failed down-regulation of this area due to deficits in top-down attentional modulation. Compensatory and top-down regulatory efforts could

therefore explain the increases in activation seen in the CoAMCI group but these interpretations are limited by the absence of behavioural measures.

Another possible but less likely explanation for the altered activation seen in CoAMCI is that they did not comply with task instructions and were engaged in other cognitive processes. These processes could have been related to the task, automatically precipitated by the stimuli, or unrelated and internally generated. The verbal and visual stimuli were highly salient and it would be very difficult to attend to any other stimuli and the likelihood of unrelated processing is therefore minimised. Furthermore, poor task compliance is not supported by our findings from the divided attention task that demonstrated good compliance but impaired performance. A potential contribution from poor compliance to the current findings cannot be excluded due to the absence of behavioural measures; however, given the characteristics of the task and participant compliance on the other tasks it is unlikely to account for the observed differences.

Thalamus

The role of the thalamus in attention has been discussed in detail above (§5.6.2.2). Greater thalamic activation during selective visual attention in CoAMCI may be related to perceived increased attentional demand and therefore compensatory efforts to overcome deficits in functional connectivity with relevant sensory areas.

Brainstem locus coeruleus and PFC

Greater activation in CoAMCI occurred in the brainstem area of the locus coeruleus. The role of the locus coeruleus in attention and memory has been discussed in detail in the introduction (§1.6.5). As the sole source of noradrenaline to the neocortex,

cerebellum, hippocampus and thalamus, it plays an important role in attentional selection and arousal related to environmental challenges (Aston-Jones, 2005; Foote et al., 1983). The modulatory effects of the locus coeruleus on attention and memory depend on its noradrenergic neurotransmission to key areas including the hippocampus, PFC and thalamus. Locus coeruleus lesions can exacerbate memory impairment caused by cholinergic deficits due to BCFS lesions, and promote amyloid deposition and neuronal loss in affected projection areas (Heneka et al., 2002; Heneka et al., 2006). Furthermore, an important role for locus coeruleus pathology early in the course of AD is suggested by the invariable and substantial neuronal loss affecting the small number of neurones of which it is comprised, and the associated significant reduction of cortical and limbic noradrenaline levels in AD that is highly correlated with disease progression, memory deficits and cognitive impairment. (Lyness et al.2003)(Grudzien et al., 2007; Heneka, 2009). Moreover, the extensive neuronal loss in the locus coeruleus correlates closer with disease progression than cholinergic cell loss in the BFCS (Forstl et al.,1994; Zarowet al., 2003). Several lines of evidence therefore indicates a central role for locus coeruleus lesions in the pathogenesis of AD and suggest that similar but less severe lesions are present in AMCI and responsible for altered activation during attention demanding tasks.

Findings from functional neuroimaging studies suggest that on challenging tasks attentional resources are controlled by a frontal-brainstem network wherein the brainstem signals the onset of a challenging attentional condition and the right PFC allocates cognitive resources (Raizada and Poldrack, 2008). Close correlation between activation in the PFC, locus coeruleus, visual, auditory and parietal areas was evident on a task that used sudden highly salient visual (flashed white disc) and auditory (bursts of noise) stimuli. The visual stimuli match those of our visual condition in as

far as they were highly salient, attended to and appeared at unpredictable intervals. Our results of increased locus coeruleus activation and decreased visual cortex activation in CoAMCI may therefore indicate compensatory increased drive from the locus coeruleus to visual cortex, that is maintained because the effects are not sufficient to down regulate locus coeruleus activity. In other words, decreased cortical noradrenaline due to reduced locus coeruleus release can hyperactivate the locus coeruleus and diminish connectivity with the PFC that controls sensory cortex responsiveness (§1.6.5) (Berridge and Waterhouse, 2003; Minzenberg et al., 2008). This interpretation is supported by finding that activation is also increased in the right PFC that we have seen above appears responsible for top down regulation of sensory cortices.

Alternatively, increased activation in the locus coeruleus may be related to anxiety in CoAMCI. The locus coeruleus also shows increased activation under other stressful situations including loud noise, punishment, pain, emotionally aversive images of snakes and angry faces (Raizada and Poldrack, 2008). Arguably, challenging attention tasks and anxiety producing stimuli overlap significantly: the latter will be processed as challenging especially if they appear relevant to survival, and the former may induce anxiety if particularly difficult. Both anxiety and task difficulty could therefore play a role in increased brainstem activation in CoAMCI. Increased “stress susceptibility” has been demonstrated in MCI. A 3-year prospective study of the association between anxiety symptoms and progression to AD in MCI found that anxiety symptoms were twice as prevalent (47%) as in the healthy elderly population; furthermore, MCI patients with anxiety symptoms were twice as likely to develop AD (83%)(Ausen et al., 2009; Palmer et al., 2007). Clinical observations therefore indicate that anxiety is more prevalent in MCI that progress to AD, therefore

most likely AMCI, and increased locus coeruleus activation is therefore very likely partly related to this.

In summary, based on the role of the locus coeruleus in attention and the presence of AD neuropathology in this area and the known effects thereof on attention, our result in CoAMCI suggest that locus coeruleus lesions may contribute to altered activation and attentional performance in AMCI. Furthermore, the increased prevalence of anxiety in prodromal AD and the established association between locus coeruleus activity and anxiety suggest that increased anxiety in the CoAMCI group – related to perceived increased task difficulty on the background of AD neuropathology- may also contribute to increased activation.

The resolution of fMRI is not sufficient to be certain that the activation in the brainstem is located in the locus coeruleus. However, Raizada and Poldrack argue persuasively that other nearby nuclei are unlikely to activate under the given task conditions and activation on the locus coeruleus under demanding and stressful attention conditions is supported by animal studies (Raizada and Poldrack, 2008). Localising activation during challenging attention tasks to the locus coeruleus is therefore supported by (1) the pattern of neural activity, (2) established functional connectivity and (3) the task conditions under which it occurs.

Behavioural data

Without behavioural data, it is not possible to determine the exact contributions from compensatory efforts and poor compliance to the altered activation in AMCI; however, the results do indicated impaired basic sensory processing in AMCI that could be associated with impaired behavioural performance similar to that seen on the divided attention task. To date, three studies have reported impaired selective visual

attention performance in AMCI and it is therefore likely that the functional differences we demonstrated in AMCI are associated with behavioural deficits (Perry and Hodges, 2003; Silveri et al., 2007; Tales et al., 2005a).

Summary

Taken together, our findings indicated altered selective visual attention processing in AMCI that may indicate compensatory mechanisms and/or failed cortical regulation. Differences were evident across the cortex and this could be caused by pathology in the BFCS and/or locus coeruleus that both have widespread afferents to the brain and influence on activation.

Selective visual attention processing appears altered in AMCI and this has potentially far reaching clinical and research implications. Altered visual processing can potentially confound tests of any cognitive domain if they require visual processing. Clinically, evidence of altered visual processing together with impaired divided attention suggest that patients with AMCI may be at risk when completing complex everyday activities that depend heavily on these functions, such as driving.

5.7.2 Functional Findings - Auditory Selective Attention

5.7.2.1 Task related activation

Robust activation in bilateral auditory (lateral temporal) and verbal processing (VLPFC, temporal pole, insula) areas, as well as lateral parietal attention areas occurred in response to auditory verbal stimulation. Activation in auditory and language processing areas were expected and increased parietal activation likely indicates that this condition had additional attentional processing demands compared

to the control condition (for a review of language processing see (Demonet et al., 2005).

5.7.2.2 Group differences

CoAMCI demonstrated greater activation in auditory association, language processing (insula, putamen), attentional orienting and alerting (thalamus), and MTL areas. Similar greater MTL (hippocampal) and thalamic activation was seen in CoAMCI on the visual condition discussed above, indicating that it persists across sensory modalities suggesting it may be generalised.

Greater activation in CoAMCI

MTL

The MTL is not strongly associated with attentional processing and greater activation in CoAMCI suggests impaired functional regulation that normally deactivates non-relevant cortices. The MTL and particularly the hippocampal formation is part of the default network that is active at rest and thought to be concerned with inwardly directed cognitive processes that included episodic memory and therefore MTL activity (§5.6.2.2). Altered activation in this area appears an increasingly consistent finding in AMCI. The magnitude of deactivation in the default network correlates with task difficulty in healthy controls indicating that inwardly directed cognitive processes are abandoned when task demands are high (for a review see (Buckner et al., 2008) (Raichle et al., 2001). Greater hippocampal activation in MCI compared to controls (hyperactivation) during episodic memory encoding (novel face name pairs, novel scenes) predicted faster rates of subsequent cognitive decline (Celone et al., 2006; Miller et al., 2008). In AD, failure to deactivate regions of the default network

also correlates with poorer cognitive performance and this has been interpreted as loss of functional regulation (Lustig et al., 2003). Taken together, these findings suggest that AD and AMCI are associated with impaired cognitive regulation resulting in maintained activation in default network that hinders processing in task relevant areas and impairs performance. Our findings suggest that this inverse correlation between hippocampal activation and cognitive performance is not specific to memory processing but also evident during attentional processing that therefore points to a more generalised deficit.

Thalamus

CoAMCI demonstrated greater activation in the thalamus and this was also evident during selective visual attention (§5.7.1.2) and divided attention (§5.6.2.2). It is therefore evident across selective and divided attention conditions, and in visual and auditory sensory modalities in CoAMCI. Activation in the thalamus is associated with the onset of attention demanding tasks and it mediates decisional processing. Greater thalamic activation can therefore be related to increased or compensated non-specific attentional arousal or to specific sensory attentional processing and decision making particular to the dA condition. Furthermore, increased thalamic activation was also evident in CoAMCI during verbal episodic encoding and the fact that this was evident across attentional conditions suggests that it may be related to attentional processing during encoding. The findings of greater thalamic activation across tasks suggest that it is a robust generalised alteration in brain activation in AMCI that predicts disease progression.

Overlap of areas of increased activation in CoAMCI across experiments.

Areas of increased activation in CoAMCI overlapped in several areas on verbal episodic encoding and selective auditory attention. These included attention (thalamus), auditory (primary, association) and language processing (insula, putamen) areas. Both experimental conditions required word processing and increased activation across conditions suggest generalised altered language processing.

Summary

CoAMCI had greater activation in visual object processing areas, attentional control areas and in language processing areas during the *visual attention* condition. These findings suggest failed top-down regulation of attentional and sensory resources. Impaired top-down regulation of attention can in turn alter activation in attentional orienting and alerting areas (brainstem, thalamus) and other visual processing areas. Greater activation in the locus coeruleus, the main source of noradrenaline to the brain, may reflect changes in arousal during challenging attention tasks and/or increased anxiety in AMCI. AD neuropathology is present in the locus coeruleus in AMCI and can potentially have widespread effects leading to impairment in attention and memory.

CoAMCI demonstrated greater activation in auditory, language, attention and MTL areas on selective *auditory attention*. Similar greater MTL and thalamic activation was seen in CoAMCI on the visual condition discussed above and this persistence across sensory modalities indicates that it is generalised. MTL activity likely reflects failed deactivation of default network areas suggesting failed cognitive regulation, as found in AD.

Greater thalamic activation may be related to increased or compensated non-specific attentional arousal or to specific sensory attentional processing and decision making. Increased thalamic activation was evident across visual, auditory and divided

attention, and also evident in CoAMCI during verbal episodic encoding. This indicates that increased thalamic activation is a robust generalised brain activation alteration in AMCI that predicts disease progression. Furthermore, several areas of increased activation overlapped between verbal episodic encoding and selective auditory attention, indicating generalised altered language processing. Taken together, these findings indicate altered cortical processing of basic visual and language stimuli in CoAMCI that may contribute significantly to altered processing *and* correlated behavioural deficits that we report on higher attention and episodic memory. These findings suggest generalised altered processing that is most likely caused by AD neuropathology in the BFCS and/or locus coeruleus noradrenergic systems that provide Ach and NA that regulate almost every aspect of cognition.

5.8 Verbal Episodic Encoding at Baseline

We studied verbal episodic memory in AMCI to determine the behavioural and functional correlates of the characteristic amnesia in prodomal AD. Available findings suggest that the episodic amnesia may in part be due to PFC dysfunction and related executive failure and we therefore employed a verbal memory paradigm sensitive to executive semantic processing. In relation to the neural correlates of amnesia, we conducted whole brain fMRI to investigate for potential contributions from abnormalities in MTL and other areas to episodic amnesia in prodromal AD.

The behavioural results revealed that verbal episodic amnesia in AMCI partly relate to executive failure (semantic elaboration deficits) at encoding and also to more liberal response bias as seen in AD. Furthermore, memory deficits correlated

positively with attention deficits, suggesting that executive failure contributes to deficits across cognitive domains in prodromal AD.

Functional comparisons revealed greater activation in CoAMCI in visual, auditory, language and attention processing areas, and in default network areas. Correlations were evident between activation in these areas, memory performance and neuropsychological measures. These findings suggest compensatory activation in task related areas and failed cognitive regulation (attenuation) in non-relevant areas in prodromal AD. These findings are discussed in detail below.

5.8.1 Behavioural Findings

Encoding success is measured indirectly by recognition tests and behavioural findings from the recognition task are therefore discussed in this section.

Corrected recognition

CoAMCI and AllAMCI were impaired on recognition as indicated by decreased corrected recognition rates and this is in keeping with the clinical diagnosis of AMCI (§4.8.1; Table 25). Corrected recognition was even further decreased in CoAMCI compared to AllAMCI, suggesting that the advanced pathology that lead to progression to AD in the former group resulted in lower corrected recognition rates.

Corrected recognition rate (Pr) is a compound score and the difference between Control and CoAMCI were predominantly due to elevated *false alarm rates* in CoAMCI, whereas the groups were comparable on *hit rates*. Equivalent *hit rates* but higher *false alarm rates* in the CoAMCI group could be explained by a response bias towards familiarity i.e. more liberal, resulting in a greater tendency to select KNOW and REMEMBER responses. This notion is supported by trends towards

liberal bias in CoAMCI and towards conservative bias in Controls. In very mild AD, unequivocal liberal bias, unrelated to impaired recognition, has been found during verbal encoding (mean MMSE= 26) and appeared related to PFC or hippocampal dysfunction (Budson et al., 2006). Available evidence supports the notion that liberal response biases may be due to PFC dysfunction in AD; normal response biases have been demonstrated in patients with isolated amnestic disorders (Korsakoff's, MTL pathology) but more liberal response biases for patients with AD that is associated with PFC dysfunction (Snodgrass and Corwin, 1988). The liberal bias observed in our AMCI groups may therefore represent an early manifestation of that found in AD.

The respective contributions from encoding and recognition processing deficits to altered response bias in prodromal AD cannot be determined from our data. It could be argued that reliable encoding processes, such as that evident in Controls, would lead to conservative recognition bias because responses would be made with greater certainty. On the other hand, unreliable encoding may lead to consciously adopted compensatory strategies that involve more guesswork as suggested by the higher false alarm rates in AMCI. Although the exact mechanism remains to be determined, prodromal AD appears to be associated with more liberal response biases similar to AD.

False recognition for lures

As a proportion of false alarms, AMCI subjects had significantly reduced *false recognition for lures.* The parameter *false recognition rate for lures* [*lure hit rate* (*lures* identified/total *lures*) – *false alarm rate* (*false alarms*/non-targets) has a value anywhere between 1 and -1. A rate of zero, as seen in the AMCI group, indicates

similar *lure hit rates* and *false alarm rates*; a value above zero, as seen in the control group, indicates a *lure hit rate* that exceeds the *false alarm rate*. Healthy memory functioning is associated with higher *lure hit rates* because it depends on semantic elaboration that in turn makes it more vulnerable to false recognition of semantically associated lures. Reduced *false recognition rates for lures* evident in AMCI have also been reported in AD and taken as evidence for semantic elaboration failure (Koutstaal et al., 2001; Schacter et al., 1997). Whether this failure occurs at encoding or recognition is not clear; nevertheless, verbal memory impairment in amnestic syndromes (due to MTL and PFC damage) appears to be mediated by semantic elaboration failure at encoding rather than failure of retrieval (Verfaellie et al., 2005). Similarities between behavioural measures and lesion locations in AMCI and amnestic syndromes suggest that semantic elaboration failure occurs at encoding in AMCI.

Semantic elaboration failure at encoding may be related to impaired generation (recall of related information), maintenance or encoding of semantic information and semantic generation deficits (impaired person naming and category fluency) have recently been demonstrated in AMCI (Artero et al., 2003; De Jager et al., 2003; Dudas et al., 2005). In AMCI, reduced *false recognition rates for lures* in the face of comparable *hit rates* indicate that correct familiarity decisions (*hits*) were made largely independently from semantic processing. We suggest this is due to failed generation and encoding of semantic information because both these processes are impaired in AMCI.

Taken together these findings indicate that verbal episodic amnesia in prodromal AD is partly related to semantic elaboration failure at encoding. More

liberal response biases at recognition, that increase the number of commission errors, may also contribute to amnesia.

Correlations between encoding and divided attention behavioural measures

Visual processing speed was impaired during divided attention in AMCI and this correlated with verbal recognition performance: faster visual processing speed correlated positively with better recognition performance and higher *false recognition rates for lures*, and negatively with *false alarm rates*. Superior recognition performance and high *false recognition rates for lures* depend on semantic elaboration and therefore executive processing. Divided attention also depends on executive processing and the positive correlation between recognition and divided attention performance indicates the presence of generalised executive deficits that contribute to attentional and memory deficits in prodromal AD. Furthermore, finding that age did not correlate with measures that differed between CoAMCI and Controls indicates that executive impairment in prodromal AD is related to neuropathology and not age.

In summary, executive failure appears to contribute significantly to verbal episodic amnesia in prodromal AD. Moreover, the correlations between memory and attention deficits in AMCI indicate a central role for executive failure in the cognitive deficits of prodromal AD. These findings suggest that pathology outside the MTL affects cognition early in the course of AD and, considering the pivotal role cholinergic neurotransmission plays in executive processing, that BFCS lesions may be responsible.

5.8.2 Functional Findings

5.8.2.1 Task related activation

For the encoding task, overt vocalisation and intentional encoding of different words were contrasted with only overt vocalisation of a repeated control that therefore did not require encoding. Encoding and control conditions were therefore matched in terms of the sensory processing and overt reading, whilst crucially differing on the requirement to encode. Although it is desirable to minimize movement during fMRI, we considered overt vocalisation desirable in order to ensure compliance throughout the relatively long duration of the task, given the cognitive deficits of the patient group. The potential difficulty of movement artefact was addressed by inserting a delay after vocalisation and prior to image acquisition, as used in similar studies (Birn et al., 2004; Shuster and Lemieux, 2005).

Activation was evident in areas related to encoding and semantic elaboration, reading and speech production, and visual attention (§4.7.2.1; Table 21). We will look at these in turn.

Encoding and semantic processing areas

Activation in PFC, MTL and cerebellar areas was expected due to encoding and semantic processing. The activation of left PFC during verbal encoding is similar to that reported in 21 out of 24 studies involving intentional or incidental verbal encoding in controls (Fletcher and Henson, 2001) and in early AD (Lustig et al., 2003). A positron emission tomography imaging study in healthy controls examined overt single word processing with an experimental design similar to the one we used. Bilateral activation was found in DLPFC when words were read aloud without further processing, and additional left VLPFC and anterior cingulate cortex activation when

semantic elaboration took place (Petersen et al., 1988). PFC activation therefore appears related to semantic and speech processing.

We have seen in the introduction (§1.4.5) that left PFC activity relates to deep meaning-based semantic processing that optimises memory and which involves the executive processes of *generating, maintaining, selecting and organising* semantically related information. Furthermore, disruption of left PFC function impairs verbal encoding and disrupting semantic elaboration results in decreased left PFC activation and reduced memory performance. These findings indicate the pivotal role that the PFC plays in semantic elaboration. Activation in the left VLPFC relates to semantic processing during verbal encoding whereas activation in DLPFC reflects the working memory processes of reorganising information and subvocal rehearsal of working memory content (for reviews see(Desgranges et al., 1998; Fletcher and Henson, 2001). Activation in both PFC regions indicates that both semantic and working memory processing took place during encoding. Semantic elaboration improves encoding and the left PFC activation seen in our groups probably reflects such a spontaneously adopted encoding strategy (Craik and Lockhart, 1972; Craik and Tulving, 1975; Logan et al., 2002).

Our findings of bilateral PFC activation are in line with the hemispheric asymmetry reduction in old adults (HAROLD) model that proposes a reduced lateralisation in older adults (Cabeza, 2002; Dolcos et al., 2002). It is not clear what causes the loss of lateralisation in older adults and possible mechanisms include compensatory activation and/or a reduction in the number of task specific networks with the result that non-specific networks are activated (Cabeza et al., 2000; Logan et al., 2002; Reuter-Lorenz et al., 2001). A study specifically designed to examine the causes of additional activation in contralateral areas concluded that increased

activation in additional areas was related to extra attentional and monitoring demands required for superior performance (Anderson et al., 2002).

Encoding and semantic elaboration interact closely as revealed by findings demonstrating that the extent of activation of the left frontal cortex and hippocampus during verbal encoding correlates positively with subsequent successful recognition (Morcom et al., 2003; Wagner et al., 1998). Furthermore, activation in MTL and PFC predicts subsequent recall performance across a variety of encoding tasks and demonstrate the interaction between these two key areas in episodic encoding (Kirchhoff et al., 2000; Sperling et al., 2003a; Staresina and Davachi, 2008; Wagner et al., 1998).

Right-sided posterior MTL activation was evident in the combined groups. Encoding verbal material is most often associated with left sided activation but several studies have revealed activation similar to what we found (Cabeza and Nyberg, 2000; Henson, 2005). Recent studies have also revealed correlations between memory performance and parietal and cerebellar activation (Brassen et al., 2006; Fliessbach et al., 2007; Staresina and Davachi, 2006).

In summary, the PFC and MTL are key nodes in the network that underpins verbal episodic memory and this network also receives contributions from other cortical areas depending on the task.

Reading and speech related areas

Activation in auditory and language processing areas were expected as the task required overt reading (for a review of language processing see (Demonet et al., 2005). Bilateral cerebellar activation, present in both our groups, has frequently been found during verbal episodic encoding and also during overt speech generation

(Cabeza and Nyberg, 2000). Additional cerebellar activation during the ENCODE condition is likely related to word form complexity and articulation differences between the various words read during the ENCODE condition and the single repeated CONTROL word. Alterations in word form have been shown to induce cerebellar activation (Shergill et al., 2003). Other areas activated in both groups are also associated with overt speech and include left primary motor cortex (BA 4), premotor cortex (BA 6), insula and superior temporal cortex (Wernicke's area) (Shuster and Lemieux, 2005).

Visual processing and attention areas

Activation in visual processing areas following letter stimuli has been discussed in detail above (§5.6.2.1). Activation in extrastriate and fusiform areas indicates letter processing that differs from the control condition (Corbetta et al., 1991; Heinze et al., 1994; Mangun et al., 1997).

5.8.2.2 Group differences

Decreased activation in AllAMCI

AllAMCI had decreased activation in the left PFC (frontal pole) and this was associated with impaired semantic processing.

In AMCI, impaired memory in association with decreased PFC activation has been demonstrated on a face-name paired associates encoding (Petrella et al., 2006), intentional picture naming encoding (Trivedi et al., 2008) and incidental picture encoding (Mandzia et al., 2007) (§1.4.6.2). These results are similar to what we report in the PFC; however, the use of visual stimuli and semantic elaboration support in these other studies disallows direct detailed comparisons. However, decreased PFC

activation occurred in AMCI across these memory tasks. Altered PFC activation therefore appears to contribute to the memory across picture and verbal encoding tasks and is likely associated with decreased semantic elaboration (§1.4.2.3).

An fMRI study of activation exclusively for words that were subsequently retrieved demonstrated greater activation in AMCI subjects in left MTL, medial PFC, anterior cingulate and post central areas (Kircher et al., 2007). Increased activation therefore appeared compensatory. Due to the differences in methodology direct comparisons are not possible; we examined all encoding attempts so our results are of activation during successful *and* failed encoding. However, altered PFC activation appears across several of the available studies of episodic memory in AMCI and therefore appears to contribute significantly to the observed amnesia. This is in line with current models of memory processing networks and the distribution of AD neuropathology; furthermore, it suggests that even at the early stages of AD, pathology outside the MTLs contribute to amnesia.

Given the multiple correlations between PFC activity and semantic elaboration (§1.4.5), the results in the AllAMCI group suggest that the semantic processing failure is closely related to decreased PFC activation which in turn may be due to local pathology or decreased cholinergic innervation from the BFCS (§1.6.2) or decreased NA from the locus coeruleus (§1.6.5; 5.7.1.2). Interestingly, in very mild AD, in-vivo PET imaging of amyloid deposition have revealed greater amyloid load in frontal cortex compared to parietal and medial temporal cortices (Klunk et al. 2004). We have already seen that AD neuropathology can alter activation in cortical areas because of local pathology, or deficits in cognitive regulation (§5.6.2.2).

Including age as a covariant produced similar results and indicates that functional differences are predominantly due to AD neuropathology.

Taken together, these findings indicate a central role for altered PFC activation and associated semantic processing failure in AMCI with unknown outcome. We next look at the results from the CoAMCI group that progressed to dementia.

Increased activation in CoAMCI

CoAMCI had greater activation in areas processing sound, language, attention, and visual stimuli. Significant correlations were evident between activation in the areas of difference, behavioural measures, and neuropsychological measures. We will look at these in turn. There are to our knowledge no published findings on verbal episodic encoding in progressive AMCI and direct comparisons of findings is therefore not possible. We therefore make comparisons with available findings.

Language and visual processing areas

CoAMCI had greater activation in right lateral temporal and insular areas that activate during overt reading, language processing, environmental noise processing and reordering of verbal items in working memory (Cabeza and Nyberg, 2000; de Zubicaray et al., 1998). All of these cognitive processes likely occur during the encoding task. It is apparent that the majority of Controls did not activate the right temporal auditory cortex whereas the majority of CoAMCI did (Figure 16). This suggests compensatory activation or impaired cognitive regulation.

Compensatory efforts could include working memory-based strategies such as subvocal word rehearsal that activate auditory cortex. Although such a strategy is possible, it would not be very practical because as the list grows there would not be enough time to rehearse it between stimuli.

Alternatively, increased activation may indicate impaired cognitive regulation similar to that seen in several cortical areas during the divided attention task in CoAMCI (§5.6.2.2). For example, AMCI may have deficient down-regulation of activation in auditory areas induced by environmental (scanner) noise, resulting in greater activation compared to Controls that have intact down-regulation. Failed cognitive regulation can interfere with the processing of relevant stimuli and this is supported by the finding that better performance correlates with greater deactivation in right temporal areas. We have seen evidence for impaired cognitive regulation on other tasks in AMCI and this is certainly the leading explanation for increased activation in task-irrelevant areas. The same holds for increased activation seen in extrastriate visual areas. It is difficult to determine if increased activation in areas that process both relevant and irrelevant stimuli, such as words and environmental noise, is due to compensatory activation or failed regulation; however, the distinction may be that compensatory activation should normalise behaviour whilst failed regulation would not.

Posterior cingulate and medial parietal areas

CoAMCI demonstrated greater activation in the posterior cingulate and precuneus during encoding and this correlated negatively with corrected recognition rates, lure recognition rates, CAMCOG scores and New Learning subscale scores (Table 24; Figure 17). Greater activation in this area in CoAMCI is therefore negatively correlated with 4/5 behavioural measures on which they are impaired. The posterior cingulate forms part of the default network (§5.6.2.2) and similar findings were evident on the divided attention task. Moreover, we have seen how the magnitude of *deactivation* in the default network correlates with task difficulty in healthy subjects

and that failure to deactivate medial parietal areas correlates with greater severity and decline in AMCI (Buckner et al., 2008; Celone et al., 2006; Miller et al., 2008; Raichle et al., 2001). This has also been found in AD, where failed deactivation of default network areas correlates with poorer cognitive performance, and it has been attributed to impaired functional regulation (Lustig et al., 2003). Our results correspond with those in AMCI and AD and this indicates that episodic memory deficits are due in part to failed cognitive regulation of the default network in AMCI.

Moreover, we have reported greater activation in the medial parietal area on two separate tasks (encoding, divided attention) in CoAMCI but not in AllAMCI. AllAMCI included non-converters that are arguably less impaired and this finding corresponds with those mentioned above indicating that medial parietal deactivation failure correlates with greater severity and decline in AMCI. Our findings therefore correspond with those from studies in AMCI and AD and indicate that failed default network deactivation, and in particular in the medial parietal areas, is a pervasive abnormality in AMCI that predicts progression to dementia.

Anterior cingulate, caudate, putamen and thalamus

Anterior cingulate activation occurs during sustained attention, verbal working memory tasks, problem solving, semantic elaboration, episodic encoding and retrieval (Cabeza and Nyberg, 2000). Activation in the caudate and putamen is also associated with verbal working memory encoding and maintenance. Activation in the right caudate appears specifically involved in verbal working memory maintenance and taken together these findings support the notion that increased activation in these areas in CoAMCI may be related to compensatory efforts that involve verbal rehearsal strategies (Chang et al., 2007).

Increased activation was also evident in the right thalamus and we have seen that the thalamus activates during attention demanding tasks and it mediates decisional processing (§5.6.2.2). Greater activation is therefore likely related to increased or compensated attentional arousal efforts due to perceived greater task difficulty in CoAMCI. Increased thalamic activation was also evident during selective visual (Table 17; §4.6.1.2), selective auditory (Table 20; §4.6.2.2) and visual-auditory divided attention (Table 13; §4.5.2.2), and this indicates altered attentional arousal processing across attention conditions. The relationship between thalamic activation and attention is supported by the positive correlation between thalamic activation and visual processing speed during divided attention (Table 14; Figure 15). It therefore appears that thalamic activation is increased across attentional conditions in AMCI due to compensatory attentional arousal efforts.

Functional-behavioural correlations

We have seen that superior recognition performance correlated with decreased activation in lateral temporal (Figure 16) and medial parietal areas (Figure 17) where CoAMCI had increased activation. We also studied correlations between activation in these areas and neurocognitive measures (Table 24). Similar negative correlations between activation in these two areas were evident with CAMCOG total scores, indicating that it generalises to out-of-scanner cognitive performance. Furthermore, activation in the medial parietal area also correlated inversely with New Learning subscale scores, illustrating that it generalises across verbal episodic memory tasks. This lends further support to the growing body of evidence that abnormality in the medial parietal areas is an early feature of AMCI that predicts conversion to AD. Finding that medial parietal activation also correlates inversely with false recognition

for lures indicates that normal default network deactivation correlates with increased semantic elaboration.

Decreased activation in CoAMCI

There were no areas of decreased activation in CoAMCI whereas decreased activation was evident in the PFC in AllAMCI. It also does not appear as if this is due to a lack of detection power as a more liberal statistical threshold did not reveal PFC differences. The only conclusion that can be drawn is that PFC activation was comparable between CoAMCI and Controls, indicating maximal activation, and that the non-converters in the AllAMCI group somehow had decreased PFC activation that contributed to the observed difference. A bi-phasic pattern of cortical activation changes have been demonstrated in AMCI in MTL areas during encoding and a similar pattern could be responsible for these findings in our groups (§1.4.6.2)(Celone et al., 2006).

Summary

Behavioural findings on the verbal episodic encoding task indicate that failed semantic elaboration contributes substantially to verbal episodic amnesia in AMCI. Furthermore, the correlations between encoding and divided attention performance indicates a central role for executive impairment in the symptomatology of AMCI and AD. This accords with findings indicating that deterioration everyday activities, which precipitate the clinical dementia syndrome, are strongly correlated with executive impairment but not amnesia (Bisiacchi et al., 2008; Royall et al., 2004; Royall et al., 2005).

Our findings suggest the presence of both compensatory and failed cognitive regulatory mechanisms during verbal episodic memory encoding in CoAMCI. Functional findings indicate that altered activation outside the MTL contributes significantly to the amnesia in AMCI. In particular, failed medial parietal deactivation was evident in AMCI that converted to AD and this correlated with episodic amnesia. Failed medial parietal deactivation was also evident and associated with impaired performance on divided attention in CoAMCI. Taken together with the findings from the divided attention and visual-auditory attention tasks, AMCI appears to be associated with failed cognitive regulation across encoding and attention conditions. Furthermore, we have now presented functional and behavioural evidence of executive impairment on two task and this suggests a much more central role for executive failure in disease progression in AD. This in turn supports disease models such as BFCS and locus coeruleus pathology that have generalised effects on cognition such as we have discussed.

5.9 Recognition at Baseline

We examined the functional correlates of verbal recognition in AMCI to (1) determine if successful recognition is associated with altered functional activation that suggests compensatory activation or strategies, and (2) to examine the functional correlates associated with failed recognition. The behavioural results from the recognition task have been discussed in detail in the above section on encoding (§5.8.1), and behavioural findings related to the discussion of functional data are provided in relevant sections and briefly summarised below.

5.9.1 Behavioural Findings

AMCI patients were impaired on recognition as indicated by lower *corrected recognition rates* (§4.8.1; Table 25). They had elevated *false alarm rates* that appear related to more liberal response bias rates, similar to that demonstrated in AD. As a proportion of false alarms, AMCI subjects displayed significantly reduced *false recognition for lures* suggesting failure of semantic elaboration at encoding. Visual processing speed was impaired during divided attention in AMCI and this correlated with verbal recognition performance: faster visual processing speed correlated (1) positively with better recognition performance and higher *false recognition rates for lures*, and (2) negatively with *false alarm rates*. These correlations of measures across tasks suggest impairment in a shared mechanism and a prime candidate is executive functioning because it controls attention and optimises memory. These correlations therefore support the notion that executive failure contributes to cognitive deficits in prodromal AD.

5.9.2 Functional Findings

5.9.2.1 Task related activation

Verbal recognition typically activates right hemispheric DLPFC, VLPFC, anterior cingulate, parietal areas (Cabeza and Nyberg, 2000). A study involving a large control group of 77 healthy young and middle-aged adults found activation in posterior cingulate, precuneus, lateral parietal lobe, right lateral temporal lobe and right prefrontal cortex (Johnson et al., 2006a); activation was lateralised to the right. These same areas showed activation during the processing of *hits* and activation was less lateralised in line with the HAROLD model as discussed above (§5.8.2.1) and in the

introduction (§1.1.3)(Tables 26, 27). Activation during the processing of *correct rejections* was similar to that reported in as healthy older adults during a similar verbal recognition task (Heun et al., 2007). Findings during recognition processing are therefore in line with published findings, which therefore indicate the task can be utilised for group comparisons in older adults.

5.9.2.2 Group differences

Hits – behavioural results

Behavioural findings suggest altered cortical processing during correct target word recognition (*hits*) in prodromal AD, as evident from two findings. Firstly, although Controls and CoAMCI were comparable on *hit rates,* a trend towards lower *hit rates* in CoAMCI suggests that impairment may be evident given larger samples. Secondly, there is some indication that CoAMCI achieved statistical behavioural equivalence via disparate cognitive processing: comparable *hit rates* were associated with increased *false alarm rates*. More liberal response *bias rates* appear to have elevated *hit rates* in CoAMCI as participants were more inclined to make familiarity responses; however, this came at the expense of increased *false alarm rates* resulting in overall poorer *corrected recognition rates*. In other words, CoAMCI appear to have comparable *hit rates* because they were more likely to choose REMEMBER or KNOW responses when they were unsure. This notion of disparate cognitive processing is supported by the imaging data that revealed altered activation in 1284 voxels. We next look at these activation differences.

***Hits* - decreased activation in CoAMCI**

CoAMCI had decreased activation in areas processing visual stimuli (visual cortex, fusiform) executive attention (PFC, thalamus, brainstem, cerebellum, posterior cingulate), working memory (caudate) and language (auditory cortex, insula, putamen) (Table 28). This could either indicate processing deficits that were insufficient to cause behavioural impairment or the use of compensatory strategies that were less reliant on the processes that activate these areas in Controls.

A recent fMRI study of recognition in AMCI found predominantly decreased left parietal and right posterior temporal activation compared to controls; however, a picture encoding task was used and activation examined during all recognition attempts and direct comparisons with our results are therefore not appropriate (Machulda et al., 2009). In spite of these methodological differences, it appears that recognition deficits are associated with altered cortical activation in prodromal AD for both verbal and visual episodic memory.

***Hits* – increased activation in CoAMCI**

Increased activation occurred in several areas including frontal polar, medial parietal, MTL, brainstem, secondary visual and auditory, caudate and putamen (Table 29). Frontal polar cortex (BA 9,10) is strongly associated with episodic memory retrieval, problem solving and sustained attention, and increased activation here may indicate that greater cognitive effort was required in task related areas to match *hit rates* of Controls (Cabeza and Nyberg, 2000).

Increased activation also occurred in bilateral MTL and medial parietal areas, that form part of the default mode network that also includes anterior polar cortex discussed above. We have seen previously (§5.6.2.2) that activation in this network appears related to inwardly directed cognitive processes and that the magnitude of

deactivation in the default network correlates with task difficulty in healthy controls and with the severity of impairment in AMCI (less impaired AMCI deactivate more and show slower cognition decline). Furthermore, greater hippocampal activation in MCI compared to controls (hyperactivation) during episodic memory appears to predict faster rates of cognitive decline (Celone et al., 2006; Miller et al., 2008). Accordingly, greater medial parietal and MTL activation in CoAMCI may be related to deactivation failure caused by impaired cognitive regulation resulting in maintained activation in default network that hinders processing in task relevant areas and impairs performance. Our findings therefore suggest that cognitive performance correlates negatively with activation in MTL and medial parietal areas across encoding, recognition and divided attention processing.

Opposite findings in the medial parietal area have been reported during picture recognition in AMCI (n=14; 8 receiving cholinesterase inhibitor treatment) compared to age-matched elderly controls (Johnson et al., 2006a). Comparisons with our results are limited because the outcome measure used by Johnson et al. (percentage correct) does not take account of false recognition and their AMCI group was not impaired on recognition, which is difficult to reconcile with a diagnosis of AMCI. Furthermore, the majority of their AMCI group received ACEI treatment that can alter cortical activation (§1.7.5).

Positive correlations between retrieval performance and activation in the MTL and medial parietal areas have been demonstrated across a large group comprised of MCI, AD and age-matched control subjects (Heun et al., 2006a). Their memory paradigm was similar to ours, requiring encoding of visually presented nouns followed by and yes/no recognition task, and it revealed similar behavioural impairments. A significant positive correlation was evident between the magnitude of activation for *hits* and

corrected recognition rates in the left hippocampus and posterior cingulate gyrus across the groups but not for the subgroups. They did not report on group differences so it is unclear if activation differed between controls and AMCI. Although not reported, their results indicate that AMCI would have demonstrated decreased activation in the MTL and medial parietal areas given the positive correlations with recognition performance, therefore the opposite of our results.

A recent study of picture recognition in AMCI found decreased MTL and increased PFC activation, the MTL findings therefore also being the opposite of what we found (Trivedi et al., 2008). Their AMCI group differed from our CoAMCI as they were older and better educated and arguably less impaired, as they were not selected because of progression to AD. Our imaging results on attention and encoding that indicate (1) greater volume (voxels) of differences between CoAMCI and Controls than AllAMCI and Controls (§4.5.2; 4.6.1.2; 4.7.2.2), and (2) the correlations between activation and performance (Figures 14-6) suggest that disease severity significantly influences activation. Moreover, other studies have also found that the degree of functional alteration correlates with the severity of cognitive impairment and speed of cognitive decline in AMCI (Celone et al., 2006; Miller et al., 2008). Therefore, the most likely explanation for the discrepancy between our results and those of Trevedi et al.is that their AMCI group was less impaired than our CoAMCI group. Task characteristics may also contribute to the different findings as picture and verbal memory engages both overlapping and different areas. Also, they compared activation during *hits* with that during *misses*, whereas we compared *hits* to all non-task activation, the contrasts generated are therefore different. Furthermore, significant results were only obtained when their analysis was limited to specific regions of interest whereas our results were evident on whole brain analysis. Direct

comparisons between our findings and those of Trevedi et al. are therefore limited by the group, task and analysis differences, and our work therefore awaits replication before firmer conclusions about can be drawn about processing of *hits* in prodromal AD.

Correct rejection

Functional differences between CoAMCI and Controls on *correct rejections* overlaps largely with that seen on *hits* suggesting that these closely related cognitive processes are affected by very similar processing changes (Table 30). CoAMCI showed increased activation in PFC (BA 6) and VLPFC (BA 44, 45) and similar findings in AMCI has lead to the suggestion that it could be used as an early marker of AD (Heun et al., 2007).

False recognition of lures

CoAMCI had decreased activation in occipitoparietal areas and increased activation in frontotemporal areas on the *false recognition for lures* contrast (Table 31). This was associated with decreased *false lure recognition rates* indicating impaired semantic elaboration at encoding. To date no other findings on *false recognition for lures* have been reported in AMCI. The findings suggest that decreased *false recognition for lures* associates with altered processing during recognition processing and not only during encoding as discussed above. Further studies, controlling for semantic elaboration during encoding, are required to determine the contribution of altered recognition processing on *false recognition for lures*.

Summary

In summary, only a few functional studies have examined recognition in prodromal AD and no firm conclusions can be drawn from their findings. Taken together, our results and published findings all indicate altered activation during recognition in AMCI and altered activation in the posterior cingulate appears the most consistent finding. We have seen in preceding sections (§5.6.2.2) that altered posterior medial parietal (cingulate) activation and atrophy appears a consistent finding in AMCI and the results discussed above supports it.

5.10 Rivastigmine Treatment Effects on Divided Attention in AMCI

We conducted a controlled treatment trial of rivastigmine treatment to determine the effects of ACEI treatment on attention in AMCI. Our results indicate that rivastigmine treatment increased visual and auditory attention processing speed during divided attention. These behavioural improvements were associated with treatment related activation decreases in relevant sensory and executive attention processing areas. These findings suggest that rivastigmine improves signal-to-noise ratios in relevant sensory cortices that consequently reduce regulatory processing requirements dependent on executive areas. These findings are discussed in detail below.

5.10.1 Rivastigmine Dosage and Tolerability

Rivastigmine treatment did not cause any serious adverse effects and the gastro-intestinal side effects (nausea, vomiting) that occurred in half of the patients at the start of treatment are in line with findings from ACEI treatment studies ((Raschetti et al., 2007) and references therein). All RivAMCI patients were able to tolerate the drug throughout the study period. Nine RivAMCI participants continued on an ACEI for

up to one year: six on rivastigmine, three on donepezil. ACEI treatment is therefore well tolerated in this patient group.

5.10.2 Demographics and Neurocognitive Results

The RivAMCI and NxAMCI groups differed significantly on MMSE and Attention subscale scores suggesting that the NxAMCI group was less impaired (Table 32). The RivAMCI was older than NxAMCI, although this did not reach statistical significance, and NxAMCI spent more years in formal education, and these findings could explain their higher MMSE and Attention subscale scores. The difference in Attention subscale scores may indicate larger cognitive reserve related to more years in formal education. Although younger age in NxAMCI may contribute to the attention difference, it is more likely related to education because available evidence indicates that education improves working memory and attention whilst age affects episodic memory (Gomez-Perez and Ostrosky-Solis, 2006). Superior Attention subscale performance did not predict better performance on the divided attention task. This is most likely due to the nature of the tasks as only accuracy is measured on the Attention subscale whereas speed is additionally measured on the divided attention task. The Attention subscale of the CAMCOG is therefore limited as it measures only one domain of importance to overall attentional processing.

Randomised treatment allocation is often not practical or ethical and pre-treatment differences occur commonly in neuropsychological research. Statistically significant differences apparent prior to treatment in non-randomised patient groups complicate the interpretation of results on the effects of treatment on group (allocation), time and interactions (group x time). However, if baseline differences favour the untreated group and the treated group then shows an improvement over

time, then any treatment effect should be considered significant because it stands to reason that the treated group improved from a more disadvantaged pre-treatment level. This is the case for the treatment comparisons between RivAMCI and NxAMCI as the later group was younger and performed better on neuropsychological measures at baseline.

5.10.3 Behavioural Findings

RivAMCI and NxAMCI were comparable on visual and auditory target processing speed, and on accuracy at baseline (§4.9.2; Table 32). This indicates that all participants had sufficient practice on the task and validates the use of reaction time as a treatment outcome measure for this experiment (§3.9.1.1). This is further supported by the absence of main time effects on behavioural measures. Following rivastigmine treatment, RivAMCI improved their visual and auditory processing speed resulting in faster speeds with large effect sizes for both (Figures 19, 20). This is indicated by faster RTs on visual and auditory targets at follow-up and interaction effects (group x time) for auditory RTs. This is in line with findings of faster visual processing speed on attentional tasks following physostigmine in healthy subjects (Bentley et al., 2004; Furey et al., 2000; Furey et al., 1997; Goekoop et al., 2004)(§1.7.5). Improvement in processing speed and accuracy have been found on the few studies of working memory in AMCI. Ten weeks of donepezil treatment improved accuracy on a verbal working memory task (n-back, letter stimuli) (Saykin et al., 2004) and five days of galantamine treatment improved speed and accuracy on a visual working memory task (n-back, letter stimuli)(Goekoop et al., 2004).

Increasing the availability of Ach following ACEI treatment was anticipated to alter speed and/or accuracy in AMCI but only a trend was evident on accuracy. The

absence of changes in accuracy is most likely related to task design as both groups performed near or at ceiling for accuracy.

Taken together, out behavioural findings indicate that rivastigmine improves attentional processing speed in AMCI during divided attention.

5.10.4 Functional Findings

5.10.4.1 Baseline between group comparisons

RivAMCI had greater activation than NxAMCI in task related primary and secondary visual processing areas (Table 33). This was associated with better Attention subscale and MMSE scores, and trends towards superior performance on on all the other neuropsychological measures in NxAMCI (Table 32). NxAMCI therefore appear less impaired than RivAMCI. Taken together these findings suggest that the activation differences between RivAMCI and NxAMCI are due to greater disease severity in the former group as indicated by poorer performance on MMSE and Attention subscale scores, and similar trends on all other neuropsychological measures at baseline.

The differences between RivAMCI and NxAMCI could be related to sampling error or pathological heterogeneity in AMCI patients. Sampling error could have occurred as the groups were recruited sequentially and we conducted post hoc analyses to exclude that activation differences were due to sampling bias (§3.9.5; 4.9.4). It could be argued that increased awareness of MCI lead to patients presenting earlier and the last group recruited (NxAMCI) may therefore include less impaired patients. However, identical recruitment procedures were followed for both groups and results indicate that the groups did not differ on the severity of cognitive impairment as measured by the deviation of individual scores from predicted

CAMCOG totals (Table 36). Patients therefore appear to have been recruited at similar severity levels given their age, education, gender and social class. The groups were therefore comparable on norm adjusted severity measures. Equivalence on severity measures does not discount the possibility that activation changes occured independent of relative severity i.e. activation could correlate with neurocognitive measures irrespective of age, education, gender and social class.

As far as the interpretation of our results is concerned, it is fortuitous that the treated group appears more impaired at baseline. If baseline differences favour the untreated group and the treated group then improves over time then they do so from a more disadvantaged pre-treatment level, adding support to any beneficial treatment related effects. Taken together, these findings indicate that the RivAMCI group may have been more impaired at baseline but that this was not due to systematic sampling error and that any behavioural improvements evident in this group occurred from a more disadvantaged starting point compared to NxAMCI.

5.10.4.2 Main effects of Time

Activation reduced over time in the combined groups in left temporal auditory areas and MTL (Table 34). Decreased activation at follow-up is likely related to repetition suppression or priming, that has been reported in temporal auditory cortical areas for spoken words similar to the verbal stimuli in the divided attention task (Badgaiyan et al., 2001; Gagnepain et al., 2008). Repetition priming refers to reduced activation in task related areas upon repeated perceptual or conceptual exposure; it appears related to increased cortical processing efficiency and is associated with maintained performance. Priming in MTL and auditory areas have been reported on several tasks

that involve verbal processing, supporting the suggestion that the observed differences over time is priming related (Cabeza and Nyberg, 2000).

Activation decreased in both groups over time in medial parietal, MTL, temporal, somatosensory, visual processing, attention processing, and cerebellar areas. These changes were not associated with significant main effects of time on any of the behavioural measures thereby limiting further interpretation.

5.10.4.3 Interaction effects

Interaction effects were evident in left hemispheric visual and verbal language processing areas, and in prefrontal executive attention processing areas (Table 35; Figures 21, 22). In these areas, RivAMCI decreased activation and NxAMCI increased activation over time indicating the treatment effects of rivastigmine. The effects of ACEI on attentional processing are mediated via top-down (task-driven) effects in prefrontal and parietal cortices and via bottom-up (signal-driven) effects in sensory cortices (§1.7.5). Decreased activation in language processing areas may indicate greater repetition suppression following rivastigmine. Similar enhanced repetition suppression has been observed in visual areas for visual stimuli following physostigmine in young controls and AD patients (Bentley et al., 2003; Riekkinen and Riekkinen, 1999). This suggest that rivastigmine enhances cortical efficiency related to attentional processing in primary sensory and association areas that in turn reduce the demands made on top-down regulating executive attention areas, resulting in decreased PFC and parietal activation. Similar findings have been reported on tasks of working memory that make heavy demands on divided attention. Improved visual working memory (faster reaction times) accompanied decreased PFC and increased extrastriate visual cortex following single dose physostigmine administration in

healthy adults (Furey et al., 2000; Furey et al., 1997). These findings indicate that improved perceptual processing in sensory cortices simplifies working memory demands that in turn required less PFC participation. This is supported by findings from animal studies demonstrating that visual cortex stimulation increases local acetylcholine release via a pathway that proceeds from visual cortex to PFC, the BFCS and back to visual cortex. Consequently, increased sensory cortex responsiveness following physostigmine could require less PFC signalling required to enhance sensory cortex excitability (Rasmusson et al., 2007). Physostigmine also improved processing speed whilst decreasing primary visual cortex activation and increasing extrastriate cortex activation during visuospatial working memory and selective visual attention tasks (Bentley et al., 2004). These results suggest that ACEI related enhanced sensory processing during working memory tasks result from (1) improved signal-noise ratios in primary sensory cortices that reduces local activation and (2) increases activation in higher visual areas leading to (3) reduced requirements of top-down regulation from the PFC resulting in decreased activation. Our finding of decreased activation in left PFC following rivastigmine is commensurate with these findings and suggested mechanism.

Only one functional study has examined ACEI treatment in AMCI by comparing treated and untreated groups. Five days of galantamine treatment improved speed and accuracy on a visual working memory task (n-back, letter stimuli) and this was associated with increased activation in right precuneus and right PFC (BA 9, 47) (Goekoop et al., 2004). The only conclusions to be drawn from this study in relation to our results are that ACEI appears to improve processing speed and that this associates with altered cortical activation.

We did not find linear correlations between activation and processing speed changes over time in RivAMCI. This suggests that treatment has a binary rather than a scalable effect on cortical activation and/or behaviour. Alternatively, our study might lack sufficient power to demonstrate linear effects.

Summary

Taken together our findings indicate improved attentional processing whilst the associated decreased cortical activation suggests enhanced processing in line with findings in controls and supported by functional circuitry identified in animals. Further studies are required if a coherent model of ACEI treatment response in AMCI is to emerge; however, the apparent lack of efficacy of ACEI treatment in AMCI argues against further studies.

5.11 Rivastigmine Treatment Effects on Visual and Auditory Selective Attention

We studied the effects of rivastigmine treatment in AMCI on selective visual and auditory attention processing in order to control for the possibility that treatment effects may generalise across attentional conditions rather than being condition and area specific. Generalised effects would suggest that treatment effects could occur via non-cortical mechanisms such as increased cardiac output or systemic effects such as vasodilatation. Our findings argue against such effects because the areas where interaction effects occurred following treatment overlapped minimally across the visual (§4.10.1.1) and auditory conditions (§4.10.1.2); moreover, there was minimal overlap with interaction areas demonstrate during the divided attention task (§4.9.3). Rivastigmine therefore appears to have task- and region- specific effects during

attentional processing and this accords with findings from ACEI studies discussed in the introduction (§1.7.5).

Interaction effects (group x time) evident on selective visual and auditory attention conditions occurred in task related areas. Following rivastigmine treatment, activation decreased in RivAMCI in auditory and language processing areas during the visual attention condition, and in visual processing areas during auditory attention. These findings suggest improved down regulation of activation in areas not relevant to a given a given attention condition following rivastigmine.

5.11.1 Behavioural Findings

Behavioural measures were not collected during the visual and auditory selective attention task and this precluded correlation analysis, thereby limiting the conclusions that can be drawn. However, including behavioural responses on the task would alter the attentional and executive processing required during the task practically rendering it a simple choice reaction time task. The findings reported therefore reflect attentional processing when participants are required to attend but not respond, similar to the processing that takes place in everyday life, that is associated with intact episodic recall in controls.

5.11.2 Visual selective attention

Rivastigmine treatment effects were evident as interactions in lateral temporoparietal areas where RivAMCI decreased activation over time whilst it remained stable in NxAMCI (Table 39; Figure 22). The right temporal cortex processes complex non-verbal sounds, such as scanner noise, and decreasing activation in RivAMCI may

indicate enhanced cognitive down regulation of non-relevant areas ((Engelien et al., 2001) and references therein). Decreased parietal activation lends further support to this suggestion as parietal activation increases during selective auditory attention and the converse could therefore indicate decreased auditory attention processing (Pugh et al., 1996). As mentioned above, the lack of behavioural measures precludes further interpretation as it is unclear if activation changes following rivastigmine causes behavioural changes; however, the results from the divided attention study indicate improved processing of visual and auditory stimuli that was associated with decreased activation in relevant auditory and visual cortices following rivastigmine. The results from the visual condition in RivAMCI may therefore indicate improved cognitive regulation following rivastigmine but future studies will need to confirm this.

A further interaction was evident in PFC where RivAMCI maintained and NxAMCI decreased activation (Table 39; Figure 24), and this may reflect enhanced activation related to top-down regulatory efforts that decrease activation in non-relevant lateral temporoparietal areas, as discussed above.

Taken together, these results indicate that rivastigmine treatment alters cortical activation during selective visual attention processing. Initial data suggests enhanced cortical down-regulation of non-relevant sensory areas that is achieved via greater PFC activation.

5.11.3 Auditory selective attention

Rivastigmine treatment effects were evident in occipitotemporal-visual areas and temporoparietal-auditory areas, where RivAMCI decreased activation relative to NxAMCI (Table 42; Figures 25 -27). Decreased activation in both these areas support the hypothesis that enhanced cognitive regulation results in down-regulation of non-

relevant cortices. Processing in posterior visual areas and in the right sided auditory areas, that process complex non-verbal sounds such as scanner noise, are not required during the verbal condition and activation in these areas are therefore down-regulated ((Engelien et al., 2001) and references therein). It is not clear why activation decreased in somatosensory areas as the task did not include specific somatosensory stimuli; however, environmental somatosensory stimulation occurred continuously during the experiments and it may be that enhanced top-down cognitive regulation also affects somatosensory signals via PFC and parietal areas.

Rivastigmine treatment effects were also evident as increased activation in RivAMCI in the brainstem, medial occipitotemporal areas (extrastriate visual, entorhinal, hippocampus, amygdala), thalamus and striatum (Table 42; Figure 28).

Environmental stress activates the brainstem locus coeruleus via the amygdala and increased activation suggests greater attentional arousal and vigilance. This is supported by increased thalamic activity that is associated with the onset of demanding attentional tasks (Kinomura et al., 1996; Raizada and Poldrack, 2008). Thalamic activity contributes to attentional orientation, sensory processing and decision making and activation is therefore associated with non-specific attentional arousal that increases during periods of increased attentional demand (for a review see (Newman, 1995)) (Newman and Mair, 2007). Consequently, increased activation in these areas may indicate enhanced attentional response following rivastigmine. The absence of behavioural measures precludes direct correlations; however, improved auditory processing during divided attention (§5.10.3) suggests that processing may also be improved during selective auditory processing.

Increased posterior cingulate activation may be related to enhanced processing of the verbal stimuli as activation in this area frequently occurs during verbal memory retrieval tasks (Cabeza and Nyberg, 2000).

Hippocampal activation is evident on tasks where participants are not instructed to encode stimuli an appears related to automatic episodic encoding, and recent work demonstrated that the level of activation remains unchanged when participants were instructed to encode the stimuli; however, it was accompanied by PFC activation (Dove et al., 2006). PFC activation therefore appears related to the processes that accompany conscious intentional encoding. In contrast to activation in non-relevant sensory cortices that may be overactive in AMCI due to impaired cognitive regulation, automatic hippocampal activation may be expected and increased activation may be related to improved cognitive down regulation elsewhere. These suggested mechanisms could not be examined further with our available data; however, the encoding paradigm employed by Dove et al. provides a potential method of study. Their task involved either passive viewing or intentional encoding of abstract images and recognition was tested after viewing. Collecting recognition data for the verbal material could clarify if enhanced hippocampal and posterior cingulate activation is associated with increased retrieval performance of incidentally encoded verbal material.

Summary

Taken together these results suggest enhanced activation in task-relevant areas and decreased activation in non-relevant areas during selective auditory attention following rivastigmine in AMCI. This in turn suggests improved cognitive regulation.

Available data do not allow behavioural correlations and further studies will be required to confirm these tentative conclusions.

5.12 Rivastigmine Treatment Effects on Verbal Episodic Memory

Rivastigmine treatment had no apparent effects on verbal episodic memory in AMCI (§ 4.11.1). Behavioural results indicate that the groups remained comparable at follow-up and interaction effects, indicative of treatment effects, were not evident on any of the behavioural measures. Furthermore, the differences between the AMCI groups and Controls evident at baseline remained at follow-up, thereby indicating that the AMCI groups did not improve compared to Controls. These findings are in line with those from the meta-analysis of rivastigmine and other ACEI treatments in AMCI-multiple domain and MCI that failed to show treatment benefits on cognition (Birks, 2006; Birks et al., 2000; Birks et al., 2009; Raschetti et al., 2007).

All participants showed increased FAR at follow-up and this may indicate that the two versions of the task used at baseline and follow-up are not matched on difficulty, alternatively it may indicate reduced motivation and compliance at follow-up that is related to the task being presented at the end of the scanning session when participants may be fatigued. Reversal of the versions in future studies can help to clarify this.

Functional data were not analysed because results were unlikely to have significant interpretive value given our behavioural findings and similar findings published during the course of our work, which failed to show any benefit of ACEI treatment on episodic memory in AD. These findings argue against further investigations of ACEI treatment effects on memory in AD. The disproportionate number of technical and compliance failures that occurred on the recognition task

indicate that it may not be suitable in its present form, or should be presented earlier to eliminate the effects of participant fatigue (§4.3). This needs to be clarified before future studies are undertaken with this task.

Summary

Taken together these findings indicate that ACEI treatment does not benefit verbal episodic memory in AMCI. The lack of treatment efficacy may be due to the faulty assumption that cortical cholinergic inputs have predominantly tonic activity as discussed in the introduction (§1.7.5). It is unlikely that the complex neuronal systems mediating episodic memory operate via tonic activity and increased synaptic Ach is therefore unlikely to compensate for the loss of presynaptic neuronal function and may in fact impair memory processing. Given the currently available findings, it appears that further study of ACEI treatment on verbal memory in AMCI is not warranted.

6. Conclusions and Future Directions

The work reported, summarised and discussed in this thesis has lead to a number of conclusions and suggestions for related future studies.

- AMCI rigorously diagnosed in specialist settings can be considered prodromal for AD and the high AD progression rate in our sample indicates it is similarly prodromal (§6.1).
- Our findings in AMCI that progressed to AD indicate altered and impaired attentional processing, leading to the conclusion that attentional deficits should be considered integral to AD at the stage of AMCI (§6.2).
- In relation to episodic memory processing in AMCI, we conclude that executive failure and altered activation in areas outside the MTL contribute significantly to the characteristic amnesia (§6.3).
- We found that rivastigmine treatment in AMCI improved attention but not memory, leading to the conclusion that treatment effects were task- and region-specific, and not sufficient to support routine treatment in AMCI (§6.4).
- Our findings also lend support to suggestions that (1) pathology affecting areas with widespread cortical influence, such as the BFCS and locus coeruleus, may be central to the development of AD, and (2) that AD neuropathology consistently affects medial parietal areas across functional and structural studies (§6.5).
- Furthermore, fMRI appears to be a valuable tool in the study of prodromal AD and further methodological advances will increase our understanding of

disease progression in AD (§6.6).

Each of these conclusions is discussed in more detail below.

6.1 AMCI as Prodromal AD

6.1.1 AMCI

AD continues to be the leading cause of dementia and the predicted increase in prevalence makes it essential to develop preventative, curative and symptomatic treatments. This will only be possible through better understanding of the disease process, from genetic- to population-level. Whilst much work has been done in established AD, relatively little is known about its prodromal stages.

At present there is no single reliable biomarker for AD, which is unsurprising as the exact pathological mechanism remains unclear and comorbidity is common (§1.1.3). Even the diagnostic gold standard of autopsy has relatively low sensitivity and specificity. Given these limitations to the construct validity of AD itself, the question arises if we can identify prodromal AD reliably enough for rigorous scientific study. Available findings indicate that it can be identified with high sensitivity and specificity using specialist clinical procedures (§1.2.3). Moreover, the high AD progression rate in our AMCI sample over a relatively short follow-up period compares favourably with the most sensitive and specific methods published and leads to the conclusion that our methods obtained sufficient sensitivity and specificity to consider our AMCI sample prodromal for AD (§ 4.1; §5.3). Furthermore, considering the relatively short follow-up period and published conversion rates, it is likely that the majority of non-converters will

eventually progress to AD (§5.1). We therefore conclude that our AMCI sample can be considered prodromal for AD and that AMCI diagnosed in specialist memory clinic settings, using rigorous procedures and norm-based neuropsychological measures, is sensitive and specific for prodromal AD whilst also being more practical than biochemical biomarkers.

We were able to investigate attention and memory in AMCI that had progressed to clinically confirmed AD (CoAMCI), thereby eliminating the diagnostic uncertainty associated with participants that remained AMCI. Although this is not practical in all studies of prodromal AD, the different results compared to the larger AllAMCI group, that contained converters and non-converters, suggest that potentially useful findings about disease progression may be evident in groups that are followed to confirm disease progression.

CoAMCI were older than Controls although this did not reach statistical significance. The potential effects of age differences were examined by correlation analyses with behavioural and neurocognitive measures on which the groups differed significantly (§4.2; §4.5.1; §4.8.1). The only significant correlation was between age and global cognition (CAMCOG total) in CoAMCI and Controls, indicating that 12% of the difference can be attributed to age and it therefore accounts for less than two of the 15-point difference on CAMCOG total means. In the absence of any significant correlations between age and any of the experimental behavioural measures that differed between Controls and CoAMCI, we conclude that age difference is unlikely to solely account for the observed functional differences.

6.1.2 Ageing

Controversy remains regarding the relationship between ageing, AMCI and AD. Distinguishing between AD and normal ageing is complicated by the lack of clarity on the exact aetiology of AD and ageing related cognitive decline (§1.3.2). Moreover, AD develops gradually and the exact time of the transition between ageing, AMCI and AD depends on the diagnosing clinician, not on accurate biomarkers (§1.2.2). The distinction between normal/healthy and disease as it pertains to AD, AMCI and ageing can therefore appear arbitrary at present. However, recent neuropathological findings indicate that AMCI and AD is qualitatively distinct from ageing (§1.3.3).

Distinguishing between AD and normal ageing is further complicated by the ambiguity of prevailing definitions and concepts of disease and normality (§1.3.1). Both these definitions depend on prevailing cultural opinions that in turn rely on the influence of environmental exposure (experience, medical intervention) on a population's genetic expression. In other words, these definitions and concepts appear to change over time as science conquers more of nature. If we take into account how an individual's opinions are influenced by their environment, society and culture - all of which in turn evolve over time- then it becomes clear that neither normal or disease are fixed definitions and that both will evolve over time. I therefore conclude that, irrespective of the relationships between ageing, AMCI and AD, efforts to prevent and treat these health states will continue as long as individuals and societies considers them unwanted.

It can therefore be conclude that AD is sufficiently distinct from normal ageing to allow separate investigation and that we can reliably identify AMCI prodromal for AD to do so. Furthermore, in spite of the continued debate about the relationship between AD and ageing and the ethical dilemmas associated with the

diagnosis (§3.1), diagnosing and studying reliably discernable prodromal stages of AD appear ethically justifiable as long as it these stages are potentially treatable. Furthermore, it appears necessary to study prodromal stages because it is here that AD progression should be arrested if it cannot be prevented.

Future directions

Since AMCI prodromal for AD can be diagnosed with increasing sensitivity and specificity, clinicians and patients will have to decide if diagnostic disclosure of AD should take place. Ethical considerations dictate that this should only be done if the stress of diagnosis can be offset by clinical gain (§3.1). At present, AD is incurable; however, available evidence strongly suggests that certain lifestyle choices and regular participation in certain activities and can reduce the risk of AD by 34% (Larson et al., 2006). It remains to be determined if these activities provide preventative benefits when prescribed as treatment in AMCI. A major obstacle in studying the effects of activities in AMCI is the poor uptake of these activities by older adults. Therefore, a delivery methods that engages people in these activities long-term is required before their clinical potential can be assessed. Working towards this, we have a study underway (ThinkingFit) that examines the effects of an evidence based motivational approach on participant compliance with an activity program. Once satisfactory participant engagement has been achieved, we plan to conduct a controlled treatment trial of the interventions.

Our AMCI group and those from several other studies were numerically older than Controls (§4.1; §5.1). Although the effects of ageing on cognition do not appear

to account for the observed differences between Controls and CoAMCI in our studies, future work should match participants closely on age in order to control for potential ageing effects.

6.2 Attention in prodromal AD

Attention is impaired early in the course of AD and the first deficits are evident in divided attention, followed by deficits in selective and then sustained attention. Attention is crucial for everyday functioning and underpins almost all other cognitive processing and attentional deficits could therefore have widespread effects on behaviour. Clinical and theoretical data predicted attention deficits early in the course of AD and our findings lead to the conclusions that attentional processing is altered during selective visual and auditory attention, and impaired on visual-auditory divided attention in prodromal AD.

6.2.1 Visual selective attention

The status of sensory attention processing in prodromal AD had not been established conclusively. We studied attention processing using fMRI to determine if there are brain activation changes evident in AMCI. One study of attention in AMCI preceded our work and reported subtle behavioural deficits in selective visual attention (§1.5.4)(Perry and Hodges, 2003).

Our result in CoAMCI revealed decreased activation in visual processing areas, and increased activation in attentional control areas (thalamic) and in non-relevant auditory and default (MTL) areas. From this it can be concluded that basic visual attention processing is altered in prodromal AD. These results suggest (1)

failure of top-down attentional control mechanisms to sufficiently activate task relevant areas leading to decreased sensory cortex activation, (2) failed down-regulation of activation in non-relevant default network and sensory areas, and (3) compensatory hyperactivation in attentional control areas, indicating increased perceived task difficulty in prodromal AD (§5.7.1.2). Due to the prominence of AD neuropathology in attentional control areas and the relative sparing of sensory cortices, we conclude that AD neuropathology affecting attentional control areas is the likely cause of altered processing in AMCI. Relevant attentional control areas that show early AD neuropathology include the PFC, anterior cingulate, lateral parietal areas, BFCS and brainstem locus coeruleus (§1.2.2; 1.6.3-5). Our findings are not supported by behavioural data as none were collected during the task; however, subsequent studies of attention in AMCI support our conclusions because they revealed attentional impairments on attentional tasks that require selective visual attention (Perry and Hodges, 2003; Silveri et al., 2007; Tales et al., 2005a).

Deficits in selective visual attention processing can have far reaching effects on cognition because of the prominence and importance of vision in everyday activities. Furthermore, it is likely to affect many other observable cognitive processes and studies of higher order cognition should take this into account. Indeed, altered visual attention processing is evident during visual-auditory divided attention processing in AMCI (§5.6) and it remains to be determined if divided attention is impaired across non-visual modalities or only when visual attention is involved.

6.2.2 Auditory selective attention

CoAMCI demonstrated greater activation in auditory, language, attention control and MTL areas during selective auditory attention. Similar greater MTL and thalamic

activation was also seen on the visual condition discussed above and supports the conclusions drawn there.

The persistence of MTL and thalamic activation changes across sensory modalities suggests that it may generalise to all selective sensory attention conditions, lending further support to the conclusions of regulatory failure and compensatory activation in prodromal AD discussed above.

Furthermore, increased thalamic activation was also evident during divided attention (§5.6.2.2) and verbal episodic encoding in CoAMCI, leading to the conclusion that it is altered across several attentional conditions and likely caused by compensatory attentional arousal efforts during times of high attentional demand. The thalamus is severely affected by neuronal and synaptic loss in AD and the presence of taupathy in the thalamus distinguishes it from age matched controls (Paskavitz et al., 1995). The thalamus also shows close functional connectivity with the locus coeruleus and nucleus basalis of Meynert that are key areas in attentional processing and that show early AD neuropathology (§1.6.4-5). Increased thalamic activation could be caused by compensatory increased cholinergic release from the BFCS, resulting in greater activation. Compensatory cholinergic release may in turn be driven by failed thalamic drive of sensory and association cortices due to local neuropathology. Altered thalamic activation in AMCI can therefore be due to local or distant AD neuropathology. Whatever the cause may be, we conclude that altered thalamic processing during attention appears to contribute significantly to the attention and memory deficits in AMCI.

6.2.3 Visual-auditory divided attention

Attention is impaired early in the course of AD and divided attention appears particularly vulnerable. Only one study of attention in AMCI preceded our initial publication and this reported impaired visual selective attention. We found impaired divided attention in AMCI that correlated with functional activation changes in a manner that suggests impaired up-regulation of activation in relevant sensory and attention areas, and deficient down-regulation in non-relevant areas. Furthermore, increased activation in the thalamus correlated inversely with processing speed suggesting disconnection between midbrain-thalamic areas that drive attention and cortical areas that process stimuli, in other words failed compensatory attentional control efforts. Increased thalamic activation was also evident on selective visual and auditory attention and episodic encoding, indicating that it contributes to functional and behavioural impairments across several tasks in prodromal AD, and the conclusions related to these findings have been discussed above (§6.2.2).

Our initial publication reported impaired divided attention and the behavioural and functional findings from this study have since been replicated in AMCI using PET imaging and a task using visually presented line drawings and auditory presented Finnish town names (Dannhauser et al., 2005; Laine et al., 2008). Divided attention deficits have also been found in AMCI with impairment in other cognitive domains (AMCI-multiple domain) and results indicate a gradual decline in divided attention as AD progresses (Belleville et al., 2007). Taken together, these findings lead to the conclusion that divided attention is impaired in prodromal AD and that the supposedly isolated amnesia in AMCI is in fact accompanied by attentional deficits. These behavioural and functional changes could be used as early markers of AD neuropathology. Clinical studies will be required to determine the diagnostic and

predictive value of divided attention in elderly patients complaining of amnesia, and we are in the process of setting up such a study (§6.6.7). Based on our results, divided attention may be used as an additional marker of AD neuropathology that can be measured to study the effects of new treatments for AD.

Finding impaired divided attention in AMCI is of further importance for patient safety as even minor deficits in divided attention can affect performance on tasks such as driving that make high demands on divided attention. Deficits on tasks requiring working memory and executive control have also been reported in AMCI (Bozoki et al., 2001; Traykov et al., 2007). Findings of executive deficits may also have bearing on cognitive rehabilitation efforts in AMCI because executive deficits, including impaired divided attention, correlate closely with deterioration in activities of daily living, whereas isolated progressive amnesia does not (§1.6.2)(Bisiacchi et al., 2008; Royall et al., 2004; Royall et al., 2005). The association between memory deficits and impaired activities of daily living is mediated via executive failure, illustrating the pivotal role of executive control in everyday activities. Therefore, cognitive rehabilitation that targets executive control may be more effective than memory training at maintaining functional independence in AD. Deficits in executive control and attention in AMCI may also partly explain impairments reported in other cognitive domains such as semantic memory, naming, digit span and verbal fluency (Bennett et al., 2006; Dudas et al., 2005).

Summary

Attentional performance and processing is impaired in AMCI prodromal for AD. The clinical conceptualisation of AMCI as comprising isolated amnesia is therefore

inaccurate. Selective sensory attention processing appears altered in AMCI and it may play a more prominent role in the symptomatology of prodromal AD.

Divided attention performance and cortical processing is impaired in prodromal AD, suggesting failed cognitive regulation and compensatory efforts. These changes appear in areas know to be affected early by AD neuropathology and support disease models proposing that widespread altered cortical processing is caused by pathology in a few key areas that are particularly vulnerable to AD neuropathology.

Future directions

Considering the relationship between memory and divided attention, the assessment of patients with AMCI will need to include sensitive behavioural measures of divided attention for a coherent picture of cognitive impairment to emerge at this stage. Impaired visual processing during divided attention can affect a broad range of everyday activities due to the predominance of visual processing in humans and it may be this dependence on visual information that renders it more vulnerable to neurodegeneration. We want to explore the relationships between memory and attention and visual attention and everyday activities further and we therefore plan to include the divided attention task in our standard clinical battery.

We have also been approached by a commercial group interested in including the divided attention task in their computerised cognitive assessment for dementia research. This will require acquiring data on the task in a large cohort to establish clinical norms. This can be conducted using our established memory clinic patient cohort and controls from relatives that attend the clinic with patients.

We also plan to include a behavioural measure in the basic visual-auditory processing paradigm for future research. This would require the use of different stimuli such as abstract images and sounds that minimises automatic semantic elaboration, followed by a post-test recognition task that provides the behavioural measure. This should allow compliance monitoring and correlations with cortical activation. This project is suitable for a master's degree and will be offered at the Institute of Psychiatry, King's College, London.

Available evidence suggests that altered cognitive processing of attention may precede behavioural impairment, similar to that demonstrated in healthy younger adults at increased genetic risk of AD (Bookheimer et al., 2000; Johnson et al., 2006b; Smith et al., 1999). Indeed, we have recently demonstrated altered cortical processing of divided attention in patients diagnosed with *subjective cognitive impairment* who are at increased risk of dementia and characterised by complaints of memory impairment in the absence of any abnormality on neuropsychological testing, medical or psychiatric assessment (Rodda et al., In press). These patients are being followed long term to determine if they progress to dementia so that we can correlate activation with clinical outcomes.

6.3 Verbal episodic memory in prodromal AD

Amnesia is characteristic of AD and AMCI and has largely been ascribed to neuropathology in the MTL. In clinical settings, memory is tested with verbal memory tasks; however, few functional neuroimaging studies have examined verbal memory in a manner that resembles clinical practice and most research studies have focused on the MTL areas and visual memory. The whole-brain neural correlates of verbal episodic amnesia in AMCI therefore remained to be

determined. Available findings indicate paradoxical correlations between MTL activity and memory performance in AMCI: increased activation associates with a higher risk of rapid decline. Away from the MTL, recent findings from functional neuroimaging studies have highlighted the role of the PFC in normal memory processing and our methods were optimised to study the whole brain and particularly the PFC and executive processing in AMCI.

AMCI had impaired verbal episodic memory performance and results revealed evidence of semantic processing deficits during encoding leading to the conclusion that executive failure contributes substantially to verbal episodic amnesia in prodromal AD (§5.8). Poor episodic memory performance correlated with increased activation in several areas including the medial parietal default mode network area (discussed below in §6.5), thereby suggesting failed cognitive down-regulation of non-task relevant areas. Decreased activation was evident in the PFC that is key to semantic processing in AllAMCI but not CoAMCI and this suggests that PFC dysfunction precedes the more widespread abnormalities found in the more severely affected CoAMCI group that progressed to AD. It also supports the notion that activation changes are non-linear in AD progression (§5.8.2.2)(Celone et al., 2006). Demonstrating executive failure in AMCI accords with findings indicating that deteriorating everyday activities, which precipitates the clinical dementia syndrome, correlate strongly with progressive executive deficits but not amnesia (Rapp and Reischies, 2005; Royall et al., 2004; Royall et al., 2005). Executive failure in AMCI therefore foreshadows that seen in the mild dementia that follows as the disease progresses. Based on our findings of altered activation in several areas outside the MTL, it can be also be concluded that studies of episodic memory should examine the whole brain if a coherent picture

of memory processing is to emerge.

We also demonstrated impaired divided attention that can contribute to memory processing deficits due to the reciprocal relationship between divided attention and memory processing (§1.6.1). Our findings support this notion as we found close correlations between episodic memory and divided attention performance in CoAMCI. Impaired divided attention during memory processing therefore likely contributes to the clinical presentation of prodromal AD.

Summary

At the time when these experiments were designed, little was known about the functional correlates of the characteristic verbal episodic memory impairment in AMCI and we were first to demonstrate the contribution of failed semantic processing and associated prefrontal cortex (PFC) dysfunction (Dannhauser et al., 2008). These findings are commensurate with those from a study of visual episodic memory in AMCI (Mandzia et al., 2007). Based on our results and the available findings it can be concluded that executive processing deficits contribute significantly to amnesia in prodromal AD at the stage of AMCI. This is associated with altered activation in areas outside the MTL indicating that neuropathology outside the MTL is partly responsible. Furthermore, the correlations between attention and memory performance indicate a central role for executive failure in the clinical presentation of AMCI. The disease mechanisms that may underpin executive failure are discussed in more detail below (§6.5).

Future directions

The associations between executive failure on attention and memory tasks and altered activation in several brain areas support the notion that neuropathology affecting brain areas that have widespread cortical influence may be pivotal in the development of AD. We have seen that neuropathology in the BFCS and locus coeruleus can affect a wide range of cognitive processes and that these areas are particularly vulnerable to AD neuropathology, and this is discussed in more detail below (§6.5). The precise contributions from neuropathology in these areas to altered brain metabolism remains to be determined; however, it is beyond the reach of current functional neuroimaging techniques due to their locations and relatively small sizes. However, considering the success of deep brain stimulation to restore function in brain disorders such as Parkinson's disease, obsessive compulsive disorder and depression, and the potential it may hold for the treatment of AD, it appears relevant to clarify the functional connectivity between key areas affected in prodromal AD (Barnikol et al., 2010; Kuhn et al., 2010).

Episodic memory can be improved by supporting semantic elaboration during encoding and this can be used to develop behavioural programs and environmental support to treat the amnesia. We have included semantic elaboration support techniques in our ThinkingFit study that examines the effects of evidence based motivational interventions on compliance with health promoting activities in MCI.

6.4 ACEI treatment in Prodromal AD

Our evaluation of rivastigmine treatment on attention and memory processing in AMCI revealed significant behavioural effects only on attention and these were accompanied by functional changes that suggest enhanced cortical processing.

6.4.1 Visual and auditory selective attention

FMRI can be used to examine the effects of medication on brain activation; however, it should be kept in mind that drugs may not only effect the signal of interest (BOLD) via neurovascular coupling but also non-specific local and global blood flow. Potential confounding effects from non-specific regional blood flow changes can be controlled by employing tasks during fMRI (paradigms) that contrast active conditions which would be equally effected by non-specific blood flow changes, rather than one active condition subtracted from a baseline resting condition (Thiel, 2003). We studied the effects of rivastigmine treatment in AMCI on selective visual and auditory attention processing to determine if treatment effects are generalised or specific to attentional conditions and task related areas. Results revealed treatment effects in task related areas and suggest enhanced cognitive regulation during selective visual and auditory attention task (§5.11.2-3). Treatment effects occurred during each attentional condition in areas that overlapped minimally across the visual and auditory conditions; moreover, there was minimal overlap with areas demonstrating treatment effects on the divided attention task (Tables 35, 39, 42). The absence of behavioural data precludes any firm conclusions on the effects of ACEI treatment on selective attention processing in AMCI; however, our results suggest enhanced cognitive regulation similar to that evident on divided attention discussed below. Taken together, these findings lead to the conclusion that the effects of ACEI

on attention is task and region specific as it occurred in task related areas and on tasks contrasting active conditions.

6.4.2 Divided attention

We studied the effects of ACEI treatment on divided attention in AMCI. Attention depends on intact cholinergic neurotransmission and it is impaired in AMCI where AD neuropathology affects the BFCS that provides cholinergic input to the brain. Attentional deficits in AMCI are therefore potentially responsive to increased synaptic Ach following ACEI treatment.

Rivastigmine treatment increased visual and auditory attention processing speed during divided attention and this was associated with decreased activation in participating sensory and executive processing areas (§5.10.3; 5.10.4.3). These findings lead to the conclusion that improved signal-to-noise ratios in sensory areas following ACEI treatment reduced the need for top-down regulatory processing in executive areas, resulting in reduced activation. This conclusion is in line with those from ACEI treatment trials in healthy adults and AMCI and supported by the identification of the proposed functional circuits in animal models (Bentley et al., 2004; Furey et al., 2000; Furey et al., 1997; Goekoop et al., 2004)(§1.7.5). ACEI treatment therefore appears to benefit divided attention in prodromal AD.

The clinical relevance of these results remains to be determined. Meta-analyses of treatment efficacy for the available ACEIs (rivastigmine, donepezil, galantamine) have revealed (1) modest effects on global cognitive measures in AD, but none specific to attention, and (2) no effects on cognition in AMCI (Birks, 2006; Birks and Melzer, 2000; Loy and Schneider, 2004; Raschetti et al., 2007). Furthermore, current national clinical guidelines based on available findings do not

recommend ACEI treatment in AMCI and recent studies suggest that side effects may be more serious and common than initially believed (Gill et al., 2009; NICE, 2006 (Amended 2007, 2009)). Trials of ACEI treatment in prodromal AD may therefore be appropriate to study disease mechanisms whilst the available evidence does not support routine treatment.

6.4.3 Verbal episodic memory

ACEIs are colloquially known as drugs that enhance memory in AD but this is not supported by available evidence that reveal no benefit to episodic memory, and that emerged after the experiments reported on in this thesis were initiated, (Birks, 2006; Birks et al., 2000; Birks et al., 2009; Loy and Schneider, 2006). Meta-analysis of ACEI treatment in AMCI also failed to demonstrate beneficial effects on episodic memory (Birks, 2006; Birks et al., 2000; Birks et al., 2009; Raschetti et al., 2007). Our results are in line with these findings and we conclude that rivastigmine treatment has no apparent effects on verbal episodic memory in prodromal AD (§ 4.11.1). These findings suggest that improved divided attention does not result in better episodic memory following ACEI treatment in AMCI.

The lack of treatment efficacy is likely due to the faulty assumption, that cortical cholinergic inputs have predominantly tonic activity, whereupon the treatment rational is based (§1.7.5). The complex neuronal systems that underpin episodic memory are unlikely to operate via tonic activity and increased synaptic Ach is therefore unlikely to compensate for the loss of presynaptic neuronal function. It therefore appears that further study of ACEI treatment on verbal memory in AMCI is not warranted.

Functional results were unavailable due to technical and compliance failures but the lack of treatment efficacy on behavioural measures and published studies argues against further studies.

It can be concluded from the disproportionate number of technical and compliance failures on the recognition task that it requires revision (§4.3).

Summary

From our results and published findings, it can be concluded that ACEI treatment improves attentional processing in prodromal AD and that this appears task- and region- specific. Our findings further illustrate the potential of fMRI to investigate subtle treatment related changes not evident on conventional behavioural assessments. Our findings on episodic memory support the general consensus that ACEI treatment does not benefit memory in prodromal AD. Taken together these results suggest a very limited scope for further study of ACEI in prodromal AD and do not support routine treatment.

6.5 Disease Mechanisms in AD

The aetiology of the sporadic form of AD remains unclear and the characteristic lesions (amyloid/senile plaques, neurofibrillary tangles, vascular lesions) of the three leading hypotheses most often appear together (§1.1.2). The presence of these lesions in key areas early in the course of AD has lead to proposals that impaired functional connectivity to and from key areas promote AD neuropathology elsewhere, causing cognitive deficits. At present, the BFCS and locus coeruleus appear to be prime candidates for promoting neuropathology due to the early appearance and gradual accumulation of AD neuropathology in these areas that are pivotal in regulating

cognition, as demonstrated by the (1) consequences of local lesions to memory and attention performance and (2) close correlations between pathological load and cognitive performance (§1.1.2; §1.1.3.2). Functional neuroimaging of brain disorders can reveal altered cortical activation and functional connectivity that indicate compensatory or failed cognitive processing caused by neuropathology. Findings from functional neuroimaging studies can therefore support neuropathological hypotheses of disease causation (§1.1.3.3). Our results support hypotheses related to disease processes in the BFCS, locus coeruleus and medial parietal default network area, and these are discussed in more detail below. The potential contributions to widespread cognitive impairment from neuropathology in the BFCS and locus coeruleus are illustrated in Figure 29.

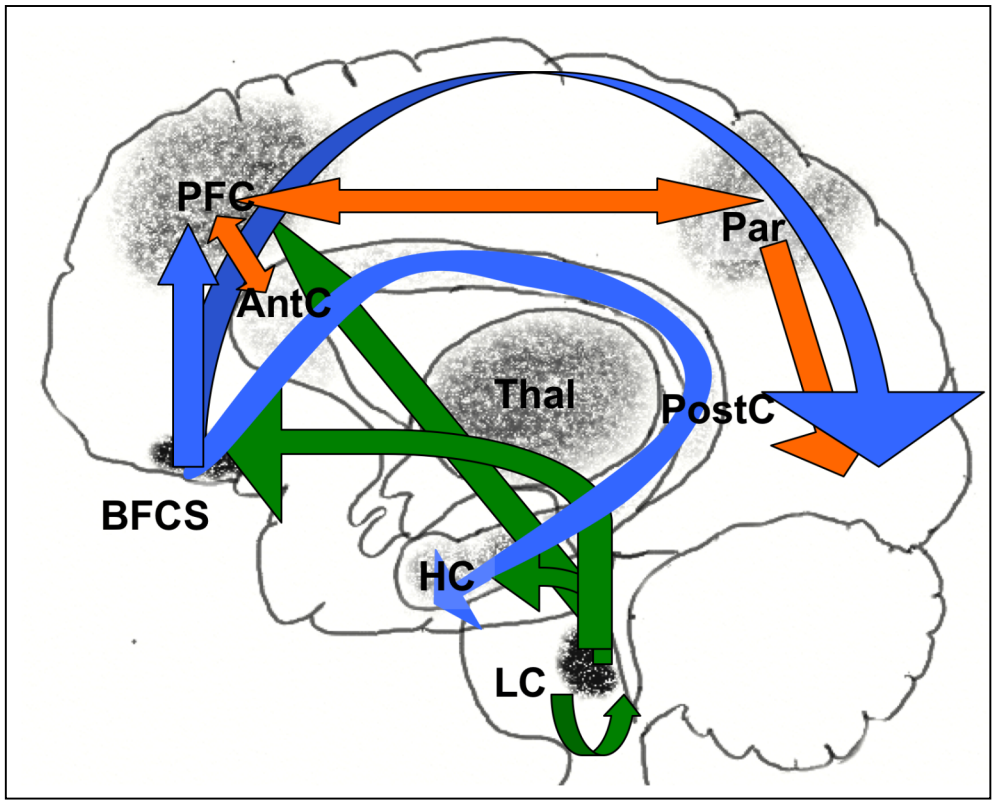


Figure 29 Disease mechanisms in AD.

The figure illustrates the pivotal role that the BFCS (BFCS; blue arrows) and locus coeruleus (LC; Green arrows) play in cognitive regulation and demonstrates how lesions of these structures can cause the range of cognitive deficits evident in AD. The BFCS provides acetylcholine to the cortex and hippocampus (HC) whilst the locus coeruleus provides noradrenaline to the BFCS, cortex, thalamus (Thal) and hippocampus. Afferents connections from both these areas contribute to executive functioning via their connectivity with the executive network that comprises the PFC, anterior cingulate (AntC) and parietal cortex (Par). The executive network optimises memory and controls attention, and both appear impaired in prodromal AD suggesting that an underlying mechanism that can impair executive functioning, such as BFCS or locus coeruleus lesions, may contribute or cause the memory and attention deficits. (PostC = posterior cingulate)

6.5.1 BFCS lesions and the cholinergic hypothesis of AD

The BFCS is the major source of acetylcholine in the brain and provides its primary cholinergic input to the hippocampus and entorhinal cortex, and it also projects to all neocortical areas and the amygdala (§1.6.4). It is therefore pivotal in cognitive regulation throughout the brain and experimentally induced lesions cause memory and attention deficits in the absence of other lesions (§1.7.1). BFCS lesions may therefore explain memory, attention and other cognitive deficits in AD and AMCI because they interrupted functional connectivity with relevant brain areas. Cholinergic neurotransmission is prominently affected in AD and findings indicate that AMCI and AD are associated with reduced cholinergic innervation and receptors on the background of very early pathology in the BFCS.

Impaired divided attention and altered cortical processing of divided and selective attention evident in AMCI indicates impaired attentional regulation, occurring across attentional conditions, suggesting interrupted cholinergic innervation from the BFCS to key areas (§5.6.1). Correlations between activation and attention performance, evident from our results, support this notion of attentional regulation via activity in key areas (§4.5.2.3). Further support for a disease mechanism involving a generalised process comes from the positive correlations between memory and attention deficits that indicate the presence of an overlapping disease mechanism, such as BFCS pathology (§4.8.1; §5.8.1). We therefore conclude that our behavioural and functional results support the cholinergic hypothesis in prodromal AD; however, confirmatory studies will need to examine the functional connectivity between the BFCS and cortical areas directly and this will require advances in fMRI methodology due to the location and small size of the BFCS nuclei.

6.5.2 Locus coeruleus lesions and noradrenergic neurotransmission deficits

The findings discussed above that support a central role for BFCS lesions in the progression of AD similarly apply to the locus coeruleus; furthermore, whilst our methods were not optimised to study BFCS activity, altered activation was evident in the locus coeruleus. AD neuropathology in the locus coeruleus has long been implicated in the pathogenesis of AD and the inevitable prominent neuronal loss and reduction in brain noradrenaline levels caused by NFTs, which correlate inversely with cognitive performance, strongly suggest that it affects cognition in AMCI (§1.6.5). The extensive neuronal loss in the locus coeruleus correlates closer with disease progression than BFCS cholinergic cell loss and it has been suggested the locus coeruleus lesions may precede and contribute to BFCS lesions in AD (Heneka, 2009). Furthermore, cholinergic release from the BFCS appears to be modulated directly from the locus coeruleus. The locus coeruleus is the sole source of noradrenaline to the neocortex, cerebellum, hippocampus and most of the thalamus and it regulates attention, arousal, and stress reactions related to environmental challenges. Locus coeruleus lesions impair learning in animal models, exacerbate the amnestic effects of cholinergic lesions and result in cognitive and metabolic changes characteristic of AD. Lesions in the locus coeruleus can therefore potentially have widespread cortical and behavioural effects. Finding increased activation in the locus coeruleus during selective visual attention provides evidence of altered locus coeruleus function in prodromal AD, and increased activation may be due to reduced noradrenaline release that otherwise attenuates locus coeruleus neurone depolarisation (§5.7.1.2). Moreover, its pivotal role in attention and memory regulation indicates that local AD neuropathology could contribute substantially to cognitive impairment and

neuropathological findings suggest it will be affected in AMCI. It can therefore be concluded that our findings provide support for the proposed role of locus coeruleus lesions in prodromal AD. The locus coeruleus has reciprocal connections from other brain areas that may affect activity in AMCI and connectivity studies will be required for further clarification.

6.5.3 Default network dysfunction

CoAMCI demonstrating greater activation in default network areas during divided attention (medial parietal; §4.5.2.2), selective auditory attention (MTL. §4.6.2.2), verbal encoding (posterior cingulate; §4.7.2.2) and recognition (medial parietal, §4.8.2.2). Furthermore, failed deactivation in these default areas correlated with performance decrements on attention and memory tasks and neurocognitive measures (Table 14, Figures 16, 17). Moreover, these changes were evident in the CoAMCI group that progressed to AD but no in the AllAMCI group that was less impaired and included non-converters. From these results, it can be concluded that increased default mode network activity, i.e. failed deactivation, correlates with cognitive deficits across tasks and predicts dementia conversion in AMCI.

The default mode network is comprised of medial PFC, MTL, medial parietal, lateral parietal and lateral temporal areas; it is active at rest and appears to process inwardly directed cognitive processes (§4.5.2.2; §5.6.2.2). Outwardly directed cognitive processes reduce default activity and the magnitude of deactivation in the network correlates with task difficulty in controls (Buckner et al., 2008; Raichle et al., 2001). Medial parietal deactivation correlates with disease severity in AMCI; less impaired AMCI deactivate more and decline slower, and similar findings have been reported in AD (Celone et al., 2006; Lustig et al., 2003; Miller et al., 2008). Default

mode network activation therefore appears to correlate with disease severity and risk of decline. Our findings support this because deactivation failure was evident in the CoAMCI group that progressed to AD but not in the less impaired AllAMCI group that included the CoAMCI and non-converters. Furthermore, the magnitude of activation, in other words, the severity of deactivation failure in the medial parietal area correlated powerfully with attention processing speed deficits in CoAMCI. It can therefore be concluded that our results support the notion that impaired regulation of default mode activity is present in prodromal AD and that its magnitude correlates with disease severity and predicts progression in AMCI.

The medial parietal area appears particularly vulnerable to AD neuropathology as it is reduced in size and suffers accelerated atrophy that predicts progression in AMCI. Functional studies reveal hypoperfusion and hypometabolism which predicts conversion and correlates with memory deficits (for a review see (Ries et al., 2008))(Xu et al., 2007). Furthermore, amyloid deposits are concentrated in default mode network areas in AD and it remains to be determined if this is cause or effect of altered default network processing (Buckner et al., 2005; Jack et al., 2008; Kemppainen et al., 2007; Lowe et al., 2009).

Functional and structural neuroimaging studies of AMCI and AD consistently reveal abnormalities in the medial parietal area; however the direction of altered functional activation appears inconsistent as resting state studies show decreased activity and studies using activation tasks show failed deactivation that correlates with performance decrements. These disparate findings could be caused by functional isolation of default network areas due to lesions affecting neurovascular coupling and connectivity with other areas. Neurones in a disconnected default network could depolarise at relatively stable rates therefore appearing altered in a specific direction

in comparison with controls depending on the experimental condition i.e. resting-state or active tasks. Taken together, these findings suggest that in prodromal AD cognitive regulation is impaired and causes maintained activation in the default mode network that could hinder processing, across cognitive tasks, in task relevant areas due to resource limitations.

Summary

Our results indicate widespread alterations to cortical processing in prodromal AD that correlate with behavioural measures on attention and memory tasks. These findings, that are present across tasks and conditions, support aetiological hypotheses proposing that AD neuropathology may arise in, and be perpetuated from key areas that regulate cognitive processing in a wide range of areas, and affecting a wide range of cognitive functions. Pathological findings indicate that lesions are already evident in the BFCS in AMCI and strongly suggest their presence in the locus coeruleus in AMCI. Lesions in these two areas could therefore cause the deficits seen in prodromal AD and these deficits in cognitive regulation may explain the persistent findings of structural and functional changes observed in default mode network areas, in particular the medial parietal areas. The explanatory models for altered cognitive processing in AMCI differ and it is not implied that one mechanism will be responsible for the functional changes reported, indeed it is more likely that several mechanisms contribute and that they interact although there may be a primary initiating disease mechanism as AD.

Future directions

The BFCS and locus coeruleus are pivotal in cognitive regulation but they are difficult to study with fMRI due to technical constraints. However, improved resolution in these areas can be achieved with optimised scanning sequences and we plan to implement these in future studies in order to determine if the proposed changes to functional connectivity with areas of abnormal activation is present. Altered functional connectivity between the BFCS, locus coeruleus and default mode network areas is of particular interest as it may explain disease progression in AD. For example, the medial parietal areas receive their cholinergic inputs via the medial cholinergic pathway from the BFCS and are the most distant supplied via this pathway (Selden et al., 1998). Functional and structural changes could therefore be related to interrupted cholinergic innervation first affecting the most distant areas (§1.6.4). This notion is supported by recent findings demonstrating microstructural pathology in the posterior cingulate, that distinguished AMCI from non-AMCI subjects with sensitivity of 80% and specificity of 60%, and connectivity studies are therefore necessary to correlate structural and functional data (Chua et al., 2009).

6.6 fMRI in AMCI

6.6.1 AMCI sample

It appears that fMRI is a valuable tool for studying the neural correlates of AD neuropathology and for evaluating interventions. Further advances in the clinical identification of prodromal AD, such as the refinement of MCI with low specificity for AD to AMCI with high specificity, has aided the interpretation and generalisability of results to clinical samples. However, further challenges to data interpretation remain and include the uncertain effects of comorbidity and varying

methodology that limits comparisons between studies. Furthermore, AMCI is a health state that is associated with relatively rapid changes in cognitive processing, that appears to proceed from a compensated to a decompensated state with different functional neuroimaging characteristics. Matching samples on their stage of progression may therefore require even further sub-classification based on their progression, similar to our CoAMCI stratification that was based on progression to AD (§4.1; §5.1).

6.6.2 Sample size

We studied a relatively small but highly selected AMCI sample rather than a larger more heterogeneous MCI sample, as the former is more likely to represent prodromal AD. Although our sample was relatively small, our results indicate a high rate of progression to AD over a short follow-up period and therefore, the loss of detection power associated with smaller samples is balanced by the higher homogeneity of the sample, achieved by our diagnostic methods. Ten to 15 participants per group was acceptable for fMRI experiments when we started the study and the number generally recommended has now increased to 15 to 20 participants, with improved reproducibility of findings demonstrated for groups up to 25 (Thirion et al., 2007). Smaller groups suffer more from the effects of outliers that can cause atypical findings; however, whilst detection power (sensitivity) for activated areas is reduced in small samples because some activated areas may not reach the set level of significance, the specificity of activation is rigorously controlled for by our statistical methods. On the other hand, reduced sensitivity due to smaller sample size is balance by the implementation of local permutation that increases detection power of group differences (3.4.8.6). The task related activation results revealed by our paradigms are

in line with published findings, leading to the conclusion that our analyses methods and sample sizes were adequate to test our hypotheses. However, our sample sizes were too small to accommodate the data loss that occurred and which rendered it impractical to analyse the recognition task data from the rivastigmine treatment study. This is not a serious limitation because our behavioural data and meta-analysis of ACEI treatment failed to show beneficial effects on memory; however, it does highlight the need for larger sample sizes to allow for data loss in future studies (§1.7.4).

6.6.3 Divided attention paradigm

Ideally, behavioural performance on a divided attention task - which conceptually is a dual-task that does not require additional processing of stimuli apart from target recognition - would be measured by calculating the *composite dual-task decrement* (Baddeley and Della, 1996; Nebes et al., 2001). To calculate this, subjects first perform each of the two sub-tasks separately to measure performance. The dual-task is then performed. The difference in reaction time during dual-task performance and sub-task performance is then calculated for each of the two sub-tasks, and difference scores are then added together to give a composite total. This allows for individual differences where subjects prioritise one of the sub-tasks. Our divided attention task did not measure performance on the sub-tasks separately due to time and design constraints during fMRI. It is likely that this reduced detection power for differences in performance. Our current methods could be altered to include the two sub-tasks of the divided attention task.

A parametric design for the divided attention task could have revealed more about areas where AMCI patients show attenuated activation and areas where

treatment is most effective. To implement this, we have already modified the task to include distractor items for the visual stimuli and degraded sound for the auditory stimuli, and pilot data collection on this task is planned for the near future.

6.6.4 Visual and auditory selective attention paradigm

This paradigm was included to study selective visual and auditory processing and the effects of ACEI treatment in AMCI. Although it appears suitable for these purposes, it may not be ideal. Auditory word stimuli automatically trigger a degree of semantic processing (recall of related items) during identification and this likely leads to activation in non-sensory areas that can complicate data interpretation. A more ideal task would use abstract sounds to minimize automatic semantic processing. We have already adapted the task along these lines and are planning to use it in future fMRI studies of attention.

We did not collect behavioural data during the passive visual-auditory processing paradigm. Behavioural data could be used to confirm compliance with task instructions and aid in interpreting the decreased visual cortex activation evident in the AllAMCI group across auditory and visual selective attention conditions. Future studies are needed to clarify this because altered basic attentional processing will likely affect processing on any experimental task dependent on sensory attention, thereby affecting data interpretation. Connectivity analysis could reveal the extent to which altered functional activation in higher cortical areas relate to altered activation in visual cortex.

6.6.5 Encoding paradigm

The encoding task was well tolerated and functional and behavioural data revealed the contribution of semantic deficits and altered activation outside the MTL in prodromal AD. It is therefore a useful and practical measure of verbal episodic memory encoding and we plan to continue using it in future.

6.6.6 Recognition paradigm

Most data were lost on the recognition task that was presented last and it may be due to participant fatigue. Presenting the task and analysing behavioural and functional data according to our methods may therefore not be suitable in AMCI. Presenting the task earlier in sessions could reduce participant fatigue. Collapsing specific response types into more general types and including more stimuli could overcome analysis problems.

All participants showed increased FAR at follow-up suggesting that the two versions of the task are not matched on difficulty although session effects may also be responsible. Reversal of the two versions can help to clarify this and can be done outside the scanner.

6.6.7 Data Analysis

Although fMRI has revolutionised neuropsychiatric research, it should be kept in mind that the BOLD signal is a proxy of cognitive processing and balanced scepticism should be maintained when interpreting results (Ekstrom, 2010). It is therefore desirable to seek conformation of conclusions from several independent

lines of enquiry, and fMRI findings are strongly supported by concomitant behavioural correlations.

FMRI has proven useful to study normal and abnormal cognitive processing in groups but has found very limited clinical application for individual patients. This is set to change with the development of *machine learning* that allows classification of data from individuals. With this method, fMRI data from an individual can be compared against standardised sets and a probability calculated for each possible diagnosis e.g. the probability of a patient having a specific disorder could be determined. Machine learning can be applied to any aspect that affects brain activation such as diagnosis, prognosis and treatment response, as long as standardised data sets are available. We are in the process of setting up collaborations to determine if machine learning applied to our existing data will have sufficient predictive power to determine the risk and rate of progression of AD in individual patients.

We used an advanced fMRI analysis method that is more sensitive in brain areas where there is relatively smaller signal. This method and the exclusion of one subject who was subsequently diagnosed with dementia with Lewy bodies revealed different functional results to what we initially reported on divided attention in AMCI (§6.2.3). We take the latter results as more accurate and this is supported by significant activation-behaviour correlations that were not evident on the initial analysis. However, our initial result has been replicated using PET imaging, although it did not involve a similar more sensitive analysis method, and the results reported in this thesis await replication.

One of the important advantages of functional neuroimaging is its potential to reveal altered brain function or treatment related changes in relatively small samples

compared to traditional behavioural studies. Furthermore, the cognitive paradigms used during functional neuroimaging are typically computer presented which reduces operator bias and allows the use of more sophisticated and sensitive behavioural measures, that in turn reduces samples sizes and therefore cost and participant exposure. On the other hand, studying smaller samples requires exceptional diagnostic rigor to reduce aetiological heterogeneity in samples, and this is aided by sensitive and specific measures. Even with these methods, larger samples may be desirable to allow for some pathological heterogeneity and for attrition of participants that are followed longitudinally to determine AD conversion rates and confirm prodromal AD retrospectively. Ideally, follow-up should continue to autopsy to allow neuropathological confirmation of AD and we could implement this as an extension of an existing brain donation program.

7. Personal Contributions

Functional neuroimaging studies involve close collaboration amongst a multidisciplinary group of researchers due to the variety of clinical and technical skills required. The work reported in this thesis involved such collaboration and the key contributors have been named in the Acknowledgments. My personal contribution to this work is detailed below.

7.1 Protocol Development, Preparation and Ethical Approval.

I developed and finalised the study protocol from a draft drawn up by my supervisors. I submitted and presented it to the local ethics committee and dealt with all necessary amendments. I prepared and submitted annual progress reports and gave notification of the end of study to the ethics committee. I secured Research and Development committee approval from the local NHS trust for the study.

7.2 Participants and Memory Clinic Procedures

I recruited all research participants and obtained their consent. I regularly assessed and reviewed patients in the memory clinic from where participants were recruited. I have continued to monitor the progress of patients in the memory clinic. During the study, I initiated and monitored rivastigmine for treated patients as per protocol. I trained all participant on the cognitive tasks in preparation for the experiments and I arranged transport and accompanied them to and from the Institute of Psychiatry where scanning took place. At the scanner, I assessed and provided participants with fMRI compatible prescription glasses and assisted in participant placement in the scanner.

7.3 FMRI Scanning Procedures and Data Analysis

I ran all the cognitive paradigms during scanning whilst a radiographer operated the scanner. I recorded and archived all behavioural data during scanning. After receiving training in the XBAM fMRI analysis package, I archived and analysed all of experimental data and prepared it for presentation and publication.

7. 4 Cognitive Paradigms

I designed the divided attention task under supervision of S, Shergill and M. Seal and conducted all of the pilot studies. C. Andrews programmed the task and assisted in designing the response box. Neuroimaging parameters were calculated and provided by the Neuroimaging Research Group, under supervision of F. Zelaya. I adapted the encoding and recognition paradigms for our study.

7.5 Statistical Analyses of Behavioural Data and Correlations with Functional Data.

I prepared data and conducted all statistical analysis reported in this thesis. For this purpose, I created Excel spreadsheets that performed the initial calculations to prepare data for statistical analysis for the divided attention task, and I modified an existing spreadsheet to do the same for the recognition task.

7.6 Procurement and Technical Maintenance of Equipment

I procured all the computer equipment required for the study, installed software and performed maintenance procedures as required.

7.7 Manuscript Preparation and Data Presentation

I authored this thesis, all oral and poster presentations, and all of the published manuscripts related to this study. I received editorial input from my supervisors and Dr R. Walker.

Bibliography

Abe K, Horiuchi M, Yoshimura K. Potentiation by DSP-4 of EEG slowing and memory impairment in basal forebrain-lesioned rats. Eur J Pharmacol 1997; 321: 149-55.

Aggleton JP, Pearce JM. Neural systems underlying episodic memory: insights from animal research. Philosophical Transactions of the Royal Society B: Biological Sciences 2001; 356: 1467-1482.

Anderson KE, Perera GM, Hilton J, Zubin N, Dela PR, Stern Y. Functional magnetic resonance imaging study of word recognition in normal elders. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2002; 26: 647-650.

Anderson ND, Ebert PL, Jennings JM, Grady CL, Cabeza R, Graham SJ. Recollection- and familiarity-based memory in healthy aging and amnestic mild cognitive impairment. Neuropsychology. 2008; 22: 177-187.

Anderton BH. Changes in the ageing brain in health and disease. Philosophical Transactions of the Royal Society B: Biological Sciences 1997; 352: 1781-1792.

Anderton BH. Ageing of the brain. Mechanisms of Ageing and Development 2002; 123: 811-817.

Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW. The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. Cereb Cortex 1991; 1: 103-16.

Artero S, Tierney MC, Touchon J, Ritchie K. Prediction of transition from cognitive impairment to senile dementia: a prospective, longitudinal study. Acta Psychiatrica Scandinavica 2003; 107: 390-393.

Aston-Jones G. Brain structures and receptors involved in alertness. Sleep Med 2005; 6 Suppl 1: S3-7.

Auld DS, Kornecook TJ, Bastianetto S, Quirion R. Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. Progress in Neurobiology 2002; 68: 209-245.

Ausen B, Edman G, Almkvist O, Bogdanovic N. Personality features in subjective cognitive impairment and mild cognitive impairment--early indicators of dementia? Dement Geriatr Cogn Disord 2009; 28: 528-35.

Backman L, Small BJ, Fratiglioni L. Stability of the preclinical episodic memory deficit in Alzheimer's disease. Brain 2001; 124: 96-102.

Baddeley A. Working memory. Oxford: Claredon Press, 1986.

Baddeley A. The episodic buffer: a new component of working memory? Trends Cogn Sci. 2000; 4: 417-423.

Baddeley A. The concept of episodic memory. Philosophical Transactions of the Royal Society B: Biological Sciences 2001; 356: 1345-1350.

Baddeley A, Della SS. Working memory and executive control. Philosophical Transactions of the Royal Society B: Biological Sciences 1996; 351: 1397-1403.

Baddeley AD, Baddeley HA, Bucks RS, Wilcock GK. Attentional control in Alzheimer's disease. Brain 2001; 124: 1492-1508.

Badgaiyan RD, Schacter DL, Alpert NM. Priming within and across modalities: exploring the nature of rCBF increases and decreases. Neuroimage. 2001; 13: 272-282.

Bagurdes LA, Mesulam MM, Gitelman DR, Weintraub S, Small DM. Modulation of the spatial attention network by incentives in healthy aging and mild cognitive impairment. Neuropsychologia 2008; 46: 2943-8.

Balota DA, Cortese MJ, Duchek JM, Adams DA, Roediger HL, McDermott KB, et al. Veridical and false memories in healthy older adults and in dementia of the Alzheimers type. Cognitive Neuropsychology 1999; 16: 361-384.

Baltes PB, Staudinger UM, Lindenberger U. Lifespan psychology: theory and application to intellectual functioning. Annu Rev Psychol 1999; 50: 471-507.

Barnikol TT, Pawelczyk NB, Barnikol UB, Kuhn J, Lenartz D, Sturm V, et al. Changes in apraxia after deep brain stimulation of the nucleus basalis Meynert in a patient with Parkinson dementia syndrome. Mov Disord 2010; 25: 1519-1520.

Bassett DS, Bullmore E. Small-world brain networks. Neuroscientist 2006; 12: 512-23.

Bassett SS, Yousem DM, Cristinzio C, Kusevic I, Yassa MA, Caffo BS, et al. Familial risk for Alzheimer's disease alters fMRI activation patterns. Brain 2006; 129: 1229-1239.

Becker JT, Mintun MA, Aleva K, Wiseman MB, Nichols T, DeKosky ST. Compensatory reallocation of brain resources supporting verbal episodic memory in Alzheimer's disease. Neurology 1996; 46: 692-700.

Becker RE, Giacobini E. Mechanisms of cholinesterase inhibition in senile dementia of the Alzheimer type: Clinical, pharamacological and therapuetic aspects. Drug Development Research 1988a; 12: 163-195.

Becker RE, Giacobini E. Pharmacokentics and pharmacodynamics of acetylcholinesterase inhibition: Can acetylcholine levels in the brain be improved in Alzheimer's disease? . Drug Development Research 1988b; 14: 235-246.

Beglinger LJ, Gaydos BL, Kareken DA, Tangphao-Daniels O, Siemers ER, Mohs RC. Neuropsychological test performance in healthy volunteers before and after donepezil administration. J Psychopharmacol 2004; 18: 102-8.

Beglinger LJ, Tangphao-Daniels O, Kareken DA, Zhang L, Mohs R, Siemers ER. Neuropsychological test performance in healthy elderly volunteers before and after donepezil administration: a randomized, controlled study. J Clin Psychopharmacol 2005; 25: 159-65.

Belleville S, Chertkow H, Gauthier S. Working memory and control of attention in persons with Alzheimer's disease and mild cognitive impairment. Neuropsychology 2007; 21: 458-69.

Benge JF, Balsis S, Geraci L, Massman PJ, Doody RS. How well do the ADAS-cog and its subscales measure cognitive dysfunction in Alzheimer's disease? Dement Geriatr Cogn Disord 2009; 28: 63-9.

Bennett IJ, Golob EJ, Parker ES, Starr A. Memory evaluation in mild cognitive impairment using recall and recognition tests. Journal of Clinical and Experimental Neuropsychology 2006; 28: 1408-1422.

Bentley P, Husain M, Dolan RJ. Effects of cholinergic enhancement on visual stimulation, spatial attention, and spatial working memory. Neuron 2004; 41: 969-982.

Bentley P, Vuilleumier P, Thiel CM, Driver J, Dolan RJ. Effects of attention and emotion on repetition priming and their modulation by cholinergic enhancement. Journal of Neurophysiology 2003; 90: 1171-1181.

Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev 2003; 42: 33-84.

Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane.Database.Syst.Rev. 2006: CD005593.

Birks J, Flicker L. Donepezil for mild cognitive impairment. Cochrane.Database.Syst.Rev. 2006; 3: CD006104.

Birks J, Grimley Evans J, Iakovidou V, Tsolaki M. Rivastigmine for Alzheimer's disease. Cochrane Database Syst Rev 2000: CD001191.

Birks J, Grimley Evans J, Iakovidou V, Tsolaki M, Holt FE. Rivastigmine for Alzheimer's disease. Cochrane Database Syst Rev 2009: CD001191.

Birks JS, Melzer D. Donepezil for mild and moderate Alzheimer's disease. Cochrane.Database.Syst.Rev. 2000: CD001190.

Birn RM, Cox RW, Bandettini PA. Experimental designs and processing strategies for fMRI studies involving overt verbal responses. Neuroimage 2004; 23: 1046-1058.

Bisiacchi PS, Borella E, Bergamaschi S, Carretti B, Mondini S. Interplay between memory and executive functions in normal and pathological aging. J Clin Exp Neuropsychol 2008; 30: 723-33.

Blackburn EH. Switching and signaling at the telomere. Cell 2001; 106: 661-73.

Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet 2005; 6: 611-22.

Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet 2006; 368: 387-403.

Blokland A. Acetylcholine: a neurotransmitter for learning and memory? Brain Research.Brain Research Reviews 1995; 21: 285-300.

Bohnen NI, Kaufer DI, Hendrickson R, Ivanco LS, Lopresti B, Davis JG, et al. Cognitive correlates of alterations in acetylcholinesterase in Alzheimer's disease. Neurosci Lett 2005a; 380: 127-32.

Bohnen NI, Kaufer DI, Hendrickson R, Ivanco LS, Lopresti BJ, Koeppe RA, et al. Degree of inhibition of cortical acetylcholinesterase activity and cognitive

effects by donepezil treatment in Alzheimer's disease. Journal of Neurology, Neurosurgery and Psychiatry 2005b; 76: 315-319.

Bondi MW, Houston WS, Eyler LT, Brown GG. fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. Neurology 2005; 64: 501-508.

Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, et al. Patterns of brain activation in people at risk for Alzheimer's disease. New England Journal of Medicine 2000; 343: 450-456.

Bopp KL, Verhaeghen P. Aging and verbal memory span: a meta-analysis. J Gerontol B Psychol Sci Soc Sci 2005; 60: P223-33.

Borkowska A, Ziolkowska-Kochan M, Rybakowski JK. One-year treatment of Alzheimer's disease with acetylcholinesterase inhibitors: improvement on ADAS-cog and TMT A, no change or worsening on other tests. Hum.Psychopharmacol. 2005; 20: 409-414.

Bowers MBJ, Goodman E, Sim MV. Some Behavioural Changes in Man Following Anticholinesterase Administration. Journal of mental and nervous disease 1964; 138: 383-9.

Bozoki A, Giordani B, Heidebrink JL, Berent S, Foster NL. Mild cognitive impairments predict dementia in nondemented elderly patients with memory loss. Archives of Neurology 2001; 58: 411-416.

Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiology of Aging 1997; 18: 351-357.

Brammer MJ, Bullmore ET, Simmons A, Williams SC, Grasby PM, Howard RJ, et al. Generic brain activation mapping in functional magnetic resonance imaging: a nonparametric approach. Magnetic Resonance Imaging 1997; 15: 763-770.

Brassen S, Weber-Fahr W, Sommer T, Lehmbeck JT, Braus DF. Hippocampal-prefrontal encoding activation predicts whether words can be successfully recalled or only recognized. Behavioural Brain Research 2006; 171: 271-278.

Breakspear M, Brammer M, Robinson PA. Construction of multivariate surrogate sets from nonlinear data using the wavelet transform. Physica.D 2003; 182: 1-22.

Broome MR, Matthiasson P, Fusar-Poli P, Woolley JB, Johns LC, Tabraham P, et al. Neural correlates of executive function and working memory in the 'at-risk mental state'. Br J Psychiatry 2009; 194: 25-33.

Brouwer WH, Waterink W, Van Wolffelaar PC, Rothengatter T. Divided attention in experienced young and older drivers: lane tracking and visual analysis in a dynamic driving simulator. Hum Factors 1991; 33: 573-82.

Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Annals of the New York Academy of Sciences 2008; 1124: 1-38.

Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, et al. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. J Neurosci 2009; 29: 1860-73.

Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci. 2005; 25: 7709-7717.

Budson AE, Daffner KR, Desikan R, Schacter DL. When false recognition is unopposed by true recognition: gist-based memory distortion in Alzheimer's disease. Neuropsychology. 2000; 14: 277-287.

Budson AE, Sitarski J, Daffner KR, Schacter DL. False recognition of pictures versus words in Alzheimer's disease: the distinctiveness heuristic. Neuropsychology. 2002; 16: 163-173.

Budson AE, Sullivan AL, Daffner KR, Schacter DL. Semantic versus phonological false recognition in aging and Alzheimer's disease. Brain and Cognition 2003; 51: 251-261.

Budson AE, Wolk DA, Chong H, Waring JD. Episodic memory in Alzheimer's disease: separating response bias from discrimination. Neuropsychologia 2006; 44: 2222-2232.

Bullmore E, Long C, Suckling J, Fadili J, Calvert G, Zelaya F, et al. Colored noise and computational inference in neurophysiological (fMRI) time series analysis: resampling methods in time and wavelet domains. Human Brain Mapping 2001; 12: 61-78.

Bullmore ET, Suckling J, Overmeyer S, Rabe-Hesketh S, Taylor E, Brammer MJ. Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. IEEE Transactions on Medical Imaging 1999; 18: 32-42.

Butler RN, Miller RA, Perry D, Carnes BA, Williams TF, Cassel C, et al. New model of health promotion and disease prevention for the 21st century. BMJ 2008; 337: a399.

Cabeza R. Hemispheric asymmetry reduction in older adults: the HAROLD model. Psychology and Aging 2002; 17: 85-100.

Cabeza R, Anderson ND, Houle S, Mangels JA, Nyberg L. Age-related differences in neural activity during item and temporal-order memory retrieval: a positron emission tomography study. Journal of Cognitive Neuroscience 2000; 12: 197-206.

Cabeza R, Nyberg L. Imaging cognition II: An empirical review of 275 PET and fMRI studies. Journal of Cognitive Neuroscience 2000; 12: 1-47.

Carnes BA, Olshansky SJ. A Realist View of Aging, Mortality, and Future Longevity. Population and Development Review 2007; 33: 367-381.

Castellani RJ. Vascular dementia and Alzheimer's disease: a waning dichotomy. J Alzheimers.Dis. 2007; 12: 343-344.

Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, et al. Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. J Neurosci. 2006; 26: 10222-10231.

Chang C, Crottaz-Herbette S, Menon V. Temporal dynamics of basal ganglia response and connectivity during verbal working memory. Neuroimage 2007; 34: 1253-69.

Chetelat G, Desgranges B, de lSV, Viader F, Berkouk K, Landeau B, et al. Dissociating atrophy and hypometabolism impact on episodic memory in mild cognitive impairment. Brain 2003; 126: 1955-1967.

Chiba AA, Bushnell PJ, Oshiro WM, Gallagher M. Selective removal of cholinergic neurons in the basal forebrain alters cued target detection. Neuroreport 1999; 10: 3119-3123.

Chua TC, Wen W, Chen X, Kochan N, Slavin MJ, Trollor JN, et al. Diffusion tensor imaging of the posterior cingulate is a useful biomarker of mild cognitive impairment. Am J Geriatr Psychiatry 2009; 17: 602-13.

Chua TC, Wen W, Slavin MJ, Sachdev PS. Diffusion tensor imaging in mild cognitive impairment and Alzheimer's disease: a review. Curr Opin Neurol 2008; 21: 83-92.

Chun MM, Turk-Browne NB. Interactions between attention and memory. Current Opinion in Neurobiology 2007; 17: 177-184.

Collins K, Mitchell JR. Telomerase in the human organism. Oncogene 2002; 21: 564-79.

Corbetta M, Miezin FM, Dobmeyer S, Shulman GL, Petersen SE. Selective and divided attention during visual discriminations of shape, color, and speed: functional anatomy by positron emission tomography. Journal of Neuroscience 1991; 11: 2383-2402.

Craik FI, Govoni R, Naveh-Benjamin M, Anderson ND. The effects of divided attention on encoding and retrieval processes in human memory. Journal of Experimental Psychology: General 1996; 125: 159-180.

Craik FI, McDowd JM. Age Differences in Recall and Recognition. Journal of Experimental Psychology: Learning, Memory and Cognition 1987; 13: 474-479.

Craik FIM, Lockhart RS. Levels of processing. A framework for memory research. Journal of Verbal Learning and Verbal Behaviour 1972; 11: 671-684.

Craik FIM, Tulving E. Depth of processing and the retention of words in episodic memory. Journal of Experimental Psychology: General 1975; 104: 168-294.

Crossley NA, Mechelli A, Fusar-Poli P, Broome MR, Matthiasson P, Johns LC, et al. Superior temporal lobe dysfunction and frontotemporal dysconnectivity in subjects at risk of psychosis and in first-episode psychosis. Human Brain Mapping 2009; 30: 4129-4137.

D'Esposito M, Detre JA, Alsop DC, Shin RK, Atlas S, Grossman M. The neural basis of the central executive system of working memory. Nature 1995; 378: 279-281.

Dannhauser TM, Shergill SS, Stevens T, Lee L, Seal M, Walker RW, et al. An fMRI study of verbal episodic memory encoding in amnestic mild cognitive impairment. Cortex 2008; 44: 869-880.

Dannhauser TM, Walker Z, Stevens T, Lee L, Seal M, Shergill SS. The functional anatomy of divided attention in amnestic mild cognitive impairment. Brain 2005; 128: 1418-1427.

Davies AD.

The influence of age on Trail Making test performance. Journal of Clinical Psychology 1968; 24: 96-98.

De Jager CA, Hogervorst E, Combrinck M, Budge MM. Sensitivity and specificity of neuropsychological tests for mild cognitive impairment, vascular cognitive impairment and Alzheimer's disease. Psychological Medicine 2003; 33: 1039-1050.

de Zubicaray GI, Williams SC, Wilson SJ, Rose SE, Brammer MJ, Bullmore ET, et al. Prefrontal cortex involvement in selective letter generation: a functional magnetic resonance imaging study. Cortex 1998; 34: 389-401.

Decarli C, Frisoni GB, Clark CM, Harvey D, Grundman M, Petersen RC, et al. Qualitative estimates of medial temporal atrophy as a predictor of progression from mild cognitive impairment to dementia. Archives of Neurology 2007; 64: 108-115.

Deese J. On the prediction of occurrence of particular verbal intrusions in immediate recall. Journal of Experimental Psychology 1959; 58: 17-22.

DeKosky ST, Ikonomovic MD, Styren SD, Beckett L, Wisniewski S, Bennett DA, et al. Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. Annals of Neurology 2002; 51: 145-155.

Demonet JF, Thierry G, Cardebat D. Renewal of the neurophysiology of language: functional neuroimaging. Physiol Rev. 2005; 85: 49-95.

Desgranges B, Baron JC, Eustache F. The functional neuroanatomy of episodic memory: the role of the frontal lobes, the hippocampal formation, and other areas. Neuroimage. 1998; 8: 198-213.

Dickerson BC. Advances in functional magnetic resonance imaging: technology and clinical applications. Neurotherapeutics 2007; 4: 360-70.

Dickerson BC, Salat DH, Bates JF, Atiya M, Killiany RJ, Greve DN, et al. Medial temporal lobe function and structure in mild cognitive impairment. Annals of Neurology 2004; 56: 27-35.

Dolcos F, Rice HJ, Cabeza R. Hemispheric asymmetry and aging: right hemisphere decline or asymmetry reduction. Neurosci Biobehav Rev 2002; 26: 819-25.

Dove A, Brett M, Cusack R, Owen AM. Dissociable contributions of the mid-ventrolateral frontal cortex and the medial temporal lobe system to human memory. Neuroimage 2006; 31: 1790-801.

Dubois B, Albert ML. Amnestic MCI or prodromal Alzheimer's disease? Lancet Neurol. 2004; 3: 246-248.

Dudas RB, Clague F, Thompson SA, Graham KS, Hodges JR. Episodic and semantic memory in mild cognitive impairment. Neuropsychologia 2005; 43: 1266-1276.

Ekstrom A. How and when the fMRI BOLD signal relates to underlying neural activity: the danger in dissociation. Brain Res Rev 2010; 62: 233-44.

Engelien A, Stern E, Silbersweig D. Functional neuroimaging of human central auditory processing in normal subjects and patients with neurological and neuropsychiatric disorders. J Clin Exp Neuropsychol 2001; 23: 94-120.

Fan J, McCandliss BD, Fossella J, Flombaum JI, Posner MI. The activation of attentional networks. Neuroimage. 2005; 26: 471-479.

Farias ST, Mungas D, Reed BR, Harvey D, Decarli C. Progression of mild cognitive impairment to dementia in clinic- vs community-based cohorts. Archives of Neurology 2009; 66: 1151-1157.

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 1997; 278: 1349-1356.

Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005; 366: 2112-2117.

Fischer P, Jungwirth S, Zehetmayer S, Weissgram S, Hoenigschnabl S, Gelpi E, et al. Conversion from subtypes of mild cognitive impairment to Alzheimer dementia. Neurology 2007; 68: 288-291.

Fleisher AS, Sowell BB, Taylor C, Gamst AC, Petersen RC, Thal LJ. Clinical predictors of progression to Alzheimer disease in amnestic mild cognitive impairment. Neurology 2007; 68: 1588-1595.

Fletcher PC, Frith CD, Grasby PM, Shallice T, Frackowiak RS, Dolan RJ. Brain systems for encoding and retrieval of auditory-verbal memory. An in vivo study in humans. Brain 1995; 118 (Pt 2): 401-416.

Fletcher PC, Henson RN. Frontal lobes and human memory: insights from functional neuroimaging. Brain 2001; 124: 849-881.

Flicker C, Ferris SH, Reisberg B. Mild cognitive impairment in the elderly: predictors of dementia. Neurology 1991; 41: 1006-1009.

Fliessbach K, Trautner P, Quesada CM, Elger CE, Weber B. Cerebellar contributions to episodic memory encoding as revealed by fMRI. Neuroimage. 2007; 35: 1330-1337.

Floel A, Poeppel D, Buffalo EA, Braun A, Wu CW, Seo HJ, et al. Prefrontal cortex asymmetry for memory encoding of words and abstract shapes. Cerebral Cortex 2004; 14: 404-409.

Foldi NS, White RE, Schaefer LA. Detecting effects of donepezil on visual selective attention using signal detection parameters in Alzheimer's disease. International Journal of Geriatric Psychiatry 2005; 20: 485-488.

Foote SL, Bloom FE, Aston-Jones G. Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. Physiol Rev 1983; 63: 844-914.

Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. Journal of Neurology, Neurosurgery and Psychiatry 1999; 66: 137-147.

Fratiglioni L, Ahlbom A, Viitanen M, Winblad B. Risk factors for late-onset Alzheimer's disease: a population-based, case-control study. Annals of Neurology 1993; 33: 258-266.

Friman O, Borga M, Lundberg P, Knutsson H. Adaptive analysis of fMRI data. Neuroimage 2003; 19: 837-45.

Furey ML, Pietrini P, Alexander GE, Schapiro MB, Horwitz B. Cholinergic enhancement improves performance on working memory by modulating the functional activity in distinct brain regions: a positron emission tomography regional cerebral blood flow study in healthy humans. Brain Res.Bull 2000; 51: 213-218.

Furey ML, Pietrini P, Haxby JV, Alexander GE, Lee HC, VanMeter J, et al. Cholinergic stimulation alters performance and task-specific regional cerebral blood flow during working memory. Proc.Natl.Acad.Sci.U.S.A 1997; 94: 6512-6516.

Fusar-Poli P, Broome MR, Matthiasson P, Williams SC, Brammer M, McGuire PK. Effects of acute antipsychotic treatment on brain activation in first episode psychosis: an fMRI study. European Neuropsychopharmacology 2007; 17: 492-500.

Gagnepain P, Chetelat G, Landeau B, Dayan J, Eustache F, Lebreton K. Spoken word memory traces within the human auditory cortex revealed by repetition

priming and functional magnetic resonance imaging. J Neurosci. 2008; 28: 5281-5289.

Ganguli M, Dodge HH, Shen C, DeKosky ST. Mild cognitive impairment, amnestic type: an epidemiologic study. Neurology 2004; 63: 115-121.

Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, et al. Mild cognitive impairment. Lancet 2006; 367: 1262-1270.

Gavrila D, Antunez C, Tormo MJ, Carles R, Garcia Santos JM, Parrilla G, et al. Prevalence of dementia and cognitive impairment in Southeastern Spain: the Ariadna study. Acta Neurologica Scandinavica 2009; 120: 300-307.

Giannakopoulos P, Gold G, Kovari E, von Gunten A, Imhof A, Bouras C, et al. Assessing the cognitive impact of Alzheimer disease pathology and vascular burden in the aging brain: the Geneva experience. Acta Neuropathol 2007; 113: 1-12.

Giannakopoulos P, Hof PR, Michel JP, Guimon J, Bouras C. Cerebral cortex pathology in aging and Alzheimer's disease: a quantitative survey of large hospital-based geriatric and psychiatric cohorts. Brain Res Brain Res Rev 1997; 25: 217-45.

Gill SS, Anderson GM, Fischer HD, Bell CM, Li P, Normand SL, et al. Syncope and its consequences in patients with dementia receiving cholinesterase inhibitors: a population-based cohort study. Arch Intern Med 2009; 169: 867-73.

Goekoop R, Rombouts SA, Jonker C, Hibbel A, Knol DL, Truyen L, et al. Challenging the cholinergic system in mild cognitive impairment: a pharmacological fMRI study. Neuroimage. 2004; 23: 1450-1459.

Golby AJ, Poldrack RA, Brewer JB, Spencer D, Desmond JE, Aron AP, et al. Material-specific lateralization in the medial temporal lobe and prefrontal cortex during memory encoding. Brain 2001; 124: 1841-1854.

Goldberg TE, Berman KF, Fleming K, Ostrem J, Van Horn JD, Esposito G, et al. Uncoupling cognitive workload and prefrontal cortical physiology: a PET rCBF study. Neuroimage 1998; 7: 296-303.

Gomez-Perez E, Ostrosky-Solis F. Attention and memory evaluation across the life span: heterogeneous effects of age and education. J Clin Exp Neuropsychol 2006; 28: 477-94.

Grady CL, Haxby JV, Horwitz B, Sundaram M, Berg G, Schapiro M, et al. Longitudinal study of the early neuropsychological and cerebral metabolic

changes in dementia of the Alzheimer type. J Clin.Exp.Neuropsychol. 1988; 10: 576-596.

Grady CL, Maisog JM, Horwitz B, Ungerleider LG, Mentis MJ, Salerno JA, et al. Age-related changes in cortical blood flow activation during visual processing of faces and location. J Neurosci. 1994; 14: 1450-1462.

Grady CL, McIntosh AR, Horwitz B, Maisog JM, Ungerleider LG, Mentis MJ, et al. Age-related reductions in human recognition memory due to impaired encoding. Science 1995; 269: 218-221.

Greenwood P, Parasuraman R. To cite this Article Greenwood, Pamela and Parasuraman, Raja(1991) 'Effects of aging on the speed and attentional cost of cognitive operations', Developmental Neuropsychology, 7: 4, 421 — 434. Developmental Neuropsychology 1991; 7: 421-434.

Grober E, Ocepek-Welikson K, Teresi JA. The Free and Cued Selective Reminding Test: evidence of psychometric adequacy Psychology Science Quarterly 2009; 51: 266-282.

Gron G, Kirstein M, Thielscher A, Riepe MW, Spitzer M. Cholinergic enhancement of episodic memory in healthy young adults. Psychopharmacology (Berl) 2005; 182: 170-179.

Grudzien A, Shaw P, Weintraub S, Bigio E, Mash DC, Mesulam MM. Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. Neurobiol Aging 2007; 28: 327-35.

Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. Archives of Neurology 2003; 60: 729-736.

Hachinski VC, Iliff LD, Zilhka E, du Boulay GH, McAllister VL, Marshall J, et al. Cerebral blood flow in dementia. Archives of Neurology 1975; 32: 632-637.

Haier RJ, Siegel BV, Nuechterlein KH, Hazlett E, Wu JC, Paek J, et al. Cortical glucose metabolic rate correlates of abstract reasoning and attention studied with positron emission tomography. Intelligence 1988; 12: 199–217.

Hamalainen A, Pihlajamaki M, Tanila H, Hanninen T, Niskanen E, Tervo S, et al. Increased fMRI responses during encoding in mild cognitive impairment. Neurobiology of Aging 2007; 28: 1889-1903.

Han SD, Bangen KJ, Bondi MW. Functional magnetic resonance imaging of compensatory neural recruitment in aging and risk for Alzheimer's disease: review and recommendations. Dement.Geriatr.Cogn Disord. 2009; 27: 1-10.

Hansson O, Buchhave P, Zetterberg H, Blennow K, Minthon L, Warkentin S. Combined rCBF and CSF biomarkers predict progression from mild cognitive impairment to Alzheimer's disease. Neurobiology of Aging 2009; 30: 165-173.

Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol. 2006; 5: 228-234.

Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297: 353-356.

Hartley A, A. . Evidence for the preservation of spatial selective attention in old age. Psychology and Ageing 1993; 8: 8.

Heinze HJ, Mangun GR, Burchert W, Hinrichs H, Scholz M, Munte TF, et al. Combined spatial and temporal imaging of brain activity during visual selective attention in humans. Nature 1994; 372: 543-6.

Heneka MT. Noradrenergic denervation facilitates the release of acetylcholine and serotonin in the hippocampus: towards a mechanism underlying upregulations described in MCI patients. Exp Neurol 2009; 217: 237-9.

Heneka MT, Galea E, Gavriluyk V, Dumitrescu-Ozimek L, Daeschner J, O'Banion MK, et al. Noradrenergic depletion potentiates beta -amyloid-induced cortical inflammation: implications for Alzheimer's disease. J Neurosci 2002; 22: 2434-42.

Heneka MT, Ramanathan M, Jacobs AH, Dumitrescu-Ozimek L, Bilkei-Gorzo A, Debeir T, et al. Locus ceruleus degeneration promotes Alzheimer pathogenesis in amyloid precursor protein 23 transgenic mice. J Neurosci 2006; 26: 1343-54.

Henson R. A mini-review of fMRI studies of human medial temporal lobe activity associated with recognition memory. Q J Exp Psychol B 2005; 58: 340-60.

Herholz K, Weisenbach S, Kalbe E, Diederich NJ, Heiss WD. Cerebral acetylcholine esterase activity in mild cognitive impairment. Neuroreport 2005; 16: 1431-1434.

Heun R, Freymann K, Erb M, Leube DT, Jessen F, Kircher TT, et al. Successful verbal retrieval in elderly subjects is related to concurrent hippocampal and posterior cingulate activation. Dement Geriatr Cogn Disord 2006a; 22: 165-72.

Heun R, Freymann K, Erb M, Leube DT, Jessen F, Kircher TT, et al. Mild cognitive impairment (MCI) and actual retrieval performance affect cerebral activation in the elderly. Neurobiology of Aging 2007; 28: 404-413.

Heun R, Kolsch H, Jessen F. Risk factors and early signs of Alzheimer's disease in a family study sample. Risk of AD. European Archives of Psychiatry and Clinical Neuroscience 2006b; 256: 28-36.

Hodges JR, Patterson K. Is semantic memory consistently impaired early in the course of Alzheimer's disease? Neuroanatomical and diagnostic implications. Neuropsychologia 1995; 33: 441-459.

Huppert FA, Brayne C, Gill C, Paykel ES, Beardsall L. CAMCOG--a concise neuropsychological test to assist dementia diagnosis: socio-demographic determinants in an elderly population sample. British Journal of Clinical Psychology 1995; 34 (Pt 4): 529-541.

Iidaka T, Anderson ND, Kapur S, Cabeza R, Craik FI. The effect of divided attention on encoding and retrieval in episodic memory revealed by positron emission tomography. J.Cogn Neurosci. 2000a; 12: 267-280.

Iidaka T, Sadato N, Yamada H, Yonekura Y. Functional asymmetry of human prefrontal cortex in verbal and non-verbal episodic memory as revealed by fMRI. Brain Res.Cogn Brain Res. 2000b; 9: 73-83.

Incisa della Rocchetta A, Milner B. Strategic search and retrieval inhibition: the role of the frontal lobes. Neuropsychologia 1993; 31: 503-524.

Iqbal K, Alonso AC, Chen S, Chohan MO, El-Akkad E, Gong CX, et al. Tau pathology in Alzheimer disease and other tauopathies. Biochimica et Biophysica Acta 2005; 1739: 198-210.

Iqbal K, Grundke-Iqbal I. Alzheimer neurofibrillary degeneration: significance, etiopathogenesis, therapeutics and prevention. J Cell Mol.Med. 2008; 12: 38-55.

Jack CR, Jr., Dickson DW, Parisi JE, Xu YC, Cha RH, O'Brien PC, et al. Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. Neurology 2002; 58: 750-757.

Jack CR, Jr., Lowe VJ, Senjem ML, Weigand SD, Kemp BJ, Shiung MM, et al. 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. Brain 2008; 131: 665-80.

Jack CR, Jr., Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, et al. Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. Brain 2009; 132: 1355-65.

Jackisch R, Gansser S, Cassel JC. Noradrenergic denervation facilitates the release of acetylcholine and serotonin in the hippocampus: towards a mechanism underlying upregulations described in MCI patients? Exp Neurol 2008; 213: 345-53.

Jaeggi SM, Buschkuehl M, Etienne A, Ozdoba C, Perrig WJ, Nirkko AC. On how high performers keep cool brains in situations of cognitive overload. Cogn Affect.Behav.Neurosci. 2007; 7: 75-89.

Janowsky JS, Shimamura AP, Kritchevsky M, Squire LR. Cognitive impairment following frontal lobe damage and its relevance to human amnesia. Behavioral Neuroscience 1989; 103: 548-560.

Jendroska K, Poewe W, Daniel SE, Pluess J, Iwerssen-Schmidt H, Paulsen J, et al. Ischemic stress induces deposition of amyloid beta immunoreactivity in human brain. Acta Neuropathol. 1995; 90: 461-466.

Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, et al. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. Archives of Neurology 2006; 63: 674-681.

Johannsen P, Jakobsen J, Bruhn P, Gjedde A. Cortical responses to sustained and divided attention in Alzheimer's disease. Neuroimage. 1999; 10: 269-281.

Johannsen P, Jakobsen J, Bruhn P, Hansen SB, Gee A, Stodkilde-Jorgensen H, et al. Cortical sites of sustained and divided attention in normal elderly humans. Neuroimage. 1997; 6: 145-155.

Johnson JA, Zatorre RJ. Neural substrates for dividing and focusing attention between simultaneous auditory and visual events. Neuroimage. 2006; 31: 1673-1681.

Johnson MK. Source monitoring and memory distortion. Philosophical Transactions of the Royal Society B: Biological Sciences 1997; 352: 1733-1745.

Johnson MK, Raye CL, Mitchell KJ, Greene EJ, Anderson AW. FMRI evidence for an organization of prefrontal cortex by both type of process and type of information. Cerebral Cortex 2003; 13: 265-273.

Johnson SC, Schmitz TW, Moritz CH, Meyerand ME, Rowley HA, Alexander AL, et al. Activation of brain regions vulnerable to Alzheimer's disease: the effect of mild cognitive impairment. Neurobiol Aging 2006a; 27: 1604-12.

Johnson SC, Schmitz TW, Trivedi MA, Ries ML, Torgerson BM, Carlsson CM, et al. The influence of Alzheimer disease family history and apolipoprotein E epsilon4 on mesial temporal lobe activation. J Neurosci. 2006b; 26: 6069-6076.

Jones BE, Yang TZ. The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. J Comp Neurol 1985; 242: 56-92.

Joubert S, Felician O, Barbeau EJ, Didic M, Poncet M, Ceccaldi M. Patterns of semantic memory impairment in Mild Cognitive Impairment. Behav.Neurol. 2008; 19: 35-40.

Kadir A, Almkvist O, Wall A, Langstrom B, Nordberg A. PET imaging of cortical 11C-nicotine binding correlates with the cognitive function of attention in Alzheimer's disease. Psychopharmacology (Berl) 2006; 188: 509-520.

Kalaria RN. Small vessel disease and Alzheimer's dementia: pathological considerations. Cerebrovascular Diseases 2002; 13 Suppl 2: 48-52.

Kelley WM, Miezin FM, McDermott KB, Buckner RL, Raichle ME, Cohen NJ, et al. Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. Neuron 1998; 20: 927-936.

Kemppainen NM, Aalto S, Wilson IA, Nagren K, Helin S, Bruck A, et al. PET amyloid ligand [11C]PIB uptake is increased in mild cognitive impairment. Neurology 2007; 68: 1603-6.

Kinomura S, Larsson J, Gulyas B, Roland PE. Activation by attention of the human reticular formation and thalamic intralaminar nuclei. Science 1996; 271: 512-5.

Kircher TT, Weis S, Freymann K, Erb M, Jessen F, Grodd W, et al. Hippocampal activation in patients with mild cognitive impairment is necessary for

successful memory encoding. J Neurol.Neurosurg.Psychiatry 2007; 78: 812-818.

Kirchhoff BA, Wagner AD, Maril A, Stern CE. Prefrontal-temporal circuitry for episodic encoding and subsequent memory. J Neurosci. 2000; 20: 6173-6180.

Klingberg T. Concurrent performance of two working memory tasks: potential mechanisms of interference. Cerebral Cortex 1998; 8: 593-601.

Knowlton BJ. The relationship between remembering and knowing: a cognitive neuroscience perspective. Acta Psychologica 1998; 98: 253-265.

Knudsen EI. Fundamental components of attention. Annual Review of Neuroscience 2007; 30: 57-78.

Koechlin E, Basso G, Pietrini P, Panzer S, Grafman J. The role of the anterior prefrontal cortex in human cognition. Nature 1999; 399: 148-151.

Kordower JH, Chu Y, Stebbins GT, DeKosky ST, Cochran EJ, Bennett D, et al. Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment. Annals of Neurology 2001; 49: 202-213.

Korf ES, Wahlund LO, Visser PJ, Scheltens P. Medial temporal lobe atrophy on MRI predicts dementia in patients with mild cognitive impairment. Neurology 2004; 63: 94-100.

Koutstaal W, Verfaellie M, Schacter DL. Recognizing identical versus similar categorically related common objects: further evidence for degraded gist representations in amnesia. Neuropsychology. 2001; 15: 268-289.

Kuhn J, Grundler TO, Lenartz D, Sturm V, Klosterkotter J, Huff W. Deep brain stimulation for psychiatric disorders. Dtsch Arztebl Int 2010; 107: 105-13.

Laine M, Tuokkola T, Hiltunen J, Vorobyev V, Bliss I, Baddeley A, et al. Central executive function in mild cognitive impairment: A PET activation study. Scand.J Psychol 2008.

Lane RM, Potkin SG, Enz A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. Int.J Neuropsychopharmacol. 2006; 9: 101-124.

Lannfelt L, Lilius L, Nastase M, Viitanen M, Fratiglioni L, Eggertsen G, et al. Lack of association between apolipoprotein E allele epsilon 4 and sporadic Alzheimer's disease. Neuroscience Letters 1994; 169: 175-178.

Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Ann Intern Med 2006; 144: 73-81.

Lawrence AD, Sahakian BJ. Alzheimer disease, attention, and the cholinergic system. Alzheimer Disease and Associated Disorders 1995; 9 Suppl 2: 43-49.

Lawton MPB, E.M. Assessment of older people: Self-maintaining and instrumental activities of daily

living. Gerontologist 1969; 9: 179-186.

Levinoff EJ, Saumier D, Chertkow H. Focused attention deficits in patients with Alzheimer's disease and mild cognitive impairment. Brain and Cognition 2005; 57: 127-130.

Lezak L. Neuropsychological assessment. Oxford: Oxford University Press, 1995.

Lindenberger U, Baltes PB. Sensory functioning and intelligence in old age: a strong connection. Psychol Aging 1994; 9: 339-55.

Lindenberger U, Baltes PB. Intellectual functioning in old and very old age: cross-sectional results from the Berlin Aging Study. Psychol Aging 1997; 12: 410-32.

Lindenberger U, Scherer H, Baltes PB. The strong connection between sensory and cognitive performance in old age: not due to sensory acuity reductions operating during cognitive assessment. Psychol Aging 2001; 16: 196-205.

Logan JM, Sanders AL, Snyder AZ, Morris JC, Buckner RL. Under-recruitment and nonselective recruitment: dissociable neural mechanisms associated with aging. Neuron 2002; 33: 827-840.

Logothetis NK. The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. Philosophical Transactions of the Royal Society B: Biological Sciences 2002; 357: 1003-1037.

Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. Nature 2001; 412: 150-157.

Loose R, Kaufmann C, Auer DP, Lange KW. Human prefrontal and sensory cortical activity during divided attention tasks. Human Brain Mapping 2003; 18: 249-259.

Low LF, Brodaty H, Edwards R, Kochan N, Draper B, Trollor J, et al. The prevalence of "cognitive impairment no dementia" in community-dwelling elderly: a pilot study. Aust.N.Z.J Psychiatry 2004; 38: 725-731.

Lowe VJ, Kemp BJ, Jack CR, Jr., Senjem M, Weigand S, Shiung M, et al. Comparison of 18F-FDG and PiB PET in cognitive impairment. J Nucl Med 2009; 50: 878-86.

Loy C, Schneider L. Galantamine for Alzheimer's disease. Cochrane.Database.Syst.Rev. 2004: CD001747.

Loy C, Schneider L. Galantamine for Alzheimer's disease and mild cognitive impairment. Cochrane.Database.Syst.Rev. 2006: CD001747.

Lucin KM, Wyss-Coray T. Immune activation in brain aging and neurodegeneration: too much or too little? Neuron 2009; 64: 110-22.

Lustig C, Snyder AZ, Bhakta M, O'Brien KC, McAvoy M, Raichle ME, et al. Functional deactivations: change with age and dementia of the Alzheimer type. Proceedings of the National Academy of Sciences of the United States of America 2003; 100: 14504-14509.

Lynch CA, Walsh C, Blanco A, Moran M, Coen RF, Walsh JB, et al. The clinical dementia rating sum of box score in mild dementia. Dement.Geriatr.Cogn Disord. 2006; 21: 40-43.

Machulda MM, Senjem ML, Weigand SD, Smith GE, Ivnik RJ, Boeve BF, et al. Functional magnetic resonance imaging changes in amnestic and nonamnestic mild cognitive impairment during encoding and recognition tasks. J Int Neuropsychol Soc 2009; 15: 372-82.

Mandzia JL, McAndrews MP, Grady CL, Graham SJ, Black SE. Neural correlates of incidental memory in mild cognitive impairment: An fMRI study. Neurobiology of Aging 2007.

Mangun GR, Hopfinger JB, Kussmaul CL, Fletcher EM, Heinze HJ. Covariations in ERP and PET measures of spatial selective attention in human extrastriated visual cortex. Human Brain Mapping 1997; 5: 273-279.

Markesbery WR, Schmitt FA, Kryscio RJ, Davis DG, Smith CD, Wekstein DR. Neuropathologic substrate of mild cognitive impairment. Archives of Neurology 2006; 63: 38-46.

Mayeux R. Epidemiology of neurodegeneration. Annual Review of Neuroscience 2003; 26: 81-104.

McGaughy J, Sarter M. Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of physostigmine and FG 7142. Behav Neurosci 1998; 112: 1519-25.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984; 34: 939-944.

Merriam W. Merriam-Webster's Medical Dictionary: Delmar Cengage Learning, 2002.

Mesulam M, Shaw P, Mash D, Weintraub S. Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. Annals of Neurology 2004; 55: 815-828.

Mesulam MM, Geula C. Acetylcholinesterase-rich pyramidal neurons in the human neocortex and hippocampus: absence at birth, development during the life span, and dissolution in Alzheimer's disease. Annals of Neurology 1988; 24: 765-773.

Metherate R, Ashe JH. Basal forebrain stimulation modifies auditory cortex responsiveness by an action at muscarinic receptors. Brain Research 1991; 559: 163-167.

Miller GA, Chapman JP. Misunderstanding analysis of covariance. Journal of Abnormal Psychology 2001; 110: 40-48.

Miller SL, Fenstermacher E, Bates J, Blacker D, Sperling RA, Dickerson BC. Hippocampal activation in adults with mild cognitive impairment predicts subsequent cognitive decline. J Neurol.Neurosurg.Psychiatry 2008; 79: 630-635.

Miller TJ, McGlashan TH, Rosen JL, Somjee L, Markovich PJ, Stein K, et al. Prospective diagnosis of the initial prodrome for schizophrenia based on the Structured Interview for Prodromal Syndromes: preliminary evidence of interrater reliability and predictive validity. Am.J Psychiatry 2002; 159: 863-865.

Minzenberg MJ, Watrous AJ, Yoon JH, Ursu S, Carter CS. Modafinil shifts human locus coeruleus to low-tonic, high-phasic activity during functional MRI. Science 2008; 322: 1700-2.

Mitchell TW, Mufson EJ, Schneider JA, Cochran EJ, Nissanov J, Han LY, et al. Parahippocampal tau pathology in healthy aging, mild cognitive impairment, and early Alzheimer's disease. Annals of Neurology 2002; 51: 182-189.

Morcom AM, Good CD, Frackowiak RS, Rugg MD. Age effects on the neural correlates of successful memory encoding. Brain 2003; 126: 213-229.

Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 1993; 43: 2412-4.

Morris JC. Mild cognitive impairment is early-stage Alzheimer disease: time to revise diagnostic criteria. Archives of Neurology 2006; 63: 15-16.

Morris JC, McKeel DW, Jr., Storandt M, Rubin EH, Price JL, Grant EA, et al. Very mild Alzheimer's disease: informant-based clinical, psychometric, and pathologic distinction from normal aging. Neurology 1991; 41: 469-478.

Morris JC, Storandt M, Miller JP, McKeel DW, Price JL, Rubin EH, et al. Mild cognitive impairment represents early-stage Alzheimer disease. Archives of Neurology 2001; 58: 397-405.

Mosby. Mosby's Medical Dictionary: Elsevier, 2009.

Mouloua M, Parasuraman R. Aging and cognitive vigilance: effects of spatial uncertainty and event rate. Exp Aging Res 1995; 21: 17-32.

Mufson EJ, Counts SE, Fahnestock M, Ginsberg SD. Cholinotrophic molecular substrates of mild cognitive impairment in the elderly. Curr.Alzheimer Res. 2007; 4: 340-350.

Mufson EJ, Ma SY, Dills J, Cochran EJ, Leurgans S, Wuu J, et al. Loss of basal forebrain P75(NTR) immunoreactivity in subjects with mild cognitive impairment and Alzheimer's disease. J.Comp Neurol. 2002; 443: 136-153.

Mumenthaler MS, Yesavage JA, Taylor JL, O'Hara R, Friedman L, Lee H, et al. Psychoactive drugs and pilot performance: a comparison of nicotine, donepezil, and alcohol effects. Neuropsychopharmacology 2003; 28: 1366-1373.

Murchison CF, Zhang XY, Zhang WP, Ouyang M, Lee A, Thomas SA. A distinct role for norepinephrine in memory retrieval. Cell 2004; 117: 131-43.

Murphy PC, Sillito AM. Cholinergic enhancement of direction selectivity in the visual cortex of the cat. Neuroscience 1991; 40: 13-20.

Nebes RD, Butters MA, Houck PR, Zmuda MD, Aizenstein H, Pollock BG, et al. Dual-task performance in depressed geriatric patients. Psychiatry Research 2001; 102: 139-151.

Nelson HE. National Adult Reading Test. Windsor, UK, 1982.

Nestor PG, Parasuraman R, Haxby JV, Grady CL. Divided attention and metabolic brain dysfunction in mild dementia of the Alzheimer's type. Neuropsychologia 1991; 29: 379-387.

Nestor PJ, Scheltens P, Hodges JR. Advances in the early detection of Alzheimer's disease. Nature Medicine 2004; 10 Suppl: S34-S41.

Neubauer AC, Fink A. Intelligence and neural efficiency. Neurosci Biobehav Rev 2009; 33: 1004-23.

Newman J. Thalamic contributions to attention and consciousness. Conscious Cogn 1995; 4: 172-93.

Newman LA, Burk JA. Effects of excitotoxic thalamic intralaminar nuclei lesions on attention and working memory. Behav Brain Res 2005; 162: 264-71.

Newman LA, Mair RG. Cholinergic modulation of visuospatial responding in central thalamus. Eur J Neurosci 2007; 26: 3543-52.

NICE. NICE technology appraisal guidance 111. In: Excellence NIfHaC, editor. London, 2006 (Amended 2007, 2009).

Nyberg L, McIntosh AR, Houle S, Nilsson LG, Tulving E. Activation of medial temporal structures during episodic memory retrieval. Nature 1996; 380: 715-717.

Nyberg L, Nilsson LG, Olofsson U, Backman L. Effects of division of attention during encoding and retrieval on age differences in episodic memory. Exp Aging Res 1997; 23: 137-43.

O'Carroll RE, Baikie EM, Whittick JE. Does the National Adult Reading Test hold in dementia? Br J Clin.Psychol 1987; 26 (Pt 4): 315-316.

Ohm TG, Muller H, Braak H, Bohl J. Close-meshed prevalence rates of different stages as a tool to uncover the rate of Alzheimer's disease-related neurofibrillary changes. Neuroscience 1995; 64: 209-217.

Ohno M, Yamamoto T, Kobayashi M, Watanabe S. Impairment of working memory induced by scopolamine in rats with noradrenergic DSP-4 lesions. Eur J Pharmacol 1993; 238: 117-20.

Ohno M, Yoshimatsu A, Kobayashi M, Watanabe S. Noradrenergic DSP-4 lesions aggravate impairment of working memory produced by hippocampal muscarinic blockade in rats. Pharmacol Biochem Behav 1997; 57: 257-61.

Okonkwo OC, Wadley VG, Ball K, Vance DE, Crowe M. Dissociations in visual attention deficits among persons with mild cognitive impairment. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn 2008; 15: 492-505.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971; 9: 97-113.

Otten LJ, Rugg MD. Task-dependency of the neural correlates of episodic encoding as measured by fMRI. Cerebral Cortex 2001; 11: 1150-1160.

Palmer K, Berger AK, Monastero R, Winblad B, Backman L, Fratiglioni L. Predictors of progression from mild cognitive impairment to Alzheimer disease. Neurology 2007; 68: 1596-602.

Parasuraman R, Greenwood PM, Alexander GE. Selective impairment of spatial attention during visual search in Alzheimer's disease. Neuroreport 1995; 6: 1861-1864.

Parasuraman R, Greenwood PM, Haxby JV, Grady CL. Visuospatial attention in dementia of the Alzheimer type. Brain 1992; 115 (Pt 3): 711-733.

Park DCS. The basic mechanisms accounting for age-related decline in cognitive function. Cognitive Ageing: A Primer. Hove: Psychology Press, 2000.

Parker ES, Landau SM, Whipple SC, Schwartz BL. Aging, recall and recognition: a study on the sensitivity of the University of Southern California Repeatable Episodic Memory Test (USC-REMT). J Clin.Exp.Neuropsychol. 2004; 26: 428-440.

Paskavitz JF, Lippa CF, Hamos JE, Pulaski-Salo D, Drachman DA. Role of the dorsomedial nucleus of the thalamus in Alzheimer's disease. J Geriatr Psychiatry Neurol 1995; 8: 32-7.

Perry E, Walker M, Grace J, Perry R. Acetylcholine in mind: a neurotransmitter correlate of consciousness? Trends in Neurosciences 1999; 22: 273-280.

Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease. A critical review. Brain 1999; 122 (Pt 3): 383-404.

Perry RJ, Hodges JR. Dissociation between top-down attentional control and the time course of visual attention as measured by attentional dwell time in patients with mild cognitive impairment. European Journal of Neuroscience 2003; 18: 221-226.

Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern.Med. 2004; 256: 183-194.

Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, et al. Current concepts in mild cognitive impairment. Archives of Neurology 2001; 58: 1985-1992.

Petersen RC, Parisi JE, Dickson DW, Johnson KA, Knopman DS, Boeve BF, et al. Neuropathologic features of amnestic mild cognitive impairment. Archives of Neurology 2006; 63: 665-672.

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Archives of Neurology 1999; 56: 303-308.

Petersen RC, Thomas RG, Grundman M, Bennett D, Doody R, Ferris S, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. New England Journal of Medicine 2005; 352: 2379-2388.

Petersen SE, Fox PT, Posner MI, Mintun M, Raichle ME. Positron emission tomographic studies of the cortical anatomy of single-word processing. Nature 1988; 331: 585-589.

Petrella JR, Krishnan S, Slavin MJ, Tran TT, Murty L, Doraiswamy PM. Mild cognitive impairment: evaluation with 4-T functional MR imaging. Radiology 2006; 240: 177-186.

Polk TA, Farah MJ. The neural development and organization the letter recognition: Evidence from functional neuroimaging, computational modelling and behavioural studies. Proc. Nat. Acad. Sci. USA 1998; 95: 847-852.

Posner MI. Chronometric explorations of mind. Hillsdale, New Jersey: Lawrence Erlbaum, 1978.

Posner MI. Cognitive neuroscience of attention. New York: Guilford Press, 2004.

Posner MI, Dehaene S, Gazzaniga MS. Attentional networks. Cognitive neuroscience: A reader. Malden, MA: Blackwell, 2000.

Power AE, Vazdarjanova A, McGaugh JL. Muscarinic cholinergic influences in memory consolidation. Neurobiology of Learning and Memory 2003; 80: 178-193.

Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Annals of Neurology 1999; 45: 358-368.

Pugh KR, offywitz BA, Shaywitz SE, Fulbright RK, Byrd D, Skudlarski P, et al. Auditory selective attention: an fMRI investigation. Neuroimage 1996; 4: 159-73.

Qi JP, Wu H, Yang Y, Wang DD, Chen YX, Gu YH, et al. Cerebral ischemia and Alzheimer's disease: the expression of amyloid-beta and apolipoprotein E in human hippocampus. J Alzheimers.Dis. 2007; 12: 335-341.

Qi Z, Wu X, Wang Z, Zhang N, Dong H, Yao L, et al. Impairment and compensation coexist in amnestic MCI default mode network. Neuroimage 2010; 50: 48-55.

Rabin LA, Pare N, Saykin AJ, Brown MJ, Wishart HA, Flashman LA, et al. Differential memory test sensitivity for diagnosing amnestic mild cognitive impairment and predicting conversion to Alzheimer's disease. Neuropsychol.Dev.Cogn B Aging Neuropsychol.Cogn 2009; 16: 357-376.

Raichle ME, Fiez JA, Videen TO, MacLeod AM, Pardo JV, Fox PT, et al. Practice-related changes in human brain functional anatomy during nonmotor learning. Cerebral Cortex 1994; 4: 8-26.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc.Natl.Acad.Sci.U.S.A 2001; 98: 676-682.

Raizada RD, Poldrack RA. Challenge-driven attention: interacting frontal and brainstem systems. Frontiers in Human Neuroscience 2008; 1.

Rand-Giovannetti E, Chua EF, Driscoll AE, Schacter DL, Albert MS, Sperling RA. Hippocampal and neocortical activation during repetitive encoding in older persons. Neurobiology of Aging 2006; 27: 173-182.

Rapp MA, Reischies FM. Attention and executive control predict Alzheimer disease in late life: results from the Berlin Aging Study (BASE). American Journal of Geriatric Psychiatry 2005; 13: 134-141.

Raschetti R, Albanese E, Vanacore N, Maggini M. Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials. PLoS.Med. 2007; 4: e338.

Rasmusson DD, Dykes RW. Long-term enhancement of evoked potentials in cat somatosensory cortex produced by co-activation of the basal forebrain and cutaneous receptors. Experimental Brain Research 1988; 70: 276-286.

Rasmusson DD, Smith SA, Semba K. Inactivation of prefrontal cortex abolishes cortical acetylcholine release evoked by sensory or sensory pathway stimulation in the rat. Neuroscience 2007; 149: 232-241.

Reuter-Lorenz PA, Marshuetz C, Jonides J, Smith EE. Neurocognitive ageing of storage and executive processes. European Journal of Cognitive Psychology 2001; 13(1/2): 257-278.

Riekkinen P, Jr., Riekkinen M. THA improves word priming and clonidine enhances fluency and working memory in Alzheimer's disease. Neuropsychopharmacology 1999; 20: 357-64.

Riekkinen P, Jr., Riekkinen M, Soininen H, Kuikka J, Laakso M, Riekkinen P, Sr. Frontal dysfunction blocks the therapeutic effect of THA on attention in Alzheimer's disease. Neuroreport 1997; 8: 1845-9.

Ries ML, Carlsson CM, Rowley HA, Sager MA, Gleason CE, Asthana S, et al. Magnetic resonance imaging characterization of brain structure and function in mild cognitive impairment: a review. J Am.Geriatr.Soc. 2008; 56: 920-934.

Riley KP, Snowdon DA, Markesbery WR. Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: findings from the Nun Study. Annals of Neurology 2002; 51: 567-577.

Rinne JO, Kaasinen V, Jarvenpaa T, Nagren K, Roivainen A, Yu M, et al. Brain acetylcholinesterase activity in mild cognitive impairment and early Alzheimer's disease. Journal of Neurology, Neurosurgery and Psychiatry 2003; 74: 113-115.

Rodda JE, Dannhauser TM, Cutinha DJ, Shergill SS, Walker Z. Subjective cognitive impairment: increased prefrontal cortex activation compared to controls during an encoding task. Int.J Geriatr.Psychiatry 2009; 24: 865-874.

Rodda JE, Dannhauser TM, Cutinha DJ, Shergill SS, Walker Z. Subjective cognitive impairment: functional MRI during a divided attention task. European Psychiatry In press.

Roediger HL, McDermott KB. Creating false memories: Remembering words that were not presented in lists. Journal of Experimental Psychology: Learning, Memory and Cognition 1995; 21: 803-814.

Rogers W, A. Attetion and ageing. In: Schwartz DPaN, editor. Cognitive Ageing: A Primer. Hove: Psychology Press, 2000.

Roman GC. Stroke, cognitive decline and vascular dementia: the silent epidemic of the 21st century. Neuroepidemiology 2003; 22: 161-164.

Roman GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. Lancet Neurol. 2002; 1: 426-436.

Rosano C, Aizenstein HJ, Cochran JL, Saxton JA, De Kosky ST, Newman AB, et al. Event-related functional magnetic resonance imaging investigation of

executive control in very old individuals with mild cognitive impairment. Biological Psychiatry 2005; 57: 761-767.

Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. Am J Psychiatry 1984; 141: 1356-64.

Roth M, Tym E, Mountjoy CQ, Huppert FA, Hendrie H, Verma S, et al. CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 1986; 149: 698-709.

Royall DR, Palmer R, Chiodo LK, Polk MJ. Declining executive control in normal aging predicts change in functional status: the Freedom House Study. Journal of the American Geriatrics Society 2004; 52: 346-352.

Royall DR, Palmer R, Chiodo LK, Polk MJ. Executive control mediates memory's association with change in instrumental activities of daily living: the Freedom House Study. Journal of the American Geriatrics Society 2005; 53: 11-17.

Rugg MD, Otten LJ, Henson RN. The neural basis of episodic memory: evidence from functional neuroimaging. Philosophical Transactions of the Royal Society B: Biological Sciences 2002; 29: 1097-1110.

Sabri O, Kendziorra K, Wolf H, Gertz HJ, Brust P. Acethylcholine receptors in dementia and mild cognitive impairment. Eur.J.Nucl.Med.Mol.Imaging 2008; 35: S30-S45.

Sahakian BJ, Owen AM, Morant NJ, Eagger SA, Boddington S, Crayton L, et al. Further analysis of the cognitive effects of tetrahydroaminoacridine (THA) in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB. Psychopharmacology (Berl) 1993; 110: 395-401.

Salloway S, Ferris S, Kluger A, Goldman R, Griesing T, Kumar D, et al. Efficacy of donepezil in mild cognitive impairment: a randomized placebo-controlled trial. Neurology 2004; 63: 651-7.

Salthouse TA. General and specific speed mediation of adult age differences in memory. J Gerontol B Psychol Sci Soc Sci 1996a; 51: P30-42.

Salthouse TA. The processing-speed theory of adult age differences in cognition. Psychol Rev 1996b; 103: 403-28.

Salthouse TA. Where in an ordered sequence of variables do independent age-related effects occur? J Gerontol B Psychol Sci Soc Sci 1996c; 51: P166-78.

Salthouse TA, Babcock RL, Shaw RJ. Effects of adult age on structural and operational capacities in working memory. Psychol Aging 1991; 6: 118-27.

Samuel W, Terry RD, DeTeresa R, Butters N, Masliah E. Clinical correlates of cortical and nucleus basalis pathology in Alzheimer dementia. Archives of Neurology 1994; 51: 772-778.

Sarazin M, Berr C, De RJ, Fabrigoule C, Pasquier F, Legrain S, et al. Amnestic syndrome of the medial temporal type identifies prodromal AD: a longitudinal study. Neurology 2007; 69: 1859-1867.

Sarter M. Neuronal mechanisms of the attentional dysfunctions in senile dementia and schizophrenia: two sides of the same coin? Psychopharmacology (Berl) 1994; 114: 539-50.

Sarter M, Bruno JP, Givens B. Attentional functions of cortical cholinergic inputs: what does it mean for learning and memory? Neurobiology of Learning and Memory 2003; 80: 245-256.

Sarter M, Hasselmo ME, Bruno JP, Givens B. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain Research.Brain Research Reviews 2005; 48: 98-111.

Sarter M, Turchi J. Age- and dementia-associated impairments in divided attention: psychological constructs, animal models, and underlying neuronal mechanisms. Dement.Geriatr.Cogn Disord. 2002; 13: 46-58.

Sato H, Hata Y, Hagihara K, Tsumoto T. Effects of cholinergic depletion on neuron activities in the cat visual cortex. J Neurophysiol 1987; 58: 781-94.

Saykin AJ, Wishart HA, Rabin LA, Flashman LA, McHugh TL, Mamourian AC, et al. Cholinergic enhancement of frontal lobe activity in mild cognitive impairment. Brain 2004; 127: 1574-1583.

Schacter DL, Verfaellie M, Anes MD. Illusory memories in amnesic patients: conceptual and perceptual false recognition. Neuropsychology. 1997; 11: 331-342.

Schneider W, Shiffrin RM. Controlled and automatic human information processing: I, Detection and attention. Psychol.Rev 1977; 84: 1-66.

Selden NR, Gitelman DR, Salamon-Murayama N, Parrish TB, Mesulam MM. Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. Brain 1998; 121 (Pt 12): 2249-2257.

Shergill SS, Brammer MJ, Fukuda R, Williams SC, Murray RM, McGuire PK. Engagement of brain areas implicated in processing inner speech in people with auditory hallucinations. British Journal of Psychiatry 2003; 182: 525-531.

Shuster LI, Lemieux SK. An fMRI investigation of covertly and overtly produced mono- and multisyllabic words. Brain and Language 2005; 93: 20-31.

Silveri MC, Reali G, Jenner C, Puopolo M. Attention and memory in the preclinical stage of dementia. J Geriatr.Psychiatry Neurol. 2007; 20: 67-75.

Siniscalchi A, Badini I, Bianchi C, Beani L. Monoamines modulate the electrically-evoked efflux of 3H-choline from slices of guinea pig nucleus basalis magnocellularis. Naunyn Schmiedebergs Arch Pharmacol 1994; 350: 10-4.

Small GW, Kepe V, Ercoli LM, Siddarth P, Bookheimer SY, Miller KJ, et al. PET of brain amyloid and tau in mild cognitive impairment. N.Engl.J Med. 2006; 355: 2652-2663.

Smiley JF, Subramanian M, Mesulam MM. Monoaminergic-cholinergic interactions in the primate basal forebrain. Neuroscience 1999; 93: 817-29.

Smith CD, Andersen AH, Kryscio RJ, Schmitt FA, Kindy MS, Blonder LX, et al. Altered brain activation in cognitively intact individuals at high risk for Alzheimer's disease. Neurology 1999; 53: 1391-1396.

Snodgrass JG, Corwin J. Pragmatics of measuring recognition memory: applications to dementia and amnesia. Journal of Experimental Psychology: General 1988; 117: 34-50.

Sorg C, Riedl V, Muhlau M, Calhoun VD, Eichele T, Laer L, et al. Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2007; 104: 18760-5.

Sorg C, Riedl V, Perneczky R, Kurz A, Wohlschlager AM. Impact of Alzheimer's disease on the functional connectivity of spontaneous brain activity. Curr Alzheimer Res 2009; 6: 541-53.

Spencer CM, Noble S. Rivastigmine. A review of its use in Alzheimer's disease. Drugs and Aging 1998; 13: 391-411.

Sperling R, Chua E, Cocchiarella A, Rand-Giovannetti E, Poldrack R, Schacter DL, et al. Putting names to faces: successful encoding of associative memories activates the anterior hippocampal formation. Neuroimage. 2003a; 20: 1400-1410.

Sperling RA, Bates JF, Chua EF, Cocchiarella AJ, Rentz DM, Rosen BR, et al. fMRI studies of associative encoding in young and elderly controls and mild Alzheimer's disease. J Neurol.Neurosurg.Psychiatry 2003b; 74: 44-50.

Squire LR, Zola SM. Structure and function of declarative and nondeclarative memory systems. Proc.Natl.Acad.Sci.U.S.A 1996; 93: 13515-13522.

Stadler MA, Roediger HL, III, McDermott KB. Norms for word lists that create false memories. Memory and Cognition 1999; 27: 494-500.

Staresina BP, Davachi L. Differential encoding mechanisms for subsequent associative recognition and free recall. J Neurosci. 2006; 26: 9162-9172.

Staresina BP, Davachi L. Selective and shared contributions of the hippocampus and perirhinal cortex to episodic item and associative encoding. J Cogn Neurosci. 2008; 20: 1478-1489.

Storandt M, Grant EA, Miller JP, Morris JC. Longitudinal course and neuropathologic outcomes in original vs revised MCI and in pre-MCI. Neurology 2006; 67: 467-73.

Streit WJ. Microglial senescence: does the brain's immune system have an expiration date? Trends Neurosci 2006; 29: 506-10.

Streit WJ, Miller KR, Lopes KO, Njie E. Microglial degeneration in the aging brain--bad news for neurons? Front Biosci 2008; 13: 3423-38.

Talairach JT, P. Co-Planar Stereotactic Atlas of the Human Brain. Stuttgart: Georg Thieme Verlag, 1988.: Georg Thieme Verlag, 1988.

Tales A, Haworth J, Nelson S, Snowden RJ, Wilcock G. Abnormal visual search in mild cognitive impairment and Alzheimer's disease. Neurocase 2005a; 11: 80-4.

Tales A, Haworth J, Wilcock G, Newton P, Butler S. Visual mismatch negativity highlights abnormal pre-attentive visual processing in mild cognitive impairment and Alzheimer's disease. Neuropsychologia 2008; 46: 1224-32.

Tales A, Snowden RJ, Haworth J, Wilcock G. Abnormal spatial and non-spatial cueing effects in mild cognitive impairment and Alzheimer's disease. Neurocase 2005b; 11: 85-92.

Terry RD, Katzman R. Life span and synapses: will there be a primary senile dementia? Neurobiology of Aging 2001; 22: 347-348.

Thiel CM. Cholinergic modulation of learning and memory in the human brain as detected with functional neuroimaging. Neurobiology of Learning and Memory 2003; 80: 234-244.

Thirion B, Pinel P, Meriaux S, Roche A, Dehaene S, Poline JB. Analysis of a large fMRI cohort: Statistical and methodological issues for group analyses. Neuroimage. 2007; 35: 105-120.

Tierney MC, Szalai JP, Snow WG, Fisher RH, Nores A, Nadon G, et al. Prediction of probable Alzheimer's disease in memory-impaired patients: A prospective longitudinal study. Neurology 1996; 46: 661-665.

Tierney MC, Yao C, Kiss A, McDowell I. Neuropsychological tests accurately predict incident Alzheimer disease after 5 and 10 years. Neurology 2005; 64: 1853-1859.

Tomasi D, Chang L, Caparelli EC, Ernst T. Different activation patterns for working memory load and visual attention load. Brain Res 2007; 1132: 158-65.

Traykov L, Raoux N, Latour F, Gallo L, Hanon O, Baudic S, et al. Executive functions deficit in mild cognitive impairment. Cogn Behav Neurol 2007; 20: 219-24.

Tremblay N, Warren RA, Dykes RW. Electrophysiological studies of acetylcholine and the role of the basal forebrain in the somatosensory cortex of the cat. II. Cortical neurons excited by somatic stimuli. J Neurophysiol. 1990; 64: 1212-1222.

Trivedi MA, Murphy CM, Goetz C, Shah RC, Gabrieli JD, Whitfield-Gabrieli S, et al. fMRI activation changes during successful episodic memory encoding and recognition in amnestic mild cognitive impairment relative to cognitively healthy older adults. Dement.Geriatr.Cogn Disord. 2008; 26: 123-137.

Troyer AK, Murphy KJ, Anderson ND, Hayman-Abello BA, Craik FI, Moscovitch M. Item and associative memory in amnestic mild cognitive impairment: performance on standardized memory tests. Neuropsychology. 2008; 22: 10-16.

Truchot L, Costes N, Zimmer L, Laurent B, Le Bars D, Thomas-Anterion C, et al. A distinct [18F]MPPF PET profile in amnestic mild cognitive impairment compared to mild Alzheimer's disease. Neuroimage 2008; 40: 1251-6.

Tulving E. Memory and consciousness. Canadian Psychology 1985; 26: 1-12.

Tulving E. Episodic memory: from mind to brain. Annu.Rev.Psychol 2002; 53: 1-25.

Uchihara T, Tsuchiya K, Kondo H, Hayama T, Ikeda K. Widespread appearance of Alz-50 immunoreactive neurons in the human brain with cerebral infarction. Stroke 1995; 26: 2145-2148.

Uchihara T, Tsuchiya K, Nakamura A, Ikeda K. Appearance of tau-2 immunoreactivity in glial cells in human brain with cerebral infarction. Neuroscience Letters 2000; 286: 99-102.

Uhlmann RF, Larson EB, Koepsell TD, Rees TS, Duckert LG. Visual impairment and cognitive dysfunction in Alzheimer's disease. J Gen Intern Med 1991; 6: 126-32.

Uhlmann RF, Larson EB, Rees TS, Koepsell TD, Duckert LG. Relationship of hearing impairment to dementia and cognitive dysfunction in older adults. JAMA 1989; 261: 1916-9.

Van der Linden M. Attention and normal ageing. In: Leclerq MZ, P., editor. Applied neuropsychology of attention: theory, diagnosis and rehabilitation. London: Taylor and Francis, 2002 205-209.

Van der Linden M, Bredart S, Beerten A. Age-related differences in updating working memory. Br J Psychol 1994; 85 (Pt 1): 145-52.

Van der Linden M, Hupet M, Feyereisen P, Schelstraete M-A, Bestgen Y, Bruyer R, et al. Cognitive Mediators of Age-Related Differences in Language Comprehension and Verbal Memory Performance. Aging, Neuropsychology, and Cognition 1999; 6: 32 - 55.

Van der Linden MC, F. Attention and normal ageing. In: Leclercq MZ, P., editor. Applied Neuropsychology of Attention: Theory, Diagnosis and Rehabilitation. Hove: Psychology Press, 2002: 205 - 229.

van Oijen M, de Jong FJ, Hofman A, Koudstaal PJ, Breteler MM. Subjective memory complaints, education, and risk of Alzheimer's disease. Alzheimers.Dement. 2007; 3: 92-97.

Vandenberghe R, Duncan J, Arnell KM, Bishop SJ, Herrod NJ, Owen AM, et al. Maintaining and shifting attention within left or right hemifield. Cereb Cortex 2000; 10: 706-13.

Vandenberghe R, Duncan J, Dupont P, Ward R, Poline JB, Bormans G, et al. Attention to one or two features in left or right visual field: a positron emission tomography study. J Neurosci 1997; 17: 3739-50.

Verfaellie M, Page K, Orlando F, Schacter DL. Impaired implicit memory for gist information in amnesia. Neuropsychology. 2005; 19: 760-769.

Verhaeghen P, Cerella J. Aging, executive control, and attention: a review of meta-analyses. Neurosci Biobehav Rev 2002; 26: 849-57.

Villringer A, Dirnagl U. Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. Cerebrovasc.Brain Metab Rev 1995; 7: 240-276.

Vohn R, Fimm B, Weber J, Schnitker R, Thron A, Spijkers W, et al. Management of attentional resources in within-modal and cross-modal divided attention tasks: an fMRI study. Human Brain Mapping 2007; 28: 1267-1275.

Wagner AD, Schacter DL, Rotte M, Koutstaal W, Maril A, Dale AM, et al. Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. Science 1998; 281: 1188-1191.

Wang H, Fan J. Human attentional networks: a connectionist model. J Cogn Neurosci. 2007; 19: 1678-1689.

Wechsler D. Wechsler Memory Scale - III (WMS-III). San Antonio: The Psychological Corporation, 1997.

Werner P, Korczyn AD. Mild cognitive impairment: conceptual, assessment, ethical, and social issues. Clin Interv Aging 2008; 3: 413-20.

Westerberg CE, Paller KA, Weintraub S, Mesulam MM, Holdstock JS, Mayes AR, et al. When memory does not fail: familiarity-based recognition in mild cognitive impairment and Alzheimer's disease. Neuropsychology. 2006; 20: 193-205.

Wheeler MA, McMillan CT. Focal retrograde amnesia and the episodic-semantic distinction. Cogn Affect.Behav.Neurosci. 2001; 1: 22-36.

Witter MP, Groenewegen HJ, Lopes da Silva FH, Lohman AH. Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. Progress in Neurobiology 1989; 33: 161-253.

Wolk DA, Signoff ED, DeKosky ST. Recollection and familiarity in amnestic mild cognitive impairment: A global decline in recognition memory. Neuropsychologia 2008; 46: 1965-1978.

Woods SW, Addington J, Cadenhead KS, Cannon TD, Cornblatt BA, Heinssen R, et al. Validity of the prodromal risk syndrome for first psychosis: findings from the North American Prodrome Longitudinal Study. Schizophrenia Bulletin 2009; 35: 894-908.

Woods SW, Miller TJ, McGlashan TH. The "prodromal" patient: both symptomatic and at-risk. CNS.Spectr. 2001; 6: 223-232.

Xu G, Antuono PG, Jones J, Xu Y, Wu G, Ward D, et al. Perfusion fMRI detects deficits in regional CBF during memory-encoding tasks in MCI subjects. Neurology 2007.

Yesavage JA, Mumenthaler MS, Taylor JL, Friedman L, O'Hara R, Sheikh J, et al. Donepezil and flight simulator performance: effects on retention of complex skills. Neurology 2002; 59: 123-125.

Yonelinas AP. The Nature of Recollection and Familiarity: A Review of 30 Years of Research. Journal of Memory and Language 2002; 46: 441-517.