Memory decline and Aβ amyloid as markers of neurodegeneration in preclinical Alzheimer’s disease

Yen Ying Lim

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Department of Psychiatry
Faculty of Medicine, Dentistry, and Health Sciences
The University of Melbourne
Abstract

Alzheimer’s disease (AD) is pathologically characterised by neurofibrillary tangles and beta-amyloid (Aβ) plaques. Clinically, it is characterised by a gradual decline in cognitive function, particularly in episodic memory. Current neuropsychological models emphasise the measurement of cognitive impairment to determine cognitive abnormality. However, as AD is a neurodegenerative disease, it has been suggested that the repeated assessment of cognitive function could provide important information about an individual’s performance over time, particularly as any changes in cognitive function in the very early stages of the disease are likely to be subtle.

The overarching aim of this thesis was to investigate the relationship between a known marker of AD, Aβ amyloid, as determined by positron emission tomography (PET) neuroimaging using \(^{11}\)C-Pittsburgh Compound B, and decline in cognitive function as potential markers of neurodegeneration in the preclinical stages of AD. Additionally, the role of genetic polymorphisms in modifying the relationship between Aβ amyloid and cognitive decline were explored.

First, the nature and magnitude of Aβ amyloid-related impairment in cognitive function was characterised cross-sectionally in both healthy older adults and adults with mild cognitive impairment (MCI). The data suggested that there were very small differences between healthy older adults with high and low levels of Aβ amyloid. Further, in adults with MCI, high Aβ amyloid was associated with a more focal impairment in episodic memory, but low Aβ amyloid was associated with additional impairments in executive function, attention and language, suggesting the presence of other underlying neurological or psychiatric processes.
The relationship between Aβ amyloid and cognitive function in both healthy older adults and adults with MCI became clearer when studied prospectively. High levels of Aβ amyloid were associated with increased rates of cognitive decline, particularly in episodic memory, and this decline occurred at the same rate in both healthy older adults and in adults with MCI. High Aβ amyloid levels were also associated with higher risk of disease progression in both healthy older adults and adults with MCI. Carriage of the apolipoprotein E (APOE) ε4 allele did not moderate this relationship between Aβ amyloid and cognitive decline; although carriage of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism Met allele did. Low levels of Aβ amyloid were associated with stable cognitive function in both healthy older adults and adults with MCI, lending strength to the hypothesis that the underlying pathological process in adults with MCI and low Aβ amyloid is non-AD in nature. These findings have important implications for future clinical trials in AD as the data strongly suggest that healthy older adults with high levels of Aβ amyloid and objectively defined decline in memory are in the preclinical stages of AD, and are promising candidates for anti-amyloid therapies aimed at halting or modifying the neurodegenerative disease process in the early stages of the disease.
Declaration of Authorship

This is to certify that:

1) The thesis comprises only my original work towards the PhD except where indicated in the Preface

2) Due acknowledgement has been made in the text to all other material used

3) The thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies, and appendices.

Yen Ying Lim

November 2012
Preface

This thesis contains no work submitted for other “qualifications”, or work carried out prior to PhD candidature.

This thesis was conducted within the context of the Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing (www.aibl.csiro.au). The AIBL Study aims to improve the understanding of the causes and diagnosis of Alzheimer’s disease (AD), examine lifestyle and diet factors that may influence the onset of AD and help develop preventative strategies. The AIBL Study is a prospective longitudinal study of ageing that has reached its initial aim of recruiting a cohort of at least 1,000 volunteers, comprised of patients with AD, mild cognitive impairment (MCI) and healthy volunteers and is now seeking to extend recruitment to compensate for the loss to follow up of individuals no longer able to participate in the study.

The AIBL Study is a collaboration cluster initiated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Preventative Health National Research Flagship. The study, launched on November 14 2006, is a joint activity between the University of Melbourne, Edith Cowan University, Neurosciences Australia, Mental Health Research Institute of Victoria, National Ageing Research Institute and CSIRO. The AIBL Study is not only one of the largest studies in AD, but it also takes a world leading approach by integrating expertise in neuroimaging, biomarkers, neuropsychology, and lifestyle interventions.

The cohort consists of over 1000 volunteers (minimum age 60 years), recruited from five population groups; 1) early AD (Clinical Dementia Rating Scale of 0.5 or 1), 2) MCI, 3) healthy volunteers who are APOE ε4 carriers, 4) healthy volunteers who are APOE ε4 non-carriers, and 5) subjective memory complainers. At baseline, each volunteer completed the International Physical Activity Questionnaire, a Food Frequency Questionnaire, a comprehensive clinical and neuropsychological test battery, and provided an 80ml blood sample for clinical pathology, biomarker analysis and storage in liquid nitrogen. In addition, 250 participants (25% from each group) received an 11C-Pittsburgh Compound B (PiB) Position Emission Tomography (PET) scan as a measure of in vivo Aβ amyloid and a Magnetic Resonance Imaging scan. These assessments have
been repeated at 18- and 36-month follow-up and have been or will be repeated again at 54 and 72 months.

The AIBL Study is led by Professor David Ames (Professor of Ageing and Health, University of Melbourne; Director of the National Ageing Research Institute) and Dr. Richard Head of CSIRO. Daily operations and scientific management are overseen by Dr. Kathryn Ellis (University of Melbourne) and Dr. Lance Macauley coordinates the CSIRO collaborative network.

Professor Colin Masters (Laureate Professor of Pathology, University of Melbourne; Director of the Mental Health Research Institute of Victoria) has a major input into the biomarkers program based on his discovery of the Aβ amyloid protein and his extensive international connections. Professor Ashley Bush (Mental Health Research Institute of Victoria) and Professor Ralph Martins (Edith Cowan University) lead the diagnostics and biomarkers program aimed at early stage identification and assesses the effectiveness of interventions.

Professor Christopher Rowe (Director, Nuclear Medicine and Centre for PET, Austin Hospital) and Associate Professor Nat Lenzo (Royal Perth Hospital and Edith Cowan University) apply state-of-the-art neuroimaging technology to develop and confirm new diagnostic tests and biomarkers. Dr. Olivier Salvado (CSIRO Team Leader) provides accurate 3D quantitative analysis of amyloid neuroimaging, including correlation of cortical thinning with amyloid deposition.

Professor Paul Maruff (Chief Science Officer, CogState Ltd.; Mental Health Research Institute of Victoria), Dr Kathryn Ellis (University of Melbourne), and Associate Professor Greg Savage (Macquarie University) lead the neuropsychological testing of the cohort to classify participants and monitor their condition.

Professor Ralph Martins (Edith Cowan University) leads the diet and lifestyle element of the research in close collaboration with key CSIRO staff.

For all articles presented in the thesis, I am the primary author in all instances, performed all statistical analyses, prepared all articles, and edited all articles in response to comments from co-authors and reviewers. Other researchers have also contributed to the work presented, and their contributions are detailed in the attached table. Due to the
large number of collaborators, my research supervisors have provided their signatures to attest that the contributions of these researchers have been listed accurately, and truly reflect the contribution of each. Additionally, collaborators who have actively contributed to the work presented in this thesis have completed and submitted the attached Collaborators Authorisation forms.

All research procedures reported in this thesis were approved by and complied with the regulations of the institutional research and ethics committees of Austin Health, St. Vincent’s Health, Hollywood Private Hospital, and Edith Cowan University

Yen Ying Lim

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Professor Paul Maruff            Professor David Ames             Dr. Kathryn Ellis
<table>
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</tr>
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<tr>
<td>Paul Maruff</td>
<td><strong>Research Supervisor</strong>, co-leader of the AIBL cognitive stream; primary investigator of AIBL-ROCS; oversaw integration of AIBL-ROCS into the AIBL study; obtained funding and contributed to design of AIBL-ROCS</td>
</tr>
<tr>
<td>David Ames</td>
<td><strong>Research Supervisor</strong>, primary investigator of the AIBL study; obtained funding for the AIBL study</td>
</tr>
<tr>
<td>Kathryn Ellis</td>
<td><strong>Research Supervisor</strong>, national coordinator of the AIBL study, co-leader of the AIBL cognitive stream</td>
</tr>
<tr>
<td>Tim Ashwood</td>
<td>Oversaw integration of AIBL-ROCS into the AIBL study; obtained funding and contributed to design of AIBL-ROCS</td>
</tr>
<tr>
<td>Pierrick Bourgeat</td>
<td>Oversaw and conducted MRI neuroimaging of AIBL participants; provided MRI neuroimaging data</td>
</tr>
<tr>
<td>Ashley Bush</td>
<td>Senior member of the AIBL management committee</td>
</tr>
<tr>
<td>David Darby</td>
<td>Senior member of the AIBL cognitive stream; obtained funding and contributed to design of AIBL-ROCS</td>
</tr>
<tr>
<td>Joanne Gale</td>
<td>Assisted with selection of appropriate statistical procedures</td>
</tr>
<tr>
<td>Karra Harrington</td>
<td>Coordinator of AIBL-ROCS</td>
</tr>
<tr>
<td>Judith Jaeger</td>
<td>Oversaw integration of AIBL-ROCS into the AIBL study; obtained funding and contributed to design of AIBL-ROCS</td>
</tr>
<tr>
<td>Adrian Kamer</td>
<td>Conducted AIBL neuropsychological assessments; AIBL-ROCS rater (Melbourne)</td>
</tr>
<tr>
<td>Rebecca Lachovitzki</td>
<td>AIBL-ROCS rater (Perth)</td>
</tr>
<tr>
<td>Simon Laws</td>
<td>Conducted <strong>BDNF</strong> Val66Met genotyping of AIBL participants</td>
</tr>
<tr>
<td>Ralph Martins</td>
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</tr>
<tr>
<td>Colin Masters</td>
<td>Senior member of the AIBL management committee; co-leader of the AIBL biomarkers stream</td>
</tr>
<tr>
<td>Pradeep Nathan</td>
<td>Contributed to concepts and study design of Chapter 10</td>
</tr>
<tr>
<td>Robert Pietrzak</td>
<td>Assisted with selection of appropriate statistical procedures</td>
</tr>
<tr>
<td>Christopher Rowe</td>
<td>Senior member of the AIBL management committee; leader of the AIBL imaging stream</td>
</tr>
<tr>
<td>Olivier Salvado</td>
<td>Oversaw and conducted MRI neuroimaging of AIBL participants; provided MRI neuroimaging data</td>
</tr>
<tr>
<td>Greg Savage</td>
<td>Co-leader of the AIBL cognitive stream</td>
</tr>
<tr>
<td>Peter Snyder</td>
<td>Contributed to concepts and study design of Chapter 10</td>
</tr>
<tr>
<td>Albrecht Stöffler</td>
<td>Oversaw integration of AIBL-ROCS into the AIBL study; obtained funding and contributed to design of AIBL-ROCS</td>
</tr>
<tr>
<td>Cassandra Szoeke</td>
<td>Senior member of the AIBL management committee</td>
</tr>
<tr>
<td>Victor Villemagne</td>
<td>Oversaw and conducted PET neuroimaging of AIBL participants; provided PET neuroimaging data</td>
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John Keats once asked, “Do you not see how necessary a world of pains and troubles is to school an intelligence and make it a soul?” Indeed, it takes not only a world of pains, troubles and sleepless nights, but a lot of financial support from the Ministry of Education, Brunei Darussalam, and also a team of people whom I will forever be indebted to – people who have been helpful, patient, and understanding during the time I indulged in my intellectual curiosities.

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Abbreviations

Aβ  beta-amyloid (abeta)
AD  Alzheimer's disease
ADAS-Cog  Alzheimer's Disease Assessment Scale – Cognitive Subscale
ADNI  Alzheimer's Disease Neuroimaging Initiative
AIBL  Australian Imaging, Biomarkers, and Lifestyle study
AIBL-ROCS  Australian Imaging, Biomarkers, and Lifestyle-Rate of Change Sub-study
ANCOVA  Analysis of covariance
ANOVA  Analysis of variance
APOE  Apolipoprotein E (gene)
BDNF  Brain-derived neurotrophic factor (gene)
BDNF  Brain-derived neurotrophic factor (protein)
BMI  Body Mass Index
CDR  Clinical Dementia Rating scale
CDR-SB  Clinical Dementia Rating scale, Sum of Boxes
CERAD  Consortium to Establish a Registry for Alzheimer's Disease
CI  Confidence intervals
CNS  Central nervous system
CPAL  Continuous Paired Associate Learning task
CR-1  Complement receptor-1 (gene)
CSF  Cerebrospinal fluid
CVLT-II  California Verbal Learning Test, Second Edition
DA  David Ames
DET  CogState Detection task
df  Degrees of freedom
EM  Episodic memory
FDG  Fluorodeoxyglucose
GDS  Geriatric Depression Score
GLM  Generalised linear model
GM  Grey matter
HA  Healthy older adults
HADS  Hospital Anxiety and Depression Scale
HIVD  Human Immunodeficiency Virus Dementia
HVLRT-R  Hopkins Verbal Learning Test-Revised
ICC  Intraclass correlation coefficient
IDN  CogState Identification task
IPAQ  International Physical Activity Questionnaire
ISLT  CogState International Shopping List Test
LMM  Linear Mixed Model
MAC-Q  Memory Complaint Questionnaire
MCI  Mild Cognitive Impairment
MCSA  Mayo Clinic Study of Ageing
MHAS  Melbourne Healthy Ageing Study
MMSE  Mini Mental State Examination
MRI  Magnetic Resonance Imaging
mRNA  Messenger ribonucleic acid
ms  Millisecond
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<td>NINCDS-ADRDA</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association</td>
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<tr>
<td>OBK</td>
<td>CogState One Back task</td>
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<td>OCL</td>
<td>CogState One Card Learning task</td>
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<td>PET</td>
<td>Position Emission Tomography</td>
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<td>PiB</td>
<td>$^{11}$C-Pittsburgh Compound B</td>
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<td>RAVLT</td>
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<td>Reliable change index</td>
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<td>SUVR</td>
<td>Standardised uptake value ratio</td>
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<td>Wechsler Adult Intelligence Scale, Third Edition</td>
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Peer reviewed publications and presentations

Published work arising from thesis

**Lim, Y.Y.,** Ellis, K.A., Harrington, K., Ames, D., Martins, R.N., Masters, C.L., Rowe, C.,
of the CogState Brief Battery in the assessment of Alzheimer’s disease related
cognitive impairment in the Australian Imaging, Biomarkers, and Lifestyle (AIBL)

**Lim, Y.Y.,** Ellis, K.A., Pietrzak, R.H., Ames, D., Darby, D., Harrington, K., Martins, R.N.,
Masters, C.L., Rowe, C., Szoeke, C., Villemagne, V.L., Maruff, P., for the AIBL Research
Group. (2012). Stronger effect of amyloid load than *APOE* genotype on cognitive

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Martins, R.N., Bush, A.I., Masters, C.L., Rowe, C.C., Villemagne, V.L., Ames, D., Darby,

**Lim, Y.Y.,** Ellis, K.A., Ames, D., Darby, D., Harrington, K., Martins, R.N., Masters, C.L., Rowe,

**Lim, Y.Y.,** Ellis, K.A., Harrington, K., Pietrzak, R.H., Gale, J., Ames, D., Bush, A.I., Darby, D.,
Martins, R.N., Masters, C.L., Rowe, C.C., Savage, G., Szoeke, C., Villemagne, V.L.,
Maruff, P., for the AIBL Research Group. (2012). Cognitive decline in adults with
amnestic mild cognitive impairment and high Aβ amyloid: Prodromal Alzheimer’s

Lim, Y.Y., Ellis, K.A., Harrington, K., Kamer, A., Pietrzak, R.H., Bush, A.I., Darby, D., Martins,
R.N., Masters, C.L., Rowe, C.C., Savage, G., Szoeke, C., Villemagne, V.L., Ames, D.,
Maruff, P., for the AIBL Research Group. (in press). Cognitive consequences of high
Aβ amyloid in mild cognitive impairment and healthy older adults: Implications for

Lim, Y.Y., Jaeger, J., Harrington, K., Ashwood, T., Ellis, K.A., Stöffler, A., Szoeke, C.,
Lachovitzki, R., Martins, R.N., Villemagne, V.L., Bush, A.I., Masters, C.L., Rowe, C.C.,
Ames, D., Darby, D. & Maruff, P. (in press). The Australian Imaging, Biomarkers, and
Lifestyle-Rate of Change sub-study (AIBL-ROCS): Rationale, design, acceptability,
and pilot data for the first three months of assessment. Archives of Clinical
Neuropsychology.

Additional published work conducted during candidature

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(2012). Short term stability of verbal memory impairment in mild cognitive
impairment and Alzheimer’s disease measured using the International Shopping

based approach to characterizing the effect of acute alprazolam challenge on visual
paired associate learning and memory in healthy older adults. Human
Psychopharmacology, 27(6), 549-558.

Thesis Presentation

This thesis is submitted as a series of nine empirical articles addressing a central theme (see Appendix A for details of each article and their respective status). Most articles presented in this thesis have been submitted to, published in, or accepted for publication in internationally refereed scientific journals. Where the article has been published, a reprint is contained in Appendix B. Where the article is in press, a copy of the acceptance letter from the journal editor is contained in Appendix B. Where the article has been submitted, a copy of the acknowledgement of receipt is contained in Appendix B if it had been received by the author at the time of submission of the thesis. Although the editorial requirements for each journal differed, all chapters presented here have been reformatted so that all references are presented in the numbered citation style, and all tables and figures are placed in text closest to the point at which they are first cited in order to facilitate reading of the thesis. This thesis was written in Australian English.

All empirical articles presented in this thesis have arisen from the ongoing Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing. Each article provides a description of the study protocols and the inclusion and exclusion criteria, and the AIBL study protocol has been previously described extensively. While all articles conformed to the initial AIBL study protocol, additional inclusion and exclusion criteria have been applied post-hoc depending upon the aims of the articles. As such, the number of participants from which data is reported may vary between articles, and the examiner is requested to be aware of this when reading the thesis.
Outline of Thesis

The thesis is organised in three main parts. The aim of the first part was to determine the utility of a computerised battery designed to measure cognitive change in detecting cognitive impairment due to Alzheimer’s disease (AD) and its prodromal stage, mild cognitive impairment (MCI). In the second part, we aimed to consider the nature and magnitude of Aβ amyloid related cognitive impairment cross-sectionally in healthy older adults and adults with MCI, and the aim of the third part was to consider the nature and magnitude of Aβ amyloid related cognitive decline prospectively in healthy older adults and adults with MCI. More specifically, the overarching aim of the thesis was to consider the combined detection of decline in memory and Aβ amyloid as markers of neurodegeneration in the very early stages of Alzheimer’s disease.

Chapter One: General Introduction

In Chapter One, we discussed a theoretical framework for the use of neuropsychological assessments, and neuroimaging techniques in the characterisation of the nature and magnitude of cognitive impairment and cognitive decline in healthy older adults and adults with MCI. In this review, we argued that most investigations of very early AD have been cross-sectional in design, and as AD is a neurodegenerative disease, prospective studies may better inform the nature of AD-related cognitive decline. We also considered the limitations of conventional neuropsychological tests in their ability to be administered repeatedly as they are time-demanding, susceptible to practice effects, and may suffer from insufficient variability, especially in identifying subtle cognitive decline in healthy populations. As such, relevant literature regarding the use of a brief computerised battery designed for the assessment of cognitive change, that is, the CogState Brief Battery, was reviewed.
Chapter Two: Use of the CogState Brief Battery in the assessment of Alzheimer’s disease related cognitive impairment in the Australian Imaging, Biomarkers and Lifestyle (AIBL) study

In Chapter Two, the CogState Brief Battery, which assesses psychomotor, attentional, working memory and visual learning functions, was validated for use in patients with MCI and AD, enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) study. In AD and MCI groups, the magnitude of impairment was greatest for tasks of working memory and memory, with a negative influence of apolipoprotein E (APOE) ε4 status on visual learning but not working memory. These results suggest that the CogState Brief Battery is able to detect AD-related cognitive impairment, and can be used to screen for AD-related cognitive changes.

Chapter Three: Aβ Amyloid, Cognition, and APOE Genotype in Healthy Older Adults

The relationship between Aβ amyloid, APOE ε4 carriage, and cognitive function in healthy older adults was explored in Chapter Three. In APOE ε4 carriers, there was a moderate negative relationship between Aβ amyloid and episodic memory. This association was not observed in ε4 non-carriers. The relationship between Aβ amyloid and episodic memory in ε4 carriers was significantly different to those in ε4 non-carriers, and the magnitude of this difference was small to moderate. This suggests that increased Aβ amyloid may signify the onset of preclinical AD, especially in healthy older adults who are genetically at risk for AD.
Chapter Four: Cognitive Consequences of High Aβ Amyloid in Mild Cognitive Impairment and Healthy Older Adults: Implications for Early Detection of Alzheimer’s Disease

Using the larger neuropsychological battery of the AIBL study, the aim of Chapter Four was to further understand the nature and magnitude of Aβ amyloid related cognitive impairment in healthy older adults and adults with amnestic mild cognitive impairment (aMCI). Participants completed an extensive neuropsychological battery assessing the cognitive domains of verbal and visual episodic memory, executive function, visuoconstruction, attention and processing speed, and language. In healthy older adults, no differences were observed between high and low Aβ amyloid groups. However, when compared to healthy older adults with low Aβ amyloid, MCI with high Aβ amyloid presented with impairments restricted to episodic memory, while the episodic memory impairments in MCI with low Aβ amyloid were accompanied by impairments in executive function, attention, visuoconstruction, and language. This suggests that MCI with high Aβ amyloid reflects preclinical AD, while MCI with low Aβ amyloid may reflect other causes of cognitive impairment, although prospective study is required to confirm this hypothesis.

Chapter Five: Stronger Effect of Amyloid Load than APOE Genotype on Cognitive Decline in Healthy Older Adults

Previously, the results of Chapter Three suggested a relationship between increasing levels of Aβ amyloid and decreasing cognitive performance, particularly in healthy older adults who carried the APOE ε4 allele. Thus, the aim of Chapter Five was to investigate the significance of APOE ε4 carriage, and high Aβ amyloid levels on longitudinal changes in cognition in healthy older adults. In this prospective study of healthy older adults,
high Aβ amyloid levels was associated with greater decline in episodic and working memory over 18 months. APOE ε4 carriage was also associated with decline in visual memory, although the effect was less than that observed for Aβ amyloid. Importantly though, APOE ε4 carriage did not moderate the relationship between Aβ amyloid and cognitive decline.

Chapter Six: Cognitive Decline in Adults with Mild Cognitive Impairment and High Aβ Amyloid: Prodromal Alzheimer’s Disease?

In order to further understand the relationship between Aβ amyloid and cognitive decline, the aim of Chapter Six was to characterise the nature and magnitude of cognitive decline in a group of adults with MCI and high and low levels of Aβ amyloid in relation to healthy older adults with low Aβ amyloid levels. Relative to healthy older adults with low Aβ amyloid, adults with MCI and high Aβ amyloid showed greater decline in working memory, and verbal and visual episodic memory at 18 months. Adults with MCI and low Aβ amyloid also showed greater decline in working memory; however did not evidence any decline in episodic memory at 18 months. The results suggest that relative to healthy older adults and adults with MCI with low Aβ amyloid, adults with MCI and high levels of Aβ amyloid showed faster rates of decline on measures of episodic memory over 18 months.

Chapter Seven: Aβ Amyloid and Cognitive Change: Examining the Preclinical and Prodromal Stages of Alzheimer’s Disease

Previously, Chapters Five and Six showed that high Aβ amyloid was associated with a faster rate of memory decline in both healthy older adults and adults with MCI. However, because these investigations were conducted over relatively short periods (i.e.,
18 months), longer prospective studies are required to determine if Aβ amyloid-related memory decline continues, and whether it is associated with an increased rate of disease progression. As such, the aim of Chapter Seven was to compare rates of episodic memory decline over 36 months between healthy older adults and adults with MCI with high and low levels of Aβ amyloid, explored the extent to which Aβ amyloid-related memory decline was associated with change in clinical status, and whether any decline was moderated by the APOE ε4 allele. Healthy older adults and adults with MCI with high Aβ amyloid showed moderate decline in episodic and working memory over 36 months. Rates of decline in episodic memory were equivalent for healthy older adults and adults with MCI who had high Aβ amyloid. Healthy older adults and adults with MCI with low Aβ amyloid did not show any cognitive decline over 36 months. Rates of disease progression were also increased in the high Aβ amyloid healthy and MCI groups.

Chapter Eight: The Australian Imaging, Biomarkers, and Lifestyle – Rate of Change Sub-Study (AIBL-ROCS): Rationale, Design, Acceptability, and Pilot Data for the first Three Months of Assessment

Large prospective studies of AD, such as the AIBL study, have sought to understand the pathological evolution of AD and factors that may influence the rate of disease progression. Estimates of rates of cognitive change are available for 12 or 24 months, but not for shorter time frames (e.g. 3 or 6 months). Most clinical drug trials seeking to reduce or modify AD symptoms have been conducted over 12 or 24 week periods. As such, the aim of Chapter Eight was to characterise the performance of a group of 105 healthy older adults, 48 adults with aMCI, and 42 adults with AD on the CogState battery of tests monthly over 3 months. The CogState battery demonstrated good test-retest reliability, stability and ability to detect AD-related cognitive impairment.
Chapter Nine: Rapid Decline in Episodic Memory in Healthy Older Adults with High Aβ Amyloid

Once the suitability of the CogState battery for repeated assessment over short test-retest intervals was established in Chapter Eight, and as estimates of rates of cognitive change over 18 and 36 months for healthy older adults and adults with MCI with high and low Aβ amyloid had been reported previously in Chapters Five to Seven, one challenge was to determine whether it was possible to detect this Aβ amyloid-related decline in memory earlier. Thus, through serial assessments, the aim of Chapter Nine was to compare rates of change in cognition over six months in healthy older adults with high and low Aβ amyloid. High Aβ amyloid was associated with greater decline in episodic memory measures over six months in healthy older adults.

Chapter Ten: Modulation of Aβ Amyloid-Related Cognitive Decline by Brain-Derived Neurotrophic Factor Val66Met Polymorphism in Preclinical Alzheimer’s Disease

A consistent observation that emerged from the results of Chapters Five and Seven was that APOE ε4 carriage did not modulate the relationship between Aβ amyloid and cognitive decline. As the brain-derived neurotrophic factor (BDNF) Val66Met (rs6265) polymorphism has previously been implicated in AD-related cognitive impairment, the aim of Chapter Ten was to determine whether the BDNF Val66Met polymorphism modulates Aβ amyloid-related cognitive decline, reduction in hippocampal volume, rates of disease progression and Aβ amyloid accumulation in otherwise healthy older adults over 36 months. In healthy older adults, BDNF Val66Met polymorphism moderated the association between high Aβ amyloid and cognitive decline, hippocampal atrophy, and rates of disease progression. In healthy older adults with low Aβ amyloid, BDNF
Val66Met polymorphism was unrelated to rates of change in cognition, hippocampal volume, or clinical classification. Further, BDNF Val66Met polymorphism did not relate to the prevalence of Aβ amyloid or rate of Aβ amyloid accumulation in either group. This result suggests that high Aβ amyloid levels coupled with Met carriage may be useful prognostic markers of accelerated cognitive decline and hippocampal degeneration in individuals in the preclinical stage of AD.

**Chapter Eleven: General Discussion**

In Chapter Eleven, a summary of the research findings, presented along three sections: (1) validation of the computerised cognitive test battery, and (2) cross-sectional, and (3) prospective characterisation of the relationship between Aβ amyloid and cognitive function in healthy older adults and adults with MCI is provided. Following this summary, implications of the research findings for current models of preclinical and prodromal AD, and for future clinical trials of AD are considered, along with a discussion of the overall limitations of the current study, and potential future directions, focusing on other biomarkers (e.g., tau) and socio-demographic factors (i.e., cognitive reserve, cardiovascular risk factors).
1.1 Clinical and cognitive characterisation of Alzheimer's disease and mild cognitive impairment

Alzheimer's disease (AD) is a debilitating neurodegenerative disease characterised pathologically by accumulation of neurofibrillary tangles and neuritic plaques.\(^1\) Early in the disease, neuropathological changes occur primarily in the hippocampus and surrounding medial temporal lobe, with later changes occurring in the frontal, temporal, and parietal association cortices, and eventually, atrophy in the limbic regions and neocortex.\(^2\)-\(^4\) While a definite diagnosis of AD can only be made post-mortem or by brain biopsy, a clinical diagnosis of probable AD is based on the detection of severe memory impairment and substantial impairment in other cognitive domains such as executive function, attention, language and visuospatial/constructional abilities.\(^3\),\(^5\) On neuropsychological tests, this impairment is often defined as performance of two standard deviations below matched controls in at least two cognitive domains as well as impairment in social and occupational function.\(^3\)

It is now widely accepted that a long transitional phase between normal ageing and clinical AD exists in which gradual decline in cognitive function is accompanied by accumulation of AD-related pathology in the brain.\(^6\)-\(^8\) Once this subtle decline has become sufficiently large, it can be classified clinically as mild cognitive impairment (MCI). In MCI, impairment in memory and other cognitive functions are detectable upon clinical assessment although at a magnitude less than that observed in AD.\(^9\)-\(^11\) For example in MCI, performance on neuropsychological tests is generally 1 or 1.5 standard deviations below matched controls, especially on tests of episodic memory and executive
While subtle cognitive impairments in older adults can reflect a variety of non-AD processes, the validity of the MCI diagnostic criteria has been supported as patients who meet clinical criteria for MCI have also been observed to develop AD at a higher rate than age-matched controls who do not meet these criteria (e.g. 10-15% per year versus 1-2% per year respectively).

1.2 Factors affecting risk of developing Alzheimer’s disease

Several factors that increase the risk of individuals developing clinically diagnosed AD have been identified. Age is the most important risk factor for AD, with the prevalence of AD estimated to double every 5 years after the age of 60. Other factors also increase the risk for developing AD-related cognitive decline and impairment. For example, conditions that predispose individuals to cardiovascular disease, such as obesity, hypertension, hypercholesterolaemia, and diabetes, have been associated with increased risk of AD, and depressive and anxious symptomatology have been also associated with increased risk of cognitive impairment.

Genetic anomalies such as autosomal dominant mutations of the presenilin 1 (PS1), presenilin 2 (PS2), and amyloid precursor protein (APP) genes have been associated with increased risk of developing very early onset (40-50 years of age) familial AD. However, these mutations are rare and account for only about 5% of all AD cases. In late-onset sporadic AD, the largest genetic risk factor known is the presence of the apolipoprotein E (APOE) ε4 allele. Carriage of at least one APOE ε4 allele in older adults increases the risk of developing AD from 20% to as much as 90% when compared to older adults who do not carry the ε4 allele. Further, this increased risk is gene-dose
dependent, with individuals who carry only one \textit{APOE} \(\varepsilon4\) allele having a lower risk of developing AD (47\%) than those who are \(\varepsilon4\) homozygous (90\%). There is also increasing interest in other genes, particularly genes related to synaptic plasticity, such as the brain-derived neurotrophic factor (\textit{BDNF}) Val66Met polymorphism\textsuperscript{27-31} and the complement receptor-1 (\textit{CRI}) gene,\textsuperscript{32,33} and their influence on late-onset AD, although no consensus currently exists as to which allele poses the larger risk of developing AD.\textsuperscript{29,34}

Aside from risk factors for developing AD, there is also growing evidence that factors such as premorbid intelligence, years of education, and occupational complexity, termed hypothetically as cognitive reserve, can act to decrease the risk of AD, as well as reduce the severity of symptoms in individuals with the disease.\textsuperscript{19,35,36} Therefore, it has been postulated that individuals with greater cognitive reserve may possess an increased ability to tolerate brain atrophy without exhibiting clinical symptoms.\textsuperscript{19,36-39}

\textbf{1.3 Neuroimaging studies of Alzheimer’s disease}

The finding that subtle changes in cognitive function precede clinical diagnosis of AD is consistent with recent neuropathological findings in patients with MCI. In particular, pathological alterations in the medial temporal lobe regions, especially in the entorhinal cortex and hippocampus, have been observed to occur early in the course of the disease,\textsuperscript{40} resulting in the episodic memory impairment that is characteristic of the cognitive profile of AD. Further, the great majority of patients with clinically diagnosed AD have been observed to have pathological levels of beta-amyloid (Abeta; A\(\beta\)).\textsuperscript{41-44}
particularly in the medial temporal and temporoparietal regions, thus suggesting that Aβ amyloid biomarkers have excellent sensitivity for detecting AD.

In patients with MCI, positron emission tomography (PET) neuroimaging studies using radiotracers such as \(^{11}\)C-Pittsburgh Compound B (PiB) that measure Aβ accumulation have reported moderate negative relationships between performance on tests of episodic memory and degree of Aβ amyloid accumulation.\(^{45-47}\) Unfortunately, there have been few prospective studies of MCI that have also measured Aβ amyloid levels, though those that do, report that the magnitude of cognitive decline over 18 to 24 months to be greatest for measures of episodic memory (magnitude of decline range from 0.32-0.52).\(^{48-51}\) In patients with AD however, studies have typically observed no relationships between Aβ amyloid levels and cognitive performance,\(^{45,47,52}\) suggesting that when individuals meet clinical criteria for AD, increased Aβ amyloid deposition may have limited effect on further deterioration in cognitive function.\(^{53}\)

Post-mortem studies suggest that Aβ amyloid plaques accumulate in the brains of individuals before clinical symptomatology is evident.\(^{54-57}\) This has recently been replicated in antemortem studies in healthy older adults who have undergone neuroimaging for Aβ amyloid,\(^{45,48,50,58-61}\) and confirmed by cerebrospinal fluid (CSF) analysis for CSF Aβ\(_{42}\).\(^{62-67}\) Structural magnetic resonance imaging (MRI) studies have also observed a strong positive correlation between the severity of cortical atrophy and cognitive impairment,\(^{68,69}\) with studies showing that early atrophy particularly in the entorhinal cortex and hippocampus is associated with an increased risk of developing symptomatic AD.\(^{7,70}\) This recent ability to detect and quantify Aβ amyloid burden using PET neuroimaging or CSF techniques and cortical atrophy using structural MRI
techniques, has given rise to suggestions that these measures may be potential biomarkers of AD.

However, in the amnestic subtype of MCI (aMCI), a classification that is generally agreed to reflect prodromal AD, the importance of Aβ amyloid in the identification of this early stage of AD is less clear as only approximately 50% of adults with aMCI have been observed to have high Aβ amyloid. The utility of Aβ amyloid in the identification of early AD pathology is further complicated by observations that approximately 20-30% of cognitively healthy older adults show high Aβ amyloid. Whilst high Aβ amyloid may indeed indicate the presence of early AD pathophysiological processes, the absence of cognitive impairments in healthy older adults with high Aβ amyloid may also reflect the potential for false positive classification of early AD when this is based on the presence of Aβ amyloid biomarkers alone. Thus, whilst the emergence of these neuroimaging and structural MRI techniques has allowed investigators to observe and understand the neuropathology of AD at different stages of the disease, neuropsychological assessments remain the cornerstone of AD diagnosis and monitoring.

1.4 Neuropsychological assessment of Alzheimer’s disease

A wide range of neuropsychological tests has been applied in the assessment of AD. Cross-sectional comparisons of cognitive function between non-demented elderly persons and those with AD indicate that impairment in episodic memory is the most frequently observed and pronounced deficit found even in the mild stages of AD, although impairment in executive function, working memory, attention, and
psychomotor speed are also common. While the nature and magnitude of AD-related cognitive impairment is well-characterised and understood, cognitive changes and impairment in individuals at risk of developing AD (i.e., healthy older adults in the preclinical stage of AD (pAD)), and even in clinically classified MCI, are less well-defined. Further, given the heterogeneity of underlying pathology in individuals with MCI it has been proposed that the underlying cause of cognitive impairment observed in MCI needs to be determined in order to clarify prognosis and treatment. An amnestic syndrome of the hippocampal type has been proposed to be the primary neuropsychological feature of prodromal AD, with the neuropsychological profile of this syndrome characterised by impairment in the free recall of information learned previously despite adequate encoding, decreased total recall due to insufficient cueing, and numerous intrusions leading to false positives during recognition tasks. Importantly though, in research settings, information from biomarkers can also be combined with clinical and cognitive information to increase the certainty of the classification of prodromal AD.

### 1.5 Defining the preclinical stage of Alzheimer’s disease

Recent attempts to define the preclinical stage of AD (pAD) have proposed that healthy older adults at risk of developing AD (i.e. individuals with pAD) are biomarker positive, carry one or more APOE ε4 allele, and demonstrate very subtle decline in cognition, although this decline is not large enough for individuals to meet standardised criteria for a diagnosis of MCI. Several studies have demonstrated that in individuals who eventually develop AD (i.e., healthy older adults with pAD at time of enrolment in the study), mean levels of cognitive performance are significantly worse than matched
controls (i.e., individuals who remain cognitively unimpaired) many years before diagnosis. However, the performance distributions on tasks of episodic memory, executive function and speed of these two groups (controls and pAD) overlap to a large degree. As diminished cognitive function in older adults may be due to factors other than dementia (e.g. demographic, educational, social, psychiatric, or metabolic factors), the classification of cognitive impairment by itself is not sufficient to define pAD. Importantly, the effect sizes for impairment between controls and individuals with pAD at this early assessment are generally small for measures of episodic memory, and executive function, indicating that it is unlikely that a single neuropsychological test or even some combination of multiple tests will be adequate for detecting pAD.

In recognising the limitations of identifying AD solely through imaging or neuropsychological assessments, investigators have sought to combine neuropathological and neuropsychological data, suggesting that individuals with pAD may be better identified by understanding how these neuropathological changes manifest in neuropsychological tests. To this end, investigators have explored the relationship between cognitive performance on neuropsychological tests and Aβ amyloid burden and between cognitive performance and hippocampal volume. These studies show that individuals with pathological levels of Aβ amyloid tend to perform worse on cognitive tests, particularly on measures of episodic memory. The strongest relationships have been observed in individuals who meet clinical criteria for MCI, although smaller but significant relationships have also been observed in a subset of healthy older adults. Similarly, episodic memory impairment has been associated strongly with loss of hippocampal volume in individuals with MCI and in
healthy older adults who have not yet developed clinically recognisable memory impairments.\textsuperscript{61,87-89}

An attempt to integrate the pathological, clinical and neuropsychological changes that characterise AD over time has been proposed by Jack and colleagues, where a long preclinical period precedes MCI, which in turn precedes progression into AD (Figure 1.1).\textsuperscript{53} Drawing on a range of biochemical, autopsy and neuropsychological studies, this model proposes that abnormal Aβ amyloid deposition (e.g. detected by PET amyloid imaging and CSF Aβ\textsubscript{42} assay) occurs first, followed by neuronal injury and degeneration (e.g. detected by CSF tau, and anatomic MRI) closer to the time when individuals start to display clinical symptomatology.\textsuperscript{53,74} Following this, the model proposes that cognitive impairment, particularly in memory, start to become evident in individuals who meet clinical criteria for MCI, and finally, functional activities of living may only start to deteriorate closer to the time when a clinical diagnosis of AD is warranted.\textsuperscript{53} However, the trajectory curves for cognitive impairment in this model are derived from the findings of rates of neuropsychological impairment from cross-sectional studies of individuals at varying stages of the disease. Recent studies which have focused on cognitive decline have observed that measurable deterioration in episodic memory is apparent very early in the disease, often years before individuals meet any clinical criteria for MCI,\textsuperscript{48,50,86,90,91} suggesting that the detection of decline, as opposed to impairment, may be more important in identifying cognitive abnormalities in the very early stages of the disease.
Figure 1.1. Model of dynamic biomarkers of the AD pathological cascade, including the preclinical stage of AD. Figure adapted from Jack and colleagues.\textsuperscript{53}
1.6 Importance of studying change in the preclinical stage of AD

As AD is a neurodegenerative disease, the hallmark of the clinical sequelae is progressive decline in memory, other cognitive functions, behaviour and ability to conduct activities of daily living.\textsuperscript{3, 9} Recent consensus statements which have attempted to define the research criteria for the identification of the preclinical stage of AD have highlighted the importance of the detection of subtle decline in cognition.\textsuperscript{74} Researchers have also argued that rather than seeking cognitive measures which might return classifications of abnormality at one point in time, the measurement of cognitive change over time may improve the ability to differentiate individuals with pAD from controls in the preclinical stages of the disease, as the best indicator of neurodegeneration in people at risk of developing AD is objective evidence of cognitive decline over time.\textsuperscript{92-96}

From an operational perspective, the terms ‘decline’ and ‘impairment’ are often used interchangeably in statements about cognitive function in AD. For example, cognitive impairment (detected by formal neuropsychological assessment in individuals with AD or MCI) is often assumed to reflect deterioration from some premorbid baseline level of cognition. This assumption is based on the premise that normative data used to classify performance on the neuropsychological tests are reliable estimates of that individual’s premorbid function. In these instances, the term ‘decline’ can be considered to be theoretical. Recent research on the direct observation of decline in cognitive function emphasises a more operational approach, whereby decline is inferred to have occurred only when some change in cognitive function has been detected on the basis of the repeated application of a scale, measure or test and depends on the objective observation of change in the individual’s performance across multiple time points (at least two).
Estimates of the rate at which cognitive impairment in pAD declines to AD have been based on assessments conducted 6 to 12 months apart over periods of many years. These studies are typically large prospective studies that follow a large group of healthy older adults at risk of developing AD for five or more years to determine who progresses to the disease. Performance at earlier assessments are then compared to determine which of the neuropsychological measures would have been the best predictors of diagnosis. Thus, the emphasis of these studies is not to measure change in cognitive function. Rather, it has been to identify which measures, made on single assessments would predict the presence of disease at a later time, that is, change in clinical status. Others have modelled changes in memory and other cognitive functions over both short (e.g. minutes, days) and long (e.g. years, decades) intervals. Several studies have also attempted to estimate the average length of the pAD period, although these have typically been conducted over long test-retest intervals (e.g. 1-3 years).

When considered together, the data from these prospective studies of early stage AD suggest that there is a group of otherwise healthy older individuals whose memory and other cognitive functions are declining over time. These individuals generally have no insight into their declining cognitive functions, as the decline is typically subtle, and their activities of daily living are unimpaired. However, when their performance on neuropsychological measures is followed for a number of years, decline is evident and this has been related to neuropathological changes or impairment (e.g. cerebral infarction in the medial temporal lobe and accumulation of neurofibrillary tangles). Importantly, at the time when decline was identified, these individuals often did not meet clinical criteria for AD or MCI, and the specificity of their impairments and absence of other risk factors (e.g. cardiovascular, endocrine, or other neurological disease)
suggest that this decline reflects incipient AD.\textsuperscript{94,109} This hypothesis is strengthened by recent studies that have shown that cognitive decline over time in otherwise healthy older adults is associated with high levels of Aβ amyloid on PET neuroimaging.\textsuperscript{48,61,84,90,91,110}

The results of these studies suggest that the study of the rate at which cognitive functions decline over time in both healthy older adults and in adults with MCI may inform behavioural models of neurodegeneration and provide a basis for clinico-pathological correlations. Laboratory and clinical trial studies have suggested that Aβ-modifying therapies may have limited effect once neuronal degeneration has occurred, but have raised the possibility that such interventions may be more likely to achieve disease modification if started earlier in the course of AD.\textsuperscript{111} Additionally, reliable estimates of the rate of change in cognitive function, and their relationship to other biomarkers and lifestyle factors will provide a firm basis for the important tasks of computing the statistical power necessary for studies investigating new treatments designed to halt or slow the progression of AD.

1.7 Studying change in cognitive function

As a general principle of measurement, the ability of a measure to detect a change in cognitive function increases with the number of measurements used to estimate that change, the size of the change itself, and the amount of error associated with the measure.\textsuperscript{112,113} Typically, studies attempting to model rates of cognitive change in the early stages of AD, that is, in patients with MCI or in individuals with pAD, have conducted assessments at retest intervals of six months to one year.\textsuperscript{82,96,98,104,105} This
has been for several reasons. First, the neuropsychological test batteries that have been used are generally very long or have been embedded in assessments that are very long (e.g. the Alzheimer’s Disease Assessment Scale-Cognitive Subscale [ADAS-Cog], and the Wechsler Memory Scale [WMS]). When sample sizes become relatively large, repeated applications of time-demanding batteries become impractical in the demand on study resources and patient burden. The administration of an extensive neuropsychological test battery can also be difficult as individuals with cognitive impairments are more prone to refuse long testing sessions than healthy older adults. Second, even if resources can be procured, most measures of cognitive and memory function cannot be repeated as this gives rise to substantial improvements in performance (i.e., practice effects), which diminish the sensitivity of these tests to any true deterioration in performance. Third, in the assessment of change over time, the Mini Mental State Examination (MMSE) and the Clinical Dementia Rating (CDR) Scale have often been used as measures of general cognition and activities of daily living respectively. However, as these measures suffer from insufficient variability in healthy controls, they are not suitable for identifying subtle cognitive declines when cognition remains within normal limits, and thus, are unlikely to be appropriate to study cognitive decline in healthy populations.

In appreciating these limitations and recognising the potential benefit of repeated assessments, some have suggested that the detection of change over time in cognitive function should be based on the repeated application of simple, repeatable and brief cognitive tests. The characteristics of such tests should be that they are simple enough to be understood easily, brief enough to not lead to fatigue or decreased motivation, and have various parallel versions. It is also crucial that these tests are able to detect small
changes in cognition in the wide range of cognitive ability of the target populations. These tests should not generate practice effects and yield outcome measures that have good metric properties (i.e., reliability, normality, homogeneity of variance, and interval level scalar properties of performance measures). Taking into account these limiting factors, it is possible to test cognitive function repeatedly by using a set of computer cognitive tests, modelled on well-known and validated experimental psychology and cognitive neuroscience paradigms. One set of tasks designed to have these characteristics is the CogState battery. Tests from the CogState battery have been shown repeatedly to be sensitive to cognitive change in preclinical AD as well as other cognitive disorders, is brief to administer, possesses good test-retest reliability, and generates little to no practice effects. Importantly, in a recent prospective study that used these tests to measure rates of cognitive change in healthy older adults, episodic memory decline was associated with accumulation of Aβ amyloid, suggesting that these tests may be useful in characterising the different stages of the AD pathophysiological process.

1.8 The Australian Imaging, Biomarkers, and Lifestyle (AIBL) Flagship Study of Ageing

Launched in 2006, the AIBL study aims to improve understanding of the causes and diagnosis of AD, examine lifestyle and dietary factors that may influence the onset of AD, and help develop preventative strategies. The study is a longitudinal study of ageing which aimed to recruit and characterise 1000 volunteers from a cross-section of Australia’s population, of which at least 200 would be adults who met clinical criteria for AD, 100 adults who met clinical criteria for MCI, and 700 cognitively healthy older adults
Participants underwent extensive neuropsychological, medical and psychiatric assessments at 18-month intervals. The total cohort size is 1112 participants, and all participants have completed their 36-month (from baseline) assessments, that is, participants have completed 3 sets of assessments of 18-month intervals.

The AIBL study is the largest study in the world involving Positron Emission Tomography (PET) scans using $^{11}$C-labelled Pittsburgh Compound-B (PiB), a PET Aβ amyloid-imaging agent. At baseline, 287 participants (177 healthy older adults, 57 MCI, and 53 AD) underwent a PiB-PET scan and initial data from these participants have now been published.\textsuperscript{48, 60, 86} The neuropsychological, medical and psychiatric assessments in the AIBL study were designed to support the classification of subjects so that the study of biomarkers and neuroimaging would have a firm and reliable classification of the clinical status of each individual.\textsuperscript{126} However, the neuropsychological battery used, whilst extensive and covering the main domains of interest in assessing for AD-related cognitive impairment, is lengthy (totalling approximately 1.5 hours to administer) and the test-retest interval between assessments is long (18 month intervals). As such, in addition to characterising the rates of cognitive change over the long term, there is an opportunity for a neuropsychological sub-study of the AIBL cohort to be conducted which could characterise rates of change over the short term.

1.9 Conclusion

The importance of the measurement and detection of cognitive change, as opposed to cognitive impairment, as a neuropsychological approach to identifying AD in the earliest
stages has recently been acknowledged as an important consideration by the National Institute on Ageing and the Alzheimer’s Association workgroup guidelines on the definition of the preclinical stage of AD, and the prodromal or MCI stage of AD. The AIBL study provides a unique opportunity to study the nature and magnitude of cognitive change in relation to known biomarkers of AD, over both the short and long-term, using both conventional neuropsychological tests, and a computerised cognitive test battery. Consequently, this thesis aims to first validate the CogState battery of tests for detection of AD-related cognitive impairment in the AIBL study (Chapter Two), and to determine the relationship between Aβ amyloid and cognitive function cross-sectionally (Chapters Three and Four) and prospectively over both the long (Chapters Five, Six, and Seven), and short (Chapter Nine) term. Genetic contributions to the relationship between Aβ amyloid and cognitive function were also investigated (Chapters Three, Five, Seven, and Ten). In all chapters, the CogState battery of tests was used to assess change in cognitive function; however, in Chapter Four, in order to further understand the nature and magnitude of Aβ amyloid-related cognitive impairment in healthy older adults and adults with aMCI, the more comprehensive neuropsychological battery of the AIBL study was used. Further, in Chapter Ten, cognitive composite scores were formed using a combination of tests from both computerised and conventional neuropsychological tests. This was done as earlier chapters had established the nature and magnitude of Aβ amyloid-related cognitive impairment and cognitive decline for each individual neuropsychological measure. Thus, by reducing the large number of neuropsychological variables into composite measures that represent a particular cognitive domain, the parsimony of conclusions that can be drawn from the data are generally improved.
Part One

Aim:

Determine the utility of a computerised battery designed to measure cognitive change in detecting cognitive impairment due to Alzheimer’s disease (AD) and its prodromal stage, mild cognitive impairment (MCI).

Chapters:

Chapter Two: Use of the CogState Brief Battery in the Assessment of Alzheimer’s Disease Related Cognitive Impairment in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study
Chapter Two: Use of the CogState Brief Battery in the Assessment of Alzheimer’s Disease Related Cognitive Impairment in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study

2.1 Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder that is characterised clinically by progressive impairment in memory, executive function, attention, language and other cognitive domains.\textsuperscript{3, 77} Neuropathological and neuropsychological evidence indicates a long prodromal period in AD with impairment in memory to be the first and most distinct cognitive abnormality.\textsuperscript{5, 90, 128, 129} The earliest stage of AD may be expressed as mild cognitive impairment (MCI) where subtle dysfunction in memory and other cognitive functions are detectable, for example classified on the basis of performance that is 1 or 1.5 standard deviation units below matched controls.\textsuperscript{9} The most important risk factor for AD is age; however factors such as the presence of the apolipoprotein (\textit{APOE}) e4 allele, elevated levels of depressive or anxiety symptoms, and premorbid intelligence or level of education also influence performance on the cognitive tests used to identify cognitive impairment in older adults.\textsuperscript{17-19}

Current approaches to the early identification of AD and MCI emphasise careful assessment of cognitive impairment; that is the identification of abnormal performance on neuropsychological tests when compared to normative data ranges.\textsuperscript{9, 11} However, the detection of decline in cognitive function, in particular memory, on the basis of serial assessments conducted over relatively short retest intervals, can also assist with the identification of degenerative disease in older people.\textsuperscript{94} For example in otherwise healthy older adults, the presence of decline in memory with continued stability in psychomotor and attentional function, measured using the CogState Brief Battery, has
been associated with elevated levels of cognitive complaints,\textsuperscript{107} decreased functional activities of daily living,\textsuperscript{130} further cognitive decline,\textsuperscript{94} and more recently with elevated levels of central nervous system (CNS) beta-amyloid (Aβ).\textsuperscript{90, 110, 131}

We have argued that the optimal characteristics of tests used to screen for change in cognitive function are brevity, reliability, the absence of practice effects and interval level scalar properties of performance measures.\textsuperscript{117, 119} In the context of cognitive screening programs for very early or preclinical AD, another important assumption is that measures of performance on the cognitive screen will be abnormal in individuals with a clinically recognizable disease. While the CogState tasks used to measure psychomotor, attentional, working memory and visual learning functions have been applied to the study of cognitive function in different groups of older adults with various levels of AD-related cognitive impairment,\textsuperscript{94, 121, 132, 133} to date, there has not been any direct and detailed exploration of differences in performance between healthy adults, adults with MCI and adults with AD.

The first aim of the current study was to examine psychomotor, attentional, working memory and visual learning functions as measured by the CogState Brief Battery in healthy older adults and determine the extent to which factors such as age, gender, education level, depressive and anxiety symptoms and premorbid intelligence, were associated with these aspects of cognitive function. The second aim of the study was to characterise the nature and magnitude of impairment in psychomotor, attentional, working memory and visual learning functions as measured by the CogState Brief Battery in individuals who met clinical criteria for MCI and AD. The third aim was to determine the extent to which the presence of the \textit{APOE} ε4 allele influenced the cognitive functions measured by the CogState Brief Battery in healthy older adults, MCI and AD.
The first hypothesis was that in healthy adults without neuropsychological evidence of cognitive impairment, psychomotor, attentional, working memory and visual learning functions as measured by the CogState Brief Battery would be worse in older compared to younger samples of people aged over 60. The second hypothesis was that in otherwise healthy older adults, the magnitude of associations between performance on the CogState Brief Battery and level of education, gender and level of depressive symptoms would be small. The third hypothesis was that, compared to healthy older adults, impairment in performance on the CogState Brief Battery would be greater for people with AD than for people with MCI. However, the nature of impaired performance would be qualitatively similar in the MCI and AD groups. The fourth hypothesis was that within the control, MCI and AD groups, carriers of the APOE ε4 allele will show worse performance than non-carriers on the CogState Brief Battery.

2.2 Methods

2.2.1 Participants.

All participants in the current study were recruited from the Australian Imaging Biomarkers and Lifestyle (AIBL) flagship study of ageing. The process of recruitment and diagnosis classification have been described in detail previously. The AIBL cohort consisted of 768 healthy older adults, 133 adults who met clinical criteria for MCI, and 211 adults who met clinical criteria for mild to moderate AD. As all participants underwent a lengthy AIBL neuropsychological, psychiatric and medical assessment on the same day, prior to assessment on the CogState Brief Battery, 115 healthy older adults, 65 adults with MCI, and 167 adults with AD were unable or unwilling to complete the CogState Brief Battery due to fatigue, frailty or time limitations. Neuropsychologists were instructed to administer psychiatric rating scales,
neuropsychological tests and other medical examinations, in their order of importance.
As the CogState Brief Battery was considered experimental in the AIBL study (i.e.,
because clinical ratings or disease staging was not based on results from the CogState
tests), it was accorded the lowest priority in the study. Participants were asked to allow
2.5 to 3 hours for the complete administration of clinical, medical and
neuropsychological tests (excluding CogState Brief Battery). As such, neuropsychologists
were instructed to use their clinical judgment to deem whether a participant was too
tired, frail, or had other time limitations to continue with additional computerised
testing. Unfortunately, the specific reason for non-administration of the CogState Brief
Battery was not recorded by the neuropsychologists. As a result, 653 healthy older
adults, 68 adults with MCI, and 44 adults with AD completed the CogState Brief Battery.
All participants with AD met the National Institute of Neurological and Communicative
Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association
(NINCDS-ADRDA) criteria for AD, and in all cases, the clinical review panel chaired by
DA reviewed all available data to ensure that the diagnosis was consistent with these
agreed criteria. Similarly, the clinical review panel reviewed available data for
participants with MCI to ensure that their classification was consistent with
internationally agreed criteria. For participants with AD, additional inclusion
criteria for inclusion in the AIBL study were a score of 18 to 26 on the Mini Mental State
Examination (MMSE). All patients with AD and MCI were rated using the Clinical
Dementia Rating scale (CDR) to provide a sum of boxes score and an overall CDR
score. Participants were excluded from the AIBL study if they had any one or more of the
following: a neurological disease other than AD that might affect cognition; a major
psychiatric disorder, systemic illness, or symptoms that could affect the patient’s ability
to complete the study; a 15-item Geriatric Depression Score (GDS) of 6 or greater; or if
they used anticonvulsant, antiparkinsonian, anticoagulant, narcotic, or immunosuppressive medications within 3 months prior to assessment. All participants practiced the CogState Brief Battery before this assessment. Demographic and clinical characteristics of the control, MCI and AD groups are shown in Table 2.1. The study complied with the regulations of institutional research and ethics committees, and all participants gave written informed consent prior to participation in the study.

Table 2.1. Demographic means (SD) for MMSE, premorbid IQ and HADS scores, and median years of education, for each clinical classification group.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n = 653)</th>
<th>MCI (n = 68)</th>
<th>AD (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage females</td>
<td>57.8%</td>
<td>54.9%</td>
<td>51.6%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>74.19 (6.78)</td>
<td>79.33 (7.21)</td>
<td>79.37 (7.85)</td>
</tr>
<tr>
<td>Education level (category)</td>
<td>13-15 years</td>
<td>9-12 years</td>
<td>9-12 years</td>
</tr>
<tr>
<td>MMSE*</td>
<td>28.72 (1.43)</td>
<td>25.75 (2.94)</td>
<td>18.88 (5.22)</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>108.27 (7.27)</td>
<td>105.94 (9.73)</td>
<td>103.63 (8.81)</td>
</tr>
<tr>
<td>HADS-D@</td>
<td>2.54 (2.12)</td>
<td>3.40 (2.46)</td>
<td>3.71 (3.18)</td>
</tr>
<tr>
<td>HADS-A#</td>
<td>4.14 (2.90)</td>
<td>4.58 (2.99)</td>
<td>4.02 (3.04)</td>
</tr>
</tbody>
</table>

*MMSE = Mini Mental State Examination  
@HADS-D = Hospital Anxiety and Depression Scale, Depression Subscale  
#HADS-A = Hospital Anxiety and Depression Scale, Anxiety Subscale

2.1.2 Measures.

2.1.2.1. CogState brief battery.

The CogState Brief Battery consists of four tasks that utilise playing-card stimuli and is presented on a laptop computer. Whilst there are other cognitive tasks listed on the CogState battery (see www.cogstate.com for details), they were not included in the AIBL
study because 1) the AIBL neuropsychological battery already contained a measure similar or identical to these (e.g. verbal list learning, paired associate learning), 2) the tests were not appropriate for use in each of the clinical groups (e.g. Groton Maze Learning Test), and 3) the functions measured were not relevant to neuropsychological models of preclinical and clinical AD (e.g. social and emotional cognition test).

The four CogState Brief Battery tasks have been described in detail previously, and are summarised here. On each trial of each task, a single playing card stimulus was presented in the centre of the computer screen. The values, color and suit of the playing cards were determined by the requirements of each task. At the presentation of each playing card stimulus, participants were required to respond either “Yes” or “No” by pressing the “K” (yes) or “D” (no) key on the computer keyboard as quickly and as accurately as possible. While these keys were identified specifically in training, during the task the keys that surrounded the “K” key (e.g. U, I, O, J, L, M, ‘,’ and ‘.’ keys) were sensitive to “Yes” responses and the keys that surrounded the “D” key (e.g. W, E, R, S, F, X, C & V keys) were sensitive to “No” responses. At the beginning of each task, task rules were presented on the computer screen, and also given verbally to the participant. This was followed by an interactive demonstration in which participants practiced the task. Once the practice trials were complete, the task began. The four tasks were presented in the same order. For each task, the speed and accuracy of each response to each trial was recorded and expressed as a mean reaction time (in milliseconds) and accuracy (proportion correct). For each task a single performance measure has been selected on the basis that it comes from a normal data distribution, has no floor or ceiling effects, does not have restricted range and has good reliability, stability and sensitivity to change. The tasks from the CogState Brief Battery are described in order below.
The Detection task is a simple reaction time test shown to measure psychomotor function. In this task, the participant must attend to the card in the center of the screen and respond to the question “has the card turned over?” Participants were instructed to press the “Yes” key as soon as the card turns face up. The face of the card is always the same generic joker card. The task ends after 35 correct trials have been recorded. Trials on which anticipatory responses occurred were excluded and another trial was given so that all participants completed the 35 trials. The primary performance measure for this task was reaction time in milliseconds (speed), which was normalised using a logarithmic base 10 ($\log_{10}$) transformation. As distributions of reaction times are typically skewed in the negative direction, estimates of central tendency (and variance) can be influenced to a greater extent. This becomes a greater issue if the number of trials on reaction time type tasks is kept to a minimum in order to limit the time for task administration. This is the case with the tasks in the CogState Brief Battery. Psychometric investigation of the outcome measures of each task in the CogState Brief Battery have identified those measures that are optimal for characterising performance on the basis that they possess high test-retest reliability, no correlation between means and standard deviations, high stability and low coefficient of variation. These characteristics have been shown to be obtained more easily with normalised reaction time data (i.e., using the logarithmic base 10 transformation) than when data are left untransformed.$^{138, 140}$

The Identification (IDN) task is a choice reaction time test shown to measure visual attention. In this task, the participant must attend to the card in the centre of the screen, and respond to the question “Is the card red?” Participants were required to press the “Yes” key if it is and the “No” key if it is not. The face of the cards displayed were either
red or black joker cards in equivalent numbers in random order. These cards were different to the generic joker card used in the DET task. The task ends after 30 correct trials. Trials on which anticipatory responses occurred were excluded and another trial was given so that all participants completed the 30 trials. The primary performance measure for this task was reaction time in milliseconds (speed), which was normalised using a $\log_{10}$ transformation.

The One Card Learning (OCL) task is a continuous visual recognition learning task that assesses visual learning within a pattern separation model. In this task, the participant must attend to the card in the center of the screen and respond to the question “have you seen this card before in this task?” If the answer was yes, participants were instructed to press the “Yes” key, and the “No” key if the answer was no. Normal playing cards were displayed (without joker cards). In this task, six cards are drawn at random from the deck and are repeated throughout the task. These six cards are interspersed with distractors (non-repeating cards). The task ends after 42 trials, without rescheduling for post-anticipatory correct trials. This version of the task was administered to the healthy and MCI groups. For the AD group, a simpler version of the task, where only four cards interspersed with distractors was used. The primary performance measure for this task was the proportion of correct answers (accuracy), which was normalised using an arcsine square-root transformation.

The One-Back (OBK) task is a task of working memory and attention. Similar in presentation to the OCL task, participants must attend to the card in the center of the screen and respond to the question “is this card the same as that on the immediately previous trial?” If the answer was yes, participants were instructed to press the “Yes” key, and the “No” key if the answer was no. The task ends after 30 correct trials. A
correct but post-anticipatory response led to scheduling of an extra trial. The primary performance measure for this task was the proportion of correct answers (accuracy), which was normalised using an arcsine square-root transformation.

2.1.2.2. Demographic and clinical characteristics.

Prior to being tested with the CogState battery, participants also underwent a series of demographic, health and cognitive tests, performed by trained research assistants under the supervision of qualified neuropsychologists. Participants’ age and educational level were based on self-report, and this information was corroborated by a family member. Additionally, the MMSE, the Wechsler Test of Adult Reading (WTAR) and the Hospital Anxiety and Depression Scale (HADS) were administered to participants to measure general cognition, premorbid IQ, and level of anxiety and depressive symptoms respectively. An 80ml blood sample was also taken from each participant, 0.5ml of which was forwarded for APOE e4 genotyping at a clinical pathology laboratory.

2.1.3 Data analysis.

For each participant, each performance measure from the four tasks in the CogState Brief Battery was computed. To test the first and second hypotheses, the first analysis examined the effect of age on each performance measure by organising participants into 5 year age groups and then comparing performance between these groups using a trend analysis set within a one-way ANOVA. The effect of educational level, premorbid IQ, and levels of depression and anxiety symptoms was then explored by examining the strength of correlations between each of these factors and each CogState performance measure with the effect of age removed statistically (i.e. partial correlations controlling age).
Because education was classified categorically, each CogState performance measure was compared between education categories using a Kruskal-Wallis test. Finally, after controlling for age, the effect of gender on each performance measure was examined using independent t-tests.

To test the third hypothesis, the second analysis examined differences between the control, MCI and AD groups controlling for the demographic and clinical characteristics shown to affect CogState performance measures identified in the first analysis. Thus, each CogState performance measure was compared between the MCI and control and AD and control groups using two planned comparisons set within an analysis of covariance (ANCOVA) in which age and level of depressive symptoms were treated as covariates. Adjusted least squares means were derived from this analysis and the magnitude of impairment on each test in the MCI and AD groups, relative to healthy older adults was expressed using Cohen’s $d$ effect size. The modulatory effect of the demographic variables was then explored by repeating these analyses and treating age as the only covariate.

To test the fourth hypothesis, the third analysis sought to determine whether $APOE \varepsilon4$ status influenced performance on any of the CogState performance measures in any of the groups by conducting a series of One-Way ANOVAs comparing performance between $APOE \varepsilon4$ carriers to non-carriers, within each clinical classification group. Cohen’s $d$ effect sizes were calculated to reflect the magnitude of differences in performance between $APOE \varepsilon4$ positive and negative individuals within each clinical group.
2.2 Results

2.2.1 Effect of demography on performance in healthy older adults.

The data in Figures 2.1a and 2.1b show the relationship between age and performance on CogState performance measures in the healthy older adults. The equation for each linear function is also shown. Statistically significant declining linear trends were observed for the speed of performance on the Detection, $F(1, 660) = 6.25, p < .001, R^2 = 0.38$, and Identification, $F(1, 661) = 11.18, p < .001, R^2 = 0.49$, tasks and for the accuracy of performance on the One-Back, $F(1, 655) = 50.65, p < .001, R^2 = 0.96$, and One Card Learning, $F(1, 651) = 13.88, p < .001, R^2 = 0.85$, tasks.

Figure 2.1a. Effect of increasing age group on speed of performance on the Detection (DET) and Identification (IDN) tasks in healthy older adults.
Figure 2.1b. Effect of increasing age group on accuracy of performance on the One Card Learning (OCL) and One-Back (OBK) tasks in healthy older adults.

Table 2.2 summarises the strength of associations observed between performance on the CogState measures and the demographic measures. The partial correlations between the CogState performance measures and the demographic variables were generally very small, with the largest showing that premorbid IQ scores explained 14% of the variance in speed of performance on the Identification task. Depressive symptoms were correlated significantly only with speed of performance on the Identification task, although the variance explained was small (10%). Premorbid IQ correlated significantly with performance on all four CogState performance measures once again with a small proportion of variance explained (8-14%). Level of education obtained was associated with the speed of performance on the Detection, $H(4) = 11.99, p < .05$, and the
Identification tasks, $H(4) = 12.41, p < .05$, but not with the accuracy of performance on the One Back, $H(4) = 7.12, p = .13$, or the One Card Learning tasks, $H(4) = 5.61, p = .23$.

With age, level of education, and premorbid IQ treated as covariates, the ANCOVAs comparing performance between males and females showed no statistically significant effects for speed of performance on the Detection $F(1, 661) = 1.30, p = .26, d = -0.09$, or Identification, $F(1, 661) = 3.02, p = .08, d = -0.14$, tasks or accuracy of performance on the One Back, $F(1, 655) = 0.43, p = .51, d = -0.05$ or One Card Learning $F(1, 651) = 2.15, p = .14, d = -0.10$, tasks.

Table 2.2. Partial correlational matrix of healthy older adults with age controlled, between the four CogState performance measures and demographic variables.

<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>Education</th>
<th>Premorbid IQ</th>
<th>HADS-D</th>
<th>HADS-A</th>
<th>MMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET Speed</td>
<td>0.02</td>
<td>-0.10*</td>
<td>-0.10*</td>
<td>-0.03</td>
<td>-0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td>IDN Speed</td>
<td>0.09</td>
<td>-0.11*</td>
<td>-0.14**</td>
<td>0.10*</td>
<td>0.07</td>
<td>-0.07</td>
</tr>
<tr>
<td>OCL Accuracy</td>
<td>0.05</td>
<td>0.04</td>
<td>0.09*</td>
<td>0.04</td>
<td>-0.06</td>
<td>0.19**</td>
</tr>
<tr>
<td>OBK Accuracy</td>
<td>0.01</td>
<td>0.05</td>
<td>0.08*</td>
<td>0.04</td>
<td>-0.03</td>
<td>0.21**</td>
</tr>
</tbody>
</table>

Note: ** indicates $p < .001$ and * indicates $p < .05$

DET = Detection task; IDN = Identification task; OBK = One Back; OCL = One Card Learning; HADS-D = Hospital Anxiety and Depression Scale, Depression Subscale; HADS-A = Hospital Anxiety and Depression Scale, Anxiety Subscale; MMSE = Mini Mental State Examination

2.2.2 Magnitude of cognitive impairment in MCI and AD.

Comparison of the demographic variables between the study groups indicated a significant difference for age, $F(2, 914) = 37.57, p < .001$; premorbid IQ, $F(2, 907) = 12.74, p < .001$; and level of depressive symptoms, $F(2, 809) = 11, p < .001$ (see Table 2.1). As
these variables had been shown to vary with performance on the CogState Brief Battery in the first analysis, they were all included as covariates in comparisons of the CogState performance measures between groups.

The results of the ANOVAs were the same for all CogState performance measures. Performance in the AD group was significantly worse than for the MCI group which was in turn significantly worse than for healthy older adults. Table 2.3 summarises the F-ratios and provides the means and standard deviations for the control group and each patient group. Figure 2 illustrates the magnitude of impairment in each patient group relative to healthy older adults. The magnitude of impairment shown in Figure 2 was the greatest for One Card Learning and One Back tasks in both the AD and MCI groups. To determine the extent to which levels of depressive symptoms and premorbid IQ influenced the magnitude of group differences, the least squared means were generated from a second set of ANCOVAs in which age was the only covariate. After this analysis, the magnitude of the impairment in the MCI and AD groups did not change substantially (MCI z-scores for Detection = -0.33, Identification = -0.51, One Back = -1.67, and One Card Learning = -1.18; AD z-scores for Detection = -0.74, Identification = -1.26, One Back = -2.90, and One Card Learning = -2.19).

2.2.3 Effect of APOE ε4 genotype on cognitive performance.

The results of the one-way ANOVAs comparing performance measures between APOE ε4 carriers and non-carriers within each group are summarised in Table 2.4. The only difference related to APOE ε4 status was for performance in the One Card Learning task in the MCI group, with the APOE ε4 carriers performing worse than non-carriers.
Table 2.3. Comparisons of means of healthy older adults to adults with MCI and AD, on the four CogState performance measures; Cohen’s $d$ provided to demonstrate the effect size of difference between MCI and AD to healthy older adults on each performance measure (larger Cohen’s $d$ indicates larger difference from healthy older adults)

<table>
<thead>
<tr>
<th></th>
<th>Healthy Older Adults</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(df) F</td>
<td>N</td>
<td>M (SD)</td>
</tr>
<tr>
<td>DET Speed</td>
<td>(2, 759) 9.60**</td>
<td>650</td>
<td>2.51 (0.15)</td>
</tr>
<tr>
<td>IDN Speed</td>
<td>(2, 756) 38.25**</td>
<td>650</td>
<td>2.71 (0.08)</td>
</tr>
<tr>
<td>OCL Accuracy</td>
<td>(2, 745) 84.80**</td>
<td>641</td>
<td>1.02 (0.13)</td>
</tr>
<tr>
<td>OBK Accuracy</td>
<td>(2, 744) 182.93**</td>
<td>644</td>
<td>1.34 (0.18)</td>
</tr>
</tbody>
</table>

Note: ** ANCOVA with age, depression and premorbid IQ as covariates ($p < .001$)

DET = Detection task; IDN = Identification task; OCL = One Card Learning; OBK = One Back
Figure 2.2. Difference in performance on the four CogState performance measures from healthy older adults; error bars represent 95% confidence intervals.
Table 2.4. Comparisons of performance between adults with and without the APOE ε4 allele within each participant group for the four CogState performance measures

<table>
<thead>
<tr>
<th></th>
<th>(df) F</th>
<th>APOE ε4 carriers</th>
<th>APOE ε4 non-carriers</th>
<th>Cohen's $d$ of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Healthy Older Adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DET Speed</td>
<td>(2, 665) 0.70</td>
<td>180</td>
<td>2.52</td>
<td>0.11</td>
</tr>
<tr>
<td>IDN Speed</td>
<td>(2, 665) 0.40</td>
<td>180</td>
<td>2.71</td>
<td>0.06</td>
</tr>
<tr>
<td>OCL Accuracy</td>
<td>(2, 665) 2.98</td>
<td>177</td>
<td>1.00</td>
<td>0.11</td>
</tr>
<tr>
<td>OBK Accuracy</td>
<td>(2, 659) 1.12</td>
<td>179</td>
<td>1.32</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>MCI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DET Speed</td>
<td>(1, 72) 0.18</td>
<td>37</td>
<td>2.56</td>
<td>0.15</td>
</tr>
<tr>
<td>IDN Speed</td>
<td>(1, 72) 2.16</td>
<td>37</td>
<td>2.76</td>
<td>0.09</td>
</tr>
<tr>
<td>OCL Accuracy</td>
<td>(1, 71) 11.28**</td>
<td>36</td>
<td>0.85</td>
<td>0.12</td>
</tr>
<tr>
<td>OBK Accuracy</td>
<td>(1, 71) 0.42</td>
<td>36</td>
<td>0.99</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>AD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DET Speed</td>
<td>(1, 50) 0.00</td>
<td>39</td>
<td>2.62</td>
<td>0.19</td>
</tr>
<tr>
<td>IDN Speed</td>
<td>(1, 47) 0.55</td>
<td>36</td>
<td>2.81</td>
<td>0.10</td>
</tr>
<tr>
<td>OCL Accuracy</td>
<td>(1, 46) 0.82</td>
<td>35</td>
<td>0.78</td>
<td>0.17</td>
</tr>
<tr>
<td>OBK Accuracy</td>
<td>(1, 41) 2.10</td>
<td>31</td>
<td>0.74</td>
<td>0.35</td>
</tr>
</tbody>
</table>

**Note:** **One-Way ANOVA ($p < .001$); larger Cohen's $d$ indicates larger differences from healthy older adults.

DET = Detection task; IDN = Identification task; OCL = One Card Learning; OBK = One Back.
2.3 Discussion

The results of this study supported the first hypothesis that psychomotor, attentional, working memory and visual learning functions as measured by the CogState Brief Battery would be worse in older than younger groups of healthy older adults aged over 60. Age-related decline was observed for each performance measure although the rate of decline in the accuracy of working memory and visual learning was greater than that for the speed of psychomotor function and attention. This cross-sectional age-related decline in performance with greater decrements in working memory and visual learning tasks has been observed almost universally.\textsuperscript{143-145} Several hypotheses have been proposed to explain this relationship between age and cognitive functioning, with neurobiological processes such as decline in health, decline in visual and auditory acuity and skeletomotor control and occult disease indicated as potential moderators.\textsuperscript{60, 143, 146, 147} These data therefore show the importance of considering age when making decisions about the presence of cognitive impairment on the basis of performance on tests from the CogState Brief Battery in cross-sectional studies.

The results of the study partially supported the second hypotheses that in healthy older adults demographic and clinical variables would affect performance on the CogState tasks. Thus, although psychomotor, attentional, working memory and visual learning functions were not different between healthy older men and women, or related to years of education or levels of anxiety symptoms, individuals with higher premorbid IQ performed slightly better on all CogState tasks. Furthermore, individuals with higher levels of depressive symptoms showed worse visual attentional function, as measured by the CogState Identification task. Interestingly though, for all statistically significant relationships observed, the magnitude was always small with the largest being that
premorbid IQ explained 14% of the variance in speed of performance on the Identification task. These relationships were small compared to the effect of age on the same tasks, where for example, age explained approximately 50% of the variance in the speed of performance on the Identification task. We believe that the very weak relationships observed between the demographic, mood and cognitive measures were rendered statistically significant because of the very large sample size studied. This hypothesis is consistent with the results of other studies that have observed small relationships between anxiety or education and attentional and memory performance measures in large epidemiological samples,\textsuperscript{148,149} although these same effects have not been observed in smaller clinical samples.\textsuperscript{39,130,131} Therefore, with the exception of age, the small effects of the demographic and clinical variables on performance on the CogState tasks observed here are unlikely to be clinically meaningful.

The results of the study supported the third hypothesis, that when compared to healthy older adults, performance on the CogState Brief Battery would be abnormal in adults with a clinical diagnosis of MCI and AD. Relative to healthy older adults, performance impairments were greater in the AD group than in the MCI group with the magnitude of impairment in working memory and visual learning greater than that in psychomotor function and attention for both groups. The presence of large (i.e. $z > 2$) impairment in higher cognitive functions such as visual learning and working memory with less severe impairment in attentional and psychomotor function in the current AD group is consistent with the results of many if not all neuropsychological studies of mild AD, and concurs with cognitive models and theories of AD which suggest that the pathophysiological process of AD exists on a continuum of increasing severity, where MCI precedes AD.\textsuperscript{9,74} For the MCI group, the qualitatively similar but quantitatively less
severe impairments in visual learning, working memory, attention and psychomotor function are also consistent with current neuropsychological models of very early AD, which show that the most reliable cognitive impairment in MCI to occur in memory and executive functions.\textsuperscript{3,150,151} Hence the results of the current study indicate that the cognitive impairments that characterise both AD and MCI manifest in performance on the CogState battery in the expected way. Importantly, statistical comparison of demographic characteristics indicated that compared to healthy older adults, both patient groups showed small but statistically significant elevations in depressive symptoms and lower premorbid IQ. However, the analysis of relationships between cognitive performance and demographic variables in the control group suggested that such variables were only weakly associated with performance on the CogState Brief Battery. Accordingly, re-characterisation of the nature and magnitude of cognitive impairment in the AD and MCI groups with variation due to depressive symptoms and premorbid IQ not statistically controlled showed that group differences remained statistically significant and that the magnitude of impairment in performance on each test in both groups did not change substantially. Hence, the results of these analyses are consistent with the conclusion drawn from the first analysis that the effects of premorbid IQ, and slight elevation in depressive symptoms are small and do not influence the performance of individuals to a clinically meaningful extent.

The results of the present study partially supported the fourth hypothesis that the \textit{APOE} \(\varepsilon4\) allele would be associated with poorer cognitive performance. Individuals with MCI who were \textit{APOE} \(\varepsilon4\) positive performed worse on the visual learning task than did individuals who were \textit{APOE} \(\varepsilon4\) negative and the magnitude of this difference was large. However, no differences between individuals with and without the \textit{APOE} \(\varepsilon4\) allele were
observed for any other CogState performance measure nor observed for any measure in the healthy older or AD groups. The finding that in MCI, \textit{APOE} ε4 positivity was associated with poorer performance on the visual learning task from the CogState battery is consistent with previous studies showing the negative influence of \textit{APOE} ε4 on learning and episodic memory,\textsuperscript{152} and with studies suggesting that \textit{APOE} ε4 may be a risk factor for cognitive impairment, by accelerating the AD disease process in MCI populations.\textsuperscript{153} Conversely, consistent with the results of this study, impairments in cognitive functioning were not associated with \textit{APOE} ε4 positivity in healthy older adults,\textsuperscript{154,155} or in adults with AD.\textsuperscript{156} The results of these studies, along with those of the current study, suggest that \textit{APOE} ε4 positivity may only present as a risk for increased memory impairment once the AD pathological process has been activated, and ceases to continue to influence its relationship with memory impairment once an individual has developed AD. We also found that \textit{APOE} ε4 positivity had no effect on tasks of psychomotor speed or attention. This is consistent with data showing that these functions are not as affected in early AD,\textsuperscript{157} and is also consistent with our results in AD where performance on tasks of psychomotor speed and attention were not as impaired as performance on tasks of memory. Lastly, we found no effects of \textit{APOE} ε4 on the One-Back task, a measure of working memory. Interestingly, other studies have also found that performance on working memory tasks are relatively spared in \textit{APOE} ε4 carriers,\textsuperscript{158} with some studies indicating that non-carriers displayed more impairment on tests of working memory and executive control than \textit{APOE} ε4 carriers.\textsuperscript{159,160} It has been postulated that independent networks serving learning and working memory may be responsible for the specificity of the effect of \textit{APOE} ε4 on tasks of learning and episodic memory but not working memory,\textsuperscript{160} and this has been similarly demonstrated in the current study.
Taken together, these results indicate that performance on the CogState Brief Battery is impaired in the prodromal and clinical stages of AD and this impairment is greatest for visual learning and working memory. Similarly, increasing age affects mainly working memory and visual learning functions. Compared to these age effects, the effects of clinical and demographic variables like mood, premorbid IQ, education and gender are small, although APOE ε4 positivity can increase the magnitude of impairment in learning in individuals with MCI. Thus, even though the tests on the CogState Brief Battery were designed for repeated use in studies that seek to identify deterioration of cognitive function within individuals over time,\textsuperscript{101} the measures themselves do show the expected levels of impairment when challenged in individuals with clinically recognisable disease, even in the early stages. Consequently, the performance measures from the CogState Brief Battery which have been shown to be sensitive to AD-related changes in otherwise healthy older adults\textsuperscript{90} could be used to follow cognitive deterioration in these individuals, even until their disease is clinically recognizable.

The impairment in performance by adults with AD-related pathology on the CogState Brief Battery in this study is consistent with a recent report that performance on the same battery was impaired in adults with schizophrenia, head injury and the dementia complex arising from infection with the Human Immunodeficiency Virus (HIV dementia; HIVD).\textsuperscript{161,162} The data from these previous studies showed that in each of these clinical groups, the magnitude of impairments in psychomotor speed and attention was almost equivalent to those in visual learning and working memory. In the current MCI and AD groups, impairment in visual learning and working memory was much greater than in psychomotor and attentional function. This difference most likely reflects the very different pathophysiology of AD-related brain changes with its predilection for cortical
and hippocampal brain regions as compared to the more generalised or mostly subcortical pathophysiologies of head injury, schizophrenia and HIVD.

The CogState Brief Battery was not designed to act as a tool for classifying cognitive impairment in adults or for diagnosing dementia. Even though differences in performance can be observed between different clinical groups, this data will not inform the extent to which a performance abnormality, or pattern of abnormalities in an individual reflects disruption to specific brain regions. The CogState Brief Battery was designed to measure change (improvement or deterioration) in cognitive function in individuals. Importantly, different tests from the battery have been shown to be sensitive to change in cognitive function that occurs in response to concussion, cardiac surgery, fatigue, low level alcohol intoxication, and particularly, for the current context, Alzheimer’s disease pathology.

The current study has several limitations. First, a simplified version of the One Card Learning task was administered to adults with AD in order to prevent a floor effect. Hence, the magnitude of impairment that would occur if the AD group performed the same test as the healthy older adults would likely be greater. However, while challenging individuals with impaired memory with difficult tests does give rise to impairment in performance, inferences about the nature of that impairment are difficult as such impairments can reflect that individuals were overwhelmed by the task generally and therefore did not understand its requirements, that there was a decrease in motivation or loss of interest as error signals became more frequent, that there was true memory impairment, or that there was a combination of these factors. In the current study, accuracy of performance on the One Card Learning task was better than
chance (chance score is 0.78, the value for an arcsine square-root transformation of 50%), suggesting that individuals’ performance was a reflection of their memory ability.

A second limitation was that we had substantial differences in sample size between the control and patient groups. Importantly, although the number of MCI and AD patients studied was relatively large by comparison, the healthy control group was very much larger. Unequal sample sizes can disrupt the assumptions of homogeneity of variance. However, in the current study, all analyses were conducted with adjustments to such sample size differences (e.g. use of ANOVAs and generation of effect sizes).

Furthermore, the use of the very large control group will have ensured that estimates of the ranges of normal performance would have high reliability, and this in turn will facilitate the recognition of abnormal performance. Third, we concluded that the effects of demography such as premorbid IQ and depressive symptomatology did not influence the performance of this cohort of adults to a clinically meaningful extent. However, this conclusion is limited in that there was only small variation in demographic characteristics in the AIBL healthy older and clinical groups. This was because clinically diagnosed depression and a history of low IQ were part of the exclusion criteria for the AIBL study. Thus the current conclusions should not be generalised to contexts where levels of premorbid IQ move into the low range or where levels of depressive symptoms become sufficient to warrant investigation for depressive illness. Given the effect of premorbid IQ on performance observed in this study, it may be important for future studies to investigate how cognitive reserve may influence performance on such tasks. Finally, it should be noted that participants from the AIBL cohort were recruited through a series of advertisements seeking volunteers for a study into memory and ageing. As such, individuals with a family history of dementia may express
more interest in participating in such research than individuals with no such history. However, proportions of APOE ε4 carriers in the AIBL cohort do not suggest an over-selection of ε4 carriers.\textsuperscript{126}

Current recommendations on the assessment of AD, especially in the preclinical and prodromal stages, emphasise the importance of change and the measurement of cognitive decline.\textsuperscript{9, 74} However, neuropsychological instruments used to measure change also need to be able to demonstrate abnormal performance in individuals with the disease of interest. Despite the limitations outlined above, this study supports the use of the CogState Brief Battery in the detection of AD-related cognitive impairment. Importantly, given its brevity, reliability, absence of practice effects and ability to detect AD-related cognitive impairment, the CogState Brief Battery may be a useful screening instrument for change in AD-related cognitive function. This may potentially inform and contribute to current knowledge about the nature of cognitive change in the very early stages of AD.
Part Two

Aim:

Determine the nature and magnitude of Aβ amyloid related cognitive impairment cross-sectionally in healthy older adults and adults with mild cognitive impairment (MCI)

Chapters:

Chapter Three: Aβ Amyloid, Cognition and APOE Genotype in Healthy Older Adults

Chapter Four: Cognitive Consequences of High Aβ Amyloid in Mild Cognitive Impairment and Healthy Older Adults: Implications for Early Detection of Alzheimer's Disease
Chapter Three: Aβ Amyloid, Cognition and APOE Genotype in Healthy Older Adults

3.1 Introduction

The development of radio-ligands that bind to β-amyloid (Aβ) in the brain (e.g. C-11 Pittsburgh Compound B [PiB]) is rapidly increasing the understanding of clinicopathological relationships in Alzheimer’s disease (AD) and its preclinical phase. While nearly all individuals who meet clinical criteria for AD have abnormally high Aβ amyloid, the relationship between Aβ amyloid burden, as measured using PiB-PET neuroimaging, and clinical ratings of disease severity such as the Clinical Dementia Rating scale (CDR), or impairment in memory determined by neuropsychological tests, is generally weak or non-existent in patients with AD.

Studies investigating levels of Aβ amyloid in healthy older adults report that approximately 30% have abnormally high levels. However, studies that compare cognition between healthy older adults with high and low levels of Aβ amyloid have found no differences, although these have been conducted in relatively small samples. These data are consistent with models of AD pathology which suggest that pathological changes precede observable impairment in cognition, although the observation of normal cognitive function in the presence of abnormally high Aβ amyloid has also been interpreted to indicate the potential for false positive classifications of AD when these are based on biomarkers alone.

In the absence of differences in cognition between healthy older adults with high and low levels of Aβ amyloid, evidence for any early cognitive effect of Aβ amyloid would be in the finding of an association between cognitive performance and Aβ amyloid level; independent of whether Aβ amyloid level was high or low. Studies of the association...
between cognition and Aβ amyloid levels in healthy older adults have been mixed, with most reporting only very small,\textsuperscript{46, 86} or no associations at all.\textsuperscript{58, 59, 84} However for most of these studies, the small sample sizes have most probably resulted in the studies being underpowered to detect relationships of a small or even moderate magnitude. Recently, a study conducted in a large subgroup of healthy older adults from the population based Mayo Clinic Study of Ageing (MCSA) cohort found that Aβ amyloid, as measured using PiB-PET neuroimaging, was negatively associated with composite scores of memory, attention, and visual-spatial function, although for all measures, the magnitude of these associations was very small (e.g. correlations ranging from 0.12 to 0.18).\textsuperscript{85} As the apolipoprotein E (\textit{APOE} $\epsilon$4 allele has been shown to increased associations between Aβ amyloid level and cognitive function in adults with MCI and healthy older adults,\textsuperscript{48, 170} the authors re-analysed their data while controlling statistically for the \textit{APOE} genotype of individuals. They observed that the magnitude of associations between Aβ amyloid and cognition increased significantly across \textit{APOE} $\epsilon$2, $\epsilon$3 and $\epsilon$4 carriers. The scatterplots provided in the study report suggested that there were no associations between Aβ amyloid and cognitive function in carriers of the \textit{APOE} $\epsilon$2 allele and small associations in carriers of the \textit{APOE} $\epsilon$3 allele with the strength of associations increasing for \textit{APOE} $\epsilon$4 carriers.\textsuperscript{85}

The observation of statistically significant associations between cognition and Aβ amyloid in \textit{APOE} $\epsilon$4 carriers led Kantarci and colleagues to conclude that in healthy older adults, increased levels of Aβ amyloid may signify the onset of AD pathological processes, at least in those with additional AD risk factors.\textsuperscript{85} However, data from the study do not provide unequivocal support for this hypothesis. First, correlations between Aβ amyloid and cognitive function were rendered non-significant after
statistical control of demographic characteristics also known to influence performance on cognitive tests (i.e., education, age and gender). Even the application of a second strategy that involved case matching ε4 carriers to non-carriers on age, gender and education and then recomputing the relationships between APOE genotype, Aβ amyloid and cognition failed to show a statistically significant effect of APOE on the relationship between Aβ amyloid load and episodic memory. Thus, it is possible that the associations between APOE ε4, Aβ amyloid load and memory observed in this study were actually secondary to differences between APOE groups in terms of their education, age, and gender. This issue was made more difficult to resolve by the study basing inferences about the presence or absence of relationships between Aβ amyloid and cognitive functioning on the basis of statistical significance alone. The magnitudes of the associations between Aβ amyloid and episodic memory were not reported for ε4 carriers and non-carriers either before or after statistical adjustment for demographic factors. Consequently, it is not possible to determine whether the loss of statistical significance with statistical control of age, education and gender differences was due to reduced statistical power, as would be the case if the magnitude of the correlations remained constant but non-significant, or whether the statistical control actually reduced the magnitude of associations.

The healthy older adults group from the Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing also provides a large sample who have undergone APOE ε4 genotyping, measurement of Aβ amyloid with PiB-PET neuroimaging, and assessment of cognitive functions known to be altered in clinical and preclinical AD. Unlike the population based MCSA study, the AIBL study uses a quasi-experimental design and has deliberately recruited individuals who carry the APOE ε4 allele.
Descriptions of the healthy older adults group from AIBL show it to be homogenous in terms of age, education, mood symptoms, and estimated premorbid intelligence. Thus, separation of the healthy older adults group into ε4 carriers and non-carriers is less likely to result in groups differing on demographic variables known to influence cognition in older adults. It is also crucial that the magnitudes of associations observed be reported, in order to accurately assess the importance of the APOE ε4 allele to the relationship between Aβ amyloid and cognition, and also allow future meta-analyses to aggregate data across the different samples from large studies in order to inform models of AD pathology.

The aim of this study was to measure the associations between Aβ amyloid burden and measures of cognitive function in healthy older adults who do and do not carry the APOE ε4 allele. The hypothesis was that the relationship between Aβ amyloid burden and cognition will be present in APOE ε4 carriers than in non-carriers, and that the aspects of cognitive function for which relationships will be found will be those known to be central to the neuropsychological presentation of AD, such as episodic memory.

3.2 Methods

3.2.1 Participants.

All participants in the current study were recruited from the Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing. The process of recruitment and diagnosis classification has been described in detail previously. The AIBL cohort consisted of 768 healthy older adults, who have undergone clinical, medical and neuroimaging assessments on three visits (baseline, 18 months and 36 months). PiB-PET neuroimaging had been conducted on 158 healthy older adults. Of
these, 144 healthy older adults had completed the CogState Brief Battery at baseline. A clinical review panel chaired by DA reviewed all available medical, psychiatric and neuropsychological information to confirm the cognitive health of individuals enrolled in the healthy controls group at each assessment time point. There was no change in the clinical status of the healthy controls group at 18 months. Participants who volunteered were excluded from the AIBL study if they had any of the following: a neurological disease other than AD that might affect cognition; a major psychiatric disorder, systemic illness, or symptoms that could affect the patient’s ability to complete the study; a 15-item Geriatric Depression Score (GDS) of 6 or greater; or if they used anticonvulsant, antiparkinsonian, anticoagulant, narcotic, or immunosuppressive medications within 3 months prior to assessment. All participants practiced the CogState Brief Battery before this assessment. The study was approved by and complied with the regulations of three institutional research and ethics committees, and all participants gave written informed consent prior to participation in the study.

3.2.2 Measures.

3.2.2.1 Cognitive assessments.

Clinical status was measured using the MMSE and the CDR scale, premorbid intelligence was estimated using the Wechsler Test of Adult Reading (WTAR) and levels of depressive and anxiety symptoms were estimated using the Hospital Anxiety and Depression Scale (HADS). Verbal episodic memory was measured using the CVLT-II and visual episodic memory was measured using the continuous visual paired associate learning task (CPAL task). All individuals also performed the CogState Brief Battery which included a test of visual learning, working memory, attention and psychomotor function, as well as providing composite measures of
learning and working memory and psychomotor function and attention.\textsuperscript{121, 132, 161, 172} All of these tests have been described in detail elsewhere and were administered according to standard protocols.

3.2.2.2 PiB neuroimaging and APOE ε4 genotyping.

PiB imaging methodology has been outlined in detail previously.\textsuperscript{45, 167} Each subject received \(~370\) MBq \(^{11}\text{C}\)-PiB intravenously over 1 minute. A 30-minute acquisition of PET standardised uptake value (SUV) data in 3D mode, consisting of 6 frames each of 5 minutes, acquired 40–70 minutes post-PiB injection were summed and normalised to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVR). An 80ml blood sample was also taken from each participant, 0.5ml of which was forwarded for APOE ε4 genotyping at a clinical pathology laboratory.

3.2.3 Data analysis.

Performance measures from the four tasks in the CogState Brief Battery, the CPAL task, CVLT-II total and delayed recall, and CDR sum of boxes (CDR-SB) were scored according to standard protocols. The CogState Brief Battery also generated two composite measures computed by first standardising performance using the mean and SD for the relevant tests from the healthy adult group. Second, the average of standardised performance accuracy scores from the One Card Learning and One Back tasks was defined as the CogState Working Memory-Learning Composite while the average of standardised performance speed scores from the Identification and Detection tasks was defined as the CogState Psychomotor-Attention Composite. Inspection of data distributions indicated that data for the CDR-SB were skewed negatively and
characterised by restriction of range. Therefore, both measures were analysed using non-parametric statistical methods.

First, the performance of APOE ε4 carriers and non-carriers on the cognitive tasks were compared using a series of one-way ANOVAs. Because of the non-normal distribution of the CDR-SB, Mann-Whitney U tests were used to compare scores between APOE ε4 carriers and non-carriers. Second, Pearson’s correlations were computed between SUVR and the outcome measures for each cognitive test. Once again, due to the non-normal distribution of data for the CDR-SB, correlations with SUVR were computed using Spearman’s correlation. For each correlation coefficient, 95% confidence intervals (CI) were computed. In order to statistically compute the differences in magnitudes of the correlations between cognitive measures and SUVR in APOE ε4 carriers and non-carriers, all correlations were transformed using Fisher’s Z-transformation.\textsuperscript{174} The test statistic used to compare groups was Fisher’s Z, and Cohen’s $q$ was used to quantify the magnitude of difference between correlations.\textsuperscript{175} Cohen’s $q$ is derived by the formula $Z_1 - Z_2$, where $Z_1$ is the Z-transformed value of the first correlation, and $Z_2$ is the value of the second correlation. According to Cohen, $q$ of less than 0.20 is considered a small effect, 0.30 a moderate effect, and 0.50 a large effect.\textsuperscript{175}

The criterion for statistical significance for all comparisons and correlations was set at $p < .05$. Although this may increase the probability of experiment-wise error, $p < .05$ was selected as research linking genes to biomarkers in early neurodegenerative disease is a new area of research, and therefore, studies should identify hypotheses for further investigation. b) although multiple cognitive tests were used to study performance in the sample, performance on these tests are correlated, especially for tests that assess the same cognitive domain, c) measures of effect size were used to guide interpretation...
about the meaningfulness of results. For example, Type I errors will be suspected where associations are statistically significant but where effect sizes are very small (e.g. \( d < 0.20 \), or \( q < 0.20 \)).

3.3 Results

3.3.1 Demographic differences between healthy older adults who are APOE \( \varepsilon 4 \) carriers and non-carriers.

Demographic and clinical characteristics of the total control group, and the APOE \( \varepsilon 4 \) carrier and non-carrier sub-groups are shown in Table 3.1. APOE \( \varepsilon 4 \) carriers did not differ on any demographic characteristic, or in performance on any of the cognitive measures (Table 3.2). APOE \( \varepsilon 4 \) carriers had a higher SUVR level than non-carriers and this difference was statistically significant, \( F(1,142) = 14.44, p < .001, d = 0.64 \) (Figure 3.1).

Table 3.1. Demographic means (SD) for MMSE, premorbid IQ and HADS scores, and median years of education, for overall group, and for APOE \( \varepsilon 4 \) carriers and non-carriers

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 144)</th>
<th>( \varepsilon 4 ) carriers (n = 61)</th>
<th>( \varepsilon 4 ) non-carriers (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) females</td>
<td>68 (47%)</td>
<td>25 (41%)</td>
<td>43 (52%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>75.09 (7.03)</td>
<td>73.90 (6.97)</td>
<td>75.96 (6.99)</td>
</tr>
<tr>
<td>Education level (category)</td>
<td>13-15 years</td>
<td>13-15 years</td>
<td>9-12 years</td>
</tr>
<tr>
<td>SUVR Neocortex</td>
<td>1.40 (0.41)</td>
<td>1.54 (0.47)</td>
<td>1.29 (0.32)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.69 (1.48)</td>
<td>28.51 (1.65)</td>
<td>28.82 (1.34)</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>108.84 (7.18)</td>
<td>107.79 (7.45)</td>
<td>109.61 (6.92)</td>
</tr>
<tr>
<td>HADS-Depression</td>
<td>2.71 (2.01)</td>
<td>2.79 (2.10)</td>
<td>2.65 (1.95)</td>
</tr>
<tr>
<td>HADS-Anxiety</td>
<td>3.92 (2.61)</td>
<td>4.07 (2.95)</td>
<td>3.82 (2.35)</td>
</tr>
</tbody>
</table>

Note: \( \chi^2 \) indicated no significant differences between groups on years of education; one-way ANOVA indicated no significant differences between APOE groups on age, premorbid IQ, and depression and anxiety levels, all \( p > .05 \)

SUVR = Standardised Uptake Value Ratio; MMSE = Mini Mental State Examination; HADS-Depression = Hospital Anxiety and Depression Scale, Depression Subscale; HADS-Anxiety = Hospital Anxiety and Depression Scale, Anxiety Subscale
Table 3.2. Comparisons of means of healthy older adults who are APOE ε4 carriers and non-carriers, on each cognitive measure and clinical rating scale.

<table>
<thead>
<tr>
<th>Test</th>
<th>APOE ε4 carrier</th>
<th>APOE ε4 non-carrier</th>
<th>ANOVA</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>M (SD)</td>
<td>N</td>
<td>M (SD)</td>
</tr>
<tr>
<td>Detection Speed</td>
<td>61</td>
<td>2.53 (0.11)</td>
<td>83</td>
<td>2.54 (0.12)</td>
</tr>
<tr>
<td>Identification Speed</td>
<td>61</td>
<td>2.71 (0.06)</td>
<td>83</td>
<td>2.72 (0.06)</td>
</tr>
<tr>
<td>One Back Accuracy</td>
<td>61</td>
<td>1.29 (0.19)</td>
<td>82</td>
<td>1.33 (0.15)</td>
</tr>
<tr>
<td>One Card Learning Accuracy</td>
<td>60</td>
<td>0.99 (0.11)</td>
<td>83</td>
<td>1.02 (0.11)</td>
</tr>
<tr>
<td>CPAL Errors</td>
<td>61</td>
<td>36.34 (24.18)</td>
<td>79</td>
<td>35.23 (22.92)</td>
</tr>
<tr>
<td>Working Memory-Learning Composite</td>
<td>61</td>
<td>-0.14 (0.68)</td>
<td>83</td>
<td>0.11 (0.77)</td>
</tr>
<tr>
<td>Psychomotor-Attention Composite</td>
<td>61</td>
<td>-0.04 (0.86)</td>
<td>83</td>
<td>0.03 (0.92)</td>
</tr>
<tr>
<td>CDR-SB®</td>
<td>61</td>
<td>0.05 (0.18)</td>
<td>83</td>
<td>0.05 (0.22)</td>
</tr>
<tr>
<td>CVLT-II Total Recall</td>
<td>61</td>
<td>50.70 (10.87)</td>
<td>83</td>
<td>49.78 (10.72)</td>
</tr>
<tr>
<td>CVLT-II Delayed Recall</td>
<td>61</td>
<td>11.30 (3.68)</td>
<td>83</td>
<td>11.92 (3.05)</td>
</tr>
</tbody>
</table>

Note: One-Way ANOVA indicated that no comparisons were significant at the $p < .05$ level; Mann-Whitney U test was used to compare groups on CDR-SB, and groups did not differ significantly at the $p < .05$ level.

CPAL = Continuous Paired Associate Learning; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes score; CVLT-II = California Verbal Learning Test, Second Edition
Figure 3.1. Scatterplot of standardised uptake value (SUVR) in healthy older adults who are APOE ε4 carriers and non-carriers.
3.3.2 Relationship between Aβ amyloid and cognition.

When considered across the entire group, statistically significant correlations with SUVR were observed for the CVLT-II delayed recall trial, the CPAL task, the CogState Working Memory-Learning Composite and the CogState OBK task although correlations were all small to moderate in magnitude, with SUVR explaining approximately only 10% of the variance in episodic memory performance (Table 3.3).

3.3.3 Effect of APOE ε4 genotype on the relationship between Aβ amyloid and cognition

Splitting the group into APOE ε4 carriers and non-carriers showed that SUVR was again correlated significantly with the CogState OBK task, the CPAL task, the CogState Working Memory-Learning Composite, and the CVLT-II delayed recall trial, with the magnitude of these associations increasing from those observed in the total group analysis. However, the magnitude of these associations remained moderate in magnitude, with SUVR explaining approximately 14% of the variance in episodic memory performance (Figures 3.2 and 3.3; Table 3.3). In APOE ε4 non-carriers, SUVR was not correlated significantly with any measure of cognition.

Statistical comparison of the correlations using Fisher’s Z indicated that the associations between SUVR and the CPAL task, the CogState Working Memory-Learning Composite, and the CVLT-II delayed recall trial were stronger in APOE ε4 carriers than in non-carriers, and the magnitude of difference in these associations was, by convention,175 small to moderate in magnitude (Table 3.3).
Figure 3.2. Relationship between Aβ amyloid (SUVR neocortex) and standardised CogState Working Memory-Visual Learning Composite Z Score in healthy older APOE ε4 carriers and non-carriers.
Figure 3.3. Relationship between Aβ amyloid (SUVR neocortex) and CVLT-II Delayed Recall score in healthy older APOE ε4 carriers and non-carriers.
Table 3.3. *Bivariate correlational matrix between measures of cognition and SUVR in the overall healthy older adults, and in APOE ε4 carriers and non-carriers.*

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 144)</th>
<th>APOE ε4 carriers (n = 61)</th>
<th>APOE ε4 non-carriers (n = 83)</th>
<th>Fisher’s Z</th>
<th>Cohen’s q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection Speed</td>
<td>-0.04 (-0.20, 0.12)</td>
<td>-0.11 (-0.32, 0.11)</td>
<td>0.04 (-0.21, 0.29)</td>
<td>-0.87</td>
<td>-0.15</td>
</tr>
<tr>
<td>Identification Speed</td>
<td>0.08 (0.24, -0.08)</td>
<td>0.11 (-0.11, 0.32)</td>
<td>0.09 (-0.17, 0.33)</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>One Back Accuracy</td>
<td>-0.30 (-0.44, -0.14)**</td>
<td>-0.37 (-0.54, -0.17)**</td>
<td>-0.17 (-0.40, 0.09)</td>
<td>-1.26</td>
<td>0.22</td>
</tr>
<tr>
<td>One Card Learning Accuracy</td>
<td>-0.13 (-0.29, 0.03)</td>
<td>-0.10 (-0.31, 0.12)</td>
<td>-0.06 (-0.31, 0.19)</td>
<td>-0.23</td>
<td>-0.04</td>
</tr>
<tr>
<td>CPAL Errors</td>
<td>0.20 (0.35, 0.03)</td>
<td>0.35 (0.15, 0.53)**</td>
<td>0.05 (-0.20, 0.30)</td>
<td>1.83*</td>
<td>0.32</td>
</tr>
<tr>
<td>Working Memory-Learning Composite</td>
<td>-0.29 (-0.43, -0.13)**</td>
<td>-0.37 (-0.54, -0.17)**</td>
<td>-0.13 (-0.37, 0.13)</td>
<td>-1.49*</td>
<td>-0.26</td>
</tr>
<tr>
<td>Psychomotor-Attention Composite</td>
<td>0.02 (0.18, -0.14)</td>
<td>-0.01 (-0.23, 0.21)</td>
<td>0.08 (-0.18, 0.33)</td>
<td>-0.52</td>
<td>-0.09</td>
</tr>
<tr>
<td>CDR-SB*</td>
<td>-0.05 (-0.21, 0.12)</td>
<td>-0.05 (-0.26, 0.17)</td>
<td>-0.01 (-0.26, 0.24)</td>
<td>-0.23</td>
<td>-0.04</td>
</tr>
<tr>
<td>CVLT-II Total Recall</td>
<td>-0.12 (-0.28, 0.04)</td>
<td>-0.24 (-0.43, -0.03)</td>
<td>-0.03 (-0.28, 0.22)</td>
<td>-1.25</td>
<td>-0.21</td>
</tr>
<tr>
<td>CVLT-II Delayed</td>
<td>-0.28 (-0.43, -0.12)**</td>
<td>-0.38 (-0.55, -0.18)**</td>
<td>-0.12 (-0.36, 0.14)</td>
<td>-1.62*</td>
<td>-0.28</td>
</tr>
</tbody>
</table>

Note: * signifies p < .05, ** signifies p < .001; CI = 95% confidence interval, upper and lower limits provided; @ Spearman’s correlation coefficient CPAL = Continuous Paired Associate Learning; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes score; CVLT-II = California Verbal Learning Test, Second edition
3.4 Discussion

The hypothesis that in healthy older adults, relationships between Aβ amyloid burden and episodic memory were stronger in individuals who were APOE ε4 carriers than in non-carriers was supported. First, healthy older adults who were APOE ε4 carriers and non-carriers from the AIBL study were equivalent in terms of age, gender, education, premorbid IQ, and anxiety and depressive symptoms. All of these demographic variables have, by themselves, been shown to exert small effects on cognitive performance in older adults.\(^{172}\) Second, Aβ amyloid was slightly higher in healthy older adults who were APOE ε4 carriers, as has been observed previously.\(^{85, 170}\) Despite the difference in Aβ amyloid, no differences in cognition were found between APOE ε4 carriers and non-carriers.\(^{128, 154}\) Most importantly, negative statistically significant associations of a moderate magnitude between Aβ amyloid load and performance on tests of episodic memory were observed in healthy older adults who carried the APOE ε4 allele. Aβ amyloid load was not associated with any measure of episodic memory in non-carriers (Table 3.3, Figures 3.2 and 3.3). Comparison of the magnitudes of associations between Aβ amyloid and episodic memory in APOE ε4 carriers and non-carriers indicated that the differences were small in magnitude. Thus, although, Aβ amyloid load explained approximately 14% of the variance in performance on the episodic memory tests in APOE ε4 carriers, this relationship was robust enough to remain present when the data for APOE ε4 carriers and non-carriers were collapsed. These data therefore suggest that increased levels of Aβ amyloid signify the onset of AD pathological processes, at least in those with additional AD risk factors.

In healthy older adults, the association between Aβ amyloid burden and cognition was small to moderate in magnitude, with the strongest observed for measures of episodic
memory (Table 3.2). This is consistent with the findings of previous studies, where small but significant relationships between levels of amyloid Aβ and episodic memory were also observed.\textsuperscript{45, 46, 85, 86} The presence, strength and statistical significance of associations with Aβ amyloid burden observed for verbal memory was equivalent to those between Aβ amyloid burden and visual recognition learning, visual paired associate learning and visual working memory. This suggests that higher amyloid Aβ burden manifests as a decreased efficiency of episodic memory generally. The absence of any association between Aβ amyloid burden and psychomotor function or visual attention suggests that early AD-related pathological changes do not manifest as impairment in visual attention or psychomotor function.\textsuperscript{9, 74} The results of the current study also suggest that the \textit{APOE} ε4 allele did influence the relationships between Aβ amyloid and cognitive function in the population based MCSA study.\textsuperscript{85} The loss of statistical significance for these relationships once education, gender and age were controlled was therefore most likely due to the loss of statistical power or to some aspect of sample bias. Thus, data on \textit{APOE} ε4 genotype, Aβ amyloid burden and cognition from healthy older adults in the MCSA and AIBL cohorts converge to show the importance of the \textit{APOE} ε4 allele in influencing early AD clinicopathological relationships.

Current models of AD suggest that accumulation of Aβ amyloid precedes changes in brain structure, which themselves precede changes in cognitive function.\textsuperscript{53, 74} The neurodegeneration in AD, and the associated cognitive impairment, has also been observed to be moderated by the presence of the \textit{APOE} ε4 allele, with investigators observing that \textit{APOE} ε4 carriers developed AD up to 6-7 years earlier than non-carriers, although the mechanism of this relationship is still unclear.\textsuperscript{176, 177} The finding that in
healthy older adults, relationships between Aβ amyloid burden and episodic memory were stronger in APOE ε4 carriers than in non-carriers is consistent with this hypothesis, and supports hypotheses that suggest that earlier Aβ amyloid deposition may occur in APOE ε4 carriers.\textsuperscript{48,86} Further, the magnitude of the difference in relationships was small to moderate in magnitude. This is the second time such a relationship has been observed and like the first study\textsuperscript{85} that observed such relationships, it is most likely due to the sample of healthy older adults being one of largest to have received Aβ amyloid neuroimaging to date. Additionally, in the recruitment of healthy older adults for PiB-PET neuroimaging, the AIBL study has deliberately biased inclusion criteria towards known risk factors for AD which include carrying at least one APOE ε4 allele and older age\textsuperscript{167,171}. Hence, in the current study, older individuals who were APOE ε4 carriers were over-represented, and as such, this relatively large sample provided the necessary statistical power for detection of the subtle relationships between Aβ amyloid burden and episodic memory in healthy older adults who were APOE ε4 carriers.

Recently, we have found stronger relationships than those observed here between episodic memory and Aβ amyloid burden in healthy older adults by measuring Aβ amyloid burden in individuals who had shown an abnormal decline in episodic memory over the previous 12 months,\textsuperscript{131} two years,\textsuperscript{90} and six years.\textsuperscript{110} In these studies, on two different samples, 50 to 70% of healthy older adults showing decline in episodic memory had abnormally high Aβ amyloid burden.\textsuperscript{90,110} One other study has also reported that decline in cognitive function is associated with elevated levels of Aβ amyloid, although they have reported that decline in measures of verbal episodic memory and not visual episodic memory are most strongly associated with Aβ amyloid
burden over an average of approximately 10 years. Another study reported that Aβ amyloid burden was strongly associated with decline over 10 years in measures of working memory but not episodic memory. The data from these prospective and retrospective studies converge with the current findings to suggest that in individuals who do not meet clinical criteria for MCI or AD, higher Aβ amyloid burden is associated with both poorer episodic memory as well as further deterioration in episodic memory. However, the process by which the subtle impairment and decline in episodic memory observed in this study, as well as previous studies, progresses to MCI-level impairment, is still not known. The current data suggest that research in healthy older individuals with combinations of memory impairment, higher Aβ amyloid burden or APOE ε4 positivity should provide a fertile area for understanding the earliest stages of AD pathophysiology.

The CDR-SB was not associated with Aβ amyloid in healthy older adults. The restricted range and negative skew that characterise the data distribution of the CDR-SB means that in healthy older adults, variability is generally limited to a small set of possible scores, thus suggesting that strategies to identify the external manifestations of AD-related brain changes early in the disease process should focus on the use of instruments with better metric characteristics than the CDR-SB. Neuropsychological instruments like verbal list-learning tests and visual paired associate learning tests have been developed and have a refined ability to detect memory impairment in older adults, with some optimised further for repeated assessments. Hence, the moderate relationships observed with Aβ amyloid burden, both across and within disease classifications, in this study most likely reflect their optimal psychometric characteristics.
One limitation of the current study is that we have explored relationships only between Aβ amyloid burden, episodic memory and APOE ε4. Other putative but validated markers of biological processes related to disease include structural analysis of the hippocampus from magnetic resonance imaging (MRI) studies and the cerebral glucose utilisation rates inferred from FDG-PET. Models of disease development in healthy older adults studied here would be improved greatly if the extent to which structural changes in the medial temporal lobe or rates of glucose utilisation are associated with the measures that have been studied here. A second limitation is that the clinicopathological inferences made about AD on the bases of observed relationships between measures of episodic memory and Aβ amyloid burden were based on cross-sectional data. Recently, we and others have argued that as AD is a neurodegenerative disease, the measurement of cognitive change over time may improve the ability to differentiate individuals at risk of developing AD (i.e., individuals in the preclinical stages of the disease) from healthy controls.
Chapter Four: Cognitive Consequences of High Aβ Amyloid in Mild Cognitive Impairment and Healthy Older Adults: Implications for Early Detection of Alzheimer’s Disease

4.1 Introduction

Levels of Aβ amyloid, detected using positron emission tomography (PET) neuroimaging and cerebrospinal fluid (CSF) analysis for Aβ42, provide useful information about the pathophysiology of Alzheimer’s disease (AD), particularly in the early stages. Using different neuroimaging agents, studies conducted in different laboratories have shown that the majority of patients with clinically-diagnosed AD have, by some validated criterion (e.g. standardised uptake value ratio (SUVR) of greater than 1.5 on PET imaging using Pittsburgh Compound B [PiB]), abnormally high levels of Aβ amyloid. This suggests that amyloid imaging biomarkers have an excellent sensitivity for classifying AD. While it is agreed that the amnestic subtype of mild cognitive impairment (aMCI), with or without additional cognitive impairment (i.e. multi domain aMCI), represents a very early or preclinical stage of AD, the importance of Aβ amyloid to the identification of this early or preclinical AD stage of the disease is less clear, as only approximately 50% of adults who meet clinical criteria for aMCI have high Aβ amyloid. This observation has led to a hypothesis that MCI with high Aβ amyloid is indicative of an incipient AD, while MCI with low Aβ amyloid may reflect other neurodegenerative or even psychiatric processes.

The hypothesis that MCI with high Aβ amyloid reflects early AD processes would be supported by the presence of relationships between Aβ amyloid levels and cognitive performance, in particular episodic memory; or by quantitative or qualitative differences in cognitive performance between patients with MCI who have high and low
Correlational studies in adults with MCI have reported negative relationships between levels of Aβ amyloid and cognitive function, although these associations have been small to moderate in magnitude, with the strongest relationships found for measures of episodic memory ($r = 0.38 - 0.60$). Comparisons of cognitive performance between MCI groups with high and low Aβ amyloid, determined by PiB-PET neuroimaging, have shown that in MCI with high Aβ amyloid, performance on measures of episodic memory is worse than in MCI with low Aβ amyloid, with the magnitudes of differences large by convention ($d = 0.60 - 1.28$); however, the sample sizes studied to date have also been small (i.e. total $n = 7$ to $20$). Others have also found small to moderate differences ($d = 0.12 - 0.56$) between groups of MCI with high and low Aβ amyloid, as measured by florbetapir F-18. With Aβ amyloid determined from CSF analysis, one study observed that adults with MCI and low CSF Aβ42 performed worse than those with high CSF Aβ42 amyloid on measures of episodic memory ($d = 0.72 - 0.85$), language ($d = 0.32 - 0.55$), attention ($d = 0.51 - 0.67$) and executive function ($d = 0.30 - 0.47$). However, an important limitation of this study was that when neuropsychological data were organised according to whether the MCI group had low CSF Aβ42, MCI with high CSF Aβ42 did not show substantial impairment in memory.

To date, the largest study of the relationship between Aβ amyloid and cognition in MCI found that individuals with MCI and pathological levels of CSF Aβ42 showed impairment relative to individuals with MCI and non-pathological levels of CSF Aβ42, on a verbal list learning test hypothesised to control the effects of deficits in attention and language (i.e., the Buschke free and cued recall test (FCRT), $d = 0.90$). The impairment detected on the FCRT was greater than that observed on a verbal list.
learning test that did not control for the potential effects of these non-memory factors (i.e., Consortium to Establish a Registry for Alzheimer’s Disease [CERAD] verbal list learning test; \( d = 0.70 \)).\textsuperscript{186} Although the difference between effect sizes for impairment on these two memory tests is not large, it does suggest indirectly that poor performance on memory tests in MCI with pathological levels of Aβ amyloid reflects specific deficits in episodic memory whilst in MCI with non-pathological levels of Aβ amyloid, poor performance of memory tests may reflect impairment in attention and language in addition to memory impairment. However, because this conclusion was based only on differences in performance on the different verbal memory tests, the hypothesis should be tested directly in individuals with MCI in whom Aβ amyloid levels have been quantified and through direct assessment of several cognitive functions such as attention, language, and executive function, in addition to episodic memory.

The importance of Aβ amyloid in non-demented adults is also complicated by observations that approximately 20-30\% of healthy older adults show high Aβ amyloid.\textsuperscript{42, 58, 59} As with MCI, high Aβ amyloid in healthy older adults may indicate the presence of early AD pathophysiological processes.\textsuperscript{53, 58, 74} However, it has also been suggested that the absence of cognitive impairments in healthy older adults with high Aβ amyloid levels may reflect the potential for false positive classification of AD when this is based on Aβ amyloid biomarkers alone.\textsuperscript{75, 76} If high Aβ amyloid does reflect early AD processes, then subtle impairments in episodic memory, qualitatively similar to those observed in MCI with high Aβ, may also occur in healthy older adults with high Aβ amyloid levels. Cross-sectional comparisons of cognitive performance between healthy older adults with high and low Aβ amyloid have typically revealed no such differences.\textsuperscript{42, 45, 49, 58} However, once again, these studies have all been conducted in
small samples (i.e. with number of high Aβ amyloid individuals ranging from 9 to 32) and therefore will have lacked the statistical power necessary for reliable comparisons of cognitive performance between healthy older adults with high and low Aβ amyloid levels, especially when the magnitudes of differences between groups with high and low Aβ amyloid levels are likely to be small.

Another issue related to the importance of high Aβ amyloid in adults with MCI is the appropriateness of the normative data against which cognitive abnormality is defined. Given that high Aβ amyloid is observed in approximately 20-30% of healthy older adults,\textsuperscript{110,187-189} and if elevated levels of Aβ amyloid in healthy older adults do indicate that AD pathological processes have begun, data from healthy older adults with high Aβ amyloid should not be included in neuropsychological normative ranges. For example, the inclusion of data from individuals with subclinical cognitive impairment in normative data may reduce estimates of normal mean performance or increase estimates of normal group variation, either of which would decrease the sensitivity of a neuropsychological test to true abnormal performance. Hence, the nature and magnitude of cognitive impairment in adults with MCI with high and low levels of Aβ amyloid may be better understood by comparing their performance to healthy older adults with low levels of Aβ amyloid.

The overarching aim of this chapter was to determine whether high Aβ amyloid influenced cognitive function in older adults who meet clinical criteria for MCI and in healthy older adults. The first hypothesis was that, compared to adults with MCI with high Aβ amyloid, those with MCI and low Aβ amyloid would show impairments in non-memory aspects of cognition such as attention, language, visuoconstruction, and executive function. The second hypothesis was that in healthy adults with high Aβ
amyloid, episodic memory would be impaired relative to healthy adults with low Aβ amyloid. The third hypothesis was that when compared to healthy older adults with low Aβ amyloid, the nature and magnitude of cognitive impairment would be qualitatively and quantitatively different in MCI with high and low Aβ amyloid.

4.2 Methods

4.2.1 Participants.

A total of 178 healthy older adults (HA) and 56 adults who met clinical criteria for amnestic MCI who had undergone PiB-PET neuroimaging for Aβ amyloid were recruited from individuals enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing. The process of recruitment and diagnosis classification of the AIBL study have been described in detail elsewhere. The AIBL clinical review panel, chaired by DA, reviewed available data for participants with MCI to ensure that their classification was consistent with internationally agreed criteria. Clinical classification was blinded to Aβ amyloid imaging data. Participants who volunteered were excluded from the AIBL study if they had any one of the following: a neurological disease other than AD that might affect cognition; a major psychiatric disorder, systemic illness, or symptoms that could affect the patient’s ability to complete the study; a 15-item Geriatric Depression Score (GDS) of 6 or greater, or if they used anticonvulsant, antiparkinsonian, anticoagulant, narcotic or immunosuppressive medications within 3 months prior to assessment. The study was approved by and complied with the regulations of the institutional research and ethics committees of Austin Health, St. Vincent’s Health, Hollywood Private Hospital, and Edith Cowan University, and all participants gave written informed consent prior to participation in the study.
In this chapter, participants were classified according to their Aβ amyloid levels, and their clinical classification. Of the 178 healthy older adults, 123 (69%) had low Aβ amyloid levels (HA low Aβ), and 55 (31%) had high Aβ amyloid levels (HA high Aβ). Similarly, of the 56 adults who met clinical criteria for MCI, 19 (34%) had low Aβ amyloid levels (MCI low Aβ), and 37 (66%) had high Aβ amyloid levels (MCI high Aβ).

4.2.2 Measures.

4.2.2.1 Demographic and clinical characteristics.

Age, gender, years of education, family history of AD, smoking history, and history of cardiovascular diseases (i.e., hypertension, angina, and diabetes), were self-reported. Depression and anxiety levels were assessed using the Hospital Anxiety and Depression Scale (HADS). The Wechsler Test of Adult Reading (WTAR) was used to estimate the premorbid intelligence of participants. The Clinical Dementia Rating (CDR) was used to rate dementia severity, as indicated by functional status for memory, orientation, problem solving, home and hobbies, community affairs and self-care. Information to determine this rating were obtained from cognitive testing, direct questioning of the participant, and from information from an informant/treating clinician for those with a diagnosis of MCI.

4.2.2.2 Neuropsychological assessment.

The AIBL neuropsychological test battery is summarised in Table 4.1. These tests were selected on the basis of their validity for demonstrating cognitive impairment in MCI, their ubiquity in the research literature, and because they covered the main domains of cognition that are affected by AD and other dementias. All tests have been described
elsewhere in detail, and all were administered according to standard protocols by trained neuropsychologists.\textsuperscript{126, 172}

Table 4.1. \textit{Summary of the Australian Imaging, Biomarkers and Lifestyle Study neuropsychological test battery and their outcome measures.}

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Measure</th>
<th>Outcome Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global cognition</td>
<td>MMSE</td>
<td>Number of correct answers</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>Logical Memory 1\textsuperscript{b}</td>
<td>Number of details correctly recalled</td>
</tr>
<tr>
<td></td>
<td>Logical Memory 2\textsuperscript{b}</td>
<td>Number of details correctly recalled</td>
</tr>
<tr>
<td></td>
<td>CVLT-II Total Recall</td>
<td>Number of words correctly recalled</td>
</tr>
<tr>
<td></td>
<td>CVLT-II Short Delay</td>
<td>Number of words correctly recalled</td>
</tr>
<tr>
<td></td>
<td>CVLT-II Long Delay</td>
<td>Number of words correctly recalled</td>
</tr>
<tr>
<td></td>
<td>CVLT-II Recognition</td>
<td>Number of true positives</td>
</tr>
<tr>
<td></td>
<td>CVLT-II d'</td>
<td>True positive minus false positive</td>
</tr>
<tr>
<td>Visual memory</td>
<td>RCFT Short Delay</td>
<td>Number of elements correctly recalled</td>
</tr>
<tr>
<td></td>
<td>RCFT Long Delay</td>
<td>Number of elements correctly recalled</td>
</tr>
<tr>
<td></td>
<td>RCFT Recognition</td>
<td>Number of elements correctly recognised</td>
</tr>
<tr>
<td></td>
<td>CogState Memory Composite (OCLOBK)</td>
<td>Accuracy (arcsine proportion correct)</td>
</tr>
<tr>
<td>Executive Function</td>
<td>Stroop C/D</td>
<td>Speed of Stroop Colours/Stroop Dots</td>
</tr>
<tr>
<td></td>
<td>Letter Fluency</td>
<td>Number of words produced in 1 minute</td>
</tr>
<tr>
<td></td>
<td>Category Switching Total</td>
<td>Number of words produced in 1 minute</td>
</tr>
<tr>
<td></td>
<td>Category Switching Accuracy</td>
<td>Number of switches made in 1 minute</td>
</tr>
<tr>
<td>Visuoconstruction</td>
<td>RCFT Copy</td>
<td>Number of elements drawn correctly</td>
</tr>
<tr>
<td></td>
<td>Clock Drawing</td>
<td>Number of elements drawn correctly</td>
</tr>
<tr>
<td>Attention and Processing</td>
<td>Digit Span\textsuperscript{a}</td>
<td>Total correct trials (forwards &amp; backwards)</td>
</tr>
<tr>
<td>Speed</td>
<td>Digit Symbol\textsuperscript{a}</td>
<td>Number of symbols correctly matched</td>
</tr>
<tr>
<td></td>
<td>CogState Speed Composite (DETIDN)</td>
<td>Speed (log_{10} milliseconds)</td>
</tr>
<tr>
<td>Language</td>
<td>Category Fluency</td>
<td>Number of words produced in 1 minute</td>
</tr>
<tr>
<td></td>
<td>Boston Naming Test</td>
<td>Number of pictures correctly named</td>
</tr>
</tbody>
</table>

\textit{Note.} MMSE = Mini Mental State Examination; CVLT-II = California Verbal Learning Test (second edition); RCFT = Rey Complex Figure Test; CogState Memory Composite was obtained by averaging standardised scores of visual learning and visual working memory tests from the CogState brief battery; CogState Speed Composite was obtained by averaging standardised scores of psychomotor function and visual attention test from the CogState brief battery.\textsuperscript{a} Sub-test of the Wechsler Adult Intelligence Scale – Third Edition (WAIS-III).\textsuperscript{b} Story A only, Subtest of the Wechsler Memory Scale.
4.2.2.3 PiB-PET neuroimaging and APOE genotyping.

PiB-PET neuroimaging methodology has been outlined in detail previously. Each subject received \(~370\) MBq \(^{11}\)C-PiB intravenously over 1 minute. A 30-minute acquisition of PET standardised uptake value (SUV) data in 3-dimensional mode, consisting of 6 frames each of 5 minutes, acquired 40-70 minutes post-PiB injection were summed and normalised to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVR). Neocortical Aβ burden was expressed as the average SUVR of the area-weighted mean of frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions. In this study, we have classified high Aβ amyloid levels based on an SUVR of 1.5 or more. An 80ml blood sample was taken from each participant upon their arrival at the study site; 0.5ml of which was forwarded for APOE ε4 genotyping at a clinical pathology laboratory.

4.2.2.4 Data analysis.

Performance measures on all neuropsychological tests were scored according to standard protocols. In healthy older adults, age was significantly different between low and high Aβ amyloid groups, and in adults with MCI, age and premorbid IQ was significantly different between low and high Aβ amyloid groups (see Table 4.2). The proportion of APOE ε4 carriers was significantly higher in the high Aβ amyloid groups, in healthy older adults and adults with MCI. Further, the proportion of adults with MCI and high Aβ amyloid with a history of hypertension was also higher than adults with MCI and low Aβ amyloid. Analyses of covariance (ANCOVA), with age and premorbid IQ as covariates, and with classification (HA, MCI), Aβ amyloid levels (high, low), and the classification x Aβ amyloid level interaction entered as fixed factors, were therefore
performed to test for differences in performance on each neuropsychological test between HA and MCI groups. We then performed two sets of planned comparisons. The first set tested the statistical significance of differences between the high and low Aβ amyloid subgroups within the MCI and HA groups separately. The second set tested the statistical significance of the differences from the HA low Aβ amyloid group to each of the other three subgroups. Cohen’s $d$ effect sizes were computed to express the magnitude of difference between groups.

The criterion for statistical significance for all comparisons or correlations was set to at least $p < .01$. This was done to balance the risk of false positive findings against identification of important relationships when a) performance on the neuropsychological outcome measures was likely to be highly correlated, especially for tests that assess the same cognitive domain, b) this is an exploratory investigation in a relatively new area of neuropsychology in which an important clinical issue has been identified, and c) measures of effect size were used to guide interpretation about the meaningfulness of results. Specifically, Type I errors were suspected where comparisons were statistically significant at the corrected level but where effect sizes were very small (e.g., $d < 0.20$).

4.3 Results

4.3.1 Demographic and cognitive differences between high and low Aβ amyloid subgroups in MCI and HA

Demographic and clinical characteristics of the MCI and HA groups are shown in Table 4.2. In the MCI group, adults with high Aβ amyloid were older, and had higher levels of
premorbid intelligence than adults with low Aβ amyloid (see Table 4.2). There were also significantly more APOE ε4 carriers in the high Aβ amyloid group than in the low Aβ amyloid group, and more adults with a history of hypertension in the MCI high Aβ amyloid group than in the low Aβ amyloid group. In healthy older adults, only the difference in age between high and low Aβ amyloid groups was statistically significant. High and low Aβ amyloid groups did not differ on any other demographic or clinical characteristic in the MCI or HA groups. Groups also did not differ on their family history of AD. Cognitive differences between the MCI and HA groups are shown in Table 4.3. Moderate to large differences on all neuropsychological measures were observed between the MCI and HA groups. Planned comparisons were then conducted to compare high and low Aβ amyloid subgroups within the MCI and HA groups.

### 4.3.2 Comparison of MCI high and low Aβ amyloid subgroups

Group means for the MCI high and low Aβ amyloid subgroups, results of statistical significance tests of the planned comparisons, and magnitudes of differences between groups are shown in the right column on Table 4.4. The MCI low Aβ amyloid subgroup performed significantly worse than the MCI high Aβ amyloid subgroup on measures of executive function, and language, and that the magnitudes of these differences were moderate to large (see Table 3b).

### 4.3.3 Comparison of HA high and low Aβ amyloid subgroups

The group means for the HA high and low Aβ amyloid subgroups, the results of statistical significance tests of the planned comparisons, and magnitudes of differences between groups are shown on the left column on Table 4.4. There were no statistically significant differences in performance between the HA high and low Aβ amyloid...
subgroups. However, small but systematic differences on some measures of episodic memory (e.g. logical memory 1, CVLT-II recognition, CogState Memory Composite, $d = 0.24$–$0.26$) and executive function (Category Switching Total and Accuracy, $d = 0.24$) were evident for the HA high Aβ amyloid subgroup.

4.3.4 Characterisation of cognitive impairment in HA high Aβ amyloid, MCI low Aβ amyloid, and MCI high Aβ amyloid subgroups relative to HA low Aβ amyloid subgroup

Magnitudes of differences in performance between the HA low Aβ amyloid subgroup and each of the three subgroups, as well as statistical comparisons of these differences, are shown in Figure 4.1. Compared to the HA low Aβ amyloid subgroup, performances on all measures of episodic memory were significantly worse in both the MCI high and low Aβ amyloid subgroups. The magnitude of impairment in episodic memory was, by convention, very large ($d > 1$). On most measures of attention and language, only the MCI low Aβ amyloid subgroup performed significantly worse than the HA low Aβ amyloid subgroup, with these impairments moderate to large in magnitude ($d = 0.45$–$1.29$).
Table 4.2. Demographic means (SD) for MMSE, CDR-SB, premorbid IQ and HADS scores, and median years of education, for overall and each Aβ amyloid group at baseline assessment

<table>
<thead>
<tr>
<th></th>
<th>Healthy Overall (n=178)</th>
<th>Healthy Low Aβ (n=123)</th>
<th>Healthy High Aβ (n=55)</th>
<th>MCI Overall (n=56)</th>
<th>MCI Low Aβ (n=19)</th>
<th>MCI High Aβ (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>89 (50%)</td>
<td>61 (50%)</td>
<td>28 (51%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE ε4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4</td>
<td>76 (43%)</td>
<td>40 (33%)</td>
<td>36 (66%)**</td>
<td></td>
<td>2 (11%)</td>
<td>28 (76%)**</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Aβ</td>
<td>71.55 (7.45)</td>
<td>69.92 (6.99)</td>
<td>75.20 (7.19)**</td>
<td>75.51 (7.48)</td>
<td>73.44 (9.29)</td>
<td>76.51 (6.32)**</td>
</tr>
<tr>
<td>High Aβ</td>
<td>1.41 (0.40)</td>
<td>1.16 (0.09)</td>
<td>1.95 (0.26)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUVR Neocortex</td>
<td>28.74 (1.23)</td>
<td>28.81 (1.23)</td>
<td>28.58 (1.23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDR-SB</td>
<td>0.04 (0.19)</td>
<td>0.05 (0.21)</td>
<td>0.03 (0.11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>111.27 (6.67)</td>
<td>111.25 (7.10)</td>
<td>112.78 (5.52)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HADS-Depression</td>
<td>2.80 (2.31)</td>
<td>2.79 (2.17)</td>
<td>2.82 (2.60)</td>
<td>3.51 (2.28)</td>
<td>3.50 (1.98)</td>
<td>3.51 (2.45)</td>
</tr>
<tr>
<td>HADS-Anxiety</td>
<td>4.14 (2.84)</td>
<td>4.06 (2.64)</td>
<td>4.33 (3.26)</td>
<td>4.71 (2.59)</td>
<td>5.00 (2.50)</td>
<td>4.57 (2.65)</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal circumference</td>
<td>92.53 (12.29)</td>
<td>92.70 (12.12)</td>
<td>92.17 (12.78)</td>
<td>92.80 (9.91)</td>
<td>95.33 (9.85)</td>
<td>91.92 (9.98)</td>
</tr>
<tr>
<td>N (%) family history of AD</td>
<td>92 (52%)</td>
<td>64 (52%)</td>
<td>28 (51%)</td>
<td>25 (45%)</td>
<td>9 (47%)</td>
<td>16 (43%)</td>
</tr>
<tr>
<td>N (%) past smokers</td>
<td>90 (51%)</td>
<td>59 (48%)</td>
<td>31 (56%)</td>
<td>24 (43%)</td>
<td>6 (32%)</td>
<td>18 (48%)</td>
</tr>
<tr>
<td>N (%) current smokers</td>
<td>6 (3%)</td>
<td>6 (5%)</td>
<td>0 (0.0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>N (%) hypertension history</td>
<td>76 (43%)</td>
<td>51 (41%)</td>
<td>25 (45%)</td>
<td>26 (46%)</td>
<td>5 (26%)</td>
<td>21 (57%)*</td>
</tr>
<tr>
<td>N (%) angina history</td>
<td>14 (8%)</td>
<td>12 (10%)</td>
<td>2 (4%)</td>
<td>3 (5%)</td>
<td>1 (5%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>N (%) diabetes history</td>
<td>13 (7%)</td>
<td>8 (7%)</td>
<td>5 (9%)</td>
<td>3 (5%)</td>
<td>1 (5%)</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>

Note: SUVR = Standardised Uptake Value Ratio; MMSE = Mini-Mental State Examination; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes Score; HADS-Depression = Hospital Anxiety and Depression Scale, Depression Subscale, HADS-Anxiety = Hospital Anxiety and Depression Scale, Anxiety Subscale

* indicates p < .01, ** indicates p < .001
Table 4.3. *Comparison of performance on neuropsychological measures between HA and MCI groups*

<table>
<thead>
<tr>
<th></th>
<th>HA</th>
<th>MCI</th>
<th>Cohen's d</th>
<th>Group SUVR</th>
<th>ANCOVA Group SUVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logical Memory 1</td>
<td>12.58 (4.11)</td>
<td>8.52 (4.17)</td>
<td>0.99**</td>
<td>(1,209) 34.32**</td>
<td>(1,209) 2.54</td>
</tr>
<tr>
<td>Logical Memory 2</td>
<td>11.08 (4.34)</td>
<td>5.40 (4.39)</td>
<td>1.31**</td>
<td>(1,207) 61.10**</td>
<td>(1,207) 0.78</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>50.87 (10.54)</td>
<td>33.47 (10.69)</td>
<td>1.65**</td>
<td>(1,211) 97.21**</td>
<td>(1,211) 2.61</td>
</tr>
<tr>
<td>CVLT-II short delay</td>
<td>11.09 (2.99)</td>
<td>4.88 (3.02)</td>
<td>2.07**</td>
<td>(1,210) 153.91**</td>
<td>(1,210) 0.49</td>
</tr>
<tr>
<td>CVLT-II long delay</td>
<td>11.63 (3.18)</td>
<td>4.72 (3.22)</td>
<td>2.17**</td>
<td>(1,211) 168.25**</td>
<td>(1,211) 0.33</td>
</tr>
<tr>
<td>CVLT-II recognition</td>
<td>14.78 (1.56)</td>
<td>12.82 (2.65)</td>
<td>1.06**</td>
<td>(1,211) 20.42**</td>
<td>(1,211) 0.25</td>
</tr>
<tr>
<td>CVLT-II d'</td>
<td>3.07 (0.74)</td>
<td>1.75 (0.77)</td>
<td>1.77**</td>
<td>(1,211) 75.38**</td>
<td>(1,211) 3.58</td>
</tr>
<tr>
<td>RCFT short recall</td>
<td>16.29 (5.92)</td>
<td>10.77 (6.00)</td>
<td>0.93**</td>
<td>(1,211) 31.04**</td>
<td>(1,211) 1.58</td>
</tr>
<tr>
<td>RCFT long recall</td>
<td>16.31 (5.90)</td>
<td>10.43 (5.96)</td>
<td>0.99**</td>
<td>(1,210) 35.67**</td>
<td>(1,210) 0.58</td>
</tr>
<tr>
<td>RCFT recognition</td>
<td>20.32 (2.29)</td>
<td>18.85 (2.34)</td>
<td>0.64**</td>
<td>(1,209) 14.02**</td>
<td>(1,209) 1.59</td>
</tr>
<tr>
<td>CogState Memory Composite</td>
<td>1.16 (0.13)</td>
<td>1.03 (0.15)</td>
<td>0.98**</td>
<td>(1,158) 15.31**</td>
<td>(1,158) 0.06</td>
</tr>
<tr>
<td>Stroop C/D</td>
<td>2.38 (0.61)</td>
<td>2.78 (0.97)</td>
<td>-0.56*</td>
<td>(1,202) 3.50</td>
<td>(1,202) 1.80</td>
</tr>
<tr>
<td>Letter Fluency</td>
<td>41.30 (12.26)</td>
<td>33.70 (12.43)</td>
<td>0.62**</td>
<td>(1,211) 13.75**</td>
<td>(1,211) 11.41**</td>
</tr>
<tr>
<td>Category Switching Total</td>
<td>13.27 (2.99)</td>
<td>10.34 (3.05)</td>
<td>0.98**</td>
<td>(1,207) 32.74**</td>
<td>(1,207) 0.01</td>
</tr>
<tr>
<td>Category Switching Accuracy</td>
<td>12.30 (3.41)</td>
<td>8.87 (3.46)</td>
<td>1.00**</td>
<td>(1,207) 34.79**</td>
<td>(1,207) 0.01</td>
</tr>
<tr>
<td>RCFT copy</td>
<td>31.34 (4.25)</td>
<td>28.46 (4.31)</td>
<td>0.68**</td>
<td>(1,211) 16.40**</td>
<td>(1,211) 0.07</td>
</tr>
<tr>
<td>Clock Drawing</td>
<td>9.87 (0.52)</td>
<td>9.69 (0.53)</td>
<td>0.34*</td>
<td>(1,207) 4.18*</td>
<td>(1,207) 1.24</td>
</tr>
<tr>
<td>Digit Span</td>
<td>17.89 (3.76)</td>
<td>16.41 (3.80)</td>
<td>0.39*</td>
<td>(1,211) 5.55*</td>
<td>(1,211) 1.78</td>
</tr>
<tr>
<td>Digit Symbol</td>
<td>56.94 (13.99)</td>
<td>51.10 (14.19)</td>
<td>0.42*</td>
<td>(1,211) 6.21*</td>
<td>(1,211) 2.41</td>
</tr>
<tr>
<td>CogState Speed Composite</td>
<td>2.62 (0.09)</td>
<td>2.67 (0.11)</td>
<td>-0.54*</td>
<td>(1,161) 4.38*</td>
<td>(1,161) 1.34</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>38.95 (8.57)</td>
<td>31.76 (8.80)</td>
<td>0.83**</td>
<td>(1,209) 24.23**</td>
<td>(1,209) 7.47**</td>
</tr>
<tr>
<td>BNT - no cues</td>
<td>28.20 (2.63)</td>
<td>25.70 (2.71)</td>
<td>0.94**</td>
<td>(1,187) 29.76**</td>
<td>(1,187) 8.71**</td>
</tr>
</tbody>
</table>

Note: * indicates $p < .01$, ** indicates $p < .001$
Table 4.4. Mean (SD) of HA high and low Aβ amyloid subgroups, and MCI high and low Aβ amyloid subgroups, and Cohen’s $d$ of difference between groups

<table>
<thead>
<tr>
<th></th>
<th>HA low Aβ Mean (SD)</th>
<th>HA high Aβ Mean (SD)</th>
<th>Cohen’s $d$</th>
<th>MCI low Aβ Mean (SD)</th>
<th>MCI high Aβ Mean (SD)</th>
<th>Cohen’s $d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logical Memory 1</td>
<td>13.09 (3.99)</td>
<td>12.07 (3.99)</td>
<td>0.26</td>
<td>9.12 (4.26)</td>
<td>7.97 (4.20)</td>
<td>0.27</td>
</tr>
<tr>
<td>Logical Memory 2</td>
<td>11.38 (4.23)</td>
<td>10.78 (4.26)</td>
<td>0.14</td>
<td>5.74 (4.49)</td>
<td>5.07 (4.42)</td>
<td>0.15</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>50.80 (10.19)</td>
<td>50.93 (10.17)</td>
<td>-0.01</td>
<td>33.82 (10.94)</td>
<td>33.11 (10.76)</td>
<td>0.07</td>
</tr>
<tr>
<td>CVLT-II short delay</td>
<td>10.99 (2.87)</td>
<td>11.18 (2.89)</td>
<td>-0.07</td>
<td>5.32 (3.09)</td>
<td>4.44 (3.05)</td>
<td>0.29</td>
</tr>
<tr>
<td>CVLT-II long delay</td>
<td>11.58 (3.07)</td>
<td>11.68 (3.07)</td>
<td>-0.03</td>
<td>5.07 (3.30)</td>
<td>4.37 (3.25)</td>
<td>0.21</td>
</tr>
<tr>
<td>CVLT-II recognition</td>
<td>14.65 (1.79)</td>
<td>15.07 (1.78)</td>
<td>-0.24</td>
<td>13.83 (1.92)</td>
<td>13.10 (1.89)</td>
<td>0.38</td>
</tr>
<tr>
<td>CVLT-II $d'$</td>
<td>3.10 (0.75)</td>
<td>3.02 (0.75)</td>
<td>0.11</td>
<td>2.13 (0.81)</td>
<td>1.72 (0.80)</td>
<td>0.51</td>
</tr>
<tr>
<td>RCFT short recall</td>
<td>16.79 (5.72)</td>
<td>15.78 (5.71)</td>
<td>0.18</td>
<td>11.49 (6.14)</td>
<td>10.04 (6.04)</td>
<td>0.24</td>
</tr>
<tr>
<td>RCFT long recall</td>
<td>16.54 (5.69)</td>
<td>16.09 (5.73)</td>
<td>0.08</td>
<td>10.94 (6.11)</td>
<td>9.91 (6.01)</td>
<td>0.17</td>
</tr>
<tr>
<td>RCFT recognition</td>
<td>20.44 (2.22)</td>
<td>20.20 (2.21)</td>
<td>0.11</td>
<td>19.22 (2.46)</td>
<td>18.49 (2.38)</td>
<td>0.30</td>
</tr>
<tr>
<td>CogState Memory Composite</td>
<td>1.18 (0.13)</td>
<td>1.15 (0.12)</td>
<td>0.24</td>
<td>1.03 (0.24)</td>
<td>1.05 (0.06)</td>
<td>-0.09</td>
</tr>
<tr>
<td>Stroop C/D</td>
<td>2.35 (0.75)</td>
<td>2.21 (0.76)</td>
<td>0.19</td>
<td>2.65 (0.89)</td>
<td>2.43 (0.79)</td>
<td>0.27</td>
</tr>
<tr>
<td>Letter Fluency</td>
<td>38.98 (11.86)</td>
<td>43.63 (11.82)</td>
<td>-0.39</td>
<td>29.17 (12.72)</td>
<td>38.22 (12.51)</td>
<td>-0.72**</td>
</tr>
<tr>
<td>Category Switching Total</td>
<td>13.61 (2.89)</td>
<td>12.92 (2.91)</td>
<td>0.24</td>
<td>9.94 (3.21)</td>
<td>10.74 (3.10)</td>
<td>-0.26</td>
</tr>
<tr>
<td>Category Switching Accuracy</td>
<td>12.71 (3.29)</td>
<td>11.90 (3.31)</td>
<td>0.25</td>
<td>8.42 (3.65)</td>
<td>9.33 (3.52)</td>
<td>-0.26</td>
</tr>
<tr>
<td>RCFT copy</td>
<td>31.31 (4.11)</td>
<td>31.37 (4.10)</td>
<td>-0.01</td>
<td>28.67 (4.41)</td>
<td>28.24 (4.34)</td>
<td>0.10</td>
</tr>
<tr>
<td>Clock Drawing</td>
<td>9.87 (0.50)</td>
<td>9.87 (0.50)</td>
<td>0.00</td>
<td>9.59 (0.55)</td>
<td>9.78 (0.52)</td>
<td>-0.36</td>
</tr>
<tr>
<td>Digit Span</td>
<td>17.56 (3.63)</td>
<td>18.21 (3.62)</td>
<td>-0.18</td>
<td>15.90 (3.89)</td>
<td>16.91 (3.83)</td>
<td>-0.26</td>
</tr>
<tr>
<td>Digit Symbol</td>
<td>56.93 (13.53)</td>
<td>56.95 (13.50)</td>
<td>0.00</td>
<td>47.52 (14.52)</td>
<td>54.69 (14.29)</td>
<td>-0.50</td>
</tr>
<tr>
<td>CogState Speed Composite</td>
<td>2.62 (0.09)</td>
<td>2.62 (0.09)</td>
<td>0.00</td>
<td>2.69 (0.17)</td>
<td>2.64 (0.04)</td>
<td>0.32</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>38.30 (8.33)</td>
<td>39.60 (8.27)</td>
<td>-0.16</td>
<td>28.46 (9.21)</td>
<td>35.05 (8.76)</td>
<td>-0.74**</td>
</tr>
<tr>
<td>BNT - no cues</td>
<td>28.06 (2.76)</td>
<td>28.33 (2.70)</td>
<td>-0.10</td>
<td>24.50 (2.83)</td>
<td>26.89 (2.71)</td>
<td>-0.87**</td>
</tr>
</tbody>
</table>

Note: * indicates $p < .01$; ** indicates $p < .001$
Figure 4.1. Magnitude of difference (Cohen’s $d$) between performance of HA high Aβ group (black circles, solid line), MCI low Aβ group (grey triangles, dotted line), and MCI high Aβ group (grey circles, solid line) relative to HA low Aβ group (represented by "0" line).

* indicates that relative to the HA low Aβ group, differences are significant at the $p < .01$ level, and ** indicates that differences are significant at the $p < .001$ level, with negative values indicating impairment.
4.4 Discussion

The first hypothesis that, compared to the MCI group with high $A\beta$ amyloid, the MCI group with low $A\beta$ amyloid would show impairments in non-memory aspects of cognition such as attention, executive function and language was supported. Compared to the MCI group with high $A\beta$ amyloid, the MCI group with low $A\beta$ amyloid showed impairment in executive function, and language; although there were no significant differences between MCI groups in episodic memory. The impairments in executive function, and language in the MCI group with low $A\beta$ amyloid were relatively large (e.g. $d \sim 1$) and consistent with the results of a previous study of MCI with high and low $A\beta$ amyloid which also observed that individuals who met clinical criteria for MCI but who have low $A\beta$ amyloid showed impairment in executive function, attention and language in addition to memory impairment.\(^{185}\)

The second hypothesis, that episodic memory would be impaired in healthy older adults with high $A\beta$ amyloid relative to healthy older adults with low $A\beta$ amyloid was not supported. No statistically significant impairment in episodic memory, or any other aspect of cognitive function, was observed in healthy older adults with high $A\beta$ amyloid. However, inspection of the differences in test performance between the two groups of healthy older adults indicated that performance on tests of episodic memory (logical memory $1 d = 0.26$, CVLT-II recognition $d = 0.24$, RCFT short recall $d = 0.18$, and the CogState Memory Composite $d = 0.24$) was slightly worse in healthy older adults with high $A\beta$ amyloid with the magnitudes of impairment sufficiently large and consistent across memory tests to suggest that the presence of some deficit in episodic memory, albeit subtle in nature. Thus, while the data from this study should be interpreted to indicate that in healthy older adults, high $A\beta$ amyloid was not associated with
impairment when cognitive function is assessed on a single occasion, they suggest strongly that further investigations using larger samples (i.e., sufficient to render differences of $d = 0.20-0.30$ statistically significant) or more sensitive measures of cognitive function should be conducted to challenge this interpretation.

The third hypothesis that when compared to healthy older adults with low Aβ amyloid, the nature and magnitude of cognitive impairment in MCI with high and low Aβ amyloid would be qualitatively and quantitatively different was supported. The qualitative differences in cognitive function between the MCI groups were illustrated more clearly in the comparison of performance in both MCI groups to healthy older adults with low Aβ amyloid. First, the magnitude of impairment in episodic memory was very large but comparable in both MCI groups. Second, while both MCI high and low Aβ amyloid groups showed impairments in language (Category Fluency, BNT), executive function (Letter Fluency, Category Switching Total, Category Switching Accuracy), and visuoconstruction (RCFT Copy, Clock) the magnitude of impairments on these tests was statistically larger for the MCI group with low Aβ amyloid levels. Finally, only the MCI group with low Aβ amyloid showed impairment in attention and psychomotor function (i.e. CogState Speed Composite, Digit Symbol, Stroop Dots). For the healthy older adults, the data showed, as discussed previously, evidence of a subtle but systematic performance deficit across the tests of episodic memory. It is important to emphasise that in none of the individuals with MCI and low Aβ amyloid was performance on the Digit Symbol or Letter Fluency task abnormal when compared to published normative data. If performance on these tests had been 1.5 standard deviations below normative ranges, in addition to memory impairment, then individuals would not have met criteria for amnestic MCI. The subtle nature of the impairment on these non-memory tasks is
also evident in that the magnitude of difference in performance between healthy older adults with low Aβ amyloid and MCI with low Aβ amyloid was less than one.

All individuals recruited to the MCI cohort of the AIBL study were referred by their treating specialist on the basis that they met internationally recognised clinical criteria for MCI. As the AIBL study was designed to maximise the detection of the earliest stages of AD, rigorous exclusion criteria were applied to the MCI cohort, in order to maximise the possibility that individuals recruited would have early AD pathology. Furthermore, additional classification of cognitive impairment in the AIBL MCI group that had undergone PiB-PET neuroimaging indicated that all satisfied criteria for aMCI. This aspect of the sample was reflected in the data from Figure 4.1 that shows both MCI subgroups to have substantial impairment in episodic memory (i.e. *d*s >1).

However, the large impairments of the MCI low Aβ amyloid group on measures of non-memory function relative to the MCI high Aβ amyloid subgroup suggest that the memory impairment observed in the MCI group with low Aβ amyloid may be due, at least in part, to the additional impairment in attentional, executive, language and visuoconstruction abilities. The qualitative differences in the nature of cognitive impairment in individuals with MCI characterised by high and low levels of Aβ amyloid is consistent with the findings of a recent study where individuals with MCI and pathological levels of CSF Aβ42 showed greater impairments on a verbal list learning test whose method of administration is hypothesised to control for deficits in attention and language, when compared to more conventional verbal list learning tests. Taken together, these findings support the hypothesis that MCI with high levels of Aβ amyloid is most indicative of prodromal AD, although prospective studies of individuals
with MCI and high and low levels of Aβ amyloid are necessary to confirm this hypothesis.

While impairment in cognitive functions in addition to memory have been reported previously in MCI with low Aβ amyloid, the current study represents the most detailed neuropsychological assessment conducted in the largest group of individuals with MCI for whom Aβ amyloid levels had been quantified. In this context, it is important to consider the nature of the pathology that may underlie cognitive impairment in MCI with low Aβ amyloid. One possibility is that this reflects subtle cardiovascular changes such that would be consistent with a classification of vascular cognitive impairment. However, we believe that this is unlikely as individuals with a history of untreated cardiovascular disease were excluded from entry to the AIBL study. Further, when the MCI low and high Aβ amyloid groups were compared on the frequency of well-established cardiovascular risk factors, the only difference present between the high and low Aβ amyloid groups was that a history of hypertension was more frequent in MCI with high Aβ amyloid group. Importantly though, although some individuals in AIBL do have cardiovascular risk factors, a condition of entry into AIBL was that all systemic illness (such as hypertension or hyperlipidaemia) were controlled medically. Finally a recent study of cardiovascular changes in the AIBL neuroimaging cohort reported that the frequency of white matter changes and lacunar infarcts were very low in the MCI and healthy older adults groups. A second possible explanation for the cognitive impairment in MCI with low Aβ amyloid is that this reflected some premorbid factor such as reduced cognitive reserve or subtle mood disorder. Previous studies have shown that individuals with low cognitive reserve are at higher risk of developing AD, and are less able to tolerate the toxic effects of Aβ amyloid.
However, although estimates of premorbid IQ were significantly lower in the MCI low Aβ amyloid group than in the MCI high Aβ amyloid group, the premorbid IQ of the MCI low Aβ amyloid group remained above average. Furthermore, premorbid IQ estimates were controlled statistically, and measures of effect size were adjusted accordingly in all comparisons of neuropsychological test performance between low and high Aβ amyloid groups. Levels of depressive and anxiety symptomatology were also unlikely to have influenced performance on neuropsychological tests in the MCI low Aβ amyloid group as individuals with clinically diagnosed depression and anxiety disorders were excluded from the AIBL study, and levels of depression and anxiety were equivalent between Aβ amyloid groups. A third possible explanation for the cognitive impairment in MCI with low Aβ amyloid in the absence of other obvious factors is that this does reflect early AD-related changes although in these individuals, the level of Aβ amyloid has yet to accumulate to levels that would be considered abnormally high (i.e., SUVR > 1.5). Cross-sectional studies indicate that correlations between Aβ amyloid levels and cognitive function in MCI are only moderate in magnitude, thus raising the possibility that in some MCI cases, cognitive impairment precedes high levels of Aβ amyloid. Through prospective study of cognition, this hypothesis would be tested by showing that cognition declines over time in the MCI low Aβ amyloid group. If, however, cognitive impairment in the MCI low Aβ amyloid group reflects subtle cardiovascular risk, then impairments in some cognitive domains may progress over time, albeit in a manner that is different to that observed in MCI with high Aβ amyloid. Finally, if the cognitive impairment in MCI with low Aβ amyloid is due to a stable insult or low premorbid IQ, then we would not expect cognitive function to decline over time.
When considered together, the results of this chapter raise an important issue regarding the classification of adults with MCI into those with an underlying AD pathology and those for whom the pathology was non-AD in nature. Previously, clinical sub-classifications such as amnestic and non-amnestic MCI have been used to make this distinction. However, as elevated levels of Aβ amyloid in adults classified with non-amnestic MCI have been observed previously, and others have observed that individuals with non-amnestic MCI progress to AD,9 there is growing evidence that the presence of cognitive impairment, especially in episodic memory, in addition to evidence of pathological levels of Aβ amyloid from some neuroimaging or CSF biomarker may provide the greatest confidence that individuals who meet clinical criteria for MCI will have early AD.\textsuperscript{40,194} The results from large prospective studies of non-demented adults with high levels of Aβ amyloid, such as the AIBL and the Alzheimer's Disease Neuroimaging Initiative (ADNI)\textsuperscript{195} studies, are necessary to confirm this hypothesis.

Even if biomarker confirmation is required for the identification of early AD, the cost and invasiveness of obtaining these samples may require a high level of clinical evidence for possible AD before such tests are ordered. The current results are consistent with data from a long history of neuropsychological studies which indicate that impairment in episodic memory is a key clinical marker of early AD.\textsuperscript{5,196,197} The current finding that MCI with high Aβ amyloid presents with impairment restricted to episodic memory accords with a suggestion by Dubois and colleagues, that the cognitive profile of MCI that reflects prodromal AD is best described as an amnestic syndrome of the hippocampal type.\textsuperscript{78} This syndrome has been proposed to be characterised primarily by poor free recall on neuropsychological tests despite adequate encoding,
numerous intrusions, and decreased total recall due to impaired recognition. Dubois and colleagues have identified this type of memory profile in patients with mild AD (i.e., MMSE score greater than 25), and from prospective studies of older adults who progressed to AD over the subsequent 5 years. The hypothesis that specific impairment in memory best identifies early AD is also consistent with structural neuroimaging and neuropathology studies which show the mesial temporal lobe and the hippocampus to be the focus of early AD pathology. Dubois and colleagues have argued strongly that only specific verbal list learning tests that control for registration and recall (i.e., Buschke free and cued recall test) are necessary to identify amnesia of the hippocampal type MCI due to AD. However, as the results from the current chapter show that MCI with high and low Aβ amyloid levels showed memory impairment to an equivalent magnitude and that the groups differed most in their additional cognitive impairments, it may be more prudent to identify purely amnestic MCI (and therefore, MCI due to AD) by showing that patients with impairment in episodic memory do not also have impairment in attention, executive function, language, and visuoconstruction through the direct assessment of these functions.

In healthy older adults, we found no differences in any cognitive performance between healthy older adults with high and low Aβ amyloid levels. This is consistent with previous studies that have also observed no differences in cognitive performance between high and low Aβ amyloid groups in healthy older adults, although the size of the samples studied have typically been small (i.e. total n ranging from 9 to 32). However, even in this sample, which is the largest sample to date of healthy older adults who had undergone PiB-PET neuroimaging for Aβ amyloid, the impairments in cognitive performance were still too subtle to reach statistical significance. Power
analysis suggests that with a larger sample (e.g., total n = 786 healthy older adults, assuming 2 groups, $d = 0.20, p < .05$ two-tailed and power = 0.80), the poorer performance observed here may have reached statistical significance. However, even if subtle differences between high and low $A\beta$ amyloid groups in healthy older adults were statistically significant, they would remain subtle and therefore unlikely to be evident to individuals, their close relatives, or even their treating clinician. Hence it may be more prudent to base the identification of very early AD by pairing evidence of high $A\beta$ amyloid with objective evidence that memory function has declined over time.$^{9,74,90}$

There were some important limitations to the current study. As described above, the AIBL study is not an epidemiological sample, and the MCI group selected was biased towards the inclusion of individuals with aMCI. As such, it would be important for these findings to be replicated in individuals with MCI and high $A\beta$ amyloid in population-based study samples, such as the Mayo Clinic Study of Ageing.$^{203}$ and the 10/66 Dementia Research Group.$^{204}$ Second, we have only compared cognitive performance of healthy older adults and adults with MCI with high and low $A\beta$ amyloid cross-sectionally. As AD is a neurodegenerative disease, it will be important for future studies to determine whether levels of $A\beta$ amyloid are associated with different rates of cognitive change. Despite this, the ability for neuropsychological assessments to detect subtle differences in cognition on the basis of a single assessment remains an important area for investigation. Third, as detailed in the data analysis section, the correction for multiple comparisons was set to $p < .01$, and this may have inflated the risk of Type I error rates. However, a more conservative approach to the management of Type I errors (e.g. the Bonferroni correction) would be unnecessary as we sought to protect
against these errors by reporting also magnitudes of effect sizes, and limiting our interpreting of any differences to those that are statistically significant, and also moderate to large in magnitude (i.e., Cohen’s d > 0.20). Further, as this is one of the first investigations of the relationship between neuropsychological test performance and Aβ amyloid, we also believe that all data should be presented. With these aspects operating, we have high confidence that the differences between groups interpreted in the current study are true because in addition to statistical significance, the effect sizes for these differences are moderate in magnitude and the nature of the impairments detected were consistent across tests of similar cognitive functions.

While some have argued for the use of composite scores to improve the parsimony from which conclusions about cognitive function can be deduced,\textsuperscript{127} we have chosen to report scores on individual tests because the combination of performance across different tests may result in a loss of important information, especially given that the current study is one of the first to examine the relationship between neuropsychological test performance and Aβ amyloid in healthy older adults and MCI. Future studies may choose to use cognitive composite measures, or limit their test batteries to those shown here to be most sensitive to the subtle cognitive impairment in MCI. In this context, this study provides a firm foundation for the design of such future studies.

Finally, it is unclear from the results of our study, whether neuropsychological normative data should only include healthy older adults who have low Aβ amyloid. The small effect sizes between healthy older adults with high and low Aβ amyloid observed in the current study suggest no differences between groups on any neuropsychological measure. However, as the impairment in episodic memory observed in healthy older adults with high Aβ amyloid was qualitatively similar to that observed in the MCI group
with high Aβ amyloid, it is possible that these small effect sizes may reflect true differences in cognitive function related to high levels of Aβ amyloid. Therefore, future studies in larger samples, or meta-analyses will be necessary to determine whether individuals with high Aβ amyloid should be excluded from normative data for healthy older adults. Nonetheless, the effect sizes reported here provide a basis for the design of future studies, and for future meta-analyses of Aβ amyloid-related cognitive impairment in healthy older adults.
Part Three

Aim:

Determine the nature and magnitude of Aβ amyloid related cognitive decline prospectively in healthy older adults and adults with MCI.

Chapters:

Chapter Five  Stronger Effect of Aβ Amyloid than APOE Genotype on Cognitive Decline in Healthy Older Adults

Chapter Six  Cognitive Decline in Adults with Mild Cognitive Impairment and High Aβ Amyloid: Prodromal Alzheimer’s Disease?

Chapter Seven  Aβ Amyloid and Cognitive Change: Examining the Preclinical and Prodromal Stages of Alzheimer’s Disease

Chapter Eight  The Australian Imaging, Biomarker and Lifestyle - Rate of Change sub-study (AIBL-ROCS): Rationale, Design, Acceptability, and Pilot Data for the First Three Months of Assessment

Chapter Nine  Rapid Decline in Episodic Memory in Healthy Older Adults with High Aβ Amyloid

Chapter Ten  Modulation of Aβ Amyloid-Related Cognitive Decline by Brain-Derived Neurotrophic Factor Val66Met Polymorphism in Preclinical Alzheimer's Disease
5.1 **Introduction**

Neuroimaging studies observe abnormally high Aβ amyloid loads in a substantial proportion of healthy older adults.\(^{45, 58, 59, 167}\) However, associations between Aβ amyloid load and cognitive function are generally only small in magnitude, with the strongest relationships found for episodic memory (see Chapter Three).\(^{48, 85, 86, 205}\) Recent cross-sectional studies of healthy older adults reported moderate associations between Aβ amyloid burden and episodic memory when analyses were restricted to carriers of the apolipoprotein (\(APOE\) \(\varepsilon4\)) allele. No such relationships were observed in non-carriers,\(^{85, 205}\) suggesting that in healthy older adults, elevated Aβ amyloid load may be a more important prognostic factor in individuals genetically at-risk for AD (see Chapter Three). However, the implications of high Aβ amyloid load for cognitive function should be determined in prospective studies. Further, while it is well-known that healthy older adults who carry the \(APOE\ \varepsilon4\) allele show accelerated cognitive decline,\(^{206, 207}\) it is unclear whether this allele may also moderate the relation between Aβ amyloid load and cognitive decline.

Two prospective PiB-PET neuroimaging studies have reported no differences between healthy older adults with high and low Aβ amyloid on the rate of cognitive change over 24-months. However, in both studies, the samples were small (\(n \leq 30\)) and neither examined the effect of \(APOE\) genotype.\(^{48, 65}\) The aim of the current study was to examine associations between Aβ amyloid load, \(APOE\) genotype, and cognitive change over 18 months in a large group of healthy older adults. We hypothesised that increased Aβ...
amyloid load and APOE ε4 genotype would be associated with episodic memory decline over 18 months.

5.2 Methods

5.2.1 Participants.

Participants were recruited from the healthy older adults group enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing. The process of recruitment and diagnostic classification of healthy older adults enrolled in the AIBL cohort has been described in detail elsewhere. Healthy older adults who volunteered were excluded from the AIBL study if they had any of the following: schizophrenia; depression (15-item Geriatric Depression Score (GDS) of 6 or greater); Parkinson’s disease; cancer (other than basal cell skin carcinoma) within the last two years; symptomatic stroke; uncontrolled diabetes; or current regular alcohol use exceeding two standard drinks per day for women or four per day for men. A clinical review panel chaired by DA reviewed all available medical, psychiatric and neuropsychological information to confirm the cognitive health of individuals enrolled in the healthy older adults group. In this study, only the sub-group of healthy older adults who had undergone PiB neuroimaging and who had completed the cognitive battery at baseline and 18-month assessment (n = 141) were included. Demographic and clinical characteristics of the healthy older adults are shown in Table 5.1. The clinical status of all participants did not change at 18 months.

The study was approved by and complied with the regulations of three institutional research and ethics committees, and all participants provided written informed consent prior to participating in the study.
5.2.2 Measures.

5.2.2.1 PiB-PET neuroimaging and APOE ε4 genotyping.

PiB-PET imaging methodology has been outlined in detail previously.\textsuperscript{110,167} PET standardised uptake value (SUV) data acquired 40-70 minutes post-PiB injection were summed and normalised to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVR). An 80ml blood sample was also taken from each participant, 0.5ml of which was forwarded for APOE genotyping at a clinical pathology laboratory.

5.2.2.2 Cognitive assessments.

All participants were assessed with the clinical rating scales and neuropsychological battery from the AIBL study (Table 5.2). These have all described in detail elsewhere and were administered according to standard protocols by trained research assistants.\textsuperscript{86,126,172} The clinical status of participants was determined by data which included the Mini Mental Status Examination (MMSE),\textsuperscript{136} and Clinical Dementia Rating (CDR) Scale.\textsuperscript{137} Premorbid intelligence was estimated using the Wechsler Test of Adult Reading (WTAR),\textsuperscript{141} and levels of depressive and anxiety symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS).\textsuperscript{142} Verbal learning and verbal episodic memory was measured using the California Verbal Learning Test, Second Edition (CVLT-II),\textsuperscript{173} and visual episodic memory was measured using the visual paired associate learning task (CPAL).\textsuperscript{122} All individuals also performed a set of computerised cognitive tests which included measures of visual learning (One Card Learning; OCL), working memory (One Back; OBK), attention (Identification; IDN) and psychomotor
(Detection; DET) function from the CogState battery.\textsuperscript{121, 132, 172} Performance on the tests from the CogState battery was not used to classify individuals' clinical status.

5.2.3 Procedure.

All healthy older adults in this study underwent an extensive medical, psychiatric, and neuropsychological assessment upon enrolment into the AIBL study. The same assessments were repeated 18 months later. In this study, we report PiB neuroimaging and APOE ε4 genotyping data obtained at baseline, and neuropsychological data obtained at baseline and 18 months in order to examine the rate of cognitive change in relation to Aβ amyloid load and APOE ε4 status.

5.2.4 Data Analysis.

Each cognitive task provided a single performance score (Table 5.2). A Working memory-Visual Learning composite score (OCL-OBK) was generated by standardising the OCL and OBK scores and then averaging them. A Psychomotor/Attention composite score (DET-IDN) was generated by standardising the DET and IDN scores and then averaging them. Consistent with observations from other studies,\textsuperscript{48, 85} the distribution of PiB SUVR data was skewed negatively and could not be normalised with data transformations. Thus, Aβ amyloid levels were classified dichotomously as either low (SUVR < 1.50) or high (SUVR ≥ 1.50) in accordance with established criteria.\textsuperscript{42, 48}

There were no statistically significant differences between Aβ amyloid and APOE groups for any demographic characteristic (see Table 5.1). A series of linear mixed model (LMM) analyses of covariance (ANCOVA) was conducted to examine the relationship between Aβ amyloid status (low Aβ amyloid vs. high Aβ amyloid), APOE ε4 status (APOE ε4 carrier vs. APOE ε4 non-carrier), and cognitive change between baseline and 18-
month assessment. The LMM procedure was used because of its ability to model both fixed and random effects, which accounts for various sources of variability, and because it provides improved estimates of within-subject coefficients (i.e., random effects) in longitudinal studies.\textsuperscript{208,209} For each task, an LMM ANCOVA with Aβ amyloid status, APOE ε4 status, and the Aβ amyloid x APOE ε4 status interaction were entered as fixed factors; participant as a random factor, baseline cognitive test score as the only covariate, and cognitive test score at the 18-month assessment as a dependent variable. For each performance measure, the magnitude of the difference in adjusted means between low and high Aβ amyloid groups and between APOE ε4 carrier and non-carrier groups at the 18-month assessment was expressed using Cohen’s $d$.\textsuperscript{184} To assist with the interpretation of the LMM, group mean and standard deviation change scores for each Aβ amyloid and APOE group, as well as for high and low Aβ amyloid groups averaged over APOE ε4 status, and APOE ε4 carriers and non-carriers averaged over Aβ amyloid status, were calculated. Although estimates of premorbid intelligence were not different between the study groups (Table 5.1), analyses were recomputed with age and premorbid intelligence included as covariates to examine the extent to which cognitive reserve may impact the relationship between Aβ amyloid load, APOE genotype and change in cognition.\textsuperscript{36}
Table 5.1. Demographic means (SD) for MMSE, CDR-SB, premorbid IQ and HADS scores, and median years of education, for overall, each Aβ amyloid group and each APOE group at baseline assessment

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 141)</th>
<th>Low Aβ amyloid (n = 96)</th>
<th>High Aβ amyloid (n = 45)</th>
<th>ε4 non-carrier (n = 78)</th>
<th>ε4 carrier (n = 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage female</td>
<td>49.65 %</td>
<td>50 %</td>
<td>48.89 %</td>
<td>51.30 %</td>
<td>47.60 %</td>
</tr>
<tr>
<td>N (percentage APOE ε4)</td>
<td>63 (44.7 %)</td>
<td>33 (34.4 %)</td>
<td>30 (66.7 %)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>76.22 (6.58)</td>
<td>75.52 (6.65)</td>
<td>77.71 (6.22)</td>
<td>76.32 (6.70)</td>
<td>74.94 (7.37)</td>
</tr>
<tr>
<td>Education level (category)</td>
<td>13-15 years</td>
<td>13-15 years</td>
<td>13-15 years</td>
<td>13-15 years</td>
<td>13-15 years</td>
</tr>
<tr>
<td>SUVR Neocortex</td>
<td>1.42 (0.42)</td>
<td>1.16 (0.08)</td>
<td>1.98 (0.27)</td>
<td>1.29 (0.32)</td>
<td>1.58 (0.48)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.78 (1.21)</td>
<td>28.84 (1.20)</td>
<td>28.64 (1.25)</td>
<td>28.83 (1.25)</td>
<td>28.71 (1.17)</td>
</tr>
<tr>
<td>CDR-SB</td>
<td>0.03 (0.16)</td>
<td>0.04 (0.18)</td>
<td>0.02 (0.11)</td>
<td>0.03 (0.18)</td>
<td>0.03 (0.12)</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>109.11 (7.31)</td>
<td>108.35 (7.91)</td>
<td>110.73 (5.63)</td>
<td>110.09 (7.14)</td>
<td>108.48 (7.40)</td>
</tr>
<tr>
<td>HADS-Depression</td>
<td>2.86 (2.20)</td>
<td>2.79 (1.92)</td>
<td>3.02 (2.73)</td>
<td>2.71 (1.91)</td>
<td>3.05 (2.52)</td>
</tr>
<tr>
<td>HADS-Anxiety</td>
<td>4.26 (2.86)</td>
<td>4.22 (2.61)</td>
<td>4.33 (3.38)</td>
<td>4.04 (2.48)</td>
<td>4.53 (3.29)</td>
</tr>
</tbody>
</table>

Note: None of the variables shown in the table differed by Aβ amyloid or APOE status, all p’s > .05

SUVR= Standardised Uptake Value Ratio; MMSE = Mini-Mental State Examination; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes Score; HADS-Depression = Hospital Anxiety and Depression Scale, Depression Subscale; HADS-Anxiety = Hospital Anxiety and Depression Scale, Anxiety Subscale
Table 5.2. *Summary of tasks, associated outcome measures, and mean (SD) scores at baseline and 18 month assessment for healthy older adults.*

<table>
<thead>
<tr>
<th>Task</th>
<th>Measure</th>
<th>Overall (n = 141)</th>
<th>Low Aβ (n = 96)</th>
<th>High Aβ (n = 45)</th>
<th>ε4 non-carrier (n = 78)</th>
<th>ε4 carrier (n = 63)</th>
<th>Overall (n = 141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET</td>
<td>Speed (log₁₀ milliseconds)</td>
<td>2.52 (0.12)</td>
<td>2.52 (0.13)</td>
<td>2.52 (0.09)</td>
<td>2.53 (0.11)</td>
<td>2.52 (0.12)</td>
<td>2.53 (0.11)</td>
</tr>
<tr>
<td>IDN</td>
<td>Speed (log₁₀ milliseconds)</td>
<td>2.72 (0.07)</td>
<td>2.72 (0.07)</td>
<td>2.73 (0.07)</td>
<td>2.73 (0.07)</td>
<td>2.72 (0.08)</td>
<td>2.72 (0.06)</td>
</tr>
<tr>
<td>OCL</td>
<td>Accuracy (arcsine proportion correct)</td>
<td>0.96 (0.11)</td>
<td>0.97 (0.10)</td>
<td>0.95 (0.11)</td>
<td>0.96 (0.11)</td>
<td>0.96 (0.11)</td>
<td>0.98 (0.11)</td>
</tr>
<tr>
<td>OBK</td>
<td>Accuracy (arcsine proportion correct)</td>
<td>1.30 (0.18)</td>
<td>1.32 (0.18)</td>
<td>1.29 (0.18)</td>
<td>1.31 (0.19)</td>
<td>1.30 (0.17)</td>
<td>1.29 (0.18)</td>
</tr>
<tr>
<td>CPAL</td>
<td>Total errors</td>
<td>43.09 (27.16)</td>
<td>38.35 (25.51)</td>
<td>47.82 (30.63)</td>
<td>40.99 (21.96)</td>
<td>45.18 (32.27)</td>
<td>48.10 (25.97)</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>Total words recalled</td>
<td>59.13 (10.45)</td>
<td>60.96 (10.22)</td>
<td>57.31 (11.71)</td>
<td>59.59 (10.37)</td>
<td>58.68 (11.23)</td>
<td>54.07 (10.70)</td>
</tr>
<tr>
<td>CVLT-II Delayed</td>
<td>Total words recalled</td>
<td>11.23 (3.11)</td>
<td>11.73 (3.04)</td>
<td>10.73 (3.18)</td>
<td>11.38 (2.98)</td>
<td>11.07 (3.22)</td>
<td>10.76 (3.35)</td>
</tr>
<tr>
<td>OCL-OBK</td>
<td>Accuracy (arcsine proportion correct)</td>
<td>1.15 (0.13)</td>
<td>1.16 (0.11)</td>
<td>1.14 (0.12)</td>
<td>1.15 (0.12)</td>
<td>1.15 (0.11)</td>
<td>1.14 (0.12)</td>
</tr>
<tr>
<td>DET-IDN</td>
<td>Speed (milliseconds)</td>
<td>2.63 (0.08)</td>
<td>2.62 (0.09)</td>
<td>2.63 (0.07)</td>
<td>2.63 (0.08)</td>
<td>2.62 (0.09)</td>
<td>2.63 (0.08)</td>
</tr>
</tbody>
</table>

Note: SD=standard deviation; DET = Detection task; IDN = Identification task; OCL = One Card Learning task; OBK = One Back task; CPAL = Continuous Paired Associate Learning task; CVLT-II = California Verbal Learning Test, Second Edition; OCL-OBK = CogState Working Memory- Visual Learning Composite, DET-IDN = CogState Psychomotor-Attention Composite; 18 month means for each Aβ amyloid group are provided in Table 5.3.
5.3 Results

Of the total sample, there was 1 non-completion for the Detection and Paired Associate Learning tasks, 2 non-completions for the One Card Learning task, and 2 non-completions for the One Back task at the 18-month assessment. Complete baseline and 18-month data were available for all of the other tasks. LMM analyses indicated that, relative to healthy older adults with low Aβ amyloid levels at baseline, healthy older adults with high baseline Aβ amyloid levels showed significantly greater decline at the 18-month assessment on all measures of verbal and visual episodic memory as well as working memory (Table 5.3). No decline at the 18-month assessment was observed for measures of psychomotor or attentional function in either Aβ amyloid group. The magnitudes of the differences in baseline-adjusted performance between the low and high Aβ amyloid groups at the 18-month assessment are shown in Figure 5.1.

Inspection of group mean raw change scores also indicate that when averaged across APOE ε4 status, individuals in the high Aβ amyloid group showed greater decline on tasks of verbal and visual episodic memory, as well as working memory, at the 18-month assessment (Table 5.4).

When APOE ε4 status was added to the LMM analyses, no statistically significant interaction between Aβ amyloid status and APOE ε4 status was observed for any cognitive measure. However, when considered by itself, the presence of the APOE ε4 allele was associated with a greater decline in visual memory after 18 months. Magnitudes of differences in baseline-adjusted performance between APOE ε4 carriers and non-carriers at the 18-month assessment are shown in Figure 5.1 (light grey bars). The greater decline in performance on memory scores in the APOE ε4 carriers, averaged over Aβ amyloid groups is also shown on Table 5.4.
Re-analysis of the data with age and premorbid intelligence included as covariates did not change the pattern of results. No interactions between Aβ amyloid and APOE ε4 status were observed, all p's > 0.5. However, statistically significant main effects of Aβ amyloid were observed for the OCL, OBK, CPAL, CVLT-II total and delayed recall, and OCL-OBK composites, all p's < .05. Statistically significant main effects of APOE ε4 status were observed for the OCL task, p < .05.

Table 5.3. Results of linear mixed model analyses examining change in cognitive performance over 18 months as a function of Aβ amyloid and APOE ε4 status in healthy older adults.

<table>
<thead>
<tr>
<th></th>
<th>DET</th>
<th>Aβ amyloid F</th>
<th>APOE F</th>
<th>Aβ amyloid x APOE F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline F</td>
<td>27.73**</td>
<td>0.42</td>
<td>0.13</td>
<td>0.30</td>
</tr>
<tr>
<td>IDN</td>
<td>62.51**</td>
<td>0.47</td>
<td>0.94</td>
<td>0.80</td>
</tr>
<tr>
<td>OCL</td>
<td>9.43**</td>
<td>5.36*</td>
<td>5.35*</td>
<td>0.20</td>
</tr>
<tr>
<td>OBK</td>
<td>4.50*</td>
<td>6.68*</td>
<td>0.22</td>
<td>0.85</td>
</tr>
<tr>
<td>CPAL</td>
<td>35.18**</td>
<td>5.69*</td>
<td>0.07</td>
<td>2.93</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>56.91**</td>
<td>6.01*</td>
<td>3.52</td>
<td>0.33</td>
</tr>
<tr>
<td>CVLT-II Delayed</td>
<td>99.31**</td>
<td>9.23**</td>
<td>0.05</td>
<td>2.05</td>
</tr>
<tr>
<td>OCL-OBK</td>
<td>10.47**</td>
<td>3.54*</td>
<td>3.46</td>
<td>0.66</td>
</tr>
<tr>
<td>DET-IDN</td>
<td>49.09**</td>
<td>0.002</td>
<td>0.14</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Note: * indicates p < .05 and ** indicates p < .001

Baseline = effect of baseline cognitive score on performance at the 18 month assessment; Aβ amyloid x APOE = interaction between Aβ amyloid and APOE on performance at the 18 month assessment; degrees of freedom for OCL-OBK is (1,138), for OCL and OBK tasks are (1,139), for DET and CPAL tasks are (1,140), and for IDN, CVLT-II total, CVLT-II delayed, and DET-IDN are (1,141).

Table 5.4. Group mean (SD) raw change scores of cognitive performance over 18 months of healthy older adults in each Aβ amyloid and APOE group.

<table>
<thead>
<tr>
<th>Test</th>
<th>Low Aβ amyloid Mean Change (SD)</th>
<th>High Aβ amyloid Mean Change (SD)</th>
<th>ε4 averaged across Aβ amyloid Mean Change (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>0.02 (0.01)</td>
<td>-0.01 (0.01)</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>-0.02 (0.01)</td>
<td>0.02 (0.02)</td>
<td>0.00 (0.02)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>0.01 (0.03)</td>
<td>0.01 (0.01)</td>
<td></td>
</tr>
<tr>
<td>IDN speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>0.00 (0.01)</td>
<td>-0.03 (0.03)</td>
<td>-0.02 (0.03)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>-0.01 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.00 (0.01)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>0.00 (0.01)</td>
<td>0.02 (0.03)</td>
<td></td>
</tr>
<tr>
<td>OCL accuracy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>0.04 (0.03)</td>
<td>0.02 (0.02)</td>
<td>-0.01 (0.03)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>0.02 (0.01)</td>
<td>-0.01 (0.01)</td>
<td>-0.03 (0.01)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>0.03 (0.03)</td>
<td>0.01 (0.01)</td>
<td></td>
</tr>
<tr>
<td>OBK accuracy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>0.01 (0.01)</td>
<td>-0.03 (0.02)</td>
<td>-0.01 (0.02)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>-0.02 (0.02)</td>
<td>-0.05 (0.02)</td>
<td>-0.03 (0.01)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>-0.01 (0.02)</td>
<td>-0.04 (0.01)</td>
<td></td>
</tr>
<tr>
<td>CPAL no. of errors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>6.08 (4.30)</td>
<td>5.94 (3.64)</td>
<td>5.62 (4.82)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>5.81 (4.11)</td>
<td>8.47 (5.99)</td>
<td>7.14 (5.99)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>5.95 (4.21)</td>
<td>7.21 (3.88)</td>
<td></td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>-4.26 (3.01)</td>
<td>-6.23 (4.41)</td>
<td>-5.25 (4.05)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>-5.23 (3.70)</td>
<td>-5.22 (3.69)</td>
<td>-5.23 (3.69)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>-4.75 (3.36)</td>
<td>-5.73 (4.05)</td>
<td></td>
</tr>
<tr>
<td>CVLT-II Delayed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>0.01 (0.01)</td>
<td>-1.75 (1.23)</td>
<td>-0.87 (0.80)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>-1.41 (0.99)</td>
<td>0.51 (0.36)</td>
<td>-0.45 (0.36)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>-0.70 (0.50)</td>
<td>-0.62 (1.11)</td>
<td></td>
</tr>
<tr>
<td>OCL-OBK accuracy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>0.00 (0.00)</td>
<td>-0.03 (0.02)</td>
<td>-0.02 (0.02)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>-0.02 (0.01)</td>
<td>-0.05 (0.03)</td>
<td>-0.03 (0.02)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>-0.01 (0.01)</td>
<td>-0.04 (0.01)</td>
<td></td>
</tr>
<tr>
<td>DET-IDN speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 non-carrier</td>
<td>0.02 (0.01)</td>
<td>0.00 (0.00)</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>-0.01 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.01 (0.02)</td>
</tr>
<tr>
<td>Aβ amyloid averaged across ε4</td>
<td>0.01 (0.02)</td>
<td>0.01 (0.01)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.1. Magnitude of decline from baseline to 18 month assessment between healthy older adults with low Aβ amyloid and high Aβ amyloid (black bars; “0” line represents low Aβ amyloid group), and between healthy older adults who are APOE ε4 carriers and non-carriers (light grey bars; “0” line represents APOE ε4 non-carriers), for each performance measure (* indicates $p < .05$; ** indicates $p < .001$); error bars represent 95% confidence intervals.
5.4 Discussion

Results of this study support the hypothesis that in healthy older adults, high Aβ amyloid load is associated with a decline in episodic memory over 18 months. Compared to the cognitive performance of healthy older adults with low Aβ amyloid load, older adults with high Aβ amyloid load showed greater decline across each aspect of episodic memory assessed and the magnitudes of these were by convention moderate\(^4\) (i.e. Cohen’s \(d = 0.40\) to \(0.60\)). A decline of comparable magnitude was observed for visual working memory (Figure 5.1). As the 95% confidence intervals for these different effect sizes showed substantial overlap, the current data is interpreted most parsimoniously as showing that high Aβ amyloid load gave rise to equivalent decline across all aspects of episodic and working memory. In contrast to memory, no effect of Aβ amyloid load was observed for psychomotor function or visual attention. Hence, the decline in episodic memory observed could not have been secondary to changes in arousal or attention.

Consistent with previous reports,\(^5,5^9,6^5,8^4\) we observed no differences between the low and high Aβ amyloid groups, or between \(APOE\varepsilon4\) carriers and non-carriers, in performance on any of the cognitive tests at the time of neuroimaging (Table 5.2). Healthy older adults with low and high Aβ amyloid, and who were \(APOE\varepsilon4\) carriers and non-carriers, were also equivalent on each demographic characteristic (Table 5.1). Furthermore, when considered as a single group, no decline from the baseline to the 18-month assessment was observed in any aspect of cognition (Table 5.2) and review at 18 months confirmed that the clinical status of the healthy adults had not changed. The relatively high estimated premorbid intelligence of the control group in the AIBL cohort has been noted previously,\(^1^2^6\) and this was also evident in the current sub-sample. It
has been suggested the high premorbid intelligence could protect against Aβ amyloid related cognitive decline. However, in the current sample, there was no difference between high and low Aβ amyloid groups in estimated premorbid intelligence. Furthermore, the effects of Aβ amyloid load and APOE ε4 on decline in episodic and working memory did not change when age and estimated premorbid intelligence were controlled statistically in the analyses. These aspects of the results support the conclusion that the decline in memory and working memory observed after 18 months in healthy older adults was related specifically to high Aβ amyloid load.

The decline in episodic and working memory associated with high Aβ amyloid load was not moderated at all by the APOE ε4 status of the healthy older adults. However, when considered by itself, the APOE ε4 allele was associated with statistically significant decline over 18 months in visual memory, although the magnitude of this was slightly less than that related to differences in Aβ amyloid load. Inspection of the magnitudes of differences between APOE ε4 carrier and non-carrier groups for the other cognitive functions indicated moderate (i.e. Cohen’s $d = 0.24 – 0.38$) but non-significant decline for visual episodic memory, verbal learning and for the CogState Working Memory/Learning Composite (OCL-OBK; Figure 5.1). This consistency suggests that these effect sizes did reflect true differences in the level of cognitive decline between APOE ε4 carriers and non-carriers, although the magnitudes of these differences were not sufficient to be rendered statistically significant in the current study. The presence of APOE ε4 allele related decline in memory in healthy older adults is consistent with previous observations, although the more subtle effect of APOE ε4 positivity observed here could reflect that the current sample was younger than some studied recently, and studied over a shorter interval than that in the benchmark study of this
issue. Thus while these data do suggest that both Aβ amyloid load and APOE ε4 status predict decline in episodic memory over 18 months, the magnitude of the effect of Aβ amyloid load was greater than that of APOE ε4 status. More importantly, though, LMM analyses and inspection of the group mean change scores (Table 5.4) suggest strongly that the presence of the APOE ε4 allele did not moderate Aβ amyloid-related decline in memory in healthy older adults.

Two small prospective studies have investigated the extent to which Aβ amyloid load, measured using PiB-PET neuroimaging, is related to change in cognitive function in healthy older adults. As in the current study, the entire group of healthy older adults from the Melbourne Healthy Ageing Study (MHAS) cohort showed no change in memory or non-memory composite scores 18 or 36 months after a baseline PiB-PET scan. However, when adults in this group were classified as having high or low Aβ amyloid load, using the same criterion as that used in the current study, healthy older adults with high Aβ amyloid load showed statistically significant decline of a moderate magnitude on both memory and non-memory composite scores 36 months after PET scanning. However, by the 36-month assessment, 25% of healthy older adults with high Aβ amyloid met clinical criteria for MCI. Thus, the general decline in cognition observed at 36 months post-scan reflected this change in clinical status. Conversely, in a subset of healthy controls recruited from the Alzheimer’s Disease Neuroimaging Initiative (ADNI), individuals with low or high Aβ amyloid load did not differ in the rate at which their global cognitive ability and verbal episodic memory deteriorated over 15 months. Given the magnitudes of the effects observed here, the sample studied in the ADNI cohort was probably too small to allow for the detection of any subtle decline in memory. Taken together, the results from these two previous prospective studies and
the current study suggest that in healthy older adults, high Aβ amyloid does increase the risk of progression to MCI and that the subtle decline in memory that characterises such progression can be detected over relatively short time intervals (i.e., 18 months), even in the absence of any change in clinical status. Our results are consistent with prospective studies of healthy older adults with increased Aβ amyloid determined from analysis of cerebral spinal fluid (CSF). These studies also report that pathological levels of CSF Aβ42 are associated with increased rates of cognitive decline, although like the neuroimaging studies, these changes were observed over much longer intervals than those observed here (e.g. 4-10 years). In the current cohort of individuals with high Aβ amyloid, we expect that the decline in memory will continue until it reaches levels that would be considered as reflecting cognitive impairment (when compared to normative data) and the clinical status of individuals changes from cognitively normal to indicative of MCI.

The finding that Aβ amyloid-related decline in memory can occur without a change in clinical status also raises an important issue about the methods for classifying levels of cognitive function in healthy older adults. It has previously been suggested that the earliest stages of AD may be most accurately detected through objective evidence for decline in cognitive function, in particular memory function, rather than from the detection of impairment in cognition. Recent recommendations for the definition of the preclinical stage of AD have also recommended that the identification of objectively defined cognitive decline may assist in the detection of preclinical AD. Thus, the combination of objectively defined cognitive decline with putative AD biomarkers should considerably strengthen the ability to detect preclinical AD.
Although these are the first studies to investigate the extent to which amyloid imaging biomarkers predict future cognitive decline, previous research has sought to determine the extent to which decline in cognitive function predicts an increase in $\text{A}\beta$ amyloid load. For example in a series of studies of healthy adults followed prospectively with brief batteries of cognitive tests, we identified that statistically reliable decline in aspects of verbal and visual episodic memory tests over 6 years,\textsuperscript{110} 2 years,\textsuperscript{90} and even one year\textsuperscript{131} was associated with increased risk of high $\text{A}\beta$ amyloid load. Other studies have observed similar declines in cognition in individuals with high $\text{A}\beta$ amyloid load.\textsuperscript{49, 84, 91} When considered with the results of the current study, these data suggest that the combination of decline in memory with parameters from amyloid imaging may be useful for the identification of the AD process in individuals who do not meet any clinical criteria for cognitive impairment. In healthy older adults with high $\text{A}\beta$ amyloid load, objectively defined decline in memory may also be a worthy target for anti-amyloid therapies.\textsuperscript{212}

An important methodological issue needs to be considered when interpreting the current results as an extensive investigation of cognitive function was not conducted. The tasks used were chosen based on their brevity, test-retest reliability, demonstrated sensitivity to change, and their ability to be applied repeatedly without the generation of practice effects.\textsuperscript{101, 132} Exploration using more detailed neuropsychological tests will be useful in further elucidating the nature of $\text{A}\beta$ amyloid-related decline in cognition.
Chapter Six: Cognitive Decline in Adults with Mild Cognitive Impairment and High Aβ Amyloid: Prodromal Alzheimer’s Disease?

6.1 Introduction

Among individuals who meet clinical criteria for mild cognitive impairment (MCI), high Aβ amyloid levels have been shown to increase the likelihood of conversion to Alzheimer’s disease (AD) and decrease the time of conversion to AD.\textsuperscript{48, 106, 213, 214} Even in individuals with MCI whose clinical status remains unchanged, high Aβ amyloid levels are associated with an increased rate of decline on measures of clinical status such as the Clinical Dementia Rating sum of boxes (CDR-SB) score.\textsuperscript{8, 49, 97, 215} Such data show the importance of Aβ amyloid biomarkers in understanding the relationship between clinically defined MCI and AD.\textsuperscript{9, 212, 216}

While changes in clinical status are important signs of disease progression, changes in cognitive function, prior to progression to AD\textsuperscript{8, 97} in MCI with high levels of Aβ amyloid, may improve understanding of early AD-related neurodegeneration.\textsuperscript{185, 197, 217} To date, the few prospective studies of MCI that have also measured Aβ amyloid levels\textsuperscript{48-51} report that the magnitude of cognitive decline (i.e., mean change over standard deviation of change) over 18-24 months is greatest for measures of episodic memory (magnitude of decline from 0.32-0.52) in patients with high Aβ amyloid. In contrast, rates of cognitive decline are uniformly low in adults with MCI and low Aβ amyloid.\textsuperscript{45, 48, 65} In most of these studies, the follow-up assessments have been conducted in groups that include individuals who have progressed to AD. Hence, it is not possible to determine how much of the decline in neuropsychological performance reported was secondary to change in clinical status.
Natural history studies of MCI, and data from placebo groups in MCI clinical trials, have estimated effect sizes for decline in global cognition, episodic memory, and working memory to range between 0.11 to 0.20.\textsuperscript{48,51,218} Taken together, these data suggest that the study of MCI groups that are well-characterised clinically but for whom A\(\beta\) biomarker data are not available may lead to an underestimation of true AD related cognitive decline. Furthermore, they also lend support to the hypothesis that patients with MCI and low A\(\beta\) amyloid may reflect neurological or psychiatric conditions other than AD.\textsuperscript{78}

Chapter Five reported that compared to healthy older adults with low A\(\beta\) amyloid levels, in whom cognition remained stable, healthy older adults with high A\(\beta\) amyloid showed moderate decline in episodic and working memory (e.g. 0.40 - 0.60) over 18 months.\textsuperscript{202} This was despite the clinical status of these healthy adults remaining unchanged. Thus, the aim of this chapter was to determine the rate of decline in episodic memory, working memory, and attention over 18 months in MCI with high and low levels of A\(\beta\) amyloid. We hypothesised that in MCI with high A\(\beta\) amyloid levels, the rate of decline in episodic memory over 18 months would be greater than that in healthy and MCI groups with low A\(\beta\) amyloid levels. In these groups we also examined the effect of A\(\beta\) amyloid on clinical status, and the extent to which disease progression altered estimates of cognitive change.

6.2 Methods

6.2.1 Participants.

Participants were recruited from the healthy older adults (HA) and MCI groups enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing. The
process of recruitment and diagnostic classification of HA and MCI groups have been described in detail previously.\textsuperscript{60,126} Exclusion criteria for the AIBL study are: schizophrenia; depression (15-item Geriatric Depression Score (GDS) of 6 or greater); Parkinson’s disease; cancer (other than basal cell skin carcinoma) within the last two years; symptomatic stroke; uncontrolled diabetes; or current regular alcohol use exceeding two standard drinks per day for women or four per day for men. A clinical review panel chaired by DA reviewed all available medical, psychiatric and neuropsychological information to confirm the cognitive health of individuals enrolled in the HA group. Similarly, all data for participants with MCI were reviewed to ensure that their clinical classification was consistent with international criteria.\textsuperscript{10,135} In this chapter, all MCI participants were classified as having amnestic MCI, although further classification into single or multiple domains was not conducted.\textsuperscript{74}

The study was approved by and complied with the regulations of the institutional ethics committees of Austin Health, St Vincent’s Health, Hollywood Private Hospital, and Edith Cowan University.\textsuperscript{126} All participants provided written informed consent prior to participating in the study.

Only HAs who had undergone PiB-PET neuroimaging and who had low levels of Aβ amyloid at baseline (n = 122) were included in the study. Similarly, adults with MCI who had undergone PiB neuroimaging and who had high levels of Aβ amyloid at baseline (n = 32) (hereafter termed MCI high Aβ), and adults with MCI and low levels of Aβ amyloid at baseline (n = 16) (hereafter termed MCI low Aβ) were included. Of the 122 HAs, 96 completed both baseline and 18-month assessments. Of the 32 participants in the MCI high Aβ group, 30 completed the 18-month assessment, and of the 16 participants in the MCI low Aβ group, 14 completed the 18-month assessment.
6.2.2 Measures.

6.2.2.1 PiB-PET neuroimaging and APOE ε4 genotyping.

The PiB-PET imaging methodology employed in this study has been outlined in detail previously.\textsuperscript{60,110} Briefly, PET standardised uptake value (SUV) data acquired 40-70 minutes post-PiB injection were summed and normalised to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVR). An 80ml blood sample was also taken from each participant, 0.5ml of which was forwarded for APOE genotyping at a clinical pathology laboratory.

6.2.2.2 Cognitive assessments.

Participants completed clinical rating scales and the neuropsychological battery from the AIBL study (Table 6.2). These measures have all been described in detail elsewhere and were administered according to standard protocols by trained research assistants.\textsuperscript{86,126,172} The clinical status of participants was determined on the basis of clinically ascertained scores on the MMSE\textsuperscript{136} and CDR scale.\textsuperscript{137} Premorbid intelligence was estimated using the Wechsler Test of Adult Reading (WTAR)\textsuperscript{141} and levels of depressive and anxiety symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS).\textsuperscript{142}

Cognition was measured using a set of computerised cognitive tests which included measures of visual paired associate learning (Continuous Paired Associate Learning; CPAL), visual pattern separation (One Card Learning; OCL), visual working memory (One Back; OBK), attention (Identification; IDN), and psychomotor function (Detection; DET) from the CogState battery, which have previously been reported elsewhere and in Chapter Two.\textsuperscript{121,132}
Performance on the tests from the CogState battery was not used to classify individuals' clinical status. Additionally, verbal episodic memory was measured using the California Verbal Learning Test, Second Edition (CVLT-II).

6.2.3 Procedure.

Participants underwent the neuropsychological and clinical assessments that comprise the AIBL study protocol at baseline and 18 months later. In this study, we report PiB-PET neuroimaging data obtained at baseline, and neuropsychological data obtained at baseline and at the 18-month assessment in order to examine the rate of cognitive change of the MCI high Aβ and MCI low Aβ groups relative to the HA group.

6.2.4 Data analysis.

Each cognitive task yielded a single performance score (Table 6.2). Consistent with observations from other studies,⁴⁸,⁸⁵ the distribution of PiB SUVR data was skewed negatively and could not be normalised with data transformations. Thus, Aβ amyloid levels were classified dichotomously as either low (SUVR < 1.50) or high (SUVR ≥ 1.50) in accordance with established criteria.⁴²,⁴⁸

As has been reported previously in AIBL, relative to the HA group, the MCI high Aβ group had lower MMSE scores, higher SUVR scores, and comprised a higher proportion of APOE ε4 carriers.⁶⁰ The MCI low Aβ group also had lower MMSE scores relative to the HA group, but did not differ on levels of SUVR or proportion of APOE ε4 carriers. Groups did not differ with respect to the proportion of females, age, depression, or anxiety scores (Table 6.1). However, the MCI low Aβ group had lower premorbid IQ than the HA and MCI high Aβ groups.
Table 6.1. Demographic means (SD) for MMSE, CDR-SB, premorbid IQ and HADS scores, for each clinical group at baseline assessment, and group mean raw scores for each performance measure at baseline.

<table>
<thead>
<tr>
<th></th>
<th>HA (n = 122)</th>
<th>MCI PiB- (n = 16)</th>
<th>MCI PiB+ (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) female</td>
<td>60 (49%)</td>
<td>8 (50%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>N (%) APOE ε4</td>
<td>33 (34%)</td>
<td>1 (6%)</td>
<td>19 (76%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>74.39 (7.34)</td>
<td>75.56 (9.68)</td>
<td>75.76 (5.89)</td>
</tr>
<tr>
<td>SUVR Neocortex</td>
<td>1.16 (0.08)</td>
<td>1.18 (0.13)</td>
<td>2.21 (0.42)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.81 (1.24)</td>
<td>27.69 (2.41)</td>
<td>26.97 (2.09)</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>108.03 (7.78)</td>
<td>103.38 (11.48)</td>
<td>108.66 (7.45)</td>
</tr>
<tr>
<td>HADS-Depression</td>
<td>2.83 (2.19)</td>
<td>3.73 (2.02)</td>
<td>3.65 (2.53)</td>
</tr>
<tr>
<td>HADS-Anxiety</td>
<td>4.06 (2.64)</td>
<td>5.27 (2.31)</td>
<td>4.78 (2.50)</td>
</tr>
<tr>
<td>Baseline CDR-SB 0/0.5/&gt;1</td>
<td>116/6/0</td>
<td>0/9/7</td>
<td>0/17/15</td>
</tr>
<tr>
<td>18 month CDR-SB 0/0.5/&gt;1</td>
<td>110/8/0</td>
<td>1/4/9</td>
<td>0/7/23</td>
</tr>
</tbody>
</table>

Note: One-way ANOVA indicated no significant differences in age or FSIQ between groups; Mann-Whitney U indicated significant differences in MMSE and CDR-SB, but no significant differences between HADS-D and HADS-A between groups; $\chi^2$ indicated a significant difference in number of APOE ε4 carriers between groups.

SUVR = Standardised Uptake Value Ratio; MMSE = Mini-Mental State Examination; HADS-Depression = Hospital Anxiety and Depression Scale, Depression Subscale; HADS-Anxiety = Hospital Anxiety and Depression Scale, Anxiety Subscale; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes score, number of participants with scores 0, 0.5, and more than 1.

A series of analyses of covariance (ANCOVA) were conducted to compare the change in cognition between baseline and 18 months, for each cognitive outcome measure, between clinical groups (HA, MCI low Aβ, MCI high Aβ). For the ANCOVA, clinical group was entered
as a fixed factor; premorbid IQ and baseline cognitive test score as the covariate, and
cognitive test score at the 18-month assessment as the dependent variable. Within each
model, a baseline x group interaction term was fitted to the model to account for between-
group differences at baseline, although this term was removed from the models if not
statistically significant. The magnitude of the difference in baseline-adjusted means
between clinical groups at the 18-month assessment was expressed using Cohen’s $d$.\textsuperscript{184} Due
to the non-normal nature of the data distributions for the CDR-SB score, the range
restriction in the MCI groups and the absence of variability in CDR-SB scores in the HA
group, a generalised linear model (GLM) was used to analyse the relationship between
clinical group and clinical change between baseline and the 18-month assessment, and to
generate the magnitude of change in performance over 18 months.\textsuperscript{219} Cohen’s $d$ expressed
the magnitude of difference between groups in baseline-adjusted 18-month CDR-SB means
from the GLM.

Finally, to remove the potential inflation of estimates of change that would occur from
disease progression in a proportion of individuals in the MCI groups, analyses were
repeated with individuals who met clinical criteria for AD at 18 months removed to
determine. This allowed determination of the extent to which any changes in
neuropsychological performance observed initially were secondary to this change in clinical
status.

6.3 Results

At the 18-month assessment, the clinical classification of all HAs remained the same as that
at baseline. For the MCI groups, 1 (7%) of the low A$\beta$ and 14 (46%) of the high A$\beta$ groups
met clinical criteria for AD at the 18 month assessment. A scatterplot of the SUVR for HAs,
MCI low A$\beta$, and MCI high A$\beta$ groups are shown in Figure 6.1.
Figure 6.1. Standardised uptake value ratio (SUVR) in HA, MCI low Aβ and MCI high Aβ groups.

ANCOVA analyses indicated that, relative to the HA group and after controlling for baseline levels of performance, the MCI high Aβ group showed significantly greater decline on measures of verbal and visual episodic memory as well as working memory, at the 18-month assessment (Table 6.2). Compared to the HA group, the MCI low Aβ group showed significantly greater decline only on measures of visual paired associate learning, and visual
working memory. No decline was observed for measures of psychomotor or attentional function in either MCI group. The GLM indicated that relative to the HA group and taking into account baseline ratings, the MCI high Aβ and low Aβ groups did not show significantly greater impairment at the 18-month assessment on the CDR-SB, Wald’s $\chi^2 (2, 6) = 5.38, p = .07$. Further, comparisons of proportion of individuals who scored 0, 0.5, or 1 using $\chi^2$ showed no change in proportions from baseline to 18 months in the MCI high Aβ, $\chi^2 (1,4) = 5.70, p = .22$, Cramér's $V = 0.31$, or MCI low Aβ groups, $\chi^2 (1,3) = 3.00, p = .39$, Cramér's $V = 0.46$. Magnitudes of differences in baseline-adjusted performance between the clinical groups at the 18-month assessment are shown in Figure 6.2 (dark grey bars for MCI low Aβ, light grey bars for MCI high Aβ).

As 46% of the MCI high Aβ, and 7% of the MCI low Aβ groups met clinical criteria for AD at the 18-month reassessment, data for the cognitive tests were reanalysed with data from the individuals who progressed to AD removed. Results of this reanalysis indicated that, relative to the HA group and taking into account performance at baseline, greater decline at the 18-month assessment on measures of verbal and visual episodic memory, as well as working memory remained significant in the MCI high Aβ group, and that the magnitudes of these differences remained comparable to that observed in the initial analysis (Figure 6.3). Additionally, no decline was observed for measures of psychomotor, attention, or clinical function (Figure 6.3). For the MCI low Aβ group, greater decline at the 18-month assessment was again observed on measures of visual paired associate learning and visual working memory. No decline was observed for any episodic memory measure, or measure of psychomotor, attention or clinical function (Figure 6.3).
Table 6.2. Results of ANCOVA with premorbid IQ as covariate, and ANCOVA estimated marginal (EM) means and standard deviation (SD) at 18 months.

<table>
<thead>
<tr>
<th>Task</th>
<th>Performance Measure</th>
<th>ANCOVA Baseline (df) F</th>
<th>ANCOVA Group (df) F</th>
<th>ANCOVA HA low Aβ amyloid (n = 96)</th>
<th>ANCOVA MCI low Aβ amyloid (n = 14)</th>
<th>ANCOVA MCI high Aβ amyloid (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET</td>
<td>Speed</td>
<td>(1,123) 9.03**</td>
<td>(2,123) 2.94</td>
<td>2.53 (0.12)</td>
<td>2.53 (0.15)</td>
<td>2.55 (0.14)</td>
</tr>
<tr>
<td>IDN</td>
<td>Speed</td>
<td>(1,123) 37.81**</td>
<td>(2,123) 1.55</td>
<td>2.72 (0.06)</td>
<td>2.73 (0.08)</td>
<td>2.74 (0.07)</td>
</tr>
<tr>
<td>OCL</td>
<td>Accuracy</td>
<td>(1,122) 8.57**</td>
<td>(2,122) 10.80**</td>
<td>1.01 (0.11)</td>
<td>1.01 (0.15)</td>
<td>0.88 (0.13)</td>
</tr>
<tr>
<td>OBK</td>
<td>Accuracy</td>
<td>(1,121) 6.67*</td>
<td>(2,121) 3.90*</td>
<td>1.32 (0.16)</td>
<td>1.17 (0.28)</td>
<td>1.20 (0.22)</td>
</tr>
<tr>
<td>CPAL</td>
<td>Total errors</td>
<td>(1,120) 58.02**</td>
<td>(2,120) 7.75**</td>
<td>38.70 (19.64)</td>
<td>56.62 (25.69)</td>
<td>57.07 (24.26)</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>Total words</td>
<td>(1,162) 62.96**</td>
<td>(2,162) 5.53**</td>
<td>46.66 (7.40)</td>
<td>50.43 (12.25)</td>
<td>39.72 (16.43)</td>
</tr>
<tr>
<td>CVLT-II Delay</td>
<td>Total words</td>
<td>(1,162) 93.63**</td>
<td>(2,162) 18.24**</td>
<td>10.64 (2.52)</td>
<td>9.85 (3.72)</td>
<td>5.64 (5.88)</td>
</tr>
</tbody>
</table>

Note: * indicates $p < .05$ and ** indicates $p < .001$; units of measurement for speed = log_{10} millisecond; units of measurement for accuracy = arcsine proportion correct.

Baseline = effect of performance at baseline on subsequent performance at 18 months; group = effect of group (HA SUVR<1.5; MCI SUVR < 1.5; MCI SUVR≥1.5) on performance at 18 months; baseline x group interaction was significant only for the measures of OBK, CVLT-II Total, and CVLT-II Delay.

HA = healthy older adults; MCI = mild cognitive impairment; Baseline = effect of baseline cognitive score on performance at the 18 month assessment.

DET = Detection task, IDN = Identification task, OCL = One Card Learning task, OBK = One Back task, CPAL = Continuous Paired Associate Learning task, CVLT-II Total = California Verbal Learning Test, Second edition, total recall Trials 1-5 (max=80)
Figure 6.2. Magnitude of change between HA (“0” line represents HA group), MCI low Aβ (dark grey bars), and MCI high Aβ (light grey bars) groups, for each cognitive and clinical measure, from baseline to 18 month assessment; error bars represent 95% confidence intervals.
Figure 6.3. Magnitude of change between HA (“0” line represents HA group), MCI low Aβ (dark grey bars), and MCI high Aβ (light grey bars) groups, for each cognitive and clinical measure, from baseline to 18 month assessment, with individuals who progressed to AD removed; error bars represent 95% confidence intervals.
6.4 Discussion

The hypothesis that in MCI with high Aβ amyloid levels, the rate of decline in episodic memory over 18 months would be greater than that in healthy and MCI groups with low Aβ amyloid levels was supported. As has been reported in Chapter Five, healthy older adults with low Aβ amyloid showed no decline on any measure of cognitive function, and the clinical status of all individuals in this group remained stable over the 18 months. Compared to healthy older adults, the MCI with high Aβ amyloid group showed substantial decline in verbal and visual episodic memory, as well as in visual working memory 18 months after the baseline assessment (Figure 6.2). When quantified in terms of a baseline-adjusted difference between groups, the decline in episodic and working memory in the MCI and high Aβ amyloid group was, by convention, large (Cohen’s $d = 0.69$-$1.45$). However in contrast to the decline in working and episodic memory, no decline was observed for psychomotor or attentional function in the MCI high Aβ amyloid group at the 18-month assessment. Similarly, with data for the CDR-SB treated as a continuous variable (albeit non-parametrically due to skew and restriction of range), no change over 18 months was observed for adults with MCI and high Aβ amyloid. Even with clinical ratings on the CDR-SB expressed as categorical outcomes, no differences in the proportion of individuals whose rating had increased in severity after 18 months were found between healthy older adults with low Aβ amyloid, and adults with MCI and high Aβ amyloid. Hence in MCI with high Aβ amyloid, decline over 18 months on the neuropsychological measures of visual and verbal episodic memory was greater than that for clinical status.

Compared to the healthy older adults, the MCI low Aβ amyloid group showed significantly greater decline only on the measures of visual working memory and visual
paired associate learning. By convention, the magnitude of this decline was moderate ($d = 0.69-0.88$). The MCI low Aβ amyloid group showed no decline in visual or verbal episodic memory and no decline in psychomotor or attentional function (Figure 6.2). When clinical ratings on the CDR-SB were treated both continuously and categorically, the MCI low Aβ amyloid group showed no change over 18 months, nor were there any differences in the proportion of individuals whose rating had increased in severity after 18 months. Taken together, these data suggest that while there is deterioration in cognition in adults with MCI and low Aβ amyloid over 18 months, the nature of this decline is different to that observed in MCI with high Aβ amyloid.

In MCI with high Aβ amyloid, decline was large and occurred for verbal episodic memory, visual pattern separation, visual paired associate learning, as well as visual working memory. In contrast, in MCI with low Aβ amyloid, decline occurred only for visual working memory and for visual paired associate learning. The absence of decline in attentional or psychomotor function in either MCI groups suggests that the episodic memory and working memory changes observed were not secondary to any changes in arousal or attention. The impairment in visual paired associate learning observed in the MCI low Aβ amyloid group could suggest impairment in episodic memory as some researchers contend that impaired performance on visual paired associate learning tasks reflects only disruption to medial temporal lobe areas. However, we have recently shown that optimal performance on the visual paired associate learning task requires intact working memory and executive function in addition to associate learning processes. Hence, one hypothesis arising from this study is that cognitive decline in MCI with low Aβ amyloid presents mainly in the domains of working memory...
and executive function, and that this deterioration is sufficient to disrupt performance on the visual paired associate learning task.

As the hallmark of AD is progressive decline in cognitive function, particularly episodic memory, the current finding of substantial memory decline in the MCI high Aβ amyloid group indicates that this decline is substantial through the prodromal stage of AD. The observation that almost half of the MCI high Aβ amyloid group progressed to AD within 18 months is consistent with the results of previous studies,\textsuperscript{48,213,214} and confirms the importance of high Aβ amyloid to the recognition of early AD. The absence of episodic memory decline, and the relatively low rate of progression to AD in the MCI low Aβ amyloid group suggests strongly that despite these individuals meeting rigorous clinical inclusion criteria for aMCI,\textsuperscript{126} their cognitive profile may reflect neurodegenerative or psychiatric processes other than AD. This is consistent with suggestions that only adults with MCI and high Aβ amyloid is indicative of incipient AD, whilst other non-AD processes may underlie the cognitive impairments in adults with MCI and low Aβ amyloid.\textsuperscript{78} Further, the results are consistent with the recent recommendations of the National Institute on Ageing and the Alzheimer’s Association workgroup, which suggest that not only do adults with MCI and high Aβ amyloid show clinical evidence of AD, but also direct evidence of AD-pathophysiological processes in the form of brain amyloidosis.\textsuperscript{74}

For the MCI high Aβ amyloid group, disease progression at 18 months was sufficient for 46% of individuals to meet clinical criteria for AD. It is possible that inclusion of data from patients with AD in the 18 month assessment would have increased estimates of the magnitude of cognitive decline in the MCI group with high Aβ amyloid. Reanalysis of the cognitive and clinical outcomes with AD data removed reduced only slightly the
magnitude of impairment in episodic and working memory in adults with MCI and high Aβ amyloid at the 18-month assessment (Figure 6.3). Further, after removal of patients who developed AD, rates of change in attention, psychomotor function and the clinical measure of disease severity (CDR-SB) across the 18 months did not differ between groups. Taken together, these data suggest strongly that the magnitude of decline in episodic memory in MCI with high Aβ amyloid levels is substantial even when clinical status does not change. Furthermore, neuropsychological measures of episodic and working memory possess greater sensitivity to this cognitive decline in MCI than do ratings of clinical disease severity.

The presence of decline on all measures of episodic memory in the MCI high Aβ amyloid group is consistent with evidence that early AD-related changes manifest across memory processing generally. The absence of decline on the learning trials of the verbal episodic memory test (CVLT-II total recall) suggests that the use of this metric may not be as sensitive to subtle AD-related changes in memory as the measure of delayed recall, and the CogState visual episodic memory and working memory tasks. The absence of decline on any other cognitive function is also consistent with a proposed concept of early AD-related memory impairment as reflecting amnesia of the hippocampal type. This model has been used to characterise the cognitive impairment observed in MCI due to AD on single assessments, and it is consistent with neurobiological studies demonstrating the predilection of early AD pathological processes for medial temporal lobe structures. Results of this chapter suggest that this neuropsychological model of early AD can be extended to describe the nature of the progression in memory impairment in prodromal AD. In this context, these results also suggest that the progression of pathological changes in AD continue to involve the
medial temporal lobe areas. This hypothesis is consistent with magnetic resonance imaging studies showing hippocampal volumes decreases by as much as 13% in MCI, prior to progression to AD.6,222

Previous studies have shown that patients with MCI with high Aβ amyloid levels demonstrate increased and faster rates of progression to AD, as well as an increased decline on clinical rating scales.48,49,213-215 In contrast, only 7% of patients with MCI and low Aβ amyloid progressed to AD. The rate of progression to AD at 18 months (46%) in this sample of MCI with high Aβ amyloid further supports the hypothesis that high Aβ amyloid among individuals with MCI is a reliable biomarker of incipient AD. Despite this high rate of progression to AD, the measure of disease severity failed to detect differences between individuals with MCI and high Aβ amyloid and cognitively healthy older adults at the 18-month assessment, when treated as either a categorical or continuous measure. The absence of statistical significance in this case most likely reflected the issue that the sample was not large enough for use of parametric analyses. Hence these data suggest that the CDR-SB has limited use as a continuous measure of disease progression in prodromal AD, particularly in smaller experimental samples. In contrast to the CDR-SB, statistically reliable and moderate decline was detected on the neuropsychological measures of episodic memory.

The magnitude of impairment in verbal and visual episodic memory, as well as working memory, observed at the 18-month assessment observed in the MCI group with high Aβ amyloid (Figure 6.2) was approximately double the magnitude of impairment in these same measures in healthy older adults with high Aβ amyloid (effect size of decline range between 0.40-0.60), as reported in Chapter Five.202 Thus, when considered together with results of Chapter Five,202 the current findings suggest that the rate of AD-related
decline in episodic memory increases once individuals meet clinical criteria for MCI. This finding accords with current models of AD in the preclinical and prodromal stages, which propose a continuum from biomarker-positive asymptomatic individuals to those who evidence subtle decline in cognition, to individuals who eventually meet clinical criteria for MCI.\textsuperscript{9,74} An important caveat when interpreting the results of this chapter is that we didn’t investigate the effect of \textit{APOE} ε4 carriage on Aβ-related cognitive decline in patients with MCI. This was due to the low numbers of \textit{APOE} ε4 carriers in the MCI low Aβ amyloid group. The low number of \textit{APOE} ε4 carriers in the MCI low Aβ amyloid group may be a result of random sampling as more healthy controls were enrolled for PiB-PET neuroimaging in the AIBL study than patients with MCI, also because the number of patients with MCI and a negative PiB scan was low. However, the finding that the number of \textit{APOE} ε4 carriers was small in the MCI low Aβ amyloid group is consistent with previous observations that Aβ amyloid levels, as measured by analysis of CSF or PET neuroimaging, is typically associated with \textit{APOE} ε4 carriage.\textsuperscript{60,170} Further, it was observed previously in Chapter Five that healthy older adults with high Aβ amyloid that \textit{APOE} ε4 carriage did not moderate the relationship between Aβ amyloid and cognitive decline.\textsuperscript{202} Despite this, it will be important for future studies to determine the influence of \textit{APOE} ε4 carriage on Aβ amyloid-related cognitive decline in adults with MCI.

In summary, the results of these previous studies, along with those of the current chapter, suggest that individuals with MCI who also possess a known biomarker of Aβ amyloid may be a worthy target for clinical trials of anti-amyloid therapies seeking to modify or alter the course of cognitive deterioration in AD.
Chapter Seven: Aβ Amyloid and Cognitive Change: Examining the Preclinical and Prodromal Stages of Alzheimer's Disease

7.1 Introduction

Aβ amyloid biomarkers provide important insights about the pathophysiology and clinical course of Alzheimer's disease (AD). Neuroimaging studies using Aβ amyloid radioligands report that Aβ amyloid levels are high in approximately 95% of patients who meet clinical criteria for AD. However, the Aβ-centric model of AD has been questioned by observations that 20-30% of healthy older adults also show high Aβ amyloid. Recent prospective studies of healthy older adults and adults with mild cognitive impairment (MCI) who have undergone neuroimaging report substantial decline in episodic memory over 18 months, but only in those with high Aβ amyloid at baseline (also see Chapters Five and Six). This decline suggests that in healthy older adults, high Aβ amyloid indicates that AD-related neurodegeneration has begun, while for MCI, high Aβ amyloid confirms that clinical abnormalities are indicative of prodromal AD. However, because most prospective studies were conducted over 24 months or less, longer studies in both healthy older and MCI groups are needed to confirm that Aβ-related memory decline is unremitting, and to determine its magnitude and association with disease progression.

Chapter Seven aimed to compare rates of episodic memory decline over 36 months between healthy older adults and adults with MCI with low and high Aβ amyloid. The first hypothesis was that cognition would remain stable in healthy older adults with low Aβ amyloid, and also in adults with MCI and low Aβ amyloid. The second hypothesis was that in healthy older adults and adults with MCI, high Aβ amyloid would be associated with episodic memory decline over 36 months. We also explored the extent
to which Aβ amyloid-related memory decline was associated with change in clinical status, and whether any decline was moderated by the apolipoprotein E (APOE) ε4 allele.

7.2 Methods

7.2.1 Participants.

Participants were recruited from the healthy older adults (HA) and MCI groups enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing. The process of recruitment and diagnostic classification of HA and MCI groups enrolled in the AIBL cohort has been described in detail elsewhere. Participants who volunteered were excluded from the AIBL study if they had any of the following: schizophrenia; depression (15-item Geriatric Depression Score (GDS) of 6 or greater); Parkinson’s disease; cancer (other than basal cell skin carcinoma) within the last two years; symptomatic stroke; uncontrolled diabetes; or current regular alcohol use exceeding two standard drinks per day for women or four per day for men. A clinical review panel chaired by DA reviewed all available medical, psychiatric and neuropsychological information to confirm the cognitive health of individuals enrolled in the healthy controls group. Similarly, all data for participants with MCI were reviewed to ensure that their clinical classification was consistent with international criteria. Clinical classification was blinded to Aβ amyloid imaging data. In this study, only HAs and adults with MCI who had undergone PiB-PET neuroimaging and who had undergone at least one cognitive testing at baseline, 18 months and 36 months were included. The number of participants who changed clinical status at each assessment time point is shown in Table 7.1. Demographic and clinical characteristics of the HA and MCI groups are shown in Table 2.
Table 7.1. Total sample size at each assessment time point.

<table>
<thead>
<tr>
<th>Baseline classification</th>
<th>Baseline N</th>
<th>N at 18M remain at baseline classification</th>
<th>N at 18M change classification</th>
<th>N 18M withdrawals</th>
<th>N at 36M remain at baseline classification</th>
<th>N at 36M change classification</th>
<th>N 36M withdrawals</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA low Aβ</td>
<td>122</td>
<td>116</td>
<td>2 HA to MCI</td>
<td>4</td>
<td>106</td>
<td>5 HA to MCI</td>
<td>5</td>
</tr>
<tr>
<td>HA high Aβ</td>
<td>55</td>
<td>47</td>
<td>4 HA to MCI</td>
<td>4</td>
<td>32</td>
<td>3 HA to MCI</td>
<td>12</td>
</tr>
<tr>
<td>MCI low Aβ</td>
<td>16</td>
<td>13</td>
<td>1 MCI to AD</td>
<td>2</td>
<td>9</td>
<td>1 MCI to AD</td>
<td>3</td>
</tr>
<tr>
<td>MCI high Aβ</td>
<td>32</td>
<td>20</td>
<td>12 MCI to AD</td>
<td>0</td>
<td>12</td>
<td>6 MCI to AD</td>
<td>2</td>
</tr>
</tbody>
</table>

*Note: Reasons for withdrawals include participant unavailability at time of assessment, hospitalisation, or disease progression*
The study was approved by and complied with the regulations of the institutional research and ethics committees of Austin Health, St. Vincent's Health, Hollywood Private Hospital, and Edith Cowan University. All participants provided written informed consent prior to participating in the study.

In this chapter, participants were classified according to their Aβ amyloid levels and their clinical classification. Of the 177 healthy controls, 122 (69%) had low Aβ amyloid levels (HA low Aβ), and 55 (31%) had high Aβ amyloid levels (HA high Aβ). Similarly, of the 48 adults who met clinical criteria for MCI, 16 (33%) had low Aβ amyloid levels (MCI low Aβ), and 32 (67%) had high Aβ amyloid levels (MCI high Aβ).

7.2.2 Measures.

7.2.2.1 PiB-PET neuroimaging and APOE ε4 genotyping.

PiB-PET imaging methodology has been described in detail elsewhere. PET standardised uptake value (SUV) data acquired 40-70 minutes post-PiB injection were summed and normalised to the cerebellar cortex SUV, resulting in a region-to-cerebellar SUV ratio (SUVR). An 80ml blood sample was also taken from each participant, 0.5ml of which was forwarded for APOE genotyping.

7.2.2.2 Cognitive assessments.

All participants were assessed with the clinical rating scales and neuropsychological battery from the AIBL study. These have all been described in detail elsewhere and were administered according to standard protocols by trained research assistants (see also Chapters Two to Six). The clinical status of participants was determined using information which included the Mini-Mental Status Examination (MMSE) and
Clinical Dementia Rating (CDR) Scale. Premorbid intelligence was estimated using the Wechsler Test of Adult Reading (WTAR), and levels of depressive and anxiety symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS). Verbal learning and verbal episodic memory was measured using the California Verbal Learning Test, Second Edition (CVLT-II). All individuals also completed a set of computerised cognitive tests, which included measures of psychomotor function (Detection; DET), attention (Identification; IDN), visual learning (One Card Learning; OCL), working memory (One Back; OBK), and visual paired associate learning (Continuous Paired Associate Learning; CPAL) from the CogState battery (see also Chapters Two to Six). Performance on the tests from the CogState battery was not used to classify individuals’ clinical status.

7.2.3 Procedure.

Participants in this study underwent an extensive medical, psychiatric, and neuropsychological assessment upon enrolment into the AIBL study. The same assessments were repeated 18 and 36 months after baseline. In this study, we report PiB-PET neuroimaging and APOE e4 genotyping data obtained at baseline, and neuropsychological data obtained at baseline, 18 months and 36 months in order to examine the rate of cognitive change in relation to Aβ amyloid load at entry to AIBL.

7.2.4 Data Analysis.

Each of the cognitive tasks yielded a single performance score. A Learning/Working Memory composite score (OCL-OBK) was generated by standardising the OCL and OBK scores and then averaging them. A Psychomotor/Attention composite score (DET-IDN) was generated by standardising the DET and IDN scores and then averaging them.
Consistent with observations from other studies, the distribution of PiB SUVR data was skewed negatively and could not be normalised with data transformations. Thus, SUVR was classified dichotomously as either low $A\beta$ (SUVR < 1.5) or high $A\beta$ (SUVR ≥ 1.5) in accordance with established criteria. To determine the relationship between $A\beta$ amyloid, clinical group and disease progression, we used $\chi^2$ tests to compare the proportion of healthy older adults in the low $A\beta$ and high $A\beta$ subgroups who met clinical criteria for MCI after 36 months, and the proportion of individuals in the MCI low $A\beta$ and high $A\beta$ subgroups who met clinical criteria for AD after 36 months.

To test the two main hypotheses, we conducted a series of repeated measures linear mixed model (LMM) analyses (using maximum likelihood estimation and an unstructured covariance matrix) to examine the relation between group (HA low $A\beta$, HA high $A\beta$, MCI low $A\beta$, and MCI high $A\beta$) and time (baseline, 18 month, and 36 month) on cognitive change. Linear mixed modeling was employed because of its ability to model both fixed and random effects, which accounts for various sources of variability, and because it provides improved estimates of within-subject coefficients (i.e., random effects) in longitudinal studies and is robust to missing data (see Table 7.1 for number of withdrawals at each time point). In these analyses, group, time, $APOE$ status, and the group x time interaction were entered as fixed factors; participant as a random factor; and cognitive test score as the dependent variable. For each performance measure, mean slope estimates were computed for each group. The magnitude of difference in the rates of change (i.e., slopes) of the HA high $A\beta$, MCI low $A\beta$, and MCI high $A\beta$ groups in relation to the HA low $A\beta$ group was expressed using Cohen’s $d$. Given that some individuals in the HA and MCI groups had progressed to meet clinical classification of MCI and AD respectively at either the 18-month or 36-month
assessment, analyses were repeated with individuals who had converted to MCI or AD at 18 or 36 months removed to determine the extent to which any changes in neuropsychological performance observed initially were secondary to this change in clinical status.

To investigate the effect of APOE ε4 on Aβ amyloid-related memory decline, and to optimise the power of this analysis, we combined data for all measures of episodic memory (OCL, OBK, CPAL, CVLT-II total recall, and CVLT-II delayed recall), which by themselves have been shown to be sensitive to the effects of Aβ amyloid-related memory decline, to form an episodic memory score.45, 48 Further, due to the small number of adults in the MCI low Aβ group who were APOE ε4 carriers (n = 1), participants were classified into high Aβ (HA high Aβ, MCI high Aβ) and low Aβ (HA low Aβ, MCI low Aβ) groups. An LMM analysis was then conducted to examine the relationship between group (high Aβ, low Aβ), APOE (ε4 carrier, ε4 non-carrier), and time (baseline, 18 month, and 36 month) on change in this episodic memory score.

7.3 Results

7.3.1 Rates of disease progression for high and low Aβ amyloid subgroups in HA and MCI.

At 36 months, the rate of progression from HA to MCI was greater in the high Aβ subgroup (18%) than in the low Aβ subgroup (6%) (Table 7.1), and this was statistically different, $\chi^2 = 4.55, p = .03$, Cramér's $V = .17$. The rate of progression from MCI to AD over 36 months was also significantly higher in the high Aβ subgroup (64%) than in the low Aβ subgroup (15%) (Table 7.1, $\chi^2 = 5.63, p = .02$, Cramér's $V = .37$).
7.3.2 Demographic differences between high and low Aβ amyloid subgroups in HA and MCI.

There were no statistically significant differences between the four groups on any of the demographic characteristics (see Table 7.2). However, there were significantly more APOE ε4 carriers in both the HA and MCI groups with high Aβ amyloid.

7.3.3 Comparison of rates of cognitive change in HA high Aβ, MCI low Aβ, and MCI high Aβ subgroups relative to HA low Aβ subgroup.

LMM analyses revealed significant group x time interactions for the IDN, OCL, and CPAL tasks, the CVLT-II total and delayed recall measures, and the Memory Composite (Table 7.3). Post-hoc comparison of slopes over 36 months for each group indicated that, relative to the HA low Aβ group, the HA high Aβ group and the MCI high Aβ group showed significantly greater rates of decline over 36 months on all measures of verbal and visual episodic memory, and that the magnitude of difference in slopes was, by convention, moderate to large (Table 7.4, Figures 7.1 and 7.2). In addition, relative to the HA low Aβ group, the HA high Aβ, and MCI high Aβ groups showed a greater rate of decline on the measure of working memory (OBK task) over 36 months, and the magnitude of difference in slopes relative to the HA low Aβ group was also moderate in magnitude, although slightly less than that observed for the measures of episodic memory (Table 7.4). Of note, the MCI low Aβ group showed no difference in slopes relative to the HA low Aβ group on any measure of episodic or working memory. However, the MCI low Aβ group showed improvement in performance in visual attention over 36 months and the magnitude of difference in the slope of the MCI low Aβ group relative to the HA low Aβ group was large (Table 7.4).
Table 7.3. Results of linear mixed model analyses examining change in cognitive performance over 36 months.

<table>
<thead>
<tr>
<th>Task</th>
<th>Group (df) F</th>
<th>p</th>
<th>Time (df) F</th>
<th>p</th>
<th>Group x Time (df) F</th>
<th>p</th>
<th>APOE (df) F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET-IDN</td>
<td>(3,211) 3.91</td>
<td>.01</td>
<td>(1,201) 0.60</td>
<td>.44</td>
<td>(3,193) 1.75</td>
<td>.16</td>
<td>(1,220) 1.36</td>
<td>.24</td>
</tr>
<tr>
<td>DET</td>
<td>(3,218) 1.25</td>
<td>.29</td>
<td>(1,202) 1.05</td>
<td>.31</td>
<td>(3,218) 0.29</td>
<td>.84</td>
<td>(1,216) 1.23</td>
<td>.27</td>
</tr>
<tr>
<td>IDN</td>
<td>(3,207) 5.90</td>
<td>.00</td>
<td>(1,192) 0.09</td>
<td>.77</td>
<td>(3,183) 3.74</td>
<td>.01</td>
<td>(1,216) 1.21</td>
<td>.27</td>
</tr>
<tr>
<td>OCL-OBK</td>
<td>(3,195) 6.51</td>
<td>.00</td>
<td>(1,180) 8.37</td>
<td>.00</td>
<td>(3,173) 7.06</td>
<td>.00</td>
<td>(1,214) 3.12</td>
<td>.08</td>
</tr>
<tr>
<td>OCL</td>
<td>(3,243) 1.71</td>
<td>.17</td>
<td>(1,234) 1.13</td>
<td>.29</td>
<td>(3,228) 4.10</td>
<td>.00</td>
<td>(1,222) 3.73</td>
<td>.06</td>
</tr>
<tr>
<td>OBK</td>
<td>(3,224) 3.75</td>
<td>.01</td>
<td>(1,207) 0.46</td>
<td>.50</td>
<td>(3,203) 2.18</td>
<td>.09</td>
<td>(1,199) 0.72</td>
<td>.40</td>
</tr>
<tr>
<td>CPAL</td>
<td>(3,231) 2.27</td>
<td>.08</td>
<td>(1,225) 4.58</td>
<td>.03</td>
<td>(3,220) 2.67</td>
<td>.04</td>
<td>(1,203) 2.23</td>
<td>.14</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>(3,240) 21.45</td>
<td>.00</td>
<td>(1,217) 7.54</td>
<td>.01</td>
<td>(3,213) 3.39</td>
<td>.02</td>
<td>(1,222) 0.38</td>
<td>.54</td>
</tr>
<tr>
<td>CVLT-II Delay</td>
<td>(3,238) 51.60</td>
<td>.00</td>
<td>(1,213) 5.98</td>
<td>.02</td>
<td>(3,210) 10.06</td>
<td>.00</td>
<td>(1,220) 0.19</td>
<td>.66</td>
</tr>
</tbody>
</table>

Note: DET = Detection task; IDN = Identification task; DET-IDN = Psychomotor/Attention composite, average of standardised DET and IDN scores; OCL = One Card Learning task; OBK = One Back task; OCL-OBK = Learning/Working Memory composite, average of standardised OCL and OBK scores; CPAL = Continuous Paired Associate Learning task; CVLT-II Total = California Verbal Learning Test, Second edition, Total Recall over Trials 1-5; CVLT-II Delay = CVLT-II 20 minute Delayed Recall; bolded values are significant at either the p < .05, or p < .001 level.
### Table 7.4. Mean slopes and Cohen’s d of the difference in slopes between HA low Aβ group, and HA high Aβ, MCI low Aβ, and MCI high Aβ groups.

<table>
<thead>
<tr>
<th>Task</th>
<th>HA low Aβ (Mean ± SD)</th>
<th>HA high Aβ (Mean ± SD)</th>
<th>MCI low Aβ (Mean ± SD)</th>
<th>MCI high Aβ (Mean ± SD)</th>
<th>HA low Aβ vs. HA high Aβ (Cohen’s d)</th>
<th>HA low Aβ vs. MCI low Aβ (Cohen’s d)</th>
<th>HA low Aβ vs. MCI high Aβ (Cohen’s d)</th>
<th>HA high Aβ vs. MCI high Aβ (Cohen’s d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET-IDN</td>
<td>0.013 ± 0.505</td>
<td>0.040 ± 0.484</td>
<td>-0.334 ± 0.368</td>
<td>0.111 ± 0.419</td>
<td>-0.05 (-0.39, 0.28)</td>
<td>0.70 (0.07, 1.32)</td>
<td>-0.20 (-0.63, 0.23)</td>
<td>-0.15 (-0.59, 0.28)</td>
</tr>
<tr>
<td>DET</td>
<td>0.016 ± 0.074</td>
<td>0.009 ± 0.071</td>
<td>-0.005 ± 0.051</td>
<td>0.012 ± 0.062</td>
<td>0.10 (-0.24, 0.43)</td>
<td>0.29 (-0.36, 0.94)</td>
<td>0.06 (-0.37, 0.48)</td>
<td>-0.04 (-0.48, 0.39)</td>
</tr>
<tr>
<td>IDN</td>
<td>0.006 ± 0.039</td>
<td>0.015 ± 0.038</td>
<td>-0.031 ± 0.027</td>
<td>0.015 ± 0.033</td>
<td>-0.23 (-0.57, 0.10)</td>
<td>0.97 (0.30, 1.62)</td>
<td>-0.24 (-0.66, 0.19)</td>
<td>0.00 (-0.44, 0.44)</td>
</tr>
<tr>
<td>OCL-OBK</td>
<td>-0.026 ± 0.538</td>
<td>-0.402 ± 0.519</td>
<td>0.137 ± 0.388</td>
<td>-0.387 ± 0.455</td>
<td>0.71 (0.36, 1.05)</td>
<td>-0.31 (-0.93, 0.32)</td>
<td>0.69 (0.25, 1.12)</td>
<td>-0.03 (-0.47, 0.41)</td>
</tr>
<tr>
<td>OCL</td>
<td>0.012 ± 0.079</td>
<td>-0.036 ± 0.077</td>
<td>0.009 ± 0.055</td>
<td>-0.022 ± 0.068</td>
<td>0.61 (0.26, 0.95)</td>
<td>0.04 (-0.61, 0.69)</td>
<td>0.44 (0.01, 0.87)</td>
<td>-0.19 (-0.62, 0.25)</td>
</tr>
<tr>
<td>OBK</td>
<td>0.031 ± 0.123</td>
<td>-0.017 ± 0.120</td>
<td>0.042 ± 0.085</td>
<td>-0.021 ± 0.103</td>
<td>0.39 (0.05, 0.73)</td>
<td>-0.09 (-0.74, 0.56)</td>
<td>0.43 (0.00, 0.87)</td>
<td>0.04 (-0.40, 0.47)</td>
</tr>
<tr>
<td>CPAL</td>
<td>-5.885 (13.738)</td>
<td>-2.378 (12.728)</td>
<td>-6.963 (9.252)</td>
<td>2.678 (10.990)</td>
<td>-0.26 (-0.68, 0.17)</td>
<td>0.08 (-0.34, 0.51)</td>
<td>-0.64 (-1.07, -0.21)</td>
<td>-0.42 (-0.85, 0.03)</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>-0.189 (5.232)</td>
<td>-2.441 (4.994)</td>
<td>-0.064 (3.861)</td>
<td>-2.880 (4.414)</td>
<td>0.44 (0.10, 0.77)</td>
<td>-0.07 (-0.62, 0.48)</td>
<td>1.15 (0.73, 1.56)</td>
<td>0.09 (-0.35, 0.53)</td>
</tr>
<tr>
<td>CVLT-II Delay</td>
<td>-0.003 (1.404)</td>
<td>-1.105 (1.341)</td>
<td>0.663 (1.047)</td>
<td>-0.910 (1.211)</td>
<td>0.80 (0.45, 1.13)</td>
<td>-0.49 (-1.04, 0.07)</td>
<td>0.66 (0.26, 1.06)</td>
<td>-0.15 (-0.59, 0.29)</td>
</tr>
</tbody>
</table>

**Note:** Negative slopes for Speed Composite, DET, IDN, and CPAL tasks indicate better performance; negative Cohen’s d values for Speed Composite, DET, IDN and CPAL tasks indicate worse performance of HA high Aβ, MCI low Aβ, or MCI high Aβ in relation to HA low Aβ.

Bolded values are significant at the p < .05 or p < .001 level.

DET = Detection task; IDN = Identification task; DET-IDN = Psychomotor/Attention composite, average of standardised DET and IDN scores; OCL = One Card Learning task; OBK = One Back task; OCL-OBK = Learning/Working Memory composite, average of standardised OCL and OBK scores; CPAL = Continuous Paired Associate Learning task; CVLT-II Total = California Verbal Learning Test, Second edition, Total Recall over Trials 1-5; CVLT-II Delay = CVLT-II 20 minute Delayed Recall.
Figure 7.1. Linear trend of performance on the Learning/Working Memory Composite (OCL-OBK) for HA high Aβ and HA low Aβ (Figure 7.1a), and MCI high Aβ and MCI low Aβ (Figure 7.1b) groups, from baseline to 36 months.
Figure 7.2. Linear trend of performance on the CVLT-II delayed recall for HA high Aβ and HA low Aβ (Figure 7.2a), and MCI high Aβ and MCI low Aβ (Figure 7.2b) groups, from baseline to 36 months.
7.3.4 Comparison of rates of cognitive change with individuals who converted to MCI or AD removed.

Re-analysis of the data with individuals who progressed to MCI or AD at either 18 month or 36 month assessment removed (see Table 7.1 for details) did not change the pattern of results. Significant group x time interactions were observed for all measures of episodic memory, all *p*'s < .05, and no effect of *APOE* was observed for any measures, all *p*'s > .05. The magnitude of difference in slopes between the HA low Aβ and HA high Aβ groups on measures of memory were slightly reduced, but remained moderate in magnitude (OCL *d* = 0.55, OBK *d* = 0.34, CPAL *d* = 0.32, CVLT-II total recall *d* = 0.45, CVLT-II delayed recall *d* = 0.81, Memory Composite *d* = 0.62). The magnitude of difference in slopes between the HA low Aβ and MCI high Aβ groups on measures of visual memory remained moderate to large in magnitude (OCL *d* = 0.54, OBK *d* = 0.76, CPAL *d* = 0.85, Memory Composite *d* = 1.07), although less pronounced on measures of verbal memory (CVLT-II total recall *d* = 0.28, CVLT-II delayed recall *d* = 0.20).

7.3.5 Exploration of the effect of *APOE* ε4 on the relationship between Aβ amyloid and change in episodic memory.

Analysis of the effect of *APOE* ε4 on the relationship between Aβ amyloid and the episodic memory score showed no significant interactions involving *APOE* (see Figure 7.3). The only statistically significant effect identified from this analysis was the expected Group (high Aβ vs. low Aβ) x time interaction, *F*(1,205) = 14.19, *p* < .001.
Figure 7.3. Linear trend of performance on the Episodic Memory Composite for low Aβ ε4 carriers, high Aβ non-ε4 carriers, low Aβ non-ε4 carriers, and low Aβ ε4 carriers, from baseline to 36 months.
7.4 Discussion

The first hypothesis that cognitive function would remain stable in healthy older adults with low Aβ amyloid levels was supported. Healthy older adults with low Aβ amyloid showed no change in episodic memory (Figures 7.1 and 7.2) or any other aspect of cognitive function over 36 months (Table 7.4). Interestingly, adults with MCI who had low Aβ amyloid also showed no change in episodic or working memory (Figures 7.1 and 7.2; Table 7.4) and even some improvement in attentional function (Table 7.4). The absence of any decline in episodic memory, or any other cognitive function, over 36 months in healthy older adults with low Aβ amyloid replicates and extends previous observations of cognitive stability over 18 months in this (see also Chapter Five), other cohorts of healthy older adults with low Aβ amyloid. The finding of no cognitive decline in the MCI with low Aβ amyloid group is consistent with the results of a recent 18-month study of a similar group, and findings that rates of progression to AD are low in MCI with low Aβ amyloid. As has been raised previously in Chapter Four, these results support the hypothesis that MCI without a positive Aβ amyloid biomarker reflects neurological or psychiatric conditions other than AD.

The second hypothesis that in non-demented adults, high Aβ amyloid would be associated with decline in memory and cognitive function over 36 months was supported. The rate of decline in episodic and working memory over 36 months in healthy older adults and MCI with high Aβ amyloid was substantial compared to cognitive changes in healthy older adults with low Aβ amyloid (Figures 7.1 and 7.2, i.e., $d = 0.4-0.8$). However, direct comparisons identified no difference between the high Aβ amyloid healthy and MCI groups in the rates of decline in episodic and working memory over 36 months. The consistently small effect sizes for these differences (Table 7.4) suggest strongly that the absence of
statistical significance did not represent Type II error. Rates of progression to MCI or AD in the low Aβ amyloid healthy and MCI groups remained low (i.e., 6% and 15% respectively). In comparison, decline in episodic and working memory after 36 months was large enough for 18% of healthy older adults with high Aβ amyloid to meet clinical criteria for MCI, and 64% of adults with MCI and high Aβ amyloid to meet clinical criteria for AD. This higher rate of disease progression has also been reported previously in Chapter Six, and in other groups of individuals with high Aβ amyloid.48, 213, 214

Importantly, re-analysis of cognitive data following removal of individuals who progressed to the next disease stage showed that the decline in memory was a general characteristic of high Aβ amyloid groups, and not merely a consequence of high Aβ groups containing more individuals for whom the disease had progressed. The finding that high Aβ amyloid was associated with substantial decline in episodic memory, is consistent with observations of decline in memory in healthy older adults over 18-24 months.49, 50, 202 However, the current results show for the first time that episodic memory decline continues at a constant rate over the preclinical and prodromal stages of AD. Together, these findings suggest strongly that AD can be identified very early in the disease course, and then monitored reliably using a combination of an Aβ amyloid imaging biomarker and cognitive assessment.

The APOE ε4 allele did not moderate the relationship between Aβ amyloid episodic memory in individuals with low Aβ (healthy older adults and adults with MCI with low Aβ amyloid) or individuals with high Aβ (healthy older adults and adults with MCI with high Aβ amyloid). This absence of any effect of the APOE ε4 allele on Aβ amyloid-related memory decline is consistent with the results of Chapter Five.202 While there is strong evidence that the presence of the APOE ε4 allele increases the risk of clinically recognised
AD,\textsuperscript{26,225} and also elevated Aβ in healthy older adults,\textsuperscript{51,60,170} the extent to which \textit{APOE} \textepsilon{4} carriage moderates relationships between neuroimaging biomarkers of Aβ amyloid and cognitive function in other cohorts of healthy older adults have yet to be determined. Thus, a hypothesis arising from the current data is that while carrying the \textit{APOE} \textepsilon{4} allele increases the risk of AD processes beginning, it does not moderate disease progression; at least at the clinical level. This hypothesis will be tested once Aβ amyloid status is considered in the large prospective studies of different \textit{APOE} allele groups.\textsuperscript{55,206}

The current study measured Aβ amyloid using the PiB-PET radioligand, although the results are consistent with prospective studies of healthy older adults and adults with MCI in which Aβ amyloid levels were determined from analysis of cerebral spinal fluid (CSF).\textsuperscript{51,66,210,226} These studies also report that pathological levels of CSF Aβ42 are associated with increased rates of cognitive decline, and these changes have been observed over similar,\textsuperscript{51} and longer intervals than those observed here (e.g. 4-10 years).\textsuperscript{66,210,215,226} However, they have typically defined cognition using general measures such as the MMSE, and the ADAS-Cog, which have known metric limitations when used in healthy older adults (i.e., range restriction, practice effects, limited sensitivity to subtle decline).\textsuperscript{115} In these studies, when the effect size of decline was characterised as mean change divided by the standard deviation of change, the MMSE and ADAS-Cog evidenced very small magnitudes of decline (e.g. $d \sim 0.10$) in healthy controls,\textsuperscript{51,227} whilst episodic memory measures demonstrated much larger magnitudes of decline (e.g. $d \sim 0.50$),\textsuperscript{51} equivalent to the magnitudes of decline observed in this study. While further consideration of more extensive cognitive measures are required, these data do suggest that a pathological level of Aβ amyloid, determined either through PET neuroimaging or analysis of CSF, does indicate increased risk for
cognitive decline, particularly in the domain of episodic memory, and crucially, increases the risk of disease progression.

A theoretical model that integrates pathological, clinical and neuropsychological changes through the preclinical and prodromal stages of AD has been proposed by Jack and colleagues. This model proposes that abnormal Aβ amyloid deposition precedes any neurodegenerative biomarker abnormalities (e.g. tau-mediated neuronal injury and neurodegeneration), which in turn precede cognitive impairment, which does not become evident until AD or MCI are diagnosed clinically. However, the trajectory curves for cognitive impairment in this model by Jack and colleagues were based on the findings of rates of neuropsychological abnormalities in cross-sectional studies of individuals at varying stages of the disease. From the current and recent studies which have focused on cognitive decline (see also Chapters Five and Six), it is now clear that detectable decline in episodic memory becomes apparent very early in the disease, often years before individuals meet any clinical criteria for MCI. Thus, if cognitive abnormality were defined as the presence of decline in cognition, as opposed to impairment relative to normative control data, the trajectory curve for cognition would be closer to those for Aβ amyloid biomarker abnormalities than to the trajectory for clinical classification. The emphasis on the measurement and detection of cognitive change, as opposed to cognitive impairment, as a neuropsychological approach to identifying AD in the earliest stages has been raised previously, and has been acknowledged recently as an important area for further consideration by the National Institute on Ageing and the Alzheimer's Association workgroup guidelines on the definition of the preclinical and prodromal stages of AD.

One caveat in interpreting the results of this study is that the AIBL study is not an epidemiological sample. Rather, the selection of MCI groups was biased towards the
inclusion of individuals with amnestic MCI. Further, in the recruitment of healthy older adults, participants in AIBL were highly educated, and had few existing or untreated medical or psychiatric illnesses. As such, it would be important for these findings to be replicated in individuals with high Aβ amyloid in population-based studies, such as the Mayo Clinic Study of Ageing,\(^2\)\(^{03}\) and the 10/66 Dementia Research Group,\(^2\)\(^{04}\) where it is possible that the Aβ-related decline may be greater than that observed here. A second caveat is that the rate of attrition (~35%) in the healthy older adults with high Aβ amyloid group was larger than in healthy older adults with low Aβ amyloid (~6%). Reasons for this were hospitalisation, and disease progression. As 54 month assessments in AIBL are currently being conducted, it remains to be seen whether these individuals return for reassessment. The high attrition rate of this group of healthy older adults lends further support to the detrimental nature of high Aβ amyloid, though currently, cognitive data to corroborate this are not available. Finally, a set of brief computerised tests was used to measure cognitive decline in the current group. These were selected because of their stability and reliability in healthy older adults and their demonstrated sensitivity to changes in memory in both healthy older adults, adults with MCI and adults with AD. However, in order to better understand the nature of memory changes, or the extent to which decline occurs in other cognitive domains known to be abnormal in early AD, the results reported here should be replicated using a more extensive battery of neuropsychological tests.

Despite these limitations, the results of this study, along with those of previous studies, suggest that memory decline is a reliable indicator of early AD. Furthermore, careful assessment of memory decline in individuals with an Aβ amyloid biomarker may provide a
sound basis for the design of clinical trials of anti-amyloid therapies in otherwise healthy older adults.

8.1 Introduction

Large prospective studies that include both biomarkers and clinical information are important to understand the pathogenesis of Alzheimer’s disease (AD). Early data from these studies show that AD-related pathophysiological changes begin prior to the clinical diagnosis of AD\(^6\), \(^8\), \(^2\), with the prodromal phase of AD now agreed to be reflected in the clinical classification of mild cognitive impairment (MCI).\(^9\), \(^4\) There is also growing evidence that decline in the cognitive domains central to AD also occurs in healthy individuals who show high levels of A\(\beta\) amyloid (also see Chapters Five and Seven),\(^5\), \(^9\), \(^4\), \(^2\) with studies showing progression over 36 months sufficient to warrant clinical criteria for MCI (also see Chapter Seven).\(^4\) Such findings raise the prospect that pharmacotherapy designed to modify or even halt AD-related pathology could be tested in both the clinical and preclinical phases of AD.\(^7\), \(^4\)

While cognitive measures that are used to inform decisions about drug efficacy in clinical trials in AD are well accepted (e.g. ADAS-Cog),\(^2\) there is also agreement that these same measures do not possess sufficient sensitivity to detect cognitive change in the earlier stages of the disease.\(^1\)\(^9\), \(^2\) For example, data from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) study show that in healthy older adults and in adults with MCI, performance on the ADAS-Cog is characterised by ceiling effects, range restriction and negative skew.\(^1\)\(^9\) These psychometric characteristics limit the sensitivity of the ADAS-Cog to any treatment-related change in mild AD and in MCI.\(^1\)\(^9\) Consequently, there is a need
for cognitive instruments that will be sensitive to cognitive change in very early as well as established AD.

In a series of recent studies (also see Chapter Two), we have found that tests from the CogState battery were sensitive to cognitive impairment (i.e. relative to matched controls) in mild to moderate AD, MCI and also in healthy older adults who carry putative AD biomarkers. Of equivalent importance, in a different sample, we also found that performance on these same CogState tasks remained stable despite repeated administration in healthy older adults, and was characterised by reliable decline over periods of 12 months or greater in MCI. Furthermore, in patients with AD, performance on the verbal list learning task of the CogState battery declined over one year. Taken together, these data suggest that the same computerised battery of cognitive tests may be used to measure cognitive function repeatedly in older individuals with normal cognition and in patients with MCI and AD. However, for use in AD groups, it has been necessary to simplify some (i.e, visual learning and associate learning) but not all (i.e., verbal learning) tests of memory in order to maximise their acceptability. Finally, the CogState battery has demonstrated sensitivity to cognitive improvement arising from treatment with current pharmacotherapies for AD (e.g. cholinesterase inhibitors), with clinical trial data showing that performance is improved after acute treatment with donepezil in healthy older adults and with daily dosing in AD. There is growing appreciation of the importance of understanding the dynamics and reliability of neuropsychological assessments used for repeated assessments. However, as yet, there has been no direct comparison of the stability and reliability of these same tests between healthy older adults and those with MCI or AD assessed over the same time intervals.
The Australian Imaging, Biomarkers and Lifestyle (AIBL) study is a prospective natural history study of over 1000 adults who are cognitively normal, or have either a diagnosis of MCI or mild AD. These individuals undergo extensive assessment using psychiatric, neuropsychological, neurological, neuroradiological and lifestyle measures at 18-month intervals. The AIBL Rate of Change (ROCS) sub-study (hereafter referred to as ROCS) was designed to leverage the care and attention used in recruiting, assessing and characterising the subjects in AIBL, by taking a subset of each clinical group and assessing them repeatedly at short retest intervals using the CogState battery to determine the extent to which any change in cognitive function could be detected in individuals with different stages of AD over intervals of 1, 2, 3, 6, 12 and 18 months. As the ROCS study is now enrolled fully and complete to the three month assessment, these data can be used to examine the acceptability of the tests in healthy older adults, and adults with MCI and AD, as well as to examine the magnitude of differences in performance between these groups. Some clinical trials of putative cognitive enhancers in AD are also conducted over three months and these trials generally measure cognitive performance at baseline and then at multiple follow-up assessments (i.e., week 4, 8 and 12). Therefore we also investigated the stability of performance on the battery over 12 weeks in each of these cognitive measures between groups. Data from this prospective study can provide estimates of the expected rate of change in cognitive function over 12 weeks, as well as estimates of associated error (i.e. test-retest reliability and stability of the different outcome measures). Such data can be useful for computing power in clinical trials conducted over the same time interval.

Whilst the use of serial assessments over short retest intervals can improve the statistical power of a study, frequent testing can also impose a substantial burden on the participants.
One way of reducing this burden is to use very brief assessments and also have assessors travel to participants' homes. Additionally, participants were allowed to miss an assessment if a convenient time could not be arranged. Analysis of rates of attrition and of missed assessments can be used to determine how these study characteristics influenced the acceptability of the ROCS design.

The first aim of this chapter was to investigate the acceptability of the ROCS study to participants over the initial three months by investigating the rates of attrition and missed assessments in each group. The first hypothesis was that the ROCS study design would have good acceptability in all groups. The second aim of this chapter was to directly compare the performance on the CogState battery between healthy older adults, adults with MCI and adults with AD who had completed three months of assessment in the ROCS study. The second hypothesis was that for tests where performance could be compared directly, healthy older adults would perform better than adults with MCI, who would in turn show performance better than adults with AD. The third aim was to determine the stability of performance on the CogState battery over the initial 12 weeks of the ROCS study in which it had been administered four times. The third hypothesis was that performance measures on the CogState battery would be reliable and remain stable over the short test-retest period in healthy, MCI and AD groups. The fourth aim was to compute estimates of variability in performance over time on the CogState battery and compare these between the three clinical groups. The fourth hypothesis was that estimates of variability in three month change scores for the ROCS CogState measures would be greater in the AD and MCI groups than in healthy controls.
8.2 Methods

8.2.1 Participants.

The sample, method of recruitment, inclusion/exclusion criteria and measures used in the AIBL study has been described in detail previously. As the ROCS sample was drawn from the AIBL group, these same criteria applied. The selection criteria for enrolment into ROCS were 1) consensus classification as either being healthy (with or without subjective memory impairment, hereafter termed healthy older adults, HA), adults with MCI, or AD, 2) existing or planned MRI and PiB-PET brain scans, 3) complete baseline neuropsychological and psychiatric ratings, 4) a willingness to allow serial assessments, and 5) ability to perform computerised cognitive tasks. HAs were classified with subjective memory impairment if they answered “Yes” to the question “Do you have difficulty with your memory?” At the completion of the pilot phase of the study, ROCS consisted of 205 enrolled subjects and all subjects had completed their first three months assessment (baseline, 1 month follow-up, 2 month follow-up and 3 month follow-up). Clinical and demographic characteristics of the healthy older, MCI and AD groups are shown in Table 8.1.

8.2.2 Measures.

The following cognitive measures were selected because they are brief to administer, can be given repeatedly without eliciting practice effects, have demonstrated ability to detect AD-related memory impairment, and because the tasks have been shown to be sensitive to cognitive change associated with existing and novel therapies for AD. Table 8.2 summarises the outcome measure for each task.
Table 8.1. Median years of education and means (standard deviations (SDs)) for participants’ demographic and clinical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>HA (N = 115)</th>
<th>MCI (N = 47)</th>
<th>AD (N = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) Female</td>
<td>66 (57%)</td>
<td>24 (52%)</td>
<td>21 (49%)</td>
</tr>
<tr>
<td>N (%) APOE ε4</td>
<td>24 (21%)</td>
<td>17 (36%)</td>
<td>30 (70%)</td>
</tr>
<tr>
<td>Age</td>
<td>73.62 (6.86)</td>
<td>78.87 (6.92)</td>
<td>79.57 (6.61)**</td>
</tr>
<tr>
<td>Years of Education</td>
<td>9-12 years</td>
<td>9-12 years</td>
<td>9-12 years</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>108.21 (7.08)</td>
<td>109.69 (5.84)</td>
<td>103.00 (10.56)**</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.06 (1.18)</td>
<td>27.95 (1.33)</td>
<td>22.16 (4.38)</td>
</tr>
<tr>
<td>CDR-SB</td>
<td>0.04 (0.14)</td>
<td>0.57 (0.70)</td>
<td>4.09 (2.60)</td>
</tr>
<tr>
<td>HADS-D</td>
<td>2.46 (2.15)</td>
<td>3.11 (2.20)</td>
<td>3.82 (2.60)**</td>
</tr>
<tr>
<td>HADS-A</td>
<td>4.05 (2.80)</td>
<td>4.05 (2.52)</td>
<td>4.73 (3.34)</td>
</tr>
<tr>
<td>CVLT-II Total</td>
<td>52.60 (9.49)</td>
<td>36.19 (8.42)</td>
<td>23.06 (9.63)</td>
</tr>
<tr>
<td>CVLT-II Delayed</td>
<td>12.01 (3.27)</td>
<td>6.35 (3.95)</td>
<td>1.79 (2.50)</td>
</tr>
<tr>
<td>Stroop C/D</td>
<td>2.33 (0.68)</td>
<td>2.68 (0.95)</td>
<td>3.20 (1.22)</td>
</tr>
<tr>
<td>BNT (No Cue)</td>
<td>28.53 (1.60)</td>
<td>27.35 (3.08)</td>
<td>22.50 (5.58)</td>
</tr>
</tbody>
</table>

** indicates group differences were significant, p < .001

Note: MMSE = Mini Mental State Examination; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes Score; HADS-D = Hospital Anxiety and Depression Scale, Depression Subscale; HADS-A = Hospital Anxiety and Depression Scale, Anxiety Subscale; CVLT-II = California Verbal Learning Test, Second edition; Stroop C/D = Stroop Interference (Colours/Dots Ratio); BNT = Boston Naming Task

Table 8.2. Number of participants enrolled in ROCS, and number (and percentage) of participants who completed each assessment time point, for the first three months of assessment.

<table>
<thead>
<tr>
<th></th>
<th>Enrolled</th>
<th>Baseline</th>
<th>Month 1</th>
<th>Month 2</th>
<th>Month 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>115</td>
<td>111 (96.5%)</td>
<td>110 (95.7%)</td>
<td>107 (93.1%)</td>
<td>103 (89.6%)</td>
</tr>
<tr>
<td>MCI</td>
<td>47</td>
<td>45 (95.7%)</td>
<td>43 (91.5%)</td>
<td>43 (91.5%)</td>
<td>40 (85.1%)</td>
</tr>
<tr>
<td>AD</td>
<td>43</td>
<td>37 (86.1%)</td>
<td>36 (83.7%)</td>
<td>34 (79.1%)</td>
<td>35 (81.4%)</td>
</tr>
</tbody>
</table>

Note: HA = healthy older adults; MCI = adults with mild cognitive impairment; AD = adults with Alzheimer’s disease
8.2.2.1 *Detection task.*

The Detection task is a measure of simple reaction time shown to measure psychomotor function. In this task, subjects must attend to the card in the centre of the screen and respond to the question: “Has the card turned over?” Subjects were instructed to press the “Yes” key as soon as the card turned face up. The face of the card was always the same generic joker card. The task ended after 35 correct trials had been recorded. Trials on which anticipatory responses occurred were excluded, and another trial was given so that all subjects completed the 35 trials. The primary performance measure for this task was reaction time in milliseconds (speed), which was normalised using a logarithmic base 10 ($\log_{10}$) transformation.

8.2.2.2 *Identification task.*

The Identification (IDN) task is a measure of choice reaction time shown to measure visual attention. In this task, the participant must attend to the card in the center of the screen, and respond to the question “Is the card red?” Participants were required to press the “Yes” key if it is and the “No” key if it is not. The face of the cards displayed were either red or black joker cards in equivalent numbers in random order. These cards were different to the generic joker card used in the DET task. The task ended after 30 correct trials. Trials on which anticipatory responses occurred were excluded and another trial was given so that all participants completed the 30 trials. The primary performance measure for this task was reaction time in milliseconds (speed), which was normalised using a $\log_{10}$ transformation.
8.2.2.3 One Card Learning task.

The One Card Learning (OCL) task is a continuous visual recognition learning task that assesses visual learning within a pattern separation model. In this task, the participant must attend to the card in the center of the screen and respond to the question “have you seen this card before in this task?” If the answer was yes, participants were instructed to press the “Yes” key, and the “No” key if the answer was no. Normal playing cards were displayed (without joker cards). In this task, six cards are drawn at random from the deck and are repeated throughout the task. These six cards are interspersed with distractors (non-repeating cards). The task ends after 42 trials, without rescheduling for postanticipatory correct trials. This version of the task was administered to the HA and MCI groups. For the AD group, a simpler version of the task was used. In this version, only four target cards were interspersed with distractors. The primary performance measure for this task was the proportion of correct answers (accuracy), which was normalised using an arcsine square-root transformation.

8.2.2.4 One-Back task.

The One-Back (OBK) task is a task of working memory and attention. Similar in presentation to the OCL task, participants must attend to the card in the center of the screen and respond to the question “is this card the same as that on the immediately previous trial?” If the answer was yes, participants were instructed to press the “Yes” key, and the “No” key if the answer was no. The task ends after 30 correct trials. A correct but post-anticipatory response led to scheduling of an extra trial. The primary performance measure for this task was the proportion of correct answers (accuracy), which was normalised using an arcsine square-root transformation.
8.2.2.5 *Continuous Paired Associate Learning task.*

The Continuous Paired Associate Learning (CPAL) task is a measure of visual learning and episodic memory. In this task, subjects must learn a series of associations between a set of simple shapes and their associated location. In HA and MCI groups, six pattern-location associations must be learned. For the mild AD group, four pattern-location associations must be learned. There are two parts to the CPAL although subjects are not made aware that the stages are different.

*In the presentation phase* of the task, the pattern appears at the location and the subject is required to acknowledge that they have seen the pattern by touching the location at which it appears. At this stage of the task the subject will also see that there are two locations at which no target appears (distractor locations). Patterns are presented in random order; however, once presented the pattern remains at the same location throughout the task.

*In the learning phase of the task* subjects must place each of the four patterns in their correct locations. They must do this in six trials. For the first trial, one of the patterns is presented in the centre location and the subject is required to remember the location at which it was shown. They indicate the location by touching it. If they touch an incorrect location, a visual and audible signal occurs (a red cross appears over the location and a buzzer sound is presented). The subject is then required to choose a second location. This process continues until the subject finds the location that has been paired with the pattern. Once the pattern has been associated with the location, the next pattern is presented in the centre location and the process continues again. This repeats until the subject has correctly placed all of the targets in their correct locations. Once all patterns have been placed correctly, the second trial begins. In the second trial, the patterns remain in the same locations, but their order of presentation in the centre of the screen is different to that of
the first trial (randomised). The process of placing each target in the correct location proceeds as it did in the first trial. When the second trial is complete the same process is repeated for trials 3 to 6. The primary performance measure for this task was the total number of errors made.

8.2.2.6 International Shopping List Test.

The International Shopping List Test (ISLT) is a four trial (three learning trials and one delayed recall trial) verbal list learning test in which individuals are instructed to remember a list of 12 items that they need to obtain from their local store. To ensure that items on the shopping list are relevant to Australian test takers, a large pool of common shopping list items (128 items) were rated by 30 Australian healthy adults using a web-based survey to indicate the ease (i.e., “very difficult,” “difficult,” “easy”, or “very easy”) with which each shopping list item could be obtained locally.\textsuperscript{236} In this study, a pool of 10 alternate forms was generated. The items on each list have been reported previously.\textsuperscript{232} For each assessment, for each participant, one of the 10 lists was chosen at random by the computer software, with the condition that if it has been selected previously for a participant, it wouldn’t be selected again. For each list, the order in which items were presented was randomised between participants by the computer software, with the order of the items remaining constant across the three learning trials.

During each assessment, the computer presented the words to the examiner one at a time at a rate of 1 word per 2 seconds. The participant was instructed to “try and remember as many items on the shopping list as possible.” The examiner then read each item to the participant as they appeared on the screen. The computer screen was never visible to the participant. Once all 12 words had been read to the participant, they were instructed to
recall as many items from the list as possible with the statement “tell me as many items on
the shopping list as you can remember.” The list of 12 words was displayed on the
computer screen and as the participant recalled each item, the examiner marked the item
by clicking on the relevant checkbox. If words were repeated, the checkbox was clicked
again. Another checkbox was clicked if the participant said a word that was not on the
original list (i.e., an intrusion). When the participant indicated that no more items could be
recalled, the trial was stopped and the same process was repeated two more times. For the
delayed recall trial, participants were asked to recall as many items as possible from the
initial list after a delay of approximately 30 minutes, during which the participant was
administered other cognitive tasks (i.e., DET, IDN, OCL, OBK, and CPAL).

8.2.3 Procedure.

All subjects were enrolled in the AIBL study before selection for ROCS began. Once
clearance by institutional ethics and research committees of Austin Health, St Vincent’s
Health, Hollywood Private Hospital and Edith Cowan University were granted, the study
coordinator contacted subjects by telephone, explained the study to them and invited them
to participate. If they agreed to participate in the study, they were informed that the
assessments were brief and that these could occur at their home, a location near their
house that was convenient or they could attend the research unit (Mental Health Research
Institute). Individual assessors organised a mutually suitable time for the visit. Participants
had also agreed to undergo at least one Positron Emission Tomography (PET)
neuroimaging scan using $^{11}$C-Pittsburgh Compound B (PiB) in the future, although at the
time of this report, the imaging aspect had not been completed for all participants.

Assessors used to gather neuropsychological data were trained on administration of the
CogState battery in accord with standard protocols. In order to facilitate communication
and encourage subjects to identify with the study, each assessor was assigned to conduct the repeated assessments on the same subjects and organised home visits directly with each subject.

### 8.2.4 Data analysis.

The acceptability of the ROCS approach was evaluated by calculating the number of subjects who completed any of the 1-, 2-, or 3-month visits, and who withdrew before the 3-month visit. A Chi-Square ($\chi^2$) analysis was used to compare rates of withdrawal at 3 months between groups. Performance on the three assessments over three months was then compared between groups using a series of 3 (group) x 4 (assessment) Linear Mixed Model (LMM) Analyses of Covariance (ANCOVA) with age, premorbid IQ, depressive symptomatology and baseline scores entered as covariates (as group comparisons of demographics identified these as being significantly different between the three groups), and participants treated as a random factor. For each test, the magnitude of difference between the MCI and AD group to the HA group was expressed using Cohen’s $d$. As simpler versions of the CPAL and OCL tasks were administered to the AD groups, performance on these tasks was not compared statistically between the HA and AD groups. Average measure intraclass correlation coefficients (ICC) were used to compute the test-retest reliability of each ROCS battery performance measure over the four assessment periods, in both the total group and in each clinical classification group separately.

Finally, to allow appreciation of the change that will occur on each of the outcome measures in the CogState battery over three months, each outcome measure from the CogState battery was submitted to an LMM ANCOVA which allowed computation of the slope of change in performance and associated standard error of this change between the first and last assessment sessions, i.e., between baseline and Month 3, for each clinical
classification group. Within-subject standard deviations (WSD) were also calculated for each clinical classification group and Hartley’s $F_{\text{max}}$ test was used to compare variances between each clinical classification group.

### 8.3 Results

#### 8.3.1 Acceptability of the ROCS design

The number of healthy older adults, adults with MCI, and adults with AD enrolled in ROCS is summarised in Table 8.2. Table 8.2 also summarises the number of participants who completed each assessment time point for the first three months of assessment. Overall, high rates of completion were observed for each clinical group, on each assessment. Reasons for missing data included participants being unavailable at the time of assessment due to vacation or other commitments, or hospitalisation due to AD and/or other illness. Thus at the 3 month assessment, 6 (5.2%) healthy older adults, 2 (4.3%) adults with MCI, and 6 (13.9%) adults with AD had withdrawn from the study. The rates of attrition were not different between the three clinical groups ($\chi^2 = 1.98, \text{df} = 2, p = .37$). All other participants returned to the study. Reasons for withdrawal from the study included progression of the disease (and subsequent hospitalisation), time constraints, and non-AD related carer commitments.

#### 8.3.2 Comparison of performance between clinical classification groups and across assessments

LMM indicated that performance of healthy older adults, adults with MCI and adults with AD differed significantly on all measures of the CogState battery. However no significant differences between assessments were identified for any of the measures and no group by assessment interaction terms were statistically significant (Table 8.3). Compared to
controls, the magnitude of impairment observed in the MCI group (averaged over
assessments) was large for the CPAL ($d = 1.53$), OCL ($d = 0.94$), OBK ($d = 0.60$), ISLT total
recall ($d = 0.82$), and ISLT delayed recall ($d = 1.36$) tasks, and small for the DET ($d = 0.20$),
and IDN ($d = 0.10$) tasks. Compared to controls, the magnitude of impairment observed in
the AD group (averaged over assessments) were large for the OBK ($d = 2.40$); ISLT total
recall ($d = 2.52$), and ISLT delayed recall ($d = 2.62$) tasks, and moderate for the DET ($d =
0.62$), and IDN ($d = 0.53$) tasks.

8.3.3 Test-retest reliability in each clinical classification group

The intraclass correlation coefficients for each outcome measure for each group are shown
in Table 8.4. When considered according to clinical classification, all measures from the
ROCS battery demonstrated high (i.e. $r > .70$) test-retest reliability and these estimates
were equivalent between the clinical groups (see Table 8.4). The highest ICCs were
observed for the overall group for the ISLT total and delayed recall score, CPAL total
errors, and accuracy of performance on the One Back task.

8.3.4 Comparison of variance in change scores and data for reliable change index

The group mean change, standard error of this change and WSDs between the first and last
assessment were computed for each measure of the ROCS battery, for each clinical
classification group (Table 8.5). For each clinical classification group, no significant
difference in mean change was observed for any of the measures of the ROCS battery.
Statistically significant differences in WSDs were observed between the HA and AD groups
for the accuracy of performance on the DET and IDN tasks, and between the HA and MCI
groups for the CPAL errors (Table 8.5).
Table B.3. Group mean (SD) for each performance measure in the HA, MCI and AD groups at each of the four assessments across three months and summary of the results of the LMM analyses for each performance measure.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Group</th>
<th>Baseline</th>
<th>1 month</th>
<th>2month</th>
<th>3month</th>
<th>FTime</th>
<th>FGroup</th>
<th>FInteraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean</td>
<td>Mean</td>
<td>(df) F</td>
<td>(df) F</td>
<td>(df) F</td>
</tr>
<tr>
<td>DET Speed (log_{10} milliseconds)</td>
<td>HA</td>
<td>2.56 (0.10)</td>
<td>2.60 (0.18)</td>
<td>2.58 (0.18)</td>
<td>2.58 (0.19)</td>
<td>(2,492) 2.98</td>
<td>(2,492) 3.67*</td>
<td>(4,492) 1.23</td>
</tr>
<tr>
<td></td>
<td>MCI</td>
<td>2.59 (0.12)</td>
<td>2.61 (0.18)</td>
<td>2.60 (0.18)</td>
<td>2.59 (0.18)</td>
<td>(2,492) 3.60*</td>
<td>(4,492) 1.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>2.64 (0.15)</td>
<td>2.61 (0.19)</td>
<td>2.62 (0.19)</td>
<td>2.64 (0.19)</td>
<td>(2,492) 1.23</td>
<td>(4,492) 0.78</td>
<td></td>
</tr>
<tr>
<td>IDN Speed (log_{10} milliseconds)</td>
<td>HA</td>
<td>2.73 (0.06)</td>
<td>2.77 (0.18)</td>
<td>2.77 (0.18)</td>
<td>2.77 (0.19)</td>
<td>(2,492) 2.05</td>
<td>(2,492) 3.60*</td>
<td>(4,492) 1.74</td>
</tr>
<tr>
<td></td>
<td>MCI</td>
<td>2.77 (0.09)</td>
<td>2.76 (0.18)</td>
<td>2.75 (0.18)</td>
<td>2.74 (0.19)</td>
<td>(2,492) 1.74</td>
<td>(4,492) 0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>2.85 (0.11)</td>
<td>2.78 (0.19)</td>
<td>2.77 (0.19)</td>
<td>2.76 (0.19)</td>
<td>(2,492) 1.74</td>
<td>(4,492) 0.78</td>
<td></td>
</tr>
<tr>
<td>OCL Accuracy (arcsine proportion correct)</td>
<td>HA</td>
<td>1.00 (0.09)</td>
<td>0.99 (0.12)</td>
<td>1.00 (0.12)</td>
<td>1.02 (0.12)</td>
<td>(2,490) 0.04</td>
<td>(2,490) 33.65**</td>
<td>(4,490) 0.78</td>
</tr>
<tr>
<td></td>
<td>MCI</td>
<td>0.94 (0.10)</td>
<td>0.94 (0.12)</td>
<td>0.93 (0.12)</td>
<td>0.93 (0.12)</td>
<td>(2,490) 0.04</td>
<td>(2,490) 33.65**</td>
<td>(4,490) 0.78</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>0.82 (0.11)</td>
<td>0.87 (0.12)</td>
<td>0.86 (0.12)</td>
<td>0.85 (0.12)</td>
<td>(2,490) 0.04</td>
<td>(2,490) 33.65**</td>
<td>(4,490) 0.78</td>
</tr>
<tr>
<td>OBK Accuracy (arcsine proportion correct)</td>
<td>HA</td>
<td>1.37 (0.16)</td>
<td>1.30 (0.16)</td>
<td>1.34 (0.16)</td>
<td>1.36 (0.16)</td>
<td>(2,473) 2.28</td>
<td>(2,473) 35.85**</td>
<td>(4,473) 1.63</td>
</tr>
<tr>
<td></td>
<td>MCI</td>
<td>1.23 (0.20)</td>
<td>1.24 (0.16)</td>
<td>1.26 (0.15)</td>
<td>1.35 (0.15)</td>
<td>(2,473) 2.28</td>
<td>(2,473) 35.85**</td>
<td>(4,473) 1.63</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>0.86 (0.30)</td>
<td>1.10 (0.20)</td>
<td>1.10 (0.20)</td>
<td>1.07 (0.20)</td>
<td>(2,473) 2.28</td>
<td>(2,473) 35.85**</td>
<td>(4,473) 1.63</td>
</tr>
<tr>
<td>CPAL Total errors</td>
<td>HA</td>
<td>31.66 (30.98)</td>
<td>36.42 (25.69)</td>
<td>35.55 (25.72)</td>
<td>30.22 (25.71)</td>
<td>(2,482) 1.82</td>
<td>(2,482) 27.63**</td>
<td>(4,482) 0.72</td>
</tr>
<tr>
<td></td>
<td>MCI</td>
<td>75.13 (42.13)</td>
<td>61.23 (25.62)</td>
<td>54.10 (25.54)</td>
<td>59.52 (25.66)</td>
<td>(2,482) 1.82</td>
<td>(2,482) 27.63**</td>
<td>(4,482) 0.72</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>67.82 (28.39)</td>
<td>48.16 (25.94)</td>
<td>40.04 (25.94)</td>
<td>39.77 (25.96)</td>
<td>(2,482) 1.82</td>
<td>(2,482) 27.63**</td>
<td>(4,482) 0.72</td>
</tr>
<tr>
<td>ISLT Total words recalled</td>
<td>HA</td>
<td>25.05 (4.50)</td>
<td>23.42 (3.95)</td>
<td>23.57 (3.93)</td>
<td>23.52 (3.93)</td>
<td>(2,491) 0.48</td>
<td>(2,491) 50.85**</td>
<td>(4,491) 0.13</td>
</tr>
<tr>
<td></td>
<td>MCI</td>
<td>17.61 (5.26)</td>
<td>21.29 (3.85)</td>
<td>21.50 (3.82)</td>
<td>21.61 (3.78)</td>
<td>(2,491) 0.48</td>
<td>(2,491) 50.85**</td>
<td>(4,491) 0.13</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>11.00 (5.16)</td>
<td>16.42 (5.30)</td>
<td>17.09 (5.31)</td>
<td>17.26 (5.31)</td>
<td>(2,491) 0.48</td>
<td>(2,491) 50.85**</td>
<td>(4,491) 0.13</td>
</tr>
<tr>
<td>ISLT Delay Total words recalled</td>
<td>HA</td>
<td>8.41 (2.32)</td>
<td>7.60 (1.92)</td>
<td>7.63 (1.91)</td>
<td>7.70 (1.91)</td>
<td>(2,481) 1.28</td>
<td>(2,481) 50.17**</td>
<td>(4,481) 0.62</td>
</tr>
<tr>
<td></td>
<td>MCI</td>
<td>4.53 (3.09)</td>
<td>6.32 (1.85)</td>
<td>5.78 (1.84)</td>
<td>5.90 (1.84)</td>
<td>(2,481) 1.28</td>
<td>(2,481) 50.17**</td>
<td>(4,481) 0.62</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>1.15 (1.40)</td>
<td>2.84 (4.03)</td>
<td>2.26 (4.05)</td>
<td>2.53 (4.05)</td>
<td>(2,481) 1.28</td>
<td>(2,481) 50.17**</td>
<td>(4,481) 0.62</td>
</tr>
</tbody>
</table>

Note: * indicates p < .05; ** indicates p < .001; the AD group received an easier version of the OCL and CPAL task than did MCI and HA groups; DET = Detection, IDN = Identification, OCL = One Card Learning, OBK = One Back, CPAL = Continuous Paired Associate Learning, ISLT = International Shopping List Test.
Table 8.4. *Summary of average measure intraclass correlation coefficients (ICC), two-way random effects model, on each CogState outcome measure in HA, MCI and AD groups, across four assessments.*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Overall</th>
<th>HA</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET Speed</td>
<td>0.79**</td>
<td>0.93**</td>
<td>0.92**</td>
<td>0.70**</td>
</tr>
<tr>
<td>IDN Speed</td>
<td>0.76**</td>
<td>0.92**</td>
<td>0.95**</td>
<td>0.71**</td>
</tr>
<tr>
<td>OCL Accuracy</td>
<td>0.87**</td>
<td>0.77**</td>
<td>0.82**</td>
<td>0.68**</td>
</tr>
<tr>
<td>OBK Accuracy</td>
<td>0.93**</td>
<td>0.75**</td>
<td>0.79**</td>
<td>0.93**</td>
</tr>
<tr>
<td>CPAL Total Errors</td>
<td>0.91**</td>
<td>0.87**</td>
<td>0.88**</td>
<td>0.81**</td>
</tr>
<tr>
<td>ISLT Total Recall</td>
<td>0.96**</td>
<td>0.86**</td>
<td>0.86**</td>
<td>0.95**</td>
</tr>
<tr>
<td>ISLT Delayed Recall</td>
<td>0.97**</td>
<td>0.87**</td>
<td>0.92**</td>
<td>0.82**</td>
</tr>
</tbody>
</table>

Note: ** indicates p < .001; DET = Detection, IDN = Identification, OCL = One Card Learning, OBK = One Back, CPAL = Continuous Paired Associate Learning, ISLT = International Shopping List Test
Table 8.5. *Mean and Standard Deviation (SD) of difference, and Within-Subject Standard Deviation (WSD) between Baseline and Month 3 for HA, MCI and AD groups.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Group mean change</th>
<th>Group SD of change</th>
<th>WSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>-0.01</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>MCI</td>
<td>-0.01</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>AD</td>
<td>-0.06</td>
<td>0.32</td>
<td>0.28*</td>
</tr>
<tr>
<td>IDN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>0.01</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>MCI</td>
<td>-0.01</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>AD</td>
<td>-0.06</td>
<td>0.32</td>
<td>0.29*</td>
</tr>
<tr>
<td>OCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>0.02</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>MCI</td>
<td>-0.01</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>AD</td>
<td>-0.01</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>OBK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>0.03</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>MCI</td>
<td>0.05</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>AD</td>
<td>-0.01</td>
<td>0.26</td>
<td>0.14</td>
</tr>
<tr>
<td>CPAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>-3.08</td>
<td>19.95</td>
<td>14.80</td>
</tr>
<tr>
<td>MCI</td>
<td>-0.96</td>
<td>38.52</td>
<td>23.71*</td>
</tr>
<tr>
<td>AD</td>
<td>-4.22</td>
<td>26.51</td>
<td>19.19</td>
</tr>
<tr>
<td>ISLT Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>0.05</td>
<td>3.32</td>
<td>2.83</td>
</tr>
<tr>
<td>MCI</td>
<td>0.17</td>
<td>4.36</td>
<td>3.31</td>
</tr>
<tr>
<td>AD</td>
<td>0.46</td>
<td>4.99</td>
<td>2.34</td>
</tr>
<tr>
<td>ISLT Delay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>0.05</td>
<td>1.72</td>
<td>1.41</td>
</tr>
<tr>
<td>MCI</td>
<td>-0.22</td>
<td>2.56</td>
<td>1.50</td>
</tr>
<tr>
<td>AD</td>
<td>-0.16</td>
<td>0.91</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Note: * indicates p < .05; DET = Detection, IDN = Identification, OCL = One Card Learning, OBK = One Back, CPAL = Continuous Paired Associate Learning, ISLT = International Shopping List Test
8.4 Discussion

The first hypothesis that the ROCS study design would be acceptable to participants was supported in part. Overall, the rate of missing data in this initial stage was 7%, which is comparable to a previous study evaluating the usability of a similar battery over five 3-month interval time points. The main reason for these missing data was complete withdrawal from the study; no individual missed an assessment. Therefore, overall, the ROCS study design can be considered acceptable to the majority of the individuals enrolled. Consideration of missing data in the individual groups suggest that while rates of missing data in the healthy older adult and MCI groups were low (~5%), rates of missing data in the AD group were somewhat higher (~10%). In the AD group, the most frequent reason for withdrawal from the study was that the patient or carer rated the patient’s disease as having increased in severity, such that all of the patients with AD who withdrew from the study had moderate disease severity. Consequently all AD patients remaining in the study at the three month period have a mild disease severity. Hence these data suggest that while the ROCS study design is acceptable in healthy older adults, MCI and mild AD it might not be so for moderate AD.

The second hypothesis that healthy older adults would perform better than adults with MCI, who would in turn perform better than adults with AD was supported. In general, performance on each measure from the CogState battery was worse in AD than in controls, although the magnitude of impairment was larger for the measures of episodic memory (ISLT total and delayed recall) than it was for the measures of attention and psychomotor function (IDN and DET). As the AD group performed simpler versions of the CPAL and OCL tasks, group mean performance on these tasks was not compared directly between AD and HA groups. For the MCI group, performance was worse than
controls only for the measures of episodic and working memory. Performance of the MCI group on measures of psychomotor function and attention was equivalent to controls. The finding that both patients with AD and MCI performed worse than controls on the ISLT total and delayed recall, CPAL and OCL is consistent with previous studies conducted in different samples (see also Chapter Two). Furthermore, we observed that the magnitude of impairment of performance on these measures of learning and memory was less in MCI than in AD. This is consistent with disease models that propose MCI to represent a disease stage intermediate between healthy ageing and dementia.

The third hypothesis that performance on the CogState battery would be reliable and remain stable over the 12 weeks in healthy adult, MCI and AD groups was supported for all of the tasks. Each of the outcome measures from the CogState battery of tests showed high test-retest reliability and the strength of these estimates of reliability did not change with increasing disease severity. Additionally, the statistical analyses showed that average performance for each of the groups remained constant across the 12 week period on each of the CogState outcome measures. Although the omnibus analyses showed no change in performance over time for any of the clinical groups, inspection of mean performance across assessments for the AD group suggests that performance improved from the first to the second assessment on tests of immediate and delayed verbal memory (ISLT), visual associate learning (CPAL), and working memory (OBK) tasks. The magnitude of this improvement from baseline to the three month assessment is summarised in Table 4. In the AD group, words recalled on the ISLT increased by half a word, errors on the CPAL decreased by 4 errors, and when expressed as change from baseline, rather than as group mean at each assessment,
accuracy of performance on the OBK did not change. We have previously reported that small improvements from the first to second assessments can occur for these same tasks in healthy older and younger adults. However, in these studies, the data for the first assessment was taken from the first time individuals performed the test (i.e., individuals had no previous practice with the test). In the current study, all individuals had completed a single practice assessment prior to the baseline assessment report here. Thus, when considered with our previous findings, there should have been no improvement in the AD group from the baseline to the second assessment. It is possible that the non-significant improvement in performance on the memory tasks in the AD group occurred because more than one practice session is required in order to achieve stable performance. This hypothesis will be tested by the extent to which performance on this battery remains stable at the six month reassessment in the AD group.

Importantly, despite these small improvements in performance on the ISLT and CPAL, the reliability of performance on each of these measures remained very high (~0.8, Table 4) in the AD group.

In healthy older adults, the stability of performance indicates that despite being given at relatively short retest intervals, performance on the battery did not improve with repeated administration (i.e. no practice effect). This stability of performance is consistent with that observed previously in healthy adults assessed repeatedly over longer time intervals (e.g. one year). The stability of performance in the healthy older adults group supports the conclusion that the absence of any improvement in performance also reflects the absence of practice effects in the MCI and AD groups. The stability of performance observed for the MCI and AD groups on all of the CogState tasks indicates also that there was no decline in any aspect of cognition in the AD group over
the 12 week test-retest period. The finding that performance in AD does not decline over 12 weeks is consistent with data from the placebo groups in recent clinical trials conducted over the same time period in which no decline in cognitive function has been observed for the ADAS-Cog\textsuperscript{238} other paper and pencil neuropsychological tests (e.g. the Neuropsychological Test Battery)\textsuperscript{239} or with some of the CogState measures studied here.

The fourth hypothesis was that estimates of variability in three month change scores for the ROCS CogState measures would be greater in the AD and MCI groups than in healthy controls. This hypothesis was supported only in part. In general within-individual variability over time was equivalent between the three groups for the majority of the outcome measures. The only exceptions to this were that variability within individuals over time was greater in the MCI group than controls for CPAL and that within-individual variability over time was greater in the AD than controls for the DET and IDN tasks. An increase in within-individual variability indicates that performance on the specific outcome measure varies more from assessment to assessment. However, in general the data show that in MCI and AD, variability in performance on the cognitive tests over time does not increase despite the lower levels of performance on these same tests.

The estimates of variability and expected mean change over time in the three groups studied here (i.e., Table 8.5), can be used to guide the design of clinical trials seeking to measure the effect of a pharmacotherapeutic intervention in groups of AD, MCI or even healthy older adults. For example, the change from baseline to week 12 on verbal memory (i.e., ISLT total recall) in the AD group was a very slight increase in words recalled of 0.46 with a standard deviation of 4.99 for this change. Thus, to plan a trial
where the improvement in performance under treatment was, for example \( d = 0.4 \) (i.e., an improvement of performance on the ISLT total recall of 2 words), then 100 patients would need to be assigned randomly to the placebo and treatment groups (assuming there are two groups, equal ratio of assignment to each group, Type I error of 0.05, and sample size required for statistical significance).\(^{240}\) For the OCL, the same expected effect size (and therefore, the same sample size), would require the accuracy of performance on this task to improve from 0.82 at baseline, to 0.85 at week 12 (refer to Table 8.5) in order for an effect to be rendered statistically significant. It is important to note that for both tests, the magnitude of improvement associated with this effect size would not increase the level of performance of the AD group to that of the MCI group.

Taken together, the results of this study suggest that the ROCS design was acceptable to healthy older adults, adults with MCI and mild AD. Acceptability of the ROCS design was lower in patients with moderate AD. All individuals in the AD group who had withdrawn were diagnosed with moderate AD and as such, became fatigued more readily by the serial assessment process than individuals with mild AD. Additionally, as they were more advanced in the AD process, hospitalisations became more frequent, and as a result, more commonly decided to withdraw from the study. All patients now remaining in the AD group of the ROCS study have AD of mild severity. We postulate that from the three month visit onwards, rates of attrition in the AD group will be lower than those observed to date. It is important to emphasise though that the majority of individuals in ROCS (88%) completed all four assessments across the three months. For these individuals, performance in the AD and MCI groups did not decline over the three month period, and this stability is consistent with that observed on other cognitive assessments in recent industry sponsored clinical trials.\(^{238, 239}\) This suggests that
clinical trials of putative cognitive enhancing drugs should be designed to detect improvement in cognition in the treatment group compared to placebo. This approach is different to those where the beneficial effects of cognitively enhancing drugs are indicated by stabile cognitive function in the treated group while performance in the placebo group declines. Alternatively, trials of drugs designed to ameliorate AD-related cognitive decline will have to be conducted over time intervals longer than 3 months if the CogState battery is used to measure cognition. In a previous 12 month study of mild to moderate AD, we observed a moderate decline ($d = 0.60$, or 2.5 words) on the ISLT total recall measure. Similarly, we observed a decline of performance over 12 months on the OCL in patients with MCI. However, future analysis of ROCS data will provide estimates of the stability of performance on this cognitive test battery over 9, 12 and 18 month periods in the clinical groups studied here.

When interpreting the results of this chapter, it is important to note that the AIBL-ROCS study is not an epidemiological sample. In the recruitment of healthy older adults, participants were highly educated, and had few existing or untreated medical or psychiatric illnesses. Further, the selection of MCI groups was biased towards the inclusion of individuals with amnestic MCI. As such, it would be important for these findings to be replicated in individuals in population-based studies, such as the Mayo Clinic Study of Ageing and the 10/66 Dementia Research Group, where it is possible that the estimates of variability in cognitive performance may be greater than that observed here. Despite this, the data presented in this chapter are important in that they are consistent with recent recommendations for estimates of stability and reliability to be reported for cognitive tests often used to measure change in cognitive function in individuals, and show that when considered over a short test-retest
interval of 3 months, each of the CogState measure has good reliability and stability, and when considered at a single time-point, is able to detect AD-related cognitive impairment.
Chapter Nine: Rapid Decline in Episodic Memory in Healthy Older Adults with High Aβ Amyloid

9.1 Introduction

Prospective positron emission tomography (PET) neuroimaging using $^{11}\text{C}$-Pittsburgh Compound B (PiB) and analysis of cerebrospinal fluid (CSF) studies show that in healthy older adults, high Aβ amyloid is associated with subsequent cognitive decline and an increased risk of conversion to mild cognitive impairment (MCI) (see also Chapters Five to Seven).\textsuperscript{48, 66, 210} It was reported in Chapter Five that healthy older adults with levels of high Aβ amyloid showed moderate decline from baseline in episodic memory after only 18 months, despite there being no change in clinical status.\textsuperscript{202} This finding supports the hypothesis that objective evidence of cognitive decline, in the context of an abnormal Aβ amyloid biomarker, provides the earliest indication of incipient Alzheimer’s Disease (AD).\textsuperscript{74} One challenge therefore is to determine how quickly cognitive decline can be identified in healthy older adults with high Aβ amyloid.

The Australian Imaging, Biomarkers and Lifestyle-Rate of Change Sub-Study (AIBL-ROCS) (see Chapter Eight for details)\textsuperscript{241} was designed to optimise sensitivity to change in cognition in non-demented adults by conducting frequent assessments with a brief computerised cognitive battery with demonstrated sensitivity to early AD. Multiple data-points for each individual provide reliable estimates of slopes of the relationships between cognitive performance and time, which can be used to model rates of change in cognition. Thus, the hypothesis of Chapter Nine was that healthy older adults with high Aβ amyloid levels would show greater decline in episodic memory than adults with low Aβ amyloid levels over six months.
9.2 Methods

9.2.1 Participants.

Forty four healthy older adults enrolled in AIBL-ROCS who had undergone PiB-PET scans and had participated in AIBL-ROCS for six months were included in this study (Table 9.1). The process of recruitment and diagnosis classification of the AIBL-ROCS study has been described in detail elsewhere (see Chapter Eight for details). The study was approved by and complied with the regulations of the institutional ethics committees of Austin Health, St Vincent’s Health, Hollywood Private Hospital, and Edith Cowan University. All participants provided written informed consent prior to participating in the study.

9.2.2 Measures.

The AIBL PiB-PET imaging methodology has been described previously. PET standardised uptake value (SUV) data acquired 40-70 minutes post-PiB injection were summed and normalised to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVR). From each participant, a 0.5ml blood sample was drawn and forwarded for APOE genotyping at a clinical pathology laboratory.

Cognitive function was measured using the CogState computerised battery which consisted of measures of visual episodic memory (One Card Learning; OCL), verbal episodic memory (International Shopping List Test; ISLT), visual paired associate learning (Continuous Paired Associate Learning; CPAL), working memory (One Back; OBK), attention (Identification; IDN) and psychomotor (Detection; DET) function. Tasks were administered according to standard instructions. Performance on the CogState battery was not used to classify clinical status. A Speed Composite was
generated by averaging the standardised DET and IDN scores, and an Episodic Memory Composite was generated by averaging the standardised OCL, CPAL, and ISLT total and delayed recall scores. All tests were administered according to standard protocol by trained assessors.

9.2.3 Procedure.

AIBL PiB-PET scanning and blood draws were conducted in a major metropolitan hospital neuroimaging centre. Trained assessors visited participants’ homes and conducted the cognitive assessments in a practice session and then at baseline and 1, 2, 3 and 6 months later.

9.2.4 Data analysis.

Aβ amyloid levels were classified dichotomously as low (SUVR < 1.5) or high (SUVR ≥ 1.5) in accordance with established criteria. Aβ amyloid groups were compared on age, depression and anxiety using analysis of variance (ANOVA), on MMSE, and CDR-SB scores using Mann-Whitney U tests, and on APOE ε4 carriers using Chi-Square (χ²).

Linear mixed model (LMM) analyses of covariance were conducted to model the rate of change in cognitive function between Aβ amyloid groups by fitting linear slopes to the relationship between performance on each cognitive task and time. For each LMM, Aβ amyloid, time (baseline, 1, 2, 3, and 6 months), and the time x Aβ amyloid interaction were entered as fixed factors; participants as a random factor; and cognitive task score as the dependent variable. Age was also included as a covariate. For each measure, the magnitude of the difference in slopes between Aβ amyloid groups was expressed using Cohen’s d.
9.3 Results

Participants with high Aβ were older than participants with low Aβ, and the frequency of APOE ε4 carriers was greater in the high Aβ amyloid group (Table 9.1). Groups did not differ on any other demographic or clinical characteristics.

Table 9.1. Demographic means (SD) for MMSE, CDR-SB, premorbid IQ and HADS scores, for overall and each Aβ amyloid group at baseline assessment

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 44)</th>
<th>Low Aβ amyloid (n = 29)</th>
<th>High Aβ amyloid (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage female</td>
<td>25 (56.82%)</td>
<td>16 (55.17%)</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>N and % APOE ε4</td>
<td>13 (29.55%)</td>
<td>5 (17.24%)</td>
<td>8 (53.33%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>77.93 (7.55)</td>
<td>75.62 (6.96)</td>
<td>82.40 (6.76)</td>
</tr>
<tr>
<td>SUVR Neocortex</td>
<td>1.43 (0.40)</td>
<td>1.16 (0.08)</td>
<td>1.95 (0.22)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.59 (1.40)</td>
<td>28.83 (1.28)</td>
<td>28.13 (1.55)</td>
</tr>
<tr>
<td>CDR-SB</td>
<td>0.10 (0.30)</td>
<td>0.10 (0.34)</td>
<td>0.10 (0.21)</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>108.82 (7.54)</td>
<td>107.62 (8.58)</td>
<td>111.13 (4.29)</td>
</tr>
<tr>
<td>HADS-Depression</td>
<td>2.76 (2.15)</td>
<td>2.89 (1.95)</td>
<td>2.50 (2.56)</td>
</tr>
<tr>
<td>HADS-Anxiety</td>
<td>3.34 (2.52)</td>
<td>3.48 (2.42)</td>
<td>3.07 (2.76)</td>
</tr>
<tr>
<td>CVLT-II Total Recall</td>
<td>47.81 (9.54)</td>
<td>48.27 (9.21)</td>
<td>46.86 (10.49)</td>
</tr>
<tr>
<td>CVLT-II Delayed Recall</td>
<td>10.50 (3.29)</td>
<td>11.07 (2.96)</td>
<td>9.36 (3.73)</td>
</tr>
<tr>
<td>Boston Naming Test</td>
<td>28.23 (1.64)</td>
<td>28.17 (1.87)</td>
<td>28.33 (1.11)</td>
</tr>
<tr>
<td>Stroop Colours/Dots</td>
<td>2.38 (0.73)</td>
<td>2.49 (0.81)</td>
<td>2.18 (0.53)</td>
</tr>
</tbody>
</table>

Note: One-way ANOVA indicated a significant difference in age between groups, p < .05, but no significant differences in HADS-Depression, HADS-Anxiety, CVLT-II total recall, CVLT-II delayed recall, Boston Naming Test, and Stroop Colours/Dots between groups; Mann-Whitney U indicated no significant differences in MMSE, and CDR-SB between groups; χ² indicated a significant difference in number of APOE ε4 carriers between groups.

SUVR = Standardised Uptake Value Ratio; MMSE = Mini-Mental State Examination; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes Score; HADS-Depression = Hospital Anxiety and Depression Scale, Depression Subscale; HADS-Anxiety = Hospital Anxiety and Depression Scale, Anxiety Subscale; CVLT-II = California Verbal Learning Test, Second edition.
Cognitive outcome measures are summarised in Table 9.2. Statistically significant Aβ amyloid x time interactions were found for the OCL, and ISLT delayed recall, where performance of the high Aβ group declined faster over six months than the low Aβ group and the difference in slopes was moderate to large in magnitude (Table 9.2). Significantly faster decline was also observed when scores from the OCL, CPAL, and ISLT total and delayed recall were combined to form an Episodic Memory Composite (Figure 9.1). No other interactions were significant, and no decline was observed for the DET, IDN and Speed Composite measures. Table 9.3 summarises group raw mean (SD) scores for each assessment time point for the high and low Aβ amyloid groups.

*Figure 9.1.* Linear trend of performance on the Episodic Memory Composite for each Aβ amyloid group, from baseline to 6 month assessment; 95% confidence intervals for each slope are shown as solid line for low Aβ amyloid group, and dotted line for high Aβ amyloid group.
Table 9.2. Summary of tasks, associated outcome measures, results of linear mixed model analyses, associated estimates of decline in each cognitive task, and mean slopes and Cohen’s d of the difference in slopes for each Aβ amyloid group.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Linear Mixed Model</th>
<th>Mean Slope (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linear Mixed Model</td>
<td>Mean Slope (SD)</td>
</tr>
<tr>
<td></td>
<td>Measure</td>
<td>Time</td>
</tr>
<tr>
<td>Detection^</td>
<td>Speed</td>
<td>(1,164) 0.10</td>
</tr>
<tr>
<td>Identification^</td>
<td>Speed</td>
<td>(1,160) 0.33</td>
</tr>
<tr>
<td>One Card Learning</td>
<td>Accuracy</td>
<td>(1,153) 0.08</td>
</tr>
<tr>
<td>One Back</td>
<td>Accuracy</td>
<td>(1,165) 3.72</td>
</tr>
<tr>
<td>CPAL^</td>
<td>Total errors</td>
<td>(1,163) 1.98</td>
</tr>
<tr>
<td>ISLT Total Recall</td>
<td>Total words</td>
<td>(1,159) 1.74</td>
</tr>
<tr>
<td>ISLT Delayed Recall</td>
<td>Total words</td>
<td>(1,152) 0.77</td>
</tr>
<tr>
<td>Speed Comp^</td>
<td>Speed</td>
<td>(1,164) 0.001</td>
</tr>
<tr>
<td>EM Comp</td>
<td>Accuracy</td>
<td>(1,153) 0.06</td>
</tr>
</tbody>
</table>

Note: * indicates p < .05, ** indicates p < .001; 95% CI = 95% confidence intervals

Speed defined as log_{10} milliseconds; Accuracy defined as arcsine proportion correct

CPAL = Continuous Paired Associate Learning task; ISLT = International Shopping List Test; Speed Comp = average of standardised DET and IDN scores; EM Comp = average of standardised OCL, CPAL, ISLT total, and ISLT delayed recall scores

^ positive scores for Detection, Identification, CPAL, and the Speed composite indicates that the low Aβ amyloid group performed worse than the high Aβ amyloid group
Table 9.3. Group raw means (SD) for each assessment time point for low Aβ amyloid and high Aβ amyloid groups.

<table>
<thead>
<tr>
<th></th>
<th>Low Aβ amyloid</th>
<th></th>
<th>Low Aβ amyloid</th>
<th></th>
<th>Low Aβ amyloid</th>
<th></th>
<th>Low Aβ amyloid</th>
<th></th>
<th>Low Aβ amyloid</th>
<th></th>
<th>Low Aβ amyloid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Month 1</td>
<td>Month 2</td>
<td>Month 3</td>
<td>Month 6</td>
<td>Baseline</td>
<td>Month 1</td>
<td>Month 2</td>
<td>Month 3</td>
<td>Month 6</td>
<td>Baseline</td>
<td>Month 1</td>
</tr>
<tr>
<td>DET</td>
<td>2.57 (0.09)</td>
<td>2.59 (0.09)</td>
<td>2.58 (0.09)</td>
<td>2.57 (0.09)</td>
<td>2.53 (0.09)</td>
<td>2.58 (0.10)</td>
<td>2.55 (0.10)</td>
<td>2.52 (0.10)</td>
<td>2.57 (0.10)</td>
<td>2.57 (0.10)</td>
<td>2.57 (0.10)</td>
<td>2.57 (0.10)</td>
</tr>
<tr>
<td>IDN</td>
<td>2.73 (0.06)</td>
<td>2.74 (0.06)</td>
<td>2.75 (0.06)</td>
<td>2.75 (0.06)</td>
<td>2.77 (0.06)</td>
<td>2.76 (0.06)</td>
<td>2.76 (0.06)</td>
<td>2.74 (0.06)</td>
<td>2.76 (0.06)</td>
<td>2.76 (0.06)</td>
<td>2.76 (0.06)</td>
<td>2.76 (0.06)</td>
</tr>
<tr>
<td>OCL</td>
<td>1.00 (0.10)</td>
<td>1.00 (0.10)</td>
<td>1.01 (0.10)</td>
<td>1.04 (0.11)</td>
<td>1.05 (0.11)</td>
<td>0.98 (0.10)</td>
<td>0.99 (0.11)</td>
<td>0.97 (0.13)</td>
<td>0.97 (0.13)</td>
<td>0.91 (0.14)</td>
<td>0.91 (0.14)</td>
<td>0.91 (0.14)</td>
</tr>
<tr>
<td>OBK</td>
<td>1.33 (0.15)</td>
<td>1.38 (0.15)</td>
<td>1.42 (0.15)</td>
<td>1.41 (0.16)</td>
<td>1.37 (0.15)</td>
<td>1.29 (0.15)</td>
<td>1.29 (0.17)</td>
<td>1.26 (0.16)</td>
<td>1.35 (0.17)</td>
<td>1.35 (0.17)</td>
<td>1.35 (0.17)</td>
<td>1.35 (0.17)</td>
</tr>
<tr>
<td>CPAL</td>
<td>36.96 (29.02)</td>
<td>24.03 (29.36)</td>
<td>29.32 (29.36)</td>
<td>27.53 (29.99)</td>
<td>28.10 (30.02)</td>
<td>48.88 (30.61)</td>
<td>74.61 (31.39)</td>
<td>53.69 (31.39)</td>
<td>46.58 (32.21)</td>
<td>52.50 (32.21)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISLT Total</td>
<td>23.99 (4.86)</td>
<td>25.96 (4.89)</td>
<td>25.76 (4.92)</td>
<td>25.22 (4.94)</td>
<td>25.55 (4.89)</td>
<td>23.16 (5.19)</td>
<td>23.46 (5.21)</td>
<td>21.99 (5.21)</td>
<td>24.31 (5.28)</td>
<td>23.54 (5.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISLT Delay</td>
<td>7.87 (2.61)</td>
<td>8.95 (2.63)</td>
<td>8.50 (2.64)</td>
<td>8.66 (2.70)</td>
<td>8.66 (2.66)</td>
<td>7.04 (2.76)</td>
<td>7.54 (2.81)</td>
<td>7.00 (2.81)</td>
<td>6.43 (2.89)</td>
<td>6.24 (3.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed Comp</td>
<td>-0.15 (0.73)</td>
<td>-0.05 (0.73)</td>
<td>-0.05 (0.73)</td>
<td>-0.07 (0.74)</td>
<td>-0.06 (0.73)</td>
<td>-0.08 (0.76)</td>
<td>0.10 (0.78)</td>
<td>-0.11 (0.78)</td>
<td>-0.34 (0.79)</td>
<td>0.04 (0.79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM Comp</td>
<td>-0.05 (0.70)</td>
<td>0.29 (0.70)</td>
<td>0.18 (0.70)</td>
<td>0.28 (0.72)</td>
<td>0.25 (0.70)</td>
<td>-0.26 (0.76)</td>
<td>-0.43 (0.75)</td>
<td>-0.47 (0.76)</td>
<td>-0.37 (0.76)</td>
<td>-0.53 (0.78)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: DET = Detection task; IDN = Identification task; OCL = One Card Learning task; OBK = One Back task; CPAL = Continuous Paired Associate Learning; ISLT Total = International Shopping List Test, Total Recall over 3 learning trials (max = 36 words); ISLT Delay = International Shopping List Test, 20 minute Delayed Recall (max = 12 words); Speed Comp = Speed Composite obtained by averaging standardised DET and IDN scores; EM Comp = Episodic Memory Composite obtained by averaging standardised OCL, CPAL, ISLT total and ISLT delay scores
9.4 Discussion

The results support the hypothesis that healthy older adults with high Aβ amyloid levels show a greater rate of decline in episodic memory than adults with low Aβ amyloid levels over a short time interval (six months). This decline was evident from frequent serial assessments of memory and the statistical comparison of slopes to healthy older adults with low Aβ amyloid levels. In the larger AIBL study cohort studied in Chapters Five and Seven, we also found that in healthy older adults, abnormally high Aβ amyloid was associated strongly with decline in visual and verbal episodic memory over 18 and 36 months. In the current chapter, we also observed that the high Aβ amyloid group showed large decline in visual and verbal episodic memory (OCL, $d = 0.95$; ISLT delayed recall, $d = 0.74$). However unlike in Chapters Five and Seven, no decline was observed for the measures of visual paired associate learning, and verbal learning suggesting that these measures may be less sensitive to AD-related changes in episodic memory over the very short term. However when all memory measures were combined to form an Episodic Memory Composite, the magnitude of difference in slopes between high and low Aβ amyloid groups remained statistically significant and large (i.e., $d = 0.76$; Table 9.2, Figure 9.1). These aspects of the results support the hypothesis that high Aβ amyloid gives rise to a general decline in episodic memory in healthy older adults.

The relatively rapid decline in episodic memory in healthy adults with high Aβ amyloid observed here is consistent with prior studies showing that in other cohorts of healthy older adults with increased Aβ amyloid, performance on these same measures decline abnormally over 12, 18, and 24 months. While the results of this study are preliminary, the rapid decline in episodic memory in healthy older adults with high Aβ amyloid strongly suggests that in these individuals, preclinical AD processes have begun
despite their normal cognitive function, and even when clinical status remains unchanged. Importantly, this can be detected over a relatively short period of time through the use of brief and frequent reassessments. As recent consensus recommendations for the definition of the preclinical stage of AD emphasise the identification of objectively defined cognitive decline,\textsuperscript{74} these results suggest that the detection of cognitive decline, particularly in episodic memory, with putative AD biomarkers, may strengthen the ability to identify early AD processes in the preclinical stage of the disease.
Chapter Ten: Modulation of Aβ Amyloid-Related Cognitive Decline by Brain-Derived Neurotrophic Factor Val66Met Polymorphism in Preclinical Alzheimer’s Disease

10.1 Introduction

Current models of Alzheimer’s disease (AD) emphasise Aβ amyloid as precipitating a cascade of events that result ultimately in synaptic loss and memory impairment, although the processes by which Aβ amyloid disrupts normal synaptic function are not well understood. Growing evidence suggests that brain-derived neurotrophic factor (BDNF) is a downstream mediator of Aβ amyloid toxicity. For example, BDNF and its main receptor, tyrosine-related kinase B (TrkB) are necessary for the synaptic excitation and neuronal plasticity that subserve memory function, and impairment in which is the earliest and most frequent AD symptom. Second, in both AD and its prodromal stage, mild cognitive impairment (MCI), BDNF messenger ribonucleic acid (mRNA) is reduced substantially in the hippocampus and temporal lobe. Furthermore the extent of BDNF loss in these areas is associated with the magnitude of cognitive impairment. Third, in AD mouse models, pharmacologic increases in BDNF can ameliorate synaptic dysfunction and improve memory deficits. Likewise, changes in BDNF secretion occur with improved memory and cognition induced by aerobic exercise in humans at risk for AD and in AD mouse models.

A polymorphism (Val66Met, rs6265, c.196G>A) that affects the secretion of mature BDNF protein has been implicated in learning and memory in healthy humans, where carriage of the Met allele is associated with poor memory performance, reduced hippocampal volumes and lower levels of activation in the hippocampus on functional
imaging.\textsuperscript{27,28,30} In AD, some cross-sectional genetic association studies show that carriage of a Met allele is associated with poorer memory and executive function,\textsuperscript{27,28} reduced regional cerebral blood flow in hippocampal and medial temporal lobe regions,\textsuperscript{27,28} and reductions in volume of hippocampal and prefrontal cortical areas.\textsuperscript{28,251,252} However, other studies observe greater cognitive impairment,\textsuperscript{29,253-255} reduced neuronal activity,\textsuperscript{29,31} and increased risk of AD\textsuperscript{29} in Val/Val homozygotes. The absence of a clear relationship between \textit{BDNF} Val66Met polymorphism and AD could be due to the small samples studied or reflect that other demographic factors such as age\textsuperscript{29} and gender\textsuperscript{256} may moderate relationships between \textit{BDNF} Val66Met polymorphism and AD.

As AD has a long preclinical phase, genetic influences may become clearer from prospective study. Furthermore, as the complexity of AD pathology increases with disease severity, associations between \textit{BDNF} Val66Met polymorphism and AD may be clearer early in the disease, where the clinical presentation is almost exclusively an amnestic syndrome.\textsuperscript{2,56} As recent neuroimaging and cerebrospinal fluid (CSF) analysis studies observed measurable decline in episodic memory (see also Chapters Five to Seven),\textsuperscript{48,50,61,202,257} reduction in hippocampal volume,\textsuperscript{42,61} and increased risk for progression to MCI and AD,\textsuperscript{48,213,214} in healthy older adults with high Aβ amyloid, the aim of this chapter was to determine the extent to which \textit{BDNF} Val66Met polymorphism influences Aβ amyloid-related memory decline, reduction in hippocampal volume, and risk of disease progression in otherwise healthy older adults. We hypothesised that Aβ amyloid-related memory decline, reduction in hippocampal volume, and disease progression in healthy adults will be larger in Met carriers. As BDNF may be a downstream mediator of Aβ amyloid toxicity,\textsuperscript{34} we also hypothesised that \textit{BDNF} Val66Met would not affect the rate of Aβ amyloid accumulation. Finally, we explored
whether changes in other areas of cognition were associated with \textit{BDNF Val66Met} and Aβ amyloid.

### 10.2 Methods

#### 10.2.1 Participants.

Participants were recruited from the healthy older adults (HA) group enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing. The process of recruitment and diagnostic classification of HAs enrolled in the AIBL cohort has been described in detail elsewhere. Participants who volunteered were excluded from the AIBL study if they had any of the following: schizophrenia; depression (15-item Geriatric Depression Score (GDS) of 6 or greater); Parkinson’s disease; cancer (other than basal cell skin carcinoma) within the last two years; symptomatic stroke; uncontrolled diabetes; or current regular alcohol use exceeding two standard drinks per day for women or four per day for men. A clinical review panel chaired by DA reviewed all available medical, psychiatric and neuropsychological information to confirm the cognitive health of individuals enrolled in the HA group. In this study, only HAs who had undergone PiB-PET neuroimaging who had undergone genotyping for \textit{BDNF} and who had undergone at least one cognitive assessment at baseline, 18 months and 36 months were included. Demographic, clinical and lifestyle characteristics of HA are shown in Table 10.1.

The study was approved by and complied with the regulations of the institutional research and ethics committees of Austin Health, St. Vincent’s Health, Hollywood Private Hospital, and Edith Cowan University. All participants provided written informed consent prior to participating in the study.
Table 10.1. Means (SD) for baseline demographic, clinical and lifestyle characteristics of the overall group, and for each Aβ amyloid and BDNF Val66Met group.

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 165)</th>
<th>Low Aβ (n = 116)</th>
<th>High Aβ (n = 49)</th>
<th>Val/Val (n = 107)</th>
<th>Met carrier (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) female</td>
<td>82 (49.70%)</td>
<td>58 (50%)</td>
<td>24 (48.98%)</td>
<td>54 (50.47%)</td>
<td>28 (48.28%)</td>
</tr>
<tr>
<td>N (%) APOE ε4</td>
<td>70 (42.42%)</td>
<td>38 (32.76%)</td>
<td>32 (65.31%)</td>
<td>43 (40.19%)</td>
<td>27 (46.55%)</td>
</tr>
<tr>
<td>N (%) Met carrier</td>
<td>58 (35.15%)</td>
<td>44 (37.93%)</td>
<td>14 (28.57%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.36 (7.15)</td>
<td>70.03 (6.90)</td>
<td>74.51 (6.81)</td>
<td>72.21 (6.96)</td>
<td>69.79 (7.30)</td>
</tr>
<tr>
<td>SUVR Neocortex</td>
<td>1.40 (0.40)</td>
<td>1.17 (0.09)</td>
<td>1.97 (0.27)</td>
<td>1.42 (0.40)</td>
<td>1.37 (0.41)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.75 (1.20)</td>
<td>28.80 (1.20)</td>
<td>28.61 (1.22)</td>
<td>28.81 (1.15)</td>
<td>28.71 (1.24)</td>
</tr>
<tr>
<td>CDR-SB</td>
<td>0.04 (0.19)</td>
<td>0.05 (0.21)</td>
<td>0.02 (0.10)</td>
<td>0.04 (0.20)</td>
<td>0.04 (0.17)</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>108.81 (7.31)</td>
<td>108.14 (7.86)</td>
<td>110.39 (5.57)</td>
<td>109.39 (6.90)</td>
<td>107.72 (7.96)</td>
</tr>
<tr>
<td>HADS-Depression</td>
<td>2.78 (2.29)</td>
<td>2.73 (2.10)</td>
<td>2.90 (2.70)</td>
<td>2.83 (2.31)</td>
<td>2.68 (2.26)</td>
</tr>
<tr>
<td>HADS-Anxiety</td>
<td>4.17 (2.88)</td>
<td>4.11 (2.68)</td>
<td>4.31 (3.32)</td>
<td>4.31 (2.98)</td>
<td>3.91 (2.69)</td>
</tr>
<tr>
<td>MAC-Q (at 36 months)</td>
<td>25.08 (4.41)</td>
<td>25.10 (4.31)</td>
<td>25.03 (4.74)</td>
<td>25.06 (4.25)</td>
<td>25.13 (4.74)</td>
</tr>
<tr>
<td>IPAQ (at 36 months)</td>
<td>4056.49 (3537.80)</td>
<td>3910.72 (3271.87)</td>
<td>4489.11 (4262.37)</td>
<td>4039.07 (3319.71)</td>
<td>4092.63 (3997.44)</td>
</tr>
</tbody>
</table>

Note: One-Way ANOVA indicated that age was significantly different between low Aβ and high Aβ groups, and premorbid IQ was significantly different between Val/Val and Met carriers, p's < .05. χ² also indicated that number of APOE ε4 carriers were higher in the high Aβ group compared to the low Aβ group, p < .001. No other variables shown in the table differed by Aβ amyloid status or BDNF Val66Met polymorphism, all p's > .05.

SUVR = Standardised Uptake Value Ratio; MMSE = Mini-Mental State Examination; CDR-SB = Clinical Dementia Rating Scale, Sum of Boxes Score; HADS-Depression = Hospital Anxiety and Depression Scale, Depression Subscale; HADS-Anxiety = Hospital Anxiety and Depression Scale, Anxiety Subscale; MAC-Q = Memory Complaint Questionnaire; IPAQ = International Physical Activity Questionnaire
10.2.2 Measures.

10.2.2.1 PiB-PET neuroimaging.

PiB-PET neuroimaging methodology has been outlined in detail previously.\textsuperscript{41,110} Each subject received ~370 MBq 11C-PiB intravenously over 1 minute. A 30-minute acquisition of PET standardised uptake value (SUV) data in 3-dimensional mode, consisting of 6 frames each of 5 minutes, acquired 40-70 minutes post-PiB injection were summed and normalised to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVR). Neocortical Aβ burden was expressed as the average SUVR of the area-weighted mean of frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions.\textsuperscript{41,110} In this study, we have classified high Aβ amyloid levels based on an SUVR of 1.5 or more.

10.2.2.2 BDNF and APOE genotyping.

An 80ml blood sample was taken from each participant, 0.5ml of which was forwarded for APOE genotyping at a clinical pathology laboratory at baseline. At the 18-month time point, a further 10ml of whole blood was used for large scale DNA extraction for AIBL bio-banking purposes. This DNA was extracted via the QIAamp\textsuperscript{®} DNA Blood Maxi Kit (QIAGEN Group) using manufacturer’s instructions (spin protocol). The BDNF Val66Met polymorphism (rs6265) was included in a custom Illumina GoldenGate assay, which included 1536 SNPs, and was performed by the Beijing Genomics Institute (BGI-Hong Kong). The BDNF Val66Met polymorphism had a call rate of greater than 99% and did not depart from Hardy-Weinberg equilibrium in the AIBL healthy older adults group ($p = .57$).
10.2.2.3 Magnetic resonance imaging.

Participants underwent a clinical magnetic resonance imaging (MRI) for screening and subsequent co-registration with the PET images. A fluid attenuated inversion recovery sequence was obtained for exclusion of subjects with cortical stroke. MRI imaging analysis has been described in detail elsewhere.\textsuperscript{48, 167} MR images were spatially normalised to the Montreal Neurological Institute (MNI) single-subject MRI brain template\textsuperscript{258} using MilxView\textsuperscript{®}, software developed by the Australian e-Health Research Centre – BioMedIA (Brisbane, Australia). As described elsewhere, T1W MR images for each subject were classified into grey matter (GM), white matter (WM) and CSF using an implementation of the expectation maximisation segmentation algorithm.\textsuperscript{259} The algorithm computed probability maps for each tissue type and was used to assign each voxel to its most likely tissue type and subsequent segmentation. To improve the accuracy of analysis of the hippocampus, a separate, manually-delineated template was drawn on the MNI single-subject every 1 mm on coronal slices, and was subsequently used for hippocampal volume. The average hippocampal volumes were normalised for head size using the total intracranial volume, defined as the sum of GM, WM and CSF volumes.

10.2.2.4 Cognitive assessments.

All participants were assessed with the clinical rating scales, neuropsychological battery, and computerised cognitive battery from the AIBL study (see Chapters Two and Four). These have all been described in detail elsewhere and were administered according to standard protocols by trained research assistants.\textsuperscript{86, 126, 172} Premorbid intelligence was estimated using the Wechsler Test of Adult Reading (WTAR),\textsuperscript{141} and
levels of depressive and anxiety symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS). \(^{142}\)

**10.2.2.5 Lifestyle assessments.**

All participants completed the Memory Complaint Questionnaire (MAC-Q) \(^{260}\) and the International Physical Activity Questionnaire (IPAQ) \(^{261}\) at 36 months.

**10.2.3 Procedure.**

Participants in this study underwent an extensive medical, psychiatric, and neuropsychological assessment upon enrolment into the AIBL study. The same assessments were repeated 18 and 36 months after baseline. In this study, we report PiB-PET neuroimaging data obtained at baseline and neuropsychological data obtained at baseline, 18 months and 36 months in order to examine the rate of cognitive change in relation to Aβ amyloid and \(BDNF\) \text{Val}66\text{Met} polymorphism at entry to AIBL. We then report PiB-PET neuroimaging data obtained at baseline, 18 months and 36 months, in order to examine the rate of Aβ amyloid accumulation in relation to \(BDNF\) \text{Val}66\text{Met} polymorphism.

**10.2.4 Data analysis.**

Composite scores were computed by standardising outcome measures on individual tests against the baseline mean and SD for the entire group and then averaging these to compute a composite score for *episodic memory* (Rey Complex Figure Test [RCFT, 3 minute and 30 minute] delay, California Verbal Learning Test, Second Edition [CVLT-II] total, short delay, and long delay recall, Logical Memory 1 and 2, and the CogState One Card Learning task); *executive function* (Letter Fluency, Category
Fluency Accuracy, Category Fluency Switching, Stroop Colours/Dots, and the CogState One Back task); *language* (Boston Naming Task, and Category Fluency Total) and *attention* (Digit Symbol, CogState Detection and Identification tasks).

Consistent with observations from other studies, the distribution of PiB SUVR data was skewed negatively and could not be normalised with data transformations. Thus, SUVR was classified dichotomously as either low Aβ (SUVR < 1.5) or high Aβ (SUVR ≥ 1.5) in accordance with established criteria.

Data for composite cognitive test scores, hippocampal volume and Aβ amyloid levels for each participant across baseline, 18 and 36 month assessments were submitted to a series of repeated-measures linear mixed model (LMM) analyses of covariance (ANCOVA). We examined the relation between *BDNF* Val66Met polymorphism (Met carrier, Val/Val homozygote), baseline Aβ amyloid level (high Aβ, low Aβ), and time (baseline, 18, and 36 month) on composite cognitive test scores, hippocampal volume, and Aβ amyloid accumulation. *BDNF*, baseline Aβ amyloid, time, *BDNF* x Aβ amyloid interaction, *BDNF* x time interaction, Aβ amyloid x time interaction, and *BDNF* x Aβ amyloid x time interaction were entered as fixed factors; participant as a random factor; age as the only covariate, and composite cognitive test score, hippocampal volume, and Aβ accumulation as dependent variables in separate analyses. These analyses generated an estimate of the rate of change (i.e., slope) over the three assessments. Consequently, for each of the outcome measure, three planned comparisons of slopes were conducted within the LMM in accordance with our study hypotheses. Specifically, slopes were compared between a) high Aβ Val/Val homozygotes and high Aβ Met carriers, b) low Aβ Val/Val homozygotes and low Aβ Met carriers and c) low Aβ Met carriers and high Aβ Met carriers. The magnitude of difference in the rates of change
(slopes) of each group was expressed using Cohen’s $d$. Because premorbid intelligence and depressive symptoms have been linked to $BDNF$ Val66Met polymorphism, all LMMs were recomputed with premorbid IQ and depression included as additional covariates. $APOE$ $\varepsilon 4$ status was not included as a covariate in these analyses because the number of high Aβ Met carriers who were $\varepsilon 4$ carriers (n=11) and non-carriers (n=3) was very small and the proportion of $APOE$ $\varepsilon 4$ carriers was equivalent in Met carriers and Val/Val homozygotes (Table 10.1). Finally, odds ratios (ORs) and their 95% confidence intervals (CIs) estimated the extent to which the $BDNF$ Met allele increased risk of progression to MCI or AD at 36 months.

10.3 Results

10.3.1 Demographic and clinical characteristics.

Demographic and clinical characteristics of the overall sample of HAs are shown in Table 10.1. Table 10.1 also shows demographic and clinical characteristics of HAs split by whether they had high or low Aβ amyloid at baseline, and whether they were Met carriers, or Val/Val homozygotes. Groups did not differ on any demographic or clinical characteristic; however, high Aβ HAs were older than low Aβ HAs, and Val/Val homozygotes had higher premorbid IQ than met carriers. While the frequency of the $APOE$ $\varepsilon 4$ allele was higher in the high Aβ group compared to the low Aβ group, $\chi^2 = 14.94, p < .001$, Cramér’s $V = .30$; there was no difference in the frequency of the $APOE$ $\varepsilon 4$ allele between Met carriers and Val/Val homozygotes, $\chi^2 = 0.62, p = .43$, Cramér’s $V = .06$. At the 36 month assessment, the study groups did not differ on their level of subjective memory complaint or level of physical activity (Table 10.1).
10.3.2 Effect of BDNF Val66Met polymorphism on cognitive decline and hippocampal atrophy in HAs with high Aβ

Relative to the high Aβ Val/Val homozygote group, high Aβ Met carriers showed a greater rate of decline on the episodic memory, executive function and language composite test scores over 36 months (Table 10.2, Figures 10.1-10.3), with effect sizes for differences between slopes moderate to large in magnitude. Groups did not differ on the rate of decline on the attention composite (Figure 10.4). High Aβ Met carriers also showed a greater rate of reduction in hippocampal volume, with the effect sizes for the difference between groups moderate in magnitude (Table 10.2, Figure 10.5).

Re-analysis with premorbid IQ and depression entered as additional covariates did not change results, with magnitudes of the difference between groups in the rates of decline increasing slightly for the episodic memory, executive function and language composites and for rate of reduction in hippocampal volume (Table 10.3).

10.3.3 Effect of BDNF Val66Met polymorphism on change in cognition and hippocampal volume in HAs with low Aβ

Relative to low Aβ Val/Val homozygotes, low Aβ Met carriers did not differ in their rate of change for any composite cognitive measure or hippocampal volume over 36 months with the effect sizes for these differences small in magnitude (Table 10.2). Re-analysis with premorbid IQ and depression entered as additional covariates did not change this result.
10.3.4 Effect of Aβ amyloid on cognitive decline and hippocampal atrophy in Met carriers

Relative to low Aβ Met carriers, high Aβ Met carriers showed a greater rate of decline on the episodic memory composite only at 36 months (Table 10.2), with the effect size for this difference large in magnitude. High Aβ Met carriers also showed greater reduction in hippocampal volume, and the effect size for this difference was large.

10.3.5 Effect of Aβ amyloid and BDNF Val66Met polymorphism on progression to MCI or AD at 36 months

At the 36 month assessment, the number of high Aβ HAs who progressed to meet clinical criteria for MCI or AD (13% [n = 5 of 39]) was higher than for low Aβ HAs (7% [n = 5 of 39]), OR (95%CI) = 2.25 (0.67, 7.54). Within the high Aβ group, progression to MCI or AD at 36 months was greater in Met carriers (25% [n = 3 of 12]) than in Val/Val homozygotes (7% [n = 2 of 27]), OR (95%CI) = 4.17 (0.60, 29.13).

10.3.6 Effect of BDNF Val66Met on the rate of Aβ amyloid accumulation

Comparison of slopes over 36 months indicated no differences in the rate of Aβ amyloid accumulation between high Aβ Met carriers and high Aβ Val/Val homozygotes, or between low Aβ Met carriers and low Aβ Val/Val homozygotes (Figure 10.6). However, high Aβ Met carriers showed a greater rate of Aβ amyloid accumulation than low Aβ Met carriers.
Table 10.2. Mean slopes (SD) for neuropsychological composites and neuroimaging measures; magnitude of difference in slopes (age as covariate)

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<tbody>
<tr>
<td>Neuropsychological</td>
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<tr>
<td>Episodic Memory</td>
<td>-0.067 (0.362)</td>
<td>-0.043 (0.378)</td>
<td>-0.230 (0.357)</td>
<td>-0.470 (0.308)</td>
<td>-0.07 (-0.44, 0.31)</td>
<td>0.70 (0.03, 1.34)</td>
<td>1.17 (0.50, 1.81)</td>
</tr>
<tr>
<td>Executive Function</td>
<td>-0.098 (0.387)</td>
<td>-0.105 (0.406)</td>
<td>0.007 (0.386)</td>
<td>-0.291 (0.320)</td>
<td>0.02 (-0.35, 0.39)</td>
<td>0.81 (0.14, 1.45)</td>
<td>0.48 (-0.15, 1.09)</td>
</tr>
<tr>
<td>Language</td>
<td>0.023 (0.018)</td>
<td>0.057 (0.155)</td>
<td>0.082 (0.144)</td>
<td>-0.015 (0.120)</td>
<td>-0.35 (-0.73, 0.02)</td>
<td>0.70 (0.04, 1.34)</td>
<td>0.49 (-0.14, 1.10)</td>
</tr>
<tr>
<td>Attention</td>
<td>0.103 (0.343)</td>
<td>0.037 (0.361)</td>
<td>0.056 (0.342)</td>
<td>-0.005 (0.288)</td>
<td>0.19 (-0.19, 0.56)</td>
<td>0.19 (-0.46, 0.82)</td>
<td>0.12 (-0.50, 0.74)</td>
</tr>
<tr>
<td>Neuroimaging</td>
<td></td>
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<tr>
<td>Hippocampal Volume</td>
<td>-0.034 (0.048)</td>
<td>-0.027 (0.048)</td>
<td>-0.047 (0.048)</td>
<td>-0.081 (0.041)</td>
<td>-0.15 (-0.54, 0.25)</td>
<td>0.73 (0.04, 1.40)</td>
<td>1.16 (0.49, 1.79)</td>
</tr>
<tr>
<td>Aβ accumulation</td>
<td>0.016 (0.045)</td>
<td>0.013 (0.047)</td>
<td>0.058 (0.046)</td>
<td>0.037 (0.040)</td>
<td>0.07 (-0.31, 0.44)</td>
<td>0.47 (-0.18, 1.11)</td>
<td>0.53 (-0.10, 1.14)</td>
</tr>
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</table>

Note: Bolded values indicate significant comparisons

Table 10.3. Mean slopes (SD) for neuropsychological composites and neuroimaging measures; magnitude of difference in slopes (age, premorbid and depression as covariates)

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<tr>
<td>Neuropsychological</td>
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<tr>
<td>Episodic Memory</td>
<td>-0.057 (0.358)</td>
<td>-0.024 (0.374)</td>
<td>-0.219 (0.355)</td>
<td>-0.468 (0.304)</td>
<td>-0.09 (-0.46, 0.28)</td>
<td>0.73 (0.06, 1.37)</td>
<td>1.23 (0.56, 1.87)</td>
</tr>
<tr>
<td>Executive Function</td>
<td>-0.095 (0.389)</td>
<td>-0.100 (0.408)</td>
<td>0.003 (0.389)</td>
<td>-0.297 (0.334)</td>
<td>0.01 (-0.36, 0.38)</td>
<td>0.80 (0.13, 0.44)</td>
<td>0.50 (-0.13, 1.12)</td>
</tr>
<tr>
<td>Language</td>
<td>0.020 (0.152)</td>
<td>0.059 (0.159)</td>
<td>0.092 (0.150)</td>
<td>-0.016 (0.128)</td>
<td>-0.25 (-0.62, 0.12)</td>
<td>0.75 (0.08, 1.39)</td>
<td>0.49 (-0.14, 1.11)</td>
</tr>
<tr>
<td>Attention</td>
<td>0.105 (0.343)</td>
<td>0.041 (0.361)</td>
<td>0.058 (0.343)</td>
<td>-0.012 (0.299)</td>
<td>0.18 (-0.19, 0.55)</td>
<td>0.21 (-0.43, 0.85)</td>
<td>0.15 (-0.47, 0.77)</td>
</tr>
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<td>Neuroimaging</td>
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<tr>
<td>Hippocampal Volume</td>
<td>-0.034 (0.049)</td>
<td>-0.027 (0.054)</td>
<td>-0.048 (0.050)</td>
<td>-0.084 (0.041)</td>
<td>-0.14 (-0.51, 0.24)</td>
<td>0.75 (0.06, 1.42)</td>
<td>1.11 (0.44, 1.74)</td>
</tr>
<tr>
<td>Aβ accumulation</td>
<td>0.018 (0.045)</td>
<td>0.012 (0.047)</td>
<td>0.058 (0.046)</td>
<td>0.036 (0.040)</td>
<td>0.13 (-0.24, 0.50)</td>
<td>0.49 (-0.16, 1.13)</td>
<td>0.50 (-0.13, 1.12)</td>
</tr>
</tbody>
</table>

Note: Bolded values indicate significant comparisons
Figure 10.1. Trajectories of change in Episodic Memory Composite for low Aβ Val/Val homozygotes, low Aβ Met carriers, high Aβ Val/Val homozygotes, and high Aβ Met carriers, with age, premorbid IQ and depression as covariates (error bars represent 95% confidence intervals)
Figure 10.2. Trajectories of change in Executive Function Composite for low Aβ Val/Val homozygotes, low Aβ Met carriers, high Aβ Val/Val homozygotes, and high Aβ Met carriers, with age, premorbid IQ and depression as covariates (error bars represent 95% confidence intervals)
Figure 10.3. Trajectories of change in Language Composite for low Aβ Val/Val homozygotes, low Aβ Met carriers, high Aβ Val/Val homozygotes, and high Aβ Met carriers, with age, premorbid IQ and depression as covariates (error bars represent 95% confidence intervals)
Figure 10.4. Trajectories of change in Attention Composite for low Aβ Val/Val homozygotes, low Aβ Met carriers, high Aβ Val/Val homozygotes, and high Aβ Met carriers, with age, premorbid IQ and depression as covariates (error bars represent 95% confidence intervals)
Figure 10.5. Trajectories of change in hippocampal volume for low Aβ Val/Val homozygotes, low Aβ Met carriers, high Aβ Val/Val homozygotes, and high Aβ Met carriers, with age, premorbid IQ and depression as covariates (error bars represent 95% confidence intervals)
Figure 10.6. Trajectories of change in Aβ amyloid accumulation for low Aβ Val/Val homozygotes, low Aβ Met carriers, high Aβ Val/Val homozygotes, and high Aβ Met carriers, with age, premorbid IQ and depression as covariates (error bars represent 95% confidence intervals)
10.4 Discussion

The hypothesis that BDNF Val66Met polymorphism would moderate Aβ amyloid-related memory decline, reduction in hippocampal volume, and risk of disease progression in HAs was supported. In HAs for whom PiB-PET neuroimaging indicated high levels of Aβ amyloid at baseline, individuals who carried a Met allele showed greater decline in episodic memory, executive function and language when compared to Val/Val homozygotes. For each aspect of cognition that showed decline, difference in the rates of decline between BDNF Val66Met polymorphism groups was, by convention, moderate to large in magnitude. In contrast, HAs with low Aβ amyloid levels showed no decline in cognition over the 36 months, irrespective of BDNF Val66Met polymorphism. Importantly, Val/Val homozygosity did not protect against Aβ amyloid-related cognitive decline, as HAs with this polymorphism who had high Aβ amyloid showed substantial decline in memory when compared to HAs with low Aβ amyloid levels (e.g. Figure 10.1). Finally, despite showing substantial decline in memory over the three years, HAs with high Aβ amyloid levels did not report higher levels of subjective memory complaints than those with low Aβ amyloid, irrespective of BDNF Val66Met polymorphism (Table 10.1). Thus, this prospective study shows that the BDNF Val66Met polymorphism moderates Aβ amyloid-related cognitive decline in HAs and this occurred despite individuals having no subjective sense of any memory impairment.

In HAs with high baseline Aβ amyloid levels, the rate of reduction in hippocampal volume was significantly greater in those who carried the Met allele than in Val/Val homozygotes. No effect of the BDNF Val66Met polymorphism on hippocampal volume was observed for adults with low Aβ amyloid. As with episodic memory, Val/Val homozygosity did not protect against Aβ amyloid-related decline. Hippocampal volume
reduced at a greater rate over 36 months in Val/Val homozygotes who also had high Aβ amyloid levels than adults with low Aβ amyloid, irrespective of their BDNF Val66Met polymorphism. Additionally, at the 36 month assessment, high Aβ amyloid and Met carriage was associated with a four-fold risk of progression to MCI or AD when compared to high Aβ amyloid Val/Val homozygotes. While Aβ amyloid-related decline in memory, reduction in hippocampal volume, and progression to MCI or AD have been reported previously in HAs (see also Chapters Five and Seven), this study shows for the first time that Aβ amyloid-related changes in cognitive function, brain structure and disease progression are worsened by BDNF Val66Met polymorphism in the very earliest stages of AD. Importantly, the finding that high Aβ amyloid was a necessary condition for BDNF Val66Met polymorphism-moderated reductions in hippocampal volume and cognitive decline suggests strongly that BDNF Val66Met is a downstream moderator of the effect of Aβ amyloid on hippocampal function.

The data also supported the hypothesis that BDNF Val66Met polymorphism would not moderate the rate of Aβ amyloid accumulation in HAs. While the rate of Aβ amyloid accumulation was greater in healthy older adults whose Aβ amyloid level was high at baseline, the rate of Aβ amyloid accumulation was not moderated by BDNF Val66Met (Tables 10.2, 10.3; Figure 10.6). Thus, while high Aβ amyloid gave rise to cognitive decline and reduction in hippocampal volume, the deleterious effects were reduced in individuals who carried the BDNF Val66Met polymorphism that has been associated with greater secretion of the BDNF protein (i.e., Val/Val homozygotes). These data accord with findings from animal studies, which have similarly found that the secretion of mature BDNF plays a crucial role in the neuronal integrity of the hippocampus.
and that Aβ decreases BDNF levels by reducing phosphorylated cAMP response element binding protein, which in turn regulates BDNF transcript expression, and by affecting BDNF retrograde axonal transport which also affects synaptic function. Furthermore, human and animal neuropathological studies show that interactions between BDNF Val66Met polymorphism and Aβ amyloid-related synaptic changes occur in the hippocampus and that these changes are related directly to memory. Finally, genetic databases do not indicate BDNF Val66Met to increase risk for AD. Collectively, the results suggest that while BDNF secretion is unrelated to levels of Aβ amyloid, it does moderate the extent to which Aβ amyloid affects brain structure and cognitive function, at least in the very early stages of AD.

Previous studies have identified no clear evidence of any relationships between BDNF Val66Met polymorphism and cognitive impairment or risk for AD. Some cross-sectional studies have reported that the Met allele is associated with impairment in memory and executive function, or with neuronal dysfunction. However, others have observed that in older adults, Val/Val homozygosity was associated with increased susceptibility to AD, and even greater cognitive decline. Importantly, because none of these studies measured Aβ amyloid levels, differences in the relationships between BDNF Val66Met polymorphism and brain structure or function may reflect variability in the number of older adults with high Aβ amyloid included in samples, especially given that the sample sizes of these studies have been small and neuroimaging studies identify that up to 30% of HAs have high Aβ amyloid levels. In this study, Aβ amyloid levels were determined through PiB-PET neuroimaging, although we expect that similar results would be observed with Aβ amyloid levels measured through cerebrospinal fluid analysis.
Several studies suggest that sociodemographic and clinical factors such as premorbid intelligence and levels of depression interact significantly with BDNF Val66Met to influence cognitive function.\textsuperscript{262} Re-analysis of the data with the effects of premorbid intelligence and depression controlled statistically did not alter the results; in fact, the strength of the relationship between BDNF Val66Met polymorphism and A\textbeta{} amyloid on cognitive function and hippocampal volume increased slightly. While this finding may suggest that the effect of BDNF Val66Met on A\textbeta{} amyloid-related cognitive decline, reductions in HV, and progression to MCI or AD is independent of these factors, this result may be because HAs enrolled in the AIBL study are generally of high intelligence, and have undergone careful screening for any recent or existing psychiatric illnesses.\textsuperscript{126}

Related to this is the interest in cognitive reserve, and the hypothesis that in some people, pre-existing brain networks are more efficient or have greater capacity and are therefore less susceptible to AD-related deterioration.\textsuperscript{19} Currently, cognitive reserve has been defined by premorbid intelligence, years of education or occupational complexity, and each of these factors has been associated with later onset of clinically diagnosed AD, as well as protection against A\textbeta{} amyloid-related cognitive impairment, although the magnitude of this protection is generally only small in magnitude (i.e., Cohen’s $d$ from 0.30 – 0.38).\textsuperscript{19,36,37,39} The results of the present study suggest that genetic factors, in particular the absence of the Met allele, may play a greater role in the brain’s ability to withstand A\textbeta{} amyloid-related neurotoxicity.

Along with the results of Chapters Five to Nine, the results of this chapter lend strong support to the importance of the detection of objectively defined decline in cognition to the early detection of AD. Models of AD have typically defined cognitive abnormality as cognitive impairment, where performance of individuals on neuropsychological tests is
compared to normative control data. However, it is clear from the current and previous chapters which have focused on cognitive decline that measurable decline in episodic memory becomes apparent very early in the disease, often years before individuals meet any clinical criteria for MCI. The emphasis on the measurement and detection of cognitive change, as opposed to cognitive impairment, as a neuropsychological approach to identifying AD in the earliest stages has been raised previously, and has been recently acknowledged as an important area for further consideration by the National Institute on Ageing and the Alzheimer’s Association workgroup guidelines on the definition of the preclinical stage of AD.

An important caveat for interpretation of these results is that HAs enrolled in AIBL were highly educated, and had few existing or untreated medical or psychiatric illnesses. As such, it would be important for these findings to be replicated in individuals with high Aβ amyloid in population-based studies, such as the Mayo Clinic Study of Ageing and the 10/66 Dementia Research Group, where it is possible that the effect of BDNF Val66Met polymorphism on Aβ-related decline may be greater than that observed here.

A second caveat is we only investigated indirect interactions between APOE, BDNF Val66Met polymorphism, Aβ amyloid and cognitive decline. This was primarily due to the small sample sizes, equivalence in the proportion of APOE ε4 carriers in Met carriers and Val/Val homozygotes, and the observation by previous studies that APOE ε4 does not interact with BDNF levels to affect cognitive function. Further, we have also observed in Chapters Five and Seven that APOE ε4 carriage did not influence cognitive decline, and did not moderate Aβ amyloid-related memory decline. The hypothesis that has arisen from this and previous studies is that while carrying the APOE ε4 allele increases the risk of AD processes beginning, it does not moderate disease progression.
at a clinical level. However, further investigation of the relationship between APOE, BDNF Val66Met polymorphism and Aβ amyloid on change in cognitive function needs to be conducted in larger prospective studies of APOE allele groups.\textsuperscript{55, 206} Finally, given the small number of Met/Met homozygotes who had high Aβ amyloid (n=3), we were unable to investigate whether there was a Met gene-dose effect on Aβ amyloid-related cognitive decline. It will be important for future studies to explore this as a greater decline in Met/Met homozygotes would further suggest of the protective effects of the Val allele on Aβ amyloid neurotoxicity in synaptic function. Notwithstanding these caveats, results of this study provide strong support that Met carriage in combination with high Aβ amyloid may serve as important prognostic markers of accelerated cognitive decline in individuals in the preclinical stage of AD, and that methods of increasing BDNF levels may be a potential therapeutic strategy for MCI and AD.
Chapter Eleven: General Discussion

Alzheimer's disease (AD) is a neurodegenerative disease characterised clinically by progressive decline in memory, other cognitive functions, behaviour, and ability to conduct activities of daily living.\textsuperscript{3,134} Although there currently exists no accepted biological test for AD, neuropathological studies indicate a long preclinical period in AD, with accumulation of brain pathology occurring up to 20 years prior to the detection of any signs of cognitive impairment.\textsuperscript{53, 268} Reviews of neuropsychological studies also suggest that when groups of cognitively healthy older adults are followed for several years, a proportion show decline in cognitive function, particularly in memory.\textsuperscript{12, 96, 128} Recent recommendations by the National Institute on Ageing and the Alzheimer's Association workgroup guidelines on the research criteria for the definition of the preclinical stage of AD also emphasise that individuals who carry a biomarker of AD and who demonstrate very subtle decline not yet meeting standardised criteria for mild cognitive impairment (MCI) are at risk for progression to AD.\textsuperscript{74} Thus, the main aim of the studies conducted in this thesis was to characterise the nature and magnitude of cognitive change in the preclinical and prodromal stages of AD and its relation to a known marker of AD pathology, beta-amyloid (A\textbeta{}).

11.1 Summary of Findings

Previous studies that have modelled rates of cognitive change in the early stages of AD, have conducted assessments over relatively long test-retest intervals.\textsuperscript{82, 96, 98, 104, 105} This has been because the neuropsychological test batteries used are generally very long,\textsuperscript{92} and most measures of cognitive function can give rise to substantial practice effects.\textsuperscript{115-119} In appreciating these limitations, a computerised cognitive battery that was brief to administer, possessed good test-retest reliability, generated little to no practice
effects,\textsuperscript{101, 117, 119} and has been shown repeatedly to be sensitive to cognitive change in preclinical AD as well as other cognitive disorders\textsuperscript{122-125} was used. However, while the computerised battery has been shown to be sensitive to cognitive change, its ability to assess impairments in cognitive function, particularly in AD and its prodromal stage, amnestic MCI (aMCI), has yet to be established. As such, the validity of the use of this battery to assess cognitive function in AD and aMCI, was first established in the Australian Imaging, Biomarkers, and Lifestyle (AIBL) study (Chapter Two).

12.1.1 Cross-sectional investigations of the relationship between Aβ amyloid and cognitive function

In healthy older adults, when cognition is considered at a single assessment, the presence of high levels of Aβ amyloid does not manifest as impairment in any aspect of cognitive function (Chapter Four). However, when levels of Aβ amyloid were considered as a continuous rather than a categorical measure, higher levels of Aβ amyloid were moderately associated with worse visual and verbal episodic memory, although this relation was only evident for healthy older adults who carried the apolipoprotein E (APOE) ε4 allele (Chapter Three). This confirms the hypothesis that APOE ε4 carriage increases the risk of Aβ amyloid-related abnormalities in cognitive performance. To further understand the nature of cognitive impairments in AD, the large AIBL neuropsychological test battery along with the brief computerised battery was used to characterise the nature and magnitude of Aβ amyloid-related cognitive impairments in healthy older adults and adults with aMCI. When compared to healthy older adults with low Aβ amyloid levels, adults with aMCI showed large impairments in memory, irrespective of whether Aβ amyloid levels were high or low. This was not surprising considering the inclusion criteria for aMCI in the AIBL study included impairment of at
least 1.5 standard deviations below population-based means on two or more memory measures. However, while cognitive impairment in adults with aMCI and high Aβ amyloid was restricted to episodic memory, aMCI with low Aβ amyloid was also characterised by large impairments in language, executive function and attention (Chapter Four). This observation accords with suggestions that only adults with aMCI and high Aβ amyloid have incipient AD, while the more extensive cognitive impairment that occurs in adults with aMCI and low Aβ amyloid suggests the existence of different or additional neurological or psychiatric processes. However, as AD is a neurodegenerative disease, the measurement of cognitive change over time may improve the ability to differentiate individuals at risk of developing AD from healthy controls in the early stages of the disease.

12.1.2 Prospective investigations of the relationship between Aβ amyloid and cognitive function

When studied prospectively over 18 and 36 months using the computerised battery, healthy older adults with a baseline classification of high Aβ amyloid showed large magnitude declines on measures of visual and verbal episodic memory when compared to healthy older adults with low Aβ amyloid, who showed no decline (Chapters Five and Seven), and this Aβ amyloid-related decline was associated with higher rates of progression to MCI, presumably as part of a progression to AD (Chapter Seven). This suggests strongly that the combination of decline in memory with parameters from amyloid imaging may be useful for the identification of AD processes in individuals who do not yet meet any clinical criteria for cognitive impairment. In order to further test the hypothesis that high Aβ amyloid levels are indicative of incipient AD, adults with aMCI were followed prospectively over 18 and 36 months. Adults with aMCI and high
Aβ amyloid showed substantial decline in visual and verbal episodic memory over 18 and 36 months (Chapters Six and Seven); however, in adults with aMCI and low Aβ amyloid, cognitive impairment remained stable over this time. This finding lends further support to the hypothesis that adults with aMCI and low Aβ amyloid may have other underlying pathology other than AD.

As Aβ amyloid-related cognitive decline has been observed to occur over 18 and 36 months even with no change in an individual’s clinical status (Chapters Five-Seven), one challenge was to determine how quickly Aβ amyloid-related cognitive decline can be identified in healthy older adults. The Australian Imaging, Biomarkers, and Lifestyle-Rate of Change Sub-study (AIBL-ROCS) was designed to optimise sensitivity to change in cognition by conducting frequent assessments with a brief computerised battery. These would form as multiple data-points for each individual provided reliable estimate of the slopes of the relationships between cognition and time (Chapter Eight). Through serial assessments of healthy older adults, relatively rapid decline of moderate magnitude in episodic memory was detected in individuals with high Aβ amyloid over 6 months, further supporting the identification of objectively defined cognitive decline in the definition of the preclinical stage of AD (Chapter Nine).

12.1.3 Genetic contributions to the relationship between Aβ amyloid and cognitive function

Consistent with the findings of previous studies, carriage of the APOE ε4 allele increased the likelihood of individuals having high Aβ amyloid levels. However, prospective study of healthy older adults and adults with aMCI indicated that APOE ε4 carriage does not moderate the relationship between Aβ amyloid and cognitive decline (Chapters Five and Seven), and does not influence cognitive decline (Chapter Seven). This suggests that
while carrying the \textit{APOE} ε4 allele increases the risk of AD processes beginning, it does not moderate disease progression; at least on a clinical level.

Another genetic influence that has received growing interest in AD studies is the brain-derived neurotrophic factor (\textit{BDNF}) Val66Met polymorphism as BDNF has been implicated as necessary for the synaptic excitation and neuronal plasticity that subserves memory function.\textsuperscript{27, 34, 245} In healthy older adults who also have high Aβ amyloid levels, carrying a Met allele was associated with moderate decline in episodic memory, reductions in hippocampal volume and increased risk of progression to MCI when compared to Val/Val homozygotes (Chapter Ten). In contrast, \textit{BDNF} Val66Met polymorphism had no effect on memory, hippocampal volume, or risk of disease progression in individuals with low Aβ amyloid. Importantly though, \textit{BDNF} Val66Met polymorphism did not affect rates of Aβ amyloid accumulation. Taken together, these results suggest that while \textit{BDNF} Val66Met is unrelated to Aβ amyloid accumulation, it does accentuate the extent to which Aβ amyloid affects brain structure and cognitive function, at least in the early stages of AD.

\textbf{12.2 \hspace{1em} Implications for the preclinical and prodromal stages of Alzheimer's disease}

The assessment of cognitive function in a single session is not sensitive enough to disambiguate the subtle effects of high Aβ amyloid levels in healthy older adults using population-based normative data, although consideration of \textit{APOE} ε4 carriage improves the Aβ amyloid signal slightly. As expected, large and reliable cognitive impairment can be detected in adults with aMCI, although it appears that aMCI with low Aβ amyloid is characterised by a different cognitive profile than aMCI with high Aβ amyloid. Most importantly though, the relationship between Aβ amyloid and cognitive function
becomes clearer in both the preclinical and prodromal stages of AD through prospective study. Repeated administration of cognitive tests allows detection of decline in episodic memory in both healthy older adults and adults with aMCI who have high Aβ amyloid levels. The consistent finding that high Aβ amyloid levels gives rise to faster decline in episodic memory suggests that high Aβ amyloid in healthy older adults indicates that the AD pathophysiological process has begun, but remains preclinical and minimally symptomatic. Further, the Aβ amyloid-related decline in episodic memory in aMCI suggests that AD pathogenesis is likely to continue at the same rate throughout the prodromal stage of the disease.

12.3 Implications for future clinical trials of preclinical Alzheimer's disease

When taken together, the results of this thesis strongly suggest that the pathophysiological processes of AD begin many years before a clinical diagnosis, and that healthy older adults with high Aβ amyloid and objectively defined decline in memory are a worthy target for anti-amyloid therapies seeking to modify or halt the progression of AD in the very early stages of the disease. Currently, no pharmacological treatment has been shown to protect neuronal degradation; however, acetylcholinesterase inhibitors (donepezil, rivastigmine, and galantamine) that inhibit the degradation of acetylcholine within neuronal synapses have been shown to slow, but not halt, the symptomatic progression of the disease.\textsuperscript{269, 270} This is compounded by the recent disappointing results of bapineuzumab in Phase III clinical trials to modify cognitive and functional endpoints of patients with mild AD, although some reduction in cognitive decline was observed for solanezumab.\textsuperscript{271, 272}

Many researchers have posited that this may be due in part to the design of clinical trials, as most are aimed directly at the mild to moderate AD stage only, where the
neurodegenerative process is already established and widespread neuronal loss is evident. As such, this has led many in the field to agree that secondary prevention, that is, diagnosing and treating the disease before symptoms become overt, but with established pathology, may be more likely to slow or halt the pathogenic process. Secondary prevention trials for sporadic AD, the Anti-Amyloid treatment in Asymptomatic AD (A4) trial, and familial AD, the Dominantly Inherited Alzheimer’s Network Trials Unit (DIAN-TU), are currently in the advanced stages of planning, and hold much promise for the hypothesis that anti-amyloid therapies are more effective when administered in healthy older adults in the preclinical stage of AD. In designing these trials, in addition to the assessment of Aβ amyloid and change in cognitive function, it will be important to consider known genetic factors such as carriage of the APOE ε4 allele, particularly as this has been shown to increase the likelihood of the presence of high levels of Aβ amyloid.

Another consideration in the design of such trials is the role of other lesser known genetic polymorphisms, such as the BDNF Val66Met polymorphism, in affecting trial outcomes. In particular, the results of this thesis demonstrated that the presence of the Met allele may moderate Aβ amyloid-related decline in episodic memory, reductions in hippocampal volume and risk of disease progression, thus questioning whether treatment approaches should include other measures beyond just the removal of excess Aβ amyloid. Some studies have reported that increasing BDNF levels through either aerobic exercise in individuals at risk for AD, or through pharmacological administration in AD mouse models can result in improved memory and other cognitive function. However, as BDNF Val66Met polymorphism does not change the rate of Aβ amyloid genesis, increasing BDNF protein secretion, or modifying the main receptor...
for BDNF (tropomyosin-related kinase B)\textsuperscript{245} as potential therapeutic strategies may only act as an adjunctive treatment.

12.4 Future Directions and Other Considerations

Aβ amyloid has received much attention in the field of AD, and this has primarily been due to the increasing wealth of evidence from large prospective studies of AD supporting the role of Aβ amyloid in preclinical AD. Nevertheless, advances in the understanding of tau-mediated neurodegeneration in AD hold great promise for the role of tau biomarkers in identifying individuals who are at risk for developing AD.\textsuperscript{9,277} One challenge is that changes in tau and phosphorylated-tau can reflect general neuronal and synaptic damage that is not specific to AD.\textsuperscript{9} Further, studies of animal models\textsuperscript{278,279} and in elderly adults followed longitudinally\textsuperscript{210,227} suggest that tau pathology may be a downstream factor of the amyloid cascade hypothesis in AD. However, there is some evidence that in healthy older adults and in patients with MCI, the combination of two biomarkers derived from cerebrospinal fluid assays (i.e., CSF Aβ\textsubscript{42} and CSF tau) provided the highest prediction of future progression to AD.\textsuperscript{226,280} As such, it remains to be investigated whether future studies of tau will contribute to models of early AD, over and above that of Aβ amyloid.

AD is endemic within older populations, and incidence rates of AD have been projected to triple by 2050.\textsuperscript{281,282} The associated financial burden of this projection is estimated to reach $83 billion or more in the USA by 2050,\textsuperscript{281} which will be greater than the cost of any other health condition. Additionally, significant physical and psychological burden is placed on the caregivers of AD patients. As such, in addition to effective treatment therapeutics, prevention strategies are equally vital to optimise the cognitive health of individuals. In particular, research into cognitive reserve suggest that higher levels of
education, occupational complexity, and cognitive stimulation or activity may increase the brain’s ability to withstand neuronal injury or insult before any clinical or cognitive symptoms become evident. Some studies have observed that in some people, higher levels of cognitive reserve are associated with later onset of clinically diagnosed AD, and even some protection against Aβ amyloid-related cognitive impairment,\(^{36,38}\) and cognitive decline.\(^{37}\)

Additionally, cardiovascular risk factors such as obesity, diabetes, and hypertension have also been shown to increase the risk of AD pathophysiological processes. In particular, it has been hypothesized that continuous exposure to cardiovascular risk factors over an extended period of time can lead to a number of changes in cerebrovascular physiology that have been observed in AD brains, such as, decreased microvascular density, diminished glucose transport across the blood-brain barrier, and cerebral amyloid angiopathy.\(^{283,284}\) However, the pathological link between the presence of cardiovascular risk factors and deposition of Aβ amyloid remains unclear, with some positing that there may be an increase in blood-brain barrier permeability in individuals with cardiovascular risk factors, which could facilitate increased Aβ amyloid deposition in the brain.\(^{285,286}\) For example, chronic hypertension has been found to induce a break in the blood-brain barrier, and this ensuing vulnerability may evoke triggers of neurodegeneration such as oxidative stress and inflammation.\(^{287}\) Relationships between cardiovascular risk factors and cognitive decline have also been observed, with higher numbers of cardiovascular risk factors being associated with greater cognitive decline.\(^{288-297}\) Given the potential link between cardiovascular risk factors and AD, a sensible preventative strategy may lie in the continued promotion of healthy lifestyles.
12.5 Limitations

For each of the studies conducted, factors that may have operated to limit the generalisability or diminish the validity of conclusions drawn from studies in this thesis have been described in detail in each chapter. To recapitulate more broadly, the main limitation associated with the findings of this thesis is that the AIBL study is not an epidemiological study, and very strict inclusion and exclusion criteria were applied in the selection of participants.\(^{126}\) In particular, healthy older adults and adults with MCI in AIBL were highly educated and had few existing or untreated medical or psychiatric conditions. Further, the sample of AIBL participants studied was almost entirely made up of individuals of Caucasian descent. As such, before the findings reported in this thesis can be generalised more globally, they will need to be replicated in individuals with high Aβ amyloid in population-based studies both in developed countries, such as the Mayo Clinic Study of Ageing,\(^{203}\) and in developing countries, such as the 10/66 Dementia Research Group,\(^{204}\) where it is possible that Aβ amyloid-related rates of cognitive decline may be different than that observed in this thesis. Further, although the healthy older adults group studied was large when compared to previous studies of Aβ amyloid in healthy older adults, there were much fewer subjects in the MCI group studied in Chapters Three, Six and Seven. As such, detailed investigation of the influence of genetic polymorphisms such as \(APOE\) ε4 and \(BDNF\) Val66Met, and their interaction with Aβ amyloid levels were unable to be conducted, and await other studies.

12.6 Conclusions

These limitations notwithstanding, data from this thesis strongly support the hypothesis that high Aβ amyloid levels in healthy older adults signify a preclinical stage of AD. Further, evidence of high levels of Aβ amyloid in adults with MCI provide
additional confirmation that it is MCI due to AD; whilst MCI with low levels of Aβ amyloid may suggest the presence of other underlying neurological or psychiatric processes. This Aβ amyloid-related decline in memory function can be detected in as little as 6 months from baseline, although the serial assessment of cognition is required in order to generate enough datapoints for a better estimation of the change trajectory. Several important genetic inferences regarding the relationship between Aβ amyloid and cognitive decline can also be drawn from this thesis. In particular, APOE ε4 carriage may increase the risk of the presence of high Aβ amyloid levels, but it does not moderate Aβ amyloid-related decline in cognition. Conversely, BDNF Val66Met polymorphism does not increase the risk of high levels of Aβ amyloid, nor does it influence the rate of Aβ amyloid accumulation, but it does moderate the relationship between Aβ amyloid and cognitive decline. Specifically, Val homozygotes with high Aβ amyloid were observed to be able to tolerate the toxic effects of Aβ amyloid better than Met carriers, suggesting that Val homozygotes may have an important biological reserve, or protective factors. However, the most important conclusion that arises from this thesis is possibly that healthy older adults with high Aβ amyloid and objectively defined decline in memory are promising candidates for clinical trials aiming to modify or halt the progression of AD in the very early stages, particularly using the techniques described in this thesis for measuring longitudinal trajectories, and that this move towards earlier intervention may ultimately result in the prevention of AD.
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## Appendix A: Article Status

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<td>Use of the CogState Brief Battery in the assessment of Alzheimer's disease related cognitive impairment in the Australian Imaging, Biomarkers, and Lifestyle (AIBL) study</td>
<td>Journal of Clinical and Experimental Neuropsychology</td>
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Appendix B: Published Work