Cerebrovasculature and Cognition in Middle-Aged Adults at Risk for Alzheimer's Disease

By

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Dedicated to the memory of Gene and Joanne Cassidy

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LIST OF ABBREVIATIONS

ACH	Amyloid Cascade Hypothesis
AD	Alzheimer's Disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
aMCI	amnestic Mild Cognitive Impairment
ANOVA	Analysis of Variance
ApoE(2,3,4)	Apolipoprotein E, three isoforms: ε2, ε3, and ε4
App	Gene for the Amyloid Precursor Protein
ASL	Arterial Spin Labeling
Aβ	Amyloid Beta
BDI	Beck's Depression Inventory
BMI	Body Mass Index
BNT	Boston Naming Test
BOLD	Blood Oxygenation Level Dependent
CA1	*Cornu Ammonis* 1, a region of the hippocampus
CA3	*Cornu Ammonis* 3, a region of the hippocampus
CBF	Cerebral Blood Flow
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CO_2	Carbon Dioxide
CVR	Cerebrovascular Reactivity
DG	Dentate Gyrus, a region of the hippocampus
DKEFS	Dellis-Kaplan Executive Function System
ERC	Entorhinal Cortex
$EtCO_2$	End-Tidal Carbon Dioxide
FDA	Food and Drug Administration
FIRST	FSL program for segmenting subcortical brain volumes
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FNO	Face/Name/Occupation Memory
IQR	Interquartile Range
JLO	Benton's Judgment of Line Orientation
LM-D	Logical Memory- Delayed Recall
LM-I	Logical Memory- Immediate Recall
LM-R	Logical Memory- Recognition
λ	Blood/Brain Partition Coefficient
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Exam
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo

MRI	Magnetic Resonance Imaging
MTL	Medial Temporal Lobe
N_2	Nitrogen
NST	Node Structure Theory
O_2	Oxygen
OP	Object/Place Memory
pCASL	Pseudocontinuous Arterial Spin Labeling
PET	Positron Emission Tomography
PLD	Post Label Delay
PMFC	Perceptual Mnemonic/Feature Conjunction
PRC	Perirhinal Cortex
REYO-C	Rey-Osterrieth Complex Figure Test- Copy
REYO-D	Rey-Osterrieth Complex Figure Test- Delayed Recall
REYO-I	Rey-Osterrieth Complex Figure Test- Immediate Recall
ROI	Region of Interest
TE	Echo Time
TFCE	Threshold Free Cluster Enhancement
TIA	Transient Ischemic Attack
TR	Repetition Time
vATL	Ventral Anterior Temporal Lobe
WBR	Within-Between Ratio
ΔM	Difference in signal intensity between control and label images in ASL

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CHAPTER I

BACKGROUND INFORMATION

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that irreversibly impairs cognitive processes, most notably in the domain of memory. AD is the most common form of dementia, constituting an estimated 60-80% of all dementia cases[1]. In 2010, roughly 4.7 million Americans were living with the disease, and that number has continued to increase[2]. It is estimated that by 2050, 13.8 million Americans will be living with the disease[2]. In addition to the significant financial and emotional burden on patients and caregivers, the cost to taxpayers is tremendous. The cost of care for AD in the US in 2015 was $226 billion, 68% of which was paid for through Medicare and Medicaid. By 2050, that amount is estimated to rise to over $1.1 trillion[3]. It is possible for this rise to become manageable. If a treatment that delayed the onset of AD by just five years were found by 2025, projections show that it would save $367 billion by 2050[1]. Unfortunately, of the top ten causes of death in the United States, only AD still has no way to prevent, cure, or significantly slow its progression. Finding a treatment for Alzheimer's disease would not only benefit patients and families, it would benefit taxpayers and the economy.

At the moment, only six drugs have been FDA approved for use in AD. All of the approved drugs are aimed only at relieving symptoms, not disease modification. From 2002-2012, 244 drugs were registered for clinical trials, and only one drug made it to market- an abysmal failure rate of 99.6%[4]. There are several potential explanations for this failure rate. Many of these drugs have been developed using animal models, but a translational gap still exists between these

animals and human physiology. No animal model naturally develops AD pathology, and genetically modified animals do not fully recapitulate the widespread neuronal loss that is seen in humans with AD[5]. In addition, the majority of these drug trials were aimed at decreasing amyloid beta levels in the brain. Studies have shown that amyloid beta does not correlate well with cognitive testing, and may not be the primary cause of neurodegeneration[6-8]. Instead, it is possible that degeneration is due to the complex interplay between amyloid beta, tau, inflammation, cerebrovascular dysfunction, and a multitude of other factors. Finally, many clinical trials recruit subjects who are already cognitively impaired, and in whom AD pathology may be beyond the point of reversibility. Future trials will benefit from research that has been designed with these limitations in mind.

A task force of AD researchers from academia, industry, and federal regulatory bodies have established that improvements need to be made to current patient recruitment strategies in clinical trials[9]. This task force concluded that trial participants should be identified in the years prior to the onset of dementia. This may be done using amnestic Mild Cognitive Impairment (aMCI) criteria combined with one or more physiological biomarkers. We propose that the diagnostic criteria for aMCI may be too broad to identify the earliest cognitive symptoms of AD, and more sensitive tests could be developed.

With that in mind, the current project has been designed to more specifically identify subtle changes in cognition and physiology in human subjects who are at risk for developing AD. Subjects in our study are middle aged (40-60 years old) because this is likely the age group in which pathology is beginning to form in individuals who will later be diagnosed with the

disease[9-11]. The overall goal of this study is improve the identification of subjects who may be at the highest risk for the development of AD, so that in the future these people may be targeted for treatment.

For this study we have chosen to focus on three primary aims. First, to better understand how AD risk may influence subtle cognitive processes using behavioral tasks that have been designed based on a thorough understanding of early AD pathology. Second, to examine the influence of AD risk on markers of cerebrovascular function. Third, to determine if there is a correlation between cerebrovascular function and cognition, and how this relationship may differ between controls and at risk subjects. Any results that are discovered in the course of this work will add to the knowledge of physiological processes that are and are not occurring in at risk subjects decades before AD is diagnosed. This work may lead to better subject screening for clinical trial design, and/or new therapeutic targets.

Alzheimer's Disease Pathology and Progression

The region of the brain primarily associated with AD is the medial temporal lobe (Figure 1) This region is comprised of hippocampal and extrahippocampal regions and it is most commonly implicated in the formation and storage of memories. For this project, we are particularly interested in the medial temporal lobe (MTL), specifically the perirhinal and entorhinal cortices, and the CA1, CA3, and Dentate Gyrus (DG) regions of the hippocampus.

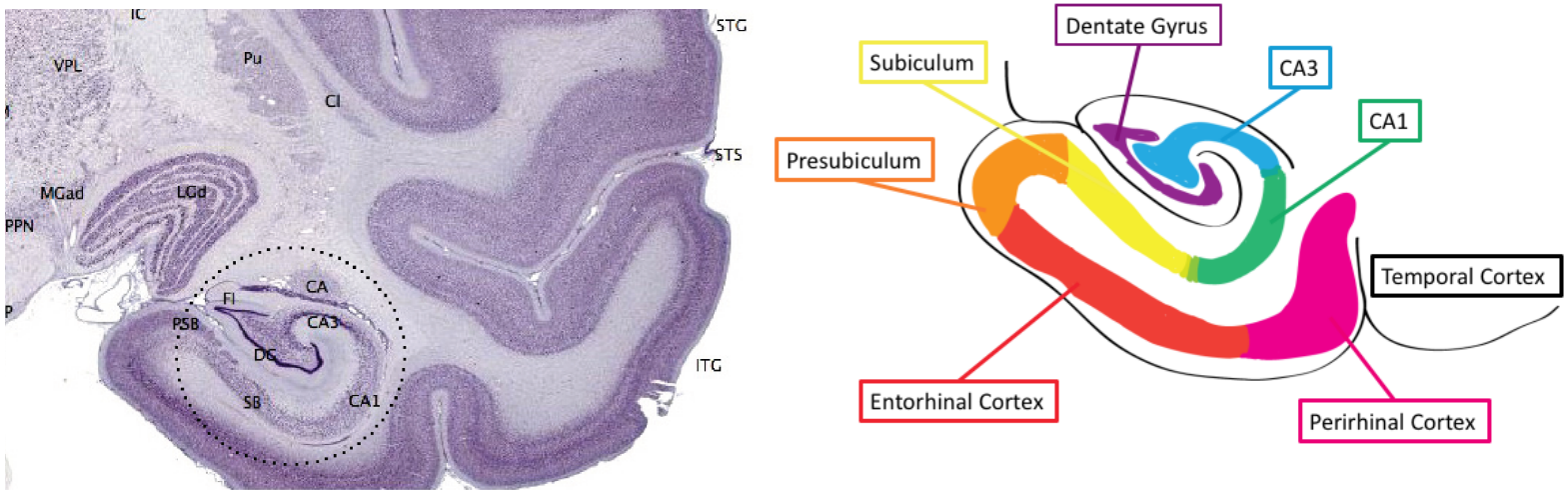

Figure 1: The medial temporal lobe. A. Coronal section of a macaque monkey brain demonstrating the major structures of the medial temporal lobe. Nissl stain, courtesy of BrainMaps.org (Mikula 2008)3. B. Cartoon delineating the boundaries of medial temporal lobe structures.

There are two primary forms of pathology associated with AD: amyloid beta plaques and neurofibrillary hyperphosphorylated tau tangles. These pathologies follow a stereotypical route throughout the cerebral cortex and the medial temporal lobe. The definitive research on AD pathology progression was accomplished in 1991 by Braak and Braak[12], who describe distinct stages of amyloid and tau accumulation.

Amyloid Pathology

Amyloid plaques are aggregates of the amyloid beta (Aβ) peptide that develop in the brain parenchyma. Although the exact mechanisms by which amyloid plaques cause neural damage haven't been fully characterized, soluble Aβ oligomers have been shown to disrupt synaptic plasticity and cause synaptic loss[13].

The distribution of early amyloid accumulation varies among individuals, thus only three stages of amyloid accumulation have been described (Figure 2). In Stage A, amyloid deposits are observed in the basal portions of the frontal, temporal, and occipital lobe. Staining in the entorhinal cortex and presubiculum has demonstrated weak amyloid accumulation with ill-defined boundaries. The hippocampus does not exhibit amyloid accumulation. In Stage B, amyloid deposits are evident in most of the cortex except the primary sensory and motor areas. Some amyloid deposition is seen in the white matter. Amyloid is present in the presubiculum and entorhinal cortex. Hippocampal deposits are primarily restricted to CA1, although other sectors may show a few deposits. In Stage C, nearly all of the cortex exhibits dense amyloid

accumulation, and subcortical structures are involved. The hippocampus still retains a relatively low level of amyloid.

Tau Pathology

Normally tau is a peptide used to create microtubules, the primary means of axonal transport. When tau is pathologically hyperphosphorylated it can oligomerize in the axon and physically prevent the transport of substances within the axon. This leads to synaptic dysfunction and neurodegeneration.

Most individuals develop tau pathology in a highly similar way, therefore tau accumulation has better defined stages than amyloid pathology (Figure 3). In Stage I, tau begins to accumulate in a lateral region of the perirhinal cortex called the transentorhinal region[14]. In Stage II, numerous tangles are apparent in the transentorhinal region, and some are evident in the CA1 region of the hippocampus. In stage I and II pathology is mostly confined to the entorhinal regions and is not evident in the cortex, therefore these stages together are called the "Transentorhinal Stages." In Stage III there is severe involvement of the transentorhinal and entorhinal regions, and modest involvement of CA1. Some individuals may exhibit limited tau pathology in the frontal, temporal, and occipital cortices and mild involvement of subcortical regions. In Stage IV, there is severe involvement of the entorhinal and transentorhinal regions, and numerous tangles are observed in the CA1. The cortex is only mildly involved, but subcortical regions are affected. Stages III and IV are referred to as the "limbic stages." Stage V exhibits severe involvement of the entorhinal cortex and all areas of the hippocampus. Subcortical regions exhibit large numbers of tau. Stage V is also evidenced by the accumulation

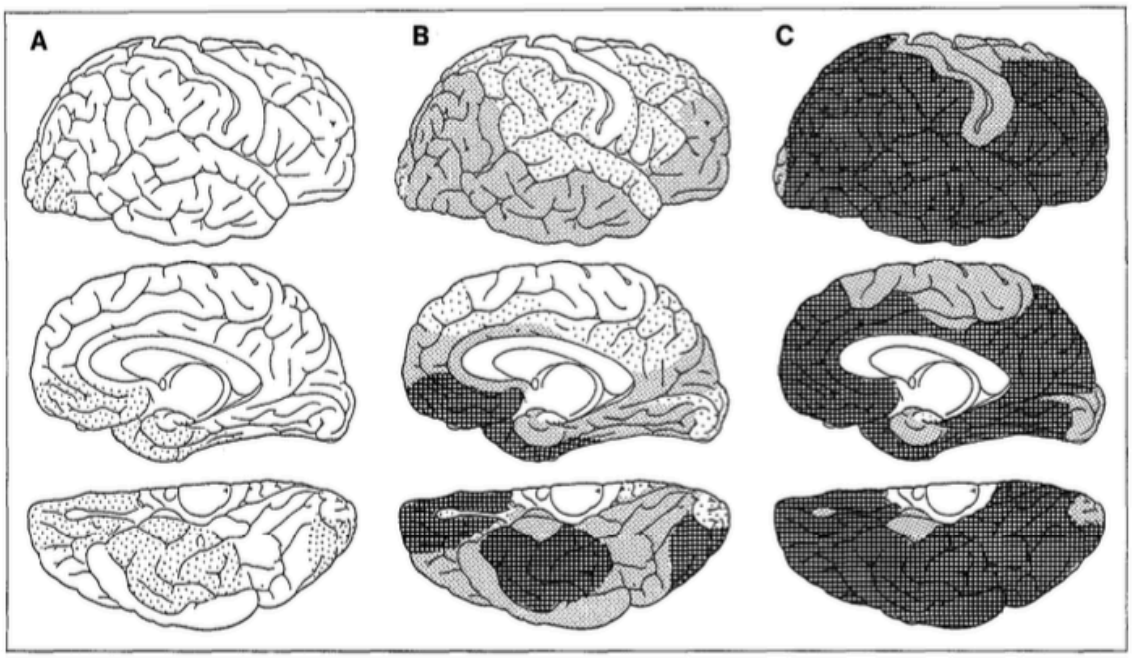

Figure 2: Braak staging of amyloid pathology. In Stage A, amyloid is only found in basal regions of the cortex. In Stage B, amyloid is present in almost all cortical association areas, but absent from motor and sensory areas. The hippocampus is only mildy involved. In Stage C, amyloid is present in all cortical regions. (Figure taken from Braak and Braak 1991)

of tau pathology in the cortex. In Stage VI, the entorhinal regions, hippocampus, subcortical regions, and the cortex exhibit severe tau pathology. Stage V and VI are referred to as the "isocortical stages."

Vascular Pathologies

There is some evidence that there may be a vascular component to the development of AD. Epidemiological studies have indicated that AD shares a number of risk factors with cardiovascular disease including hypertension[15-17], hypercholesterolemia[18,19], diabetes mellitus[20,21], atherosclerosis[22,23], smoking[24], and obesity[25]. Post mortem studies have indicated that most AD brains exhibit vascular pathology such as microvascular disease, periventricular white matter lesions, and cerebral amyloid angiopathy[26,27]. Furthermore Aβ interferes with vascular function[28,29]. Subjects with AD have a reported 40% lower rate of cerebral blood flow (CBF) compared to cognitively normal controls throughout the brain[30,31], and evidence has shown that hypoperfusion may precede clinical symptoms[32]. Mounting evidence has indicated that cerebrovascular dysfunction may occur decades before a diagnosis. Wierenga et. al. have posited that there may be a biphasic curve of CBF function in AD with increased CBF early in life (figure 4), followed by reduced CBF as the disease advances[33]. A number of previous studies have shown that cerebrovascular reactivity (CVR), an index of vascular functionm is also impaired in subjects diagnosed with AD[34-42].

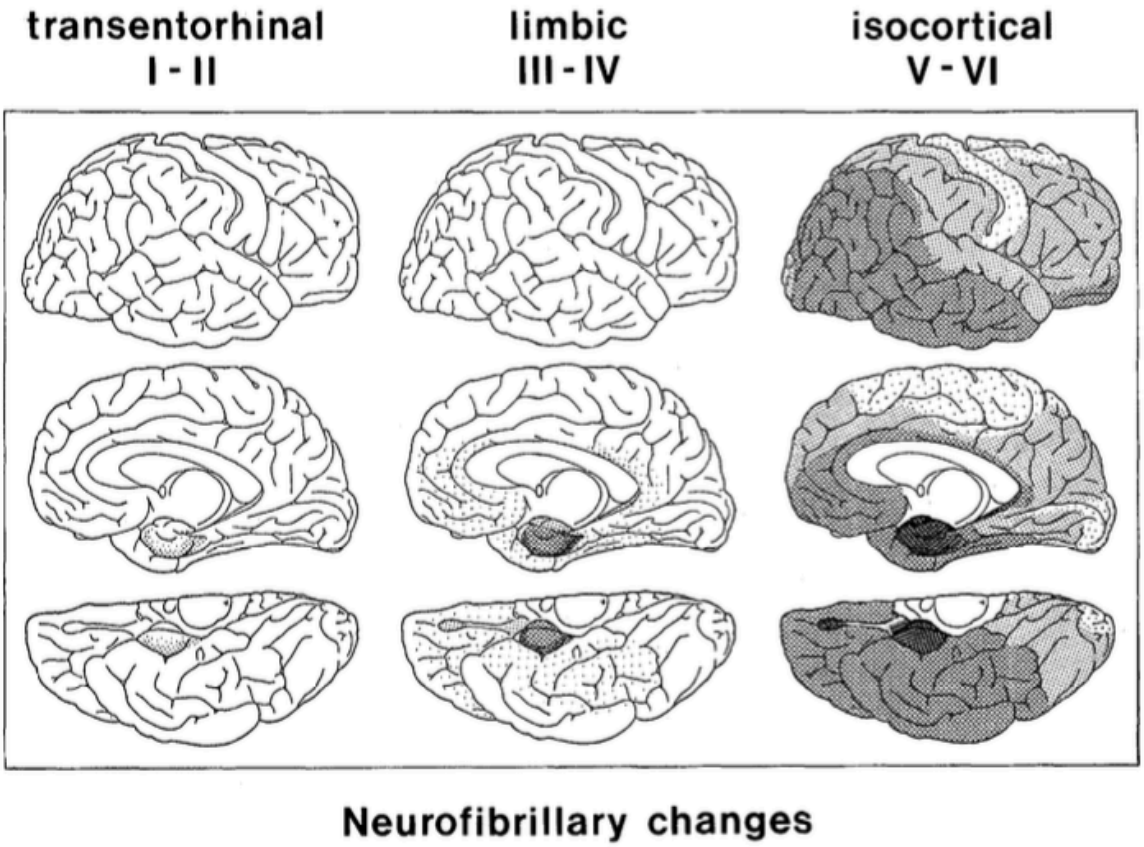

Figure 3: Distribution of neurofibrillary (tau) tangles. In stages I-II pathology is confined to the transentorhinal region, in stage III-IV pathology severely involves the entorhinal regions and enters the hippocampus, and in stages V-VI pathology is seen in the cortex. (Figure taken from Braak and Braak 1991).

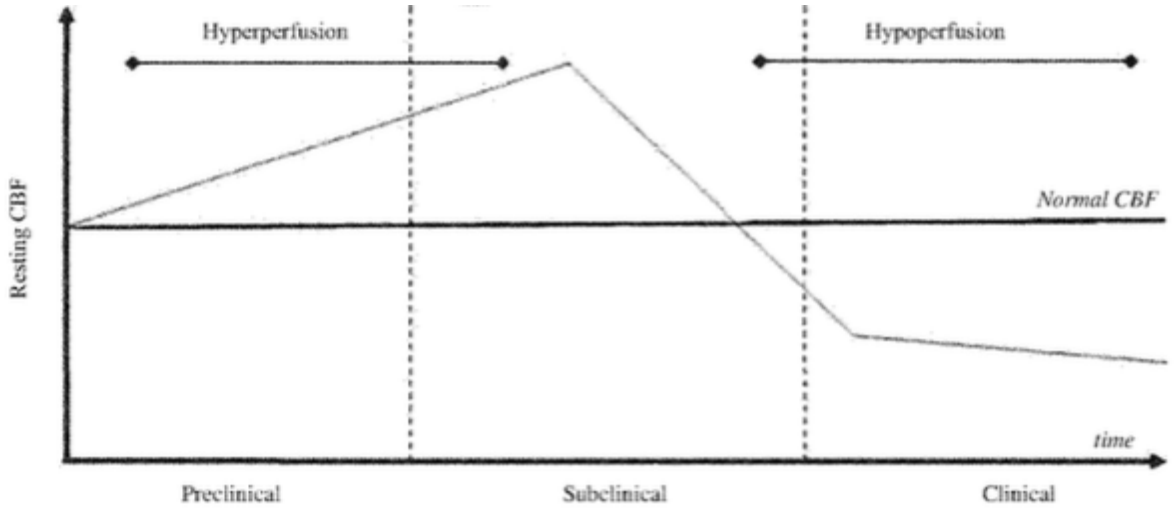

Figure 4: Hypothetical model of CBF in AD. The brain is hyperperfused in the pre-clinical stages of AD, then becomes hypoperfused in the later stages. (Figure taken from Wierenga et. Al. 2014).

Other Pathologies

Human and animal studies have shown a number of other pathophysiologies associated with AD, including neuroinflammation[43], oxidative stress[44], mitochondrial dysfunction[45], and Ca_2^+ dysregulation[46]. The temporal order of events and relative contribution of each to the AD phenotype is still unclear.

Hypotheses Regarding the Development of Alzheimer's Disease

For two decades, the overwhelming hypothesis regarding the development of AD was the Amyloid Cascade Hypothesis (ACH). This hypothesis, based on the best known biochemical, neuropathological, and genetic information available at the time, states that the accumulation of Aβ is the first step in the development of AD and initiates all other pathologies. The ACH has fallen into criticism in recent years with some researchers suggesting that it should be abandoned altogether[47]. Autopsy studies and Positron Emission Tomorgraphy (PET) studies have indicated that many cognitively normal older adults have evidence of amyloid in their brains[48]. Clinical trials in which AD patients were immunized against Aβ were halted due to adverse events, but the patients whose plaque burdens were successfully reduced during the trial did not show any improvements in their cognitive symptoms[7]. These data indicate that amyloid pathology is neither necessary nor sufficient for the cognitive symptoms of AD.

AD may also be characterized as a primarily tau-based disease. As previously mentioned, tau pathology follows a more stereotypical route through the brain than amyloid pathology[49]. Tau

pathology precedes amyloid pathology[50,51] and is more closely correlated with neuronal loss than amyloid, both spatially and temporally[52]. Studies have shown that tau pathology also correlates more strongly with cognitive function than amyloid pathology[53]. Tau may be a better target for understanding the earliest stages of AD than amyloid. Studying tau in humans *in vivo* has been limited because until recently tau could only be detected using a lumbar puncture, which limits spatial information, or after death. A PET ligand for tau is currently being developed[54] that will help elucidate its spatial and temporal properties in the early stages of AD.

Despite the evidence of a vascular component to AD, it is unclear whether vascular or amyloid pathology arises first. The two-hit theory of AD posits that some vascular insult (the first hit) which may be related to aging or vascular risk factors results in changes to the blood brain barrier and hypoperfusion. This contributes to the production, accumulation, and impaired clearance of Aβ (second hit), which ultimately leads to the tissue damage and dementia associated with AD[55]. Lending support to this theory is the observation that in transgenic mice overexpressing the Swedish mutation of the gene for amyloid precursor protein (*App)* cerebrovascular dysfunction develops earlier than cognitive changes and Aβ plaque formation[56,57].

Role of Family History, Aging, and Genetics on Disease Risk

In order to better understand the earliest stages of AD pathology, we must look at people who are likely to develop the disease in the future decades before a diagnosis can currently be made. Risk of AD increases with age, and most people who are diagnosed with AD are over 65 years old[1,2], therefore we chose to study middle aged subjects between 40 and 60 years old.

Family history of AD also increases risk for the disease, and may be the largest determinant of overall risk. Heritability of AD was estimated at approximately 58%, and people who have a first degree family history of AD have 2-4 times the lifetime risk as people with no family history[58]. Therefore, the primary characteristic we used to stratify subjects into "At risk" and "control" groups was family history.

Researchers have worked to identify specific risk genes that contribute to the development of AD, and genome-wide association studies have identified over 100 genes that may confer AD risk[58]. The most common risk gene for AD is a cholesterol-processing gene called apolipoprotein E. There are three possible isotypes of Apolipoprotein, ε2 (ApoE2), ε3 (ApoE3), and ε4 (ApoE4). Most of the general population (60%) has two copies of ApoE3, however, 23% of the population is estimated to carry one or two copies of ApoE4[59]. This gene has been associated with an increased incidence of AD, a decreased age of onset, and reduced survival[60]. An alternate allele of apolipoprotein, ε2(ApoE2) has been identified as a protective gene for the development of AD[61]. Subjects were genotyped for Apolipoprotein E to further assess their overall risk of AD.

Summary

AD is a devastating disease and a growing public health risk. In order to combat the anticipated rise in AD rates, patients with AD need to be identified as early as possible. This study aims to identify the earliest cognitive and physiological features of AD by intensely examining subjects at risk for AD.

CHAPTER II

STUDY OVERVIEW AND SUBJECT CHARACTERISTICS

Specific Aims and Hypotheses

This study had three primary hypotheses:

Hypothesis 1: Middle-aged individuals at risk for AD will have reduced performance on sensitive cognitive tests of MTL function compared to age-matched controls.

Aim 1: MTL function of middle aged at risk subjects and age-matched controls will be examined using cognitive tests that target specific subregions of the MTL. These tests have been designed to follow the route of AD pathology progression, beginning in the entorhinal cortex (oddity detection), continuing into the CA1 region of the hippocampus (face-name association memory and object-place association memory), and moving into the dentate gyrus and CA3 (pattern separation). We hypothesize that performance differences between groups will follow the trajectory of AD pathology. In this scenario, we hypothesize that the first task to be affected would be the oddity detection task. The second task would be the associational memory tasks, and the third task would be pattern separation. We predict that there will be no differences in groups in associational memory or pattern separation if there are no differences in oddity detection. Standard experimental data (e.g., accuracy, reaction time, etc.) from this aim will also be used for correlational analyses in Aim 3.

Hypothesis 2: Middle-aged individuals at risk for AD will have increased CBF and reduced CVR in the MTL/hippocampus area compared to age-matched controls.

Aim 2: CVR as well as CBF and volume will be quantified in middle-aged adults at risk for AD and age-matched controls using arterial spin labeling (ASL) combined with a vasoactive gas challenge. We expect increased CBF and decreased CVR in at risk subjects compared to controls in the entorhinal cortex and hippocampus, but not in a region unaffected by AD, the occipital lobe. Most studies to date have either investigated whole brain CBF or have registered blood flow maps to a standard atlas for regional analysis. However, these methods may suffer from inaccurate registration due to individual volume differences, particularly in our population of interest. Importantly, we plan to use established algorithms and manual segmentation techniques to outline regions of interest (gray matter, hippocampi, entorhinal cortices, and occipital lobe) individually for each subject. CBF and CVR values will also be used for correlational analyses in Aim 3.

Hypothesis 3: CVR and CBF will correlate with performance on MTL-targeted cognitive tasks

The data collected in Aim 1 and Aim 2 will be drawn from the same pool of subjects. Correlations between CVR and cognitive testing scores from Aim 1 will be calculated. These correlations will be separately investigated in at risk subjects and control subjects to determine if these relationships differ with risk for AD.

Subjects

Recruitment Goals

We aimed to recruit a total of 75 subjects: 50 subjects with a first degree family history of AD ("At Risk") and 25 subjects with no known family history of AD ("Control"). We anticipated

that after genetic testing, the at risk group would be divided roughly equally into carriers and non-carriers of ApoE ε4, thus creating three groups: at risk subjects with ApoE ε4 (n~25), at risk subjects without ApoE ε4 (n~25) and control subjects (n=25)

Recruitment and Screening

Subjects were recruited through mass email lists, ResearchMatch.org, the Vanderbilt Memory and Alzheimer's Center, and word-of-mouth. After initial contact, subjects answered a series of questions to determine eligibility for the study based on the inclusion and exclusion criteria (Table 1). If a subject was deemed eligible, he or she provided informed consent according to the Vanderbilt University Institutional Review Board. A total of 76 subjects were recruited; 47 who had a parent diagnosed with AD and 29 who did not. Several subjects were removed from the study due to abnormal performance in neuropsychological testing (n=19), incompatibility with the MRI (n=2) or because they no longer wished to participate (n=2). A total of 55 subjects completed the study.

Inclusion Criteria

Male and female subjects were recruited who were between the ages of 40 and 60 years old, spoke English fluently, and demonstrated no cognitive impairments on standard tests of neuropsychological functioning.

Exclusion Criteria

Subjects were excluded from the study if they were knowingly unable to do all three study sessions, had a history of traumatic brain injury, stroke, transient ischemic attack (TIA), degenerative or neurological disease, psychiatric illness or mood disorder, a diagnosed learning

disability, uncorrected vision (<20/30), colorblindness, hearing problems, a history of alcohol or substance abuse, or any MRI contraindications. Subjects were asked to indicate the medications that they were on and were excluded if they were on anticonvulsants, antipsychotics, benzodiazepines, psychostimulants, opioids, tricyclic antidepressants, some norepinephrine reuptake inhibitors. Subjects could not be pregnant or have need for continuous oxygen, nor could they have respiratory disease, chronic angina, or any other unstable cardiac condition.

Demographic Data

Subjects were asked to self-report age, sex race, ethnicity, and years of education. Due to the vascular component of this study, subjects were also asked to self-report history of heart disease, sleep apnea, diabetes (type 1 or 2), recent smoking history and lifetime smoking history, medication, and estimated exercise frequency. Body Mass Index (BMI) was calculated using subjects' self-reported height and weight. Subjects' handedness was determined using the Edinburgh Handedness Scale.

Neuropsychological Tests

To make sure that subjects were cognitively normal for his or her age, subjects underwent a battery of neuropsychological testing. The battery included the Mini-Mental State Exam, the Logical Memory Exam (immediate recall, delayed recall, and recognition) from the Delis-Kaplan Executive Function System (DKEFS), the Rey-Osterrieth Complex Figure Test (copy, immediate recall, and delayed recall), DKEFS verbal fluency (letter fluency and category fluency), Trails A and B, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) word list learning task, the Benton Judgment of Line Orientation Test, and the Boston

Naming Test. Any subject who scored >1.5 standard deviations outside the norms in any test was removed from the study. Subjects were also given the Beck Depression Inventory to rule out mood disorders.

Apolipoprotein E Genotyping

Apolipoprotein E ε4 (ApoE4) is the most common risk gene for Alzheimer's disease[59] and we anticipated that the At Risk group would have more carriers of ApoE4 than the control group. Subjects were genotyped for carrier status of Apolipoprotein E (ε2, ε3, ε4). Buccal cells were collected from each subject (Oragene Discover OGR-500 kit, DNA Genotek Inc., Ottowa, ON, Canada) and DNA was isolated and genotyped by Vanderbilt Technologies for Advanced Genomics using Applied Biosystems Taqman 7900 HT Instrument (Life Technologies, Grand Island, NY, USA) with primers to rs7412 and rs429358.

Study Timeline

If a subject expressed interest in the study, he or she was first given information about the study and asked a series of questions to determine eligibility (pre-screening). Following pre-screening, subjects were asked to attend three separate study sessions. The first session was approximately one hour. During this session informed consent was obtained, demographic data was collected, and subjects underwent a rigorous battery of neuropsychological testing. The second session lasted approximately one hour and involved an MRI scan. The third session lasted approximately three hours during which subjects completed cognitive tasks designed to challenge different regions of the medial temporal lobe. All sessions for this study were completed within three months of initial screening.

Not all subjects completed each cognitive and/or neuroimaging test, therefore demographic, neuropsychological, and genetic results are reported separately for each of the following chapters.

Figure 5: Timeline of study

CHAPTER III

COGNITIVE TESTING REFLECTS THE ROUTE OF ALZHEIMER'S DISEASE PATHOLOGY

Introduction

While there are many cognitive tests that have been developed to study AD, most of these tests were developed to detect gross impairment due to stroke, trauma, or large lesions. The clinical standards are broadly based on cognitive function rather than a thorough understanding of the underlying anatomy of AD pathology. For this study, we proposed that creating anatomically targeted cognitive tasks could more fully elucidate the cognitive functions that are impacted by the very earliest stages of AD.

Amyloid pathology varies widely among AD patients, however tau pathology follows a stereotypical route through the brain[49]. According to Braak and Braak's staging it begins in the perirhinal (transentorhinal) and entorhinal cortices and presubiculum, moves to the CA1 region, and finally enters the CA3 region and dentate gyrus. It is only in the final stages of AD that tau pathology enters the isocortex. We chose to use a battery of cognitive tasks that are sensitive to 1) the perirhinal cortex (PRC), 2) the CA1 region of the hippocampus, and 3) the CA3 and dentate gyrus regions of the hippocampus. We anticipated that the order of changes that we would see in these tasks would reflect AD pathology, i.e. we would first see changes in tasks targeted to the perirhinal cortex. If there was a difference between subject groups in that task we may see a difference between groups in tasks targeted to the CA1 region, and if that were true we

would then see differences in tasks targeted to the CA1 region and dentate gyrus. We would not anticipate that these differences would occur in a different order.

Perirhinal Cortex Task

The PRC is involved in visually processing complex objects. According to the perceptual mnemonic/feature conjunction (PMFC) model of Bussey and Saksida, the PRC is critical for combining complex features of an object into a unified representation, and any PRC abnormalities would result in difficulty distinguishing complex objects while leaving memory and lower level visual processes relatively intact[62]. One way to probe the function of the PRC is a task known as oddity detection[63]. In this task, subjects are asked to identify the "odd man out" from several perceptually similar items. Previous work has shown that damage to the PRC results in a reduced ability to distinguish similar complex objects, while damage to the hippocampus results in a reduced ability to distinguish similar scenes[64,65]. This deficit is particularly notable when the objects to be compared contain a high degree of feature ambiguity, and when subjects are shown unfamiliar objects such as Greebles[63]. Because the PRC is associated with the comparison of features within a single item[62,65,66], we hypothesized that at risk subjects would be impaired at distinguishing perceptually similar stimuli featuring a single object (faces, familiar objects, and Greebles) compared to controls.

Some studies have hypothesized that it may be possible to measure binding capacity in subjects using eye-tracking[64]. Based on this theory, subjects who are able to bind more features of a complex visual object will spend more time looking at a single item before comparing it to the next item. This is referred to as a higher number of fixations within an object area. Subjects who bind fewer features will spend more time looking from one object to another and back. This is

referred to as a higher amount of fixations between object areas. The ratio of within item fixations and between-item fixations, or the within-between ratio (WBR) is an index of binding capacity (Figure 6)

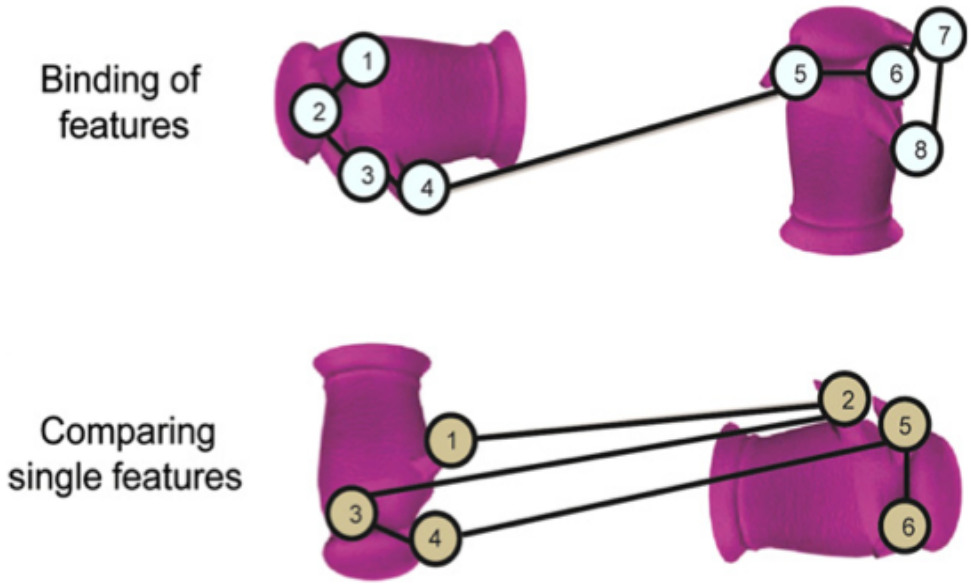

Figure 6: Within-between ratio. Subjects who bind more features will spend more time looking within an object (a) while subjects who bind fewer features will spend more time looking between objects (b).

CA1 Tasks

One of the roles of the CA1 is binding information together in memory[67]. For example, associating a name with a face. We chose to approach testing the CA1 in two different ways: face/name/occupation (FNO) memory, and object/place (OP) memory.

FNO memory has been shown to be impaired in subjects with aMCI and AD. In particular, it is notable that the association between faces and names is often more impaired than the association between faces and occupations[68]. Performance on an FNO task has been shown to correlate with amyloid load in cognitively normal subjects[69]. We anticipated that subjects at risk for AD would be impaired on the face/name component of FNO, but not the face/occupation component.

One of the most common complaints heard in the memory clinic is that subjects forget where they put everyday objects, for example forgetting where they put their keys. While there have been impairments observed in OP memory in aMCI patients[70], most tasks involve subjects remembering the location of objects on a screen. We thought that it would be interesting to see how subjects' memory was impacted by having control over where the objects were placed on the screen. By giving the subjects the option of where to place the objects, we were more fully replicating the real world experience of object/place memory. We anticipated that subjects at risk for AD may have more trouble remembering where they had placed the objects than controls.

CA3 and Dentate Gyrus Tasks

One of the functions of the CA3 and dentate gyrus (DG) is to perform pattern separation, i.e. to

create a unique memory for similar, but different objects. In a pattern separation task subjects are shown a series of objects. After a delay, subjects are shown objects that are either novel, repeated, or visually similar lures, and asked to describe them as "old," "new," or "similar." Novel objects have never been seen before, repeated objects are identical to the first presentation of the object, and lure objects are visually similar to an object that was previously presented, but not identical. An example of correct pattern separation would be responding "similar" to a lure, while an example of incorrect pattern separation would be responding "old" to a lure. We refer to incorrect pattern separation as "pattern completion," but note that the term is used in other contexts to describe retrieving an intact memory from a degraded cue. Pattern separation has been shown by our lab and others to be impaired with aging and in subjects with aMCI and AD[71,72]. We predicted that subjects at risk for AD would demonstrate impaired pattern separation.

Methods

Subjects

Methods for recruitment and screening, demographic data collection, neuropsychological data collection, and genetic data collection are described in Chapter II.

Oddity Detection

Participants' primary task was to identify the "odd man out" from four visually similar items, i.e. the one item (target) that was different from the other three (foils). Four sets of stimuli were used for this task: faces, objects, Greebles, and scenes. The faces, objects and Greebles were drawn from the TarrLab database (courtesy of Michael J. Tarr, Center for the Neural Basis of Cognition and Department of Psychology, Carnegie Mellon University, http://www.tarrlab.org/. Funding

provided by NSF award 0339122.) Additional objects were drawn from the Amsterdam Library of Object Images[73]. Scene stimuli were photographs taken in the Nashville area.

The stimuli for each category were organized into pairs according to visual similarity with one member of the pair acting as the target item and the second member acting as the foil. The use of each pair member as the target stimulus was counterbalanced across participants. For faces, objects and scenes, visual similarity was subjectively judged by the authors and the foil was presented from three different viewpoints. The Greeble pairs were members of the same 'family' and 'gender', and each target Greeble was presented with three rotations of the foil Greeble on each trial (Figure 6). Rotations were randomly assigned as 0°, 90°, 180°, or 270°, with a unique rotation for each of the four Greebles within a single trial. All stimuli images were scaled to 300x300 pixels.

The on screen position of the target item was randomized for each trial. Beneath each item was a digit (1, 2, 3, or 4) (Figure 6). Participants verbally reported the digit that corresponded to the item they believed to be the target to the experimenter, who then entered the response. Participants performed four blocks each consisting of a different stimulus set. For faces and Greebles there were 32 trials per block and for objects and scenes there were 28 trials per block. The order of the blocks was partially counterbalanced across participants using a Latin Square routine.

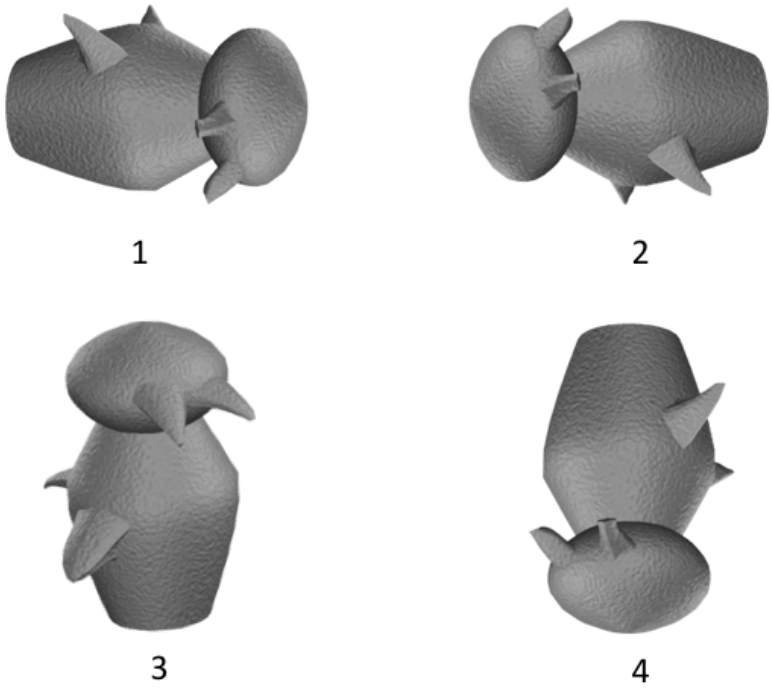

Figure 7. Example of one trial of oddity detection. Subjects were asked to state the number below the item that was different from the other three. In this example, the correct answer is 3.

During the task, eye tracking data was collected using an EyeLink 1000 tracker (SR Research Ltd., Ontario, Canada) at a sampling rate of 250 Hz. Saccades were defined as any movement of at least 0.1 degree visual angle (°) that exceeded a velocity threshold of 30°/s and an acceleration threshold of 8000°/s^2. Blinks were periods of activity with missing pupil data that exceeded 3 consecutive samples. Fixations consisted of all other recordings. The data were filtered such that fixations that were not on one of the four stimuli were removed from the analysis. The within-between ratio was defined as the number of fixations within a single item divided by the number of fixations between items[64]. Each trial began with a small dot at the center of the screen used as a drift correction stimulus. When the participant's fixation to the dot was satisfactory, the experimenter triggered the onset of the target item and three foil items positioned in the four quadrants of the screen.

The stimuli were displayed on a flat screen monitor (1024×768 pixel resolution, 75 Hz refresh rate) using Experiment Builder (SR Research Ltd.). Participants were seated comfortably in a chair with their heads on a chin rest that was positioned such that their eyes were approximately 57 cm away from the monitor.

Face/Name/Occupation Memory

The Face/Name/Occupation study protocol was adapted from Amariglio et. al. 2012[68] Subjects were shown a series of 32 black and white pictures of neutral faces. First, subjects were asked to

observe the faces and try to identify any distinguishing characteristics, in order to familiarize themselves with the faces. After the subject had seen all 32 faces, they were shown the faces a second time, but this time a name and a job were displayed underneath each face. Subjects were asked to remember both the name and the job that went with each face. In both the familiarization and encoding stages, the faces were presented for 3 seconds. Between faces a white "+" would appear on the screen for 1 second.

Following a delay of approximately 10 minutes, subjects were shown all of the faces again, but this time each face had either two names or two jobs listed below. The subjects were asked to identify which name or job had gone with the face earlier (Figure 7). The number of total correct, names correct, and jobs correct were analyzed as well as reaction times for each category.

Object Place Memory

Object place memory was assessed using an internally developed protocol. Subjects were shown a picture of a common household room on a computer screen (kitchen, bathroom, bedroom, etc.). Subjects were presented with a common household object semantically appropriate for the room (spoon, floss, alarm clock, etc.), and asked to use the computer mouse to place this object wherever they think it should go (Figure 9). Only one object appeared on the screen at a time. There were a total of 32 scenes and 128 objects to be placed. Following a ten-minute delay, subjects were presented with the same scenes and the same objects and asked to place the object in the same exact location that s/he placed it earlier. Subjects were scored primarily on accuracy (placement within 100 pixels of original placement), however on incorrect trials subjects were also scored on distance from original placement.

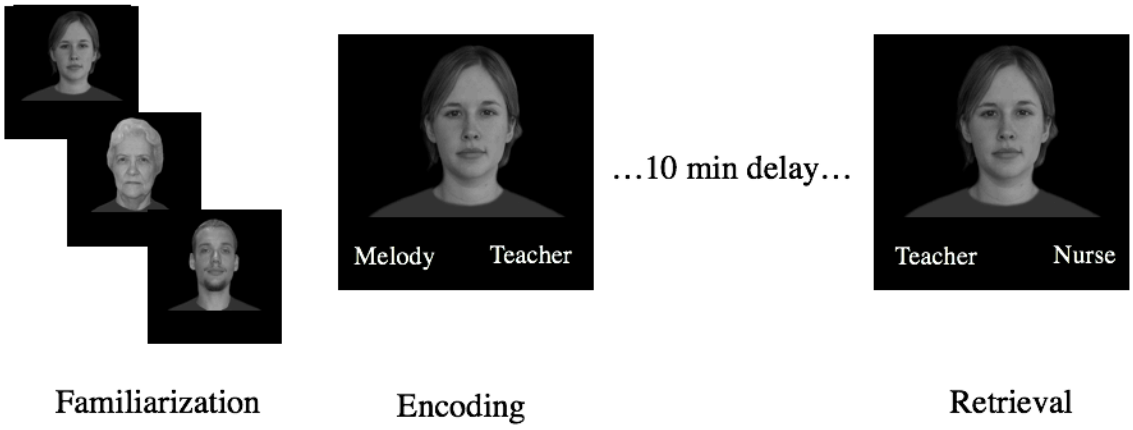

Figure 8: Face/Name/Occupation Memory. Subjects were first familiarized with the faces, then shown faces with a name and job listed below. Following a 10-minute delay subjects were shown the faces again, but with either two names or two jobs below the face. Subjects were asked to select which name or job had been presented with that face earlier.

Figure 9: Object place task. Subjects were presented with a real world scene and an object and asked to place the object anywhere in the room that they wanted. Following a delay, subjects were given the same room and object and asked to put it in the same location they had earlier. If the object was placed more than 100 pixels from the original placement it was considered incorrect, and the distance from the original placement was recorded.

Pattern Separation

Pattern separation was adapted from a paradigm developed in-house, detailed methods can be found in Ally et. al. (2013)[72]. Briefly, subjects were shown a series of real-world objects and asked to determine if the object would fit in a shoebox or not. Following a delay of approximately 10 minutes, subjects are shown a series of objects (Figure 9). One third of the objects was identical to objects that were shown earlier ("Repeated"), one third of objects was not shown before ("Novel") and one third of objects was visually similar, but not identical to objects shown earlier ("Lure"). Subjects are asked to determine if the object is old, new, or similar.

Table 1 represents the various item types and responses and what they mean. The primary analyses were focused on rates of bias-corrected pattern separation (pattern separation rate – similar bias rate) and the bias corrected pattern completion score (pattern completion rate – false alarm rate).

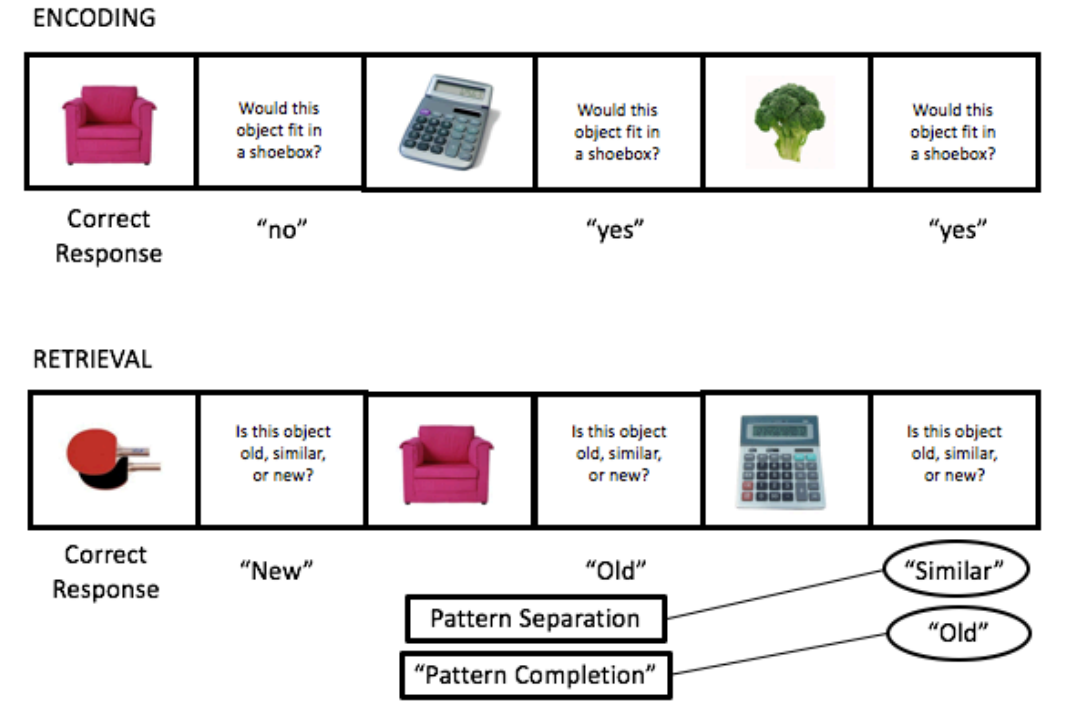

Figure 10: The pattern separation task. Subjects were shown real-world color objects and asked to determine if they would fit in a shoebox or not. Following a delay, subjects were again shown objects, but this time were asked to endorse the item as "old," "new," or "similar." Correctly responding "Similar" to a lure item is pattern separation, and incorrectly responding "old" to a lure item is pattern completion,

	Response		
Item Type	**"New"**	**"Similar"**	**"Old"**
Novel	Correct Rejection Rate	Similar bias rate	False alarm rate
Lure	Incorrect	**Pattern separation rate**	**Pattern completion rate**
Repeated	Miss rate	Incorrect	Hit rate

Table 1: Pattern separation item types and responses. Bias-corrected pattern separation is defined as pattern separation rate – similar bias rate, and bias-corrected pattern completion rate is defined as pattern completion rate – false alarm rate.

Results

Subjects

A total of 34 subjects with a family history of AD ("At Risk") and 23 subjects with no family history of AD ("Control") completed the study. Subjects were matched on age, sex, race, and education (Table 2). As expected, the at risk group had more ApoE4 carriers than the control group (p=0.002).

Neuropsychological Testing

All subjects were cognitively normal at the time of testing, i.e. they did not score > 1.5 S.D. below the expected range of scores for his or her age. Subjects did not significantly differ on any neuropsychological test (Table 3), although there was a trend toward a difference in the memory portions of the Rey-Osterrieth Complex Visual Figure Test (immediate recall p=0.05, delayed recall p=0.08).

Oddity Detection

A correct trial was defined as a trial where the subject correctly identified the target item. The proportion of correct trials were subjected to a mixed-design repeated measures ANOVA with a within-subject factors of Stimulus Type (faces, scenes, objects, and Greebles) and a between-subject factor of Group (at risk and control) (Figure 2). This analysis showed a main effect of Stimulus Type ($F_{(3,56)}$=13.8, p<.001) and an interaction of Group and Stimulus Type($F_{(3,156)}$=3.8, p=.01), but no main effect of Group ($F_{(1,52)}$ =0.174, p=.68). After further analysis of the interaction using independent t-tests, a significant difference between groups was found in the Greebles condition (t(52)=2.28, p=0.03). When data was re-analyzed with a within-subject factor

of Stimulus Type (faces, scenes, objects, and Greebles) and a between subject factor of ApoE4 Carrier Status (carrier and non-carrier), we did not find an interaction of Carrier Status and Stimulus Type ($F_{3,150}$=0.111, p=0.954) or a main effect of Carrier Status ($F_{1,50}$=0.073, p=0.788).

	At Risk (n=34)	**Control (n=22)**	**p-value**
Age (yrs ± s.d.)	53 ± 6	53 ± 6	0.888
Sex (% female)	79	59	0.104
Race (% non-white)	6	18	0.151
Education (yrs ± s.d.)	17 ± 2	18 ± 4	0.077
ApoE4 Carrier (% carrier)	63	18	0.001**

Table 2: Demographic data. There were no significant differences between groups in age, sex, race, or education, although there was a trend toward a difference between groups in education (p-0.08). There were significantly more ApoE4 characters in the at risk group (p=0.001).

	At Risk (n=34) Mean (SEM)	Control (n=22) mean (SEM)	p-value
MMSE	29.88 (0.06)	29.8 (0.1)	0.34
LM-I	29 (1)	28 (1)	0.63
LM-D	24 (1)	24 (1)	0.89
LM-R	26.3 (0.4)	26.4 (0.4)	0.84
REYO-C	34.9 (0.2)	35.2 (0.3)	0.48
REYO-I	21 (1)	23.6 (0.9)	0.07
REYO-D	21 (1)	23.3 (0.9)	0.09
FAS	43 (2)	48 (3)	0.19
CAT	58 (2)	61 (2)	0.30
Trails A	26 (1)	26 (1)	0.29
Trails B	56 (2)	60 (2)	0.19
CERAD-I	24.1 (0.5)	25.0 (0.6)	0.28
CERAD-D	7.9 (0.3)	8.5 (0.3)	0.15
CERAD-R	9.85 (0.07)	9.91 (0.06)	0.60
JLO	23.9 (0.7)	25.4 (0.9)	0.21
BNT	57.3 (0.5)	57.4 (0.7)	0.90
Tower	480 (32)	476 (35)	0.93

Table 3: Neuropsychological testing. There were no significant differences between groups in any of the neuropsychological tests. MMSE = Mini Mental State Exam, LM-I = Delis Kaplan Executive Function System (DKEFS) Logical Memory Test Immediate Recall, LM-D = DKEFS Logical Memory Test Delayed Recall, LM-R – DKEFS Logical Memory Test Recognition, REYO-C = Rey Osterrieth Complex Figure Test (REYO) Copy, REYO-I = REYO Immediate Recall, REYO-D = REYO Delayed Recall, FAS = DKEFS Verbal Fluency, CAT = DKEFS Semantic Fluency, CERAD-I= Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word List Learning- Immediate Recall, CERAD-D = CERAD Word List Learning Delayed Recall, CERAD-R = CERAD Word List Learning Recognition, J-LO = Benton Judgment of Line Orientation, BNT = Boston Naming Test, Tower = DKEFS Tower Test

Several subjects reported that it was difficult at the beginning of the Greebles condition to know what details to look for, but after a number of trials it became easier. We wanted to determine if there was indeed a learning effect in this condition and, if so, whether the two subject groups demonstrated different rates of learning. To accomplish this, accuracy data from the Greebles condition was split into tertiles for each group (Figure 3) and subjected to a mixed-design repeated measures ANOVA with a within-subject factor of Tertile (first tertile, second tertile, and third tertile) and a between-subject factor of Group (at risk and control). There was a main effect of Tertile ($F_{(2,90)}$=6.50, p<0.01) and of Group ($F_{(1,45)}$=6.30, p=0.02). However there was no interaction ($F_{(2,90)}$=0.45, p=0.64). This indicates that there was a learning effect in both groups, and although at risk group was consistently less accurate than the control group, the two groups did not learn at different rates.

Eye tracking was used to determine if there were any behavioral differences in viewing behavior, specifically a difference in the within-between ratio (WBR). A repeated measures ANOVA was performed with a within-subjects factor of Stimulus Type (faces, scenes, objects, and Greebles) and a between-subjects factor of Group (at risk and control). The ANOVA revealed a main effect of Stimulus Type ($F_{(3,156)}$=203.5, p<.001) with Greebles showing the highest WBR followed by scenes, faces, and objects. There was a trend towards a main effect of Group ($F_{(1,52)}$=3.0, p=0.09). However, there was no interaction between Stimulus Type and Group ($F_{(3,156)}$=0.87 p=0.46). This indicates that, although not significant, there is a trend toward at risk subjects having a lower binding capacity than control subjects. Further investigation into this binding capacity is warranted. When data were reanalyzed with a within-subjects factor of Stimulus Type (faces,

scenes, objects, and Greebles) and a between-subjects factor of ApoE4 Carrier Status (carrier or non-carrier) we found no interaction between Stimulus Type and Carrier Status ($F_{3,150}$=0.824, p=0.483) or a main effect of Carrier Status ($F_{1,50}$=1.708, p=0.197).

Face/Name/Occupation Memory

The primary outcome measure of the FNO task was name accuracy and occupation accuracy. Performance in FNO was non-parametric, so a Mann-Whitney U test was used to test name and occupation accuracy separately. There was no difference between groups in name accuracy (Z=0.29, p=0.77), but there was a significant difference between groups in occupation accuracy (Z=-2.34, p=0.019). There were no differences between groups in total accuracy (Z=0.71, p=0.48) or reaction time (name: z=0.50, p=0.62 occupation: Z=0.65, p=0.51 total: Z=0.03, p=0.97). When subjects were classified based on carrier status of ApoE4 rather than family history of AD, there were no significant differences between groups in name accuracy (Z=0.247, p=0.80), occupation accuracy (Z=0.027, p=0.98), or total accuracy (Z=0.255, p=0.80). Using a paired student's t-test, we found that performance on face/name memory was worse than face/occupation memory for all subjects (t(55)=7.54, p<0.0001).

Object/Place Memory

There were no differences between groups in object place accuracy (t(52)=0.79, p=0.44). There were also no differences in all distances from the target (t(52)=1.11, p=0.27) or incorrect distances from the target (t(52)=0.34, p=0.74). Finally, there were no differences in reaction times between groups (t(52)=0.087, p=0.93). When the groups were divided based on ApoE4 carrier status rather than family history we found no differences between groups in object place

accuracy (t(50)=1.34, p=0.19), distances from the target (t(50)=1.07, p=0.29), incorrect distance from the target (t(50)=1.46, p=0.15), or reaction time (t(50)=0.53, p=0.60).

Pattern Separation

There were no statistically significant differences between groups in bias-corrected pattern separation (t(54)=0.70, 0.49) or bias-corrected pattern completion, although there was a trend toward a difference (t(54)=1.56, p=0.13). There were also no differences between groups in overall accuracy (t(54)=0.11, p=0.91). When groups were divided based on carrier status of ApoE4, we found no significant differences between groups in bias-corrected pattern separation (t(52)=0.047, p=0.96) bias-corrected pattern completion (t(52)=1.27, p=0.21), or overall accuracy (t(52)=0.79, p=0.43).

Discussion

Current neuropsychological testing fails to identify individuals with AD until pathology is relatively advanced and likely irreversible. Research has shown that AD pathology is present in the brain decades before most diagnoses, and this pathology is likely disrupting neuronal function in subtle ways[51]. The goal of this study was to determine whether more sensitive and specific cognitive testing would recapitulate the route by which tau pathology is known to infiltrate the MTL, i.e. beginning in the entorhinal and perirhinal cortices, then entering the CA1, and finally entering the CA3 and dentate gyrus. We accomplished this by using anatomically-designed cognitive tests targeted to each of these areas.

Oddity Detection

Previous studies have shown that the perirhinal cortex is involved in distinguishing stimuli with complex visual features within a single item (i.e., faces and objects), while the hippocampus is involved in the perceptual processing of scenes. We did not see a difference between the groups with respect to performance in scenes, indicating that it is possible that if there is early AD pathology in the at-risk group, hippocampal functioning has been left intact. While we did not observe a difference between groups in real world objects or faces, we did see a modest difference in the processing of Greebles. One reason for this may be the relative novelty of Greebles compared to objects and faces. Work done in humans, monkeys, and rats have shown that perirhinal neurons preferentially fire in response to novel objects[74,75]. Human subjects with damage to the perirhinal cortex perform worse on this task as feature ambiguity within items increases. Of the stimuli that were used, subjects reported that the Greebles contained the highest degree of feature ambiguity, which may further explain why this stimulus in particular showed an effect between groups.

There was an element of learning to this task, and an argument could be made that the at risk subjects simply took longer to learn the task than control subjects. However, this was not the case. Both groups became more accurate with time, indicating that there is a learning effect to this task. The at risk group consistently remained less accurate across time compared to the control group, but learned at a similar rate.

Unlike the other stimuli, the Greebles required mental rotation of the images both around the x-axis (the object remained in the same upright orientation but was rotated left or right) and along

the z-axis (the object was turned 90° or 180° from the upright orientation). Many studies have suggested that the more an object needs to be mentally rotated for a task, the higher the neural activity observed[76-78]. Mentally rotating an object 180° may require more mental effort than rotating it laterally, so it is possible that this extra effort may influence performance in this task. In contrast, a previous study demonstrated that rotations around the z axis resulted in better performance than rotations around the x- or y- axes[79]. A previous study examined mental rotation ability in subjects at risk for AD and found that there were no behavioral differences in reaction time or accuracy, but there were increases in activation in the right superior parietal lobule, the right insula, the right middle frontal gyrus, and the right inferior frontal gyrus[80]. In the future, the oddity detection task could be paired with fMRI to determine if there are any differences in activation in any of the brain areas associated with mental rotation.

We used eyetracking to determine if there were any behavioral differences in viewing behavior during this task. We found that although there was a modest difference in accuracy when viewing Greeble stimuli, there were no differences in the within-between ratio during the Greeble portion of the task. This indicates that the difference in accuracy is not due to a change in the number of complex features that the subject is able to hold in memory at once.

Because there was a difference between groups in this task, it is possible that there is some early pathology present in the perirhinal cortex in this group.

Face/Name/Occupation Memory

The FNO task was designed to target the CA1 region of the hippocampus, which is activated during paired association tasks[67]. Previous studies have indicated that face/name memory is

impaired in aMCI and AD patients[68] and performance on a face/name memory task correlates with amyloid load in cognitively normal older adults[81]. We were surprised to see that there was no difference between groups in the face/name component of the task, but there was a statistically significant difference between groups in the face/occupation component of the task. This is especially surprising because both groups of subjects performed worse at face/name memory than face/occupation memory.

The node structure theory (NST)[82] posits that there are semantic and phonological nodes underlying lexical nodes (words or names). These nodes connect to each other, and priming is transmitted through these connections. In order to activate a node, it needs to be primed through its connections. When a node has been sufficiently primed, its contents become available. In order to produce a word, a thought must first activate semantic nodes, then these transmit priming to the lexical node, which in turn activates the phonological nodes to form the word.

It is easier to remember occupational information because there are more semantic nodes connected to the idea of an occupation. For example, to remember that someone is a farmer you might remember that he or she wears overalls or wakes up early. Names are largely connected to phonological nodes, therefore there are fewer routes through which name information can be accessed.

Previous studies have shown that both older and younger adults make more name errors than occupation errors but older adults make more name errors than younger adults[83]. Older and younger adults make the same number of occupation errors. In this study we found that people at

risk for AD make more occupation errors compared to name errors compared to controls. It is possible that the at risk subjects are having more difficulty accessing multiple nodes. Trouble accessing nodes may be a sign of inadequate priming, and a weakened connection between nodes.

Interestingly, a recent study has shown that an area of the anterior temporal lobe, the ventral anterior temporal lobe face area (vATL), responds to conceptual information about an unknown face such as name and occupation[84,85]. In particular, the right vATL was sensitive to face/occupation information in an fMRI multivoxel pattern analysis[86]. The vATL is in or near the perirhinal cortex, the region of the brain first impacted by AD pathology. Since there was a difference in occupation memory but not face memory between groups, it is possible that the right vATL is being disproportionately affected by early AD pathology.

Object/Place Memory

The object/place task was also chose to test visuospatial associational memory in the CA1 region of the hippocampus[67,87]. Visuospatial associative learning is impaired in the earliest stages of AD, and is able to discriminate older adults who will develop AD in the future[88,89]We aimed to make this task as ecologically valid as possible by replicating the context of a common memory complaint, i.e. forgetting where an object was placed. We found that there was no difference between subject groups in any of the parameters that were examined.

Previous studies indicating that visuospatial abilities decline prior to an AD diagnosis have shown that this decline occurs approximately three years before diagnosis[90]. For this study,

subjects were middle aged, and diagnosis would not be anticipated for a decade or more. It is possible that this brain region has not begun to be affected by AD pathology. In animal studies, widespread networks have been associated with spatial association memory including the hippocampus, the perirhinal cortex, and the prefrontal cortex[87,91]. Because of the diffuse brain regions involved in this task and the unlikelihood that AD pathology is present in all of the regions, this task may not be able to detect differences in this age group.

One limitation to the ecological validity of this task is that subjects are using a two-dimensional screen to accomplish a task that is done in a three-dimensional world. Different neural systems are likely involved in processing a two-dimensional scene vs. navigating a complex three-dimensional environment. This issue can be addressed in future studies using virtual reality systems[92]. Subjects would be able to navigate and place objects in a virtual environment, and then be tested for the memory of those object locations.

Pattern Separation

Pattern separation was chosen to evaluate the function of the dentate gyrus and CA3 regions of the brain[93], These are among the last regions of the medial temporal lobe affected by AD pathology[94], therefore we did not anticipate that at risk subjects would perform more poorly than control subjects. As expected, we did not find a significant difference in subjects in pattern separation.

Interestingly, we did find a trend toward a difference in our definition of pattern completion, i.e. calling a similar item "old". We found that at risk subjects tend to have a higher rate of pattern

completion than controls. There has been some debate in the literature over whether pattern separation and pattern completion operate completely independently, or if there is competition in the hippocampus for each, resulting in a functional trade-off. Some studies have shown that typically when rates of pattern separation go down, rates of pattern completion rise, particularly in older adults[95], while others have shown that a decrease in pattern separation due to hippocampal damage does not always result in an increase in pattern separation[72,96].

There are two potential mechanisms by which pattern completion would occur: the first is that the memory for the object to be remembered has been degraded in some way, and the second is that the object was insufficiently encoded. Using eye-tracking, a previous study has demonstrated that pattern completion is likely due to insufficient encoding[97]. Successful encoding depends on a complex interaction between the prefrontal cortex and medial temporal lobe structures, and the connectivity of this network has been shown to be impaired in MCI[98]. Future studies in this population would benefit from using eye-tracking and/or fMRI to better understand the contribution of encoding processes underlying an increase in pattern completion.

Overall Discussion

This work has indicated that there are detectible cognitive differences between subjects at risk for AD and age-matched controls. AD pathology, and in particular tau pathology, begins in the perirhinal and entorhinal cortices, then enters the CA1, before finally entering the CA3 and dentate gyrus. Our hypothesis was that the at risk subjects would perform worse in oddity detection, which is dependent on intact perirhinal cortex function. If they performed worse in oddity detection, they would perform worse in face/name and/or object/place memory, which are

dependent on intact CA1 function. Finally, if they performed worse in the other tasks they may perform worse in pattern separation, which is dependent on intact CA3 and dentate gyrus function. We did not anticipate that group differences would occur in any other order.

We found that subjects did perform differently in the oddity detection task and the face/name memory task, but not in the object/place memory task or the pattern separation task. This result fits with our hypothesis and indicates that AD pathology may already be present in the perirhinal cortex and possibly the CA1 region of the hippocampus, but not in the dentate gyrus or CA3 region of the hippocampus. Due to previous work examining the earliest stages of AD pathology, we believe that these changes are likely due to underlying tau pathology and not amyloid pathology.

Novel PET ligands have been developed that are able to detect tau pathology *in vivo*[48]. Future studies should use this PET scan in conjunction with the cognitive tasks developed here to better understand the impact tau pathology may have on these cognitive tasks.

While our primary method of categorizing at risk subjects was based on family history, we also did secondary analyses where we examined carrier status of ApoE4, the most common risk gene for AD. We did not find any significant differences in any cognitive test when we analyzed data this way. This provides further evidence that family history of AD may be a stronger risk factor than ApoE4 carrier status.

Conclusion

We found that the at risk group performed worse than the controls in the oddity detection task and the FNO task, although only for select stimuli. Subjects did not differ in the object/place task or in the pattern separation task, although there was a trend toward a difference in bias-corrected pattern completion. Together, these results indicate that AD pathology may impact cognitive function in subtle ways in the earliest stages of the disease.

CHAPTER IV

NEUROVASCULAR IMAGING METHODS DO NOT PREDICT RISK OF ALZHEIMER'S DISEASE IN MIDDLE AGED SUBJECTS

Introduction

Multiple studies have suggested that vascular physiology likely plays a role in the progression of AD[55,99,100]. Epidemiological studies have indicated that AD shares a number of risk factors with cardiovascular disease including hypertension[15-17], hypercholesterolemia[18,19], diabetes mellitus[20,21], atherosclerosis[22,23], smoking[24], and obesity[25]. Post mortem studies have indicated that most AD brains exhibit vascular pathology such as microvascular disease, periventricular white matter lesions, and cerebral amyloid angiopathy[26,27]. Furthermore amyloid beta (Aβ), one of the hallmark characteristics of AD, interferes with vascular function[28,29]. Despite this evidence, it is unclear whether vascular or amyloid pathology arises first. The two-hit theory of AD posits that some vascular insult (the first hit) which may be related to aging or vascular risk factors results in changes to the blood brain barrier and hypoperfusion. This contributes to the production, accumulation, and impaired clearance of Aβ (second hit), which ultimately leads to the tissue damage and dementia associated with AD[55]. Lending support to this theory is the observation that in transgenic mice overexpressing the Swedish mutation of *App* cerebrovascular dysfunction develops earlier than cognitive changes and Aβ plaque formation[56,57].

If vascular abnormalities are present early in the pathogenesis of AD, this would have a two-fold significance. First, it would highlight the need to better manage vascular risk factors, and potentially lead to more aggressive medical treatment of cerebrovascular and cardiovascular disease, as is currently being investigated as part of a large-scale multi-site trial[101]. Secondly, *in vivo* knowledge of functional neurodegeneration is largely based on Positron Emission Tomography (PET) and more recently functional Magnetic Resonance Imaging (fMRI), both of which do not measure neuronal activity directly but rather indirectly through either metabolic (^{18}F-fluorodeoxyglucose PET) or blood flow and oxygenation changes (^{15}O PET, blood oxygenation level dependent [BOLD] fMRI, and arterial spin labeling [ASL] fMRI). In recent years BOLD fMRI has become a common method used to study brain function in living humans due to its inexpensive and noninvasive nature compared to PET. During neural activation there is a moderate increase in oxygen consumption followed by a larger increase in cerebral blood flow (CBF) to the activated region. BOLD fMRI takes advantage of this mismatch in CBF and oxygen consumption to identify areas of neural activation, usually while the subject is performing a specific task.

Since its introduction in the early 1990s thousands of papers have been published using BOLD fMRI to better understand AD, and these studies have identified both increases and decreases in BOLD signal change compared to the normal population during various tasks[102]. The results of these studies have been particularly inconclusive in studies of healthy subjects at risk for AD[103] The reported changes in BOLD response have often been attributed to either neural dysfunction or compensatory mechanisms, however these changes may instead be due to regional alterations

in cerebrovascular function. A full understanding of vascular changes associated with AD throughout the lifespan is necessary for the accurate interpretation of these studies.

Subjects with AD have a reported 40% lower rate of cerebral blood flow (CBF) compared to cognitively normal controls throughout the brain[30,31], and evidence has shown that hypoperfusion may precede clinical symptoms[32]. Mounting evidence has indicated that cerebrovascular dysfunction may occur decades before a diagnosis. Young adults who carry the epsilon 4 variant of the apolipoprotein E gene (ApoE4+), a known risk gene for AD[59,60], have shown increases, decreases, or no differences in cerebral blood flow (CBF) depending on the brain region studied[104-106]. In middle-aged subjects at risk for AD based on ApoE status and/or family history, the results have also been mixed with both hypoperfusion[107] and hyperperfusion[108] being observed. Wierenga et. al. have posited that there may be a biphasic curve of CBF function in AD with increased CBF early in life, followed by reduced CBF as the disease advances[33]. It is currently unclear whether these early changes in CBF are due to vascular factors, neural factors, or some combination of the two. In order to address this, it would be valuable to measure a factor that is strictly vascular (i.e. not neuronal), in conjunction with a behavioral task that relies on intact neuronal function without depending on vascular factors as a surrogate for neural activity. If subjects in the earliest stages of AD have compromised vascular function, but there is no evidence of a behavioral impairment, that indicates that perhaps vascular damage precedes neuronal damage. Conversely, if subjects in the earliest stages of AD demonstrate behavioral changes but no differences in vasculature, that indicates that neuronal damage precedes vascular damage.

Cerebrovascular reactivity, i.e. the responsiveness of cerebral blood vessels, can be quantified in the brain by comparing rates of blood flow at rest to blood flow during a vasoactive stimulus. Typically, the vasoactive stimulus is hypercapnia induced by breath holding, the inhalation of a low concentration of carbon dioxide, or administration of acetazolamide. The value of a hypercapnic stimulus is that it operates by activating endothelial nitric oxide synthase in vascular endothelial cells, and thus it bypasses any neural influences. The majority of previous research has shown that CVR is impaired in subjects diagnosed with AD[34-42]. Two studies have failed to find a difference in AD patients compared to controls[109,110], however both of these studies quantified CVR using Positron Emission Tomography (PET) imaging. Studies examining CVR in subjects with Mild Cognitive Impairment (MCI), a prodromal form of AD, have also demonstrated a reduction in CVR in patients compared to controls[38,39].

If cerebrovascular dysfunction is present early in the AD disease process, it is possible that CVR is impaired many years before a diagnosis. Interestingly, a recent study has shown that young ApoE4+ subjects have reduced cerebrovascular reactivity compared to carriers of the neuroprotective ApoE2 allele[111]. To our knowledge, no study has been done comparing CVR in middle aged adults at risk for AD to age-matched controls.

In the present study, CBF and CVR are examined in cognitively healthy middle-aged (40-60 yrs) subjects at increased risk for AD. Although we collected data on carrier status for Apolipoprotein E, the most common risk gene for AD, we based our risk status on first degree family history as there is evidence that family history is a stronger predictor of disease[58,112]. We focused our *a priori* region of interest (ROI) analyses specifically on the area of the brain primarily affected by

AD, i.e. the hippocampus, and total gray matter. ROI analyses were done in native space to avoid any registration deficits as a result of aging and/or AD-related tissue atrophy. We also used a discovery-driven approach to identify any other regions that were different between at risk and control subjects. We hypothesized that subjects at risk for AD would demonstrate increased CBF and decreased CVR compared to control subjects, but no differences in brain volume.

Methods

Subjects

Methods for recruitment and screening, demographic data collection, neuropsychological data collection, and genetic data collection are described in Chapter II.

Scanning Session

All scans were completed using a scanner with a field strength of 3.0T (Philips Medical Systems, Best, The Netherlands) using a 32-channel receive array head coil and a body coil for radiofrequency transmission. The entire MRI protocol took approximately 45 minutes. The subject wore an oxygen mask, nasal cannula, and pulse oximeter for the duration of the scanning session. For all scans except those used for cerebrovascular reactivity (CVR) calculation, the subject inhaled medical air (21% O_2/79% N_2). Subjects were continually monitored for heart rate, pulse oxygenation level, and end-tidal CO_2 ($EtCO_2$).

Image Acquisition

i. Structural Imaging

For image registration and volumetric analyses, a T1-weighted image was acquired (MPRAGE, TE=3.7, TR=8 ms, spatial resolution=1x1x1 mm^3).

ii. Perfusion imaging

A gradient-echo single-shot echo planar imaging (factor=37) pseudo-continuous arterial spin labeling (pCASL) sequence was used with total labeling duration=1650 ms. TR/TE = 4000/13 ms; spatial resolution = 3x3x7 mm^3; slices=17. Background suppression was used concomitantly to suppress signal from gray and white matter static tissue. A total of 30 tag/control pairs were collected for each pCASL scan. An equilibrium magnetization image (M_0) was acquired with identical spatial resolution and readout as the pCASL scan, but with repetition time=20 seconds and all background suppression and labeling pulses removed.

iii. Hypercapnia Challenge

To quantify CVR, subjects inhaled a hypercapnic gas mixture (5% CO_2/21% O_2/74% N_2) for approximately 4 minutes. The sequences and parameters used for the baseline and hypercapnic scans were identical.

Image Analysis

i. Cerebral blood flow

For pCASL scans acquired at baseline and during the hypercapnia challenge, the difference between control and label images was pair-wise subtracted after which the mean difference image was calculated. CBF values were then quantified using the optimized method described by Alsop et al[113]:

$$CBF = \frac{6000 \times \lambda \times \Delta M \times e^{\frac{PLD}{T_{1,blood}}}}{2 \times \alpha \times T_{1,blood} \times M_0 \times (1 - e^{\frac{-\tau}{T_{1,blood}}})}$$

Where λ is the blood/brain partition coefficient and is equal to 0.9 mL/g[114], ΔM is the difference in signal intensities in the control and label images, PLD is the post-labeling delay and is equal to 1525 ms, $T_{1, blood}$ is the longitudinal relaxation time of blood at 3.0T and is equal to 1650 ms[115], α is the labeling efficiency and is equal to 0.85[116], M_0 is the signal intensity of the equilibrium magnetization image, and τ is the label duration and is equal to 1650 ms. The units of CBF are mL blood/100g tissue/minute.

CBF maps were linearly registered to T1 images using FSL FLIRT[117,118], CBF is significantly reduced in white matter compared to gray matter, however due to the limited spatial resolution of ASL (3x3x7 mm^3) many voxels contain both tissue types[119]. To reduce partial volume effects, each subject's ASL data was co-registered to their T_1-weighted structural image and the fractional tissue volume was calculated in each voxel using FMRIB's Automated Segmentation Tool (FAST)[120]. Only voxels containing >85% gray matter were included in total gray matter analysis. All ROI calculations were performed in native space. Left and right hippocampal masks were created for each subject using FSL FIRST[121]. For the discovery-based approach, subjects' CBF map was registered to the Montreal Neurological Institute 2 mm standard brain and entered into Randomise, FSL's tool for nonparametric permutation inference, and analyzed with threshold-free cluster enhancement (TFCE)[122]. Data were analyzed using 500 permutations.

ii. Cerebrovascular reactivity

CVR was defined as the difference in CBF between the scans with and without hypercapnia normalized by baseline signal ($\frac{CBF_{gas}-CBF_{air}}{CBF_{air}}$). To correct for respiration differences, that value was divided by the change in $EtCO_2$ during hypercapnia (~5-10 mmHg). CVR maps were analyzed by region of interest and permutation analysis as described in the previous section.

iii. Volume and Cortical Thickness

The volume of the perirhinal cortex (PRC), the entorhinal cortex (ERC), and the hippocampus, and the cortical thickness of the PRC and ERC were calculated using FreeSurfer (version 5.3.0). FreeSurfer methods for segmentation of brain volumes have been previously described[123-135]. ROIs were chosen based on regions that are known to show the earliest AD pathology. All ROIs were defined using atlases implemented in FreeSurfer. Left and right hippocampal volumes were defined using the automated segmentation routine, left and right entorhinal volumes and cortical thickness were parcellated using the Desikan-Killiany Atlas[136], and left and right perirhinal cortex volumes and cortical thickness were parcellated using the ex-vivo Broadmann Area Atlas[137].Volumes were normalized by total brain volume.

Results

Subjects

A total of 32 subjects with a family history of AD ("At Risk") and 22 subjects with no family history of AD ("control") completed the study. Subjects were matched on age, sex, race, diabetes, smoking history (lifetime and recent), exercise, resting heart rate, and body mass index (Table 4). The at risk group had significantly more carriers of the ε4 variant of Apolipoprotein E (p=0.001, Table 4). At the time of testing, all subjects were cognitively normal, i.e., they did not

score >1.5 S.D. below the expected scores for his or her age on any neuropsychological test. There was a significant difference between groups in the immediate memory portion of the Rey-Osterrieth Complex Figure Test, but there were no significant group differences in any other neuropsychological test (Table 5).

Cerebral Blood Flow

Blood flow maps were visually inspected before and after image registration to confirm image quality (Figure 11). No differences between groups were observed in the gray matter ($F_{3,51}$=2.59, p=0.06), left hippocampus ($F_{3,51}$=1.53, p=0.21), or right hippocampus ($F_{3,51}$=0.95, p=0.42) after correcting for age, and sex (Figure 12), although there was a trend for a difference in gray matter. If groups were divided based on carrier status for ApoE4 rather than family history, there were again no differences between groups observed in the gray matter ($F_{3,50}$=2.46, p=0.07), left hippocampus ($F_{3,50}$=1.40, p=0.26), or right hippocampus ($F_{3,50}$=0.81, p=0.49). The permutation test using TFCE did not identify any significant clusters.

Cerebrovascular Reactivity

After correcting for age and sex there were no CVR differences observed between groups in gray matter ($F_{3,42}$=0.78, p=0.51), the left hippocampus ($F_{3,44}$=1.5, p=0.24), or the right hippocampus ($F_{3,47}$=0.54, p=0.66) (Figure 13). After dividing groups based on carrier status of ApoE4, we

	At Risk (n=32)	Control (n=22)	p-value
Age (yrs ± s.d.)	53±6	53±6	0.94
Sex (% female)	81	60	0.07
Race (% non-white)	6	18	0.16
ApoE4 (% carriers)	63	18	0.001**
Diabetes (%)	3	0	0.419
Smoking in the past month (%)	9	9	1
Lifetime smoking (%)	27	27	1
Exercise (rating out of 4 ± s.d.)	3.5±0.9	3.2±1	0.24
Resting heart rate (beats per minute ± s.d.)	70±12	71±11	0.861
Body mass index (mean ± s.d.)	28±5	27±6	0.453

Table 4: Demographic data. There were no significant differences between groups in age, sex, or race. There were also no differences between groups in history of diabetes, smoking, exercise, resting heart rate, or body mass index. There were significantly more ApoE4 carriers in the at risk group (p=0.001).

	At Risk (n=32) Mean (SEM)	Control (n=22) Mean (SEM)	p-value
MMSE	29.89 (0.06)	29.8 (0.1)	0.36
LM-I	28 (1)	28 (1)	0.85
LM-D	24 (1)	24 (1)	0.92
LM-R	26.1 (0.4)	26.4 (0.4)	0.61
REYO-C	34.8 (0.3)	35.2 (0.3)	0.32
REYO-I	21 (1)	23.6 (0.9)	0.045*
REYO-D	21 (1)	23.3 (0.9)	0.09
FAS	42 (2)	48 (3)	0.10
CAT	57 (2)	61 (2)	0.23
Trails A	26 (1)	28 (1)	0.21
Trails B	56 (2)	60 (2)	0.23
CERAD-I	24.1 (0.5)	25 (0.6)	0.24
CERAD-D	7.8 (0.3)	8.5 (0.3)	0.11
CERAD-R	9.85 (0.08)	9.91 (0.06)	0.58
JLO	23.6 (0.7)	25.4 (0.9)	0.14
BNT	57.1 (0.6)	57.4 (0.7)	0.72
Tower	493 (32)	476 (35)	0.72

Table 5: Neuropsychological testing. There were a significant difference between groups in the immediate memory portion of the Rey-Osterrieth Complex Figure Test. No other significant differences were found. MMSE = Mini Mental State Exam, LM-I = Delis Kaplan Executive Function System (DKEFS) Logical Memory Test Immediate Recall, LM-D = DKEFS Logical Memory Test Delayed Recall, LM-R – DKEFS Logical Memory Test Recognition, REYO-C = Rey Osterrieth Complex Figure Test (REYO) Copy, REYO-I = REYO Immediate Recall, REYO-D = REYO Delayed Recall, FAS = DKEFS Verbal Fluency, CAT = DKEFS Semantic Fluency, CERAD-I= Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word List Learning- Immediate Recall, CERAD-D = CERAD Word List Learning Delayed Recall, CERAD-R = CERAD Word List Learning Recognition, J-LO = Benton Judgment of Line Orientation, BNT = Boston Naming Test, Tower = DKEFS Tower Test

again found no differences between groups in gray matter ($F_{3,39}$=1.20, p=0.33), the left hippocampus ($F_{3,39}$=0.78, p=0.51), or the right hippocampus ($F_{3,39}$=1.05, p=0.38) The permutation test using TFCE did not identify any significant clusters.

Volume and Cortical Thickness

After segmenting the brain into pre-defined regions of interest (left and right PRC, ERC, and hippocampus), volumes were calculated and normalized by total brain volume. The cortical thickness of each cortical region (the PRC and ERC) were also calculated. After correcting for age and sex no differences were found between groups in any region studied (Figure 14). There were also no cortical thickness or volume differences observed in any brain region when groups were divided based on carrier status of ApoE4 (Figure 15).

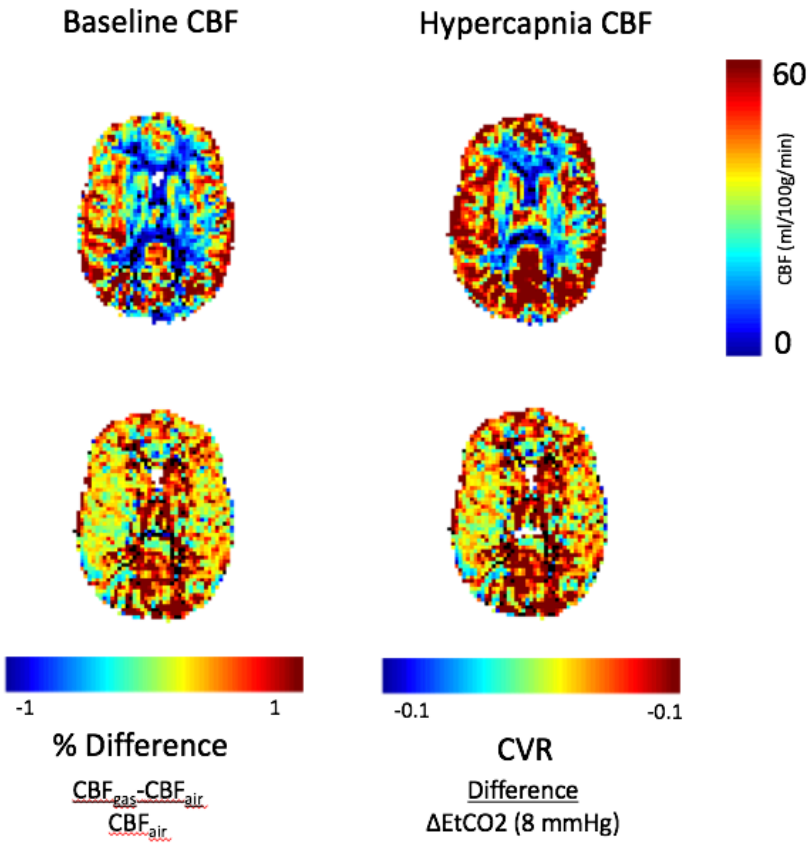

Figure 11: Representative images of neurovascular imaging. All CBF and CVR maps were visually inspected and registered to the subjects' T1 image.

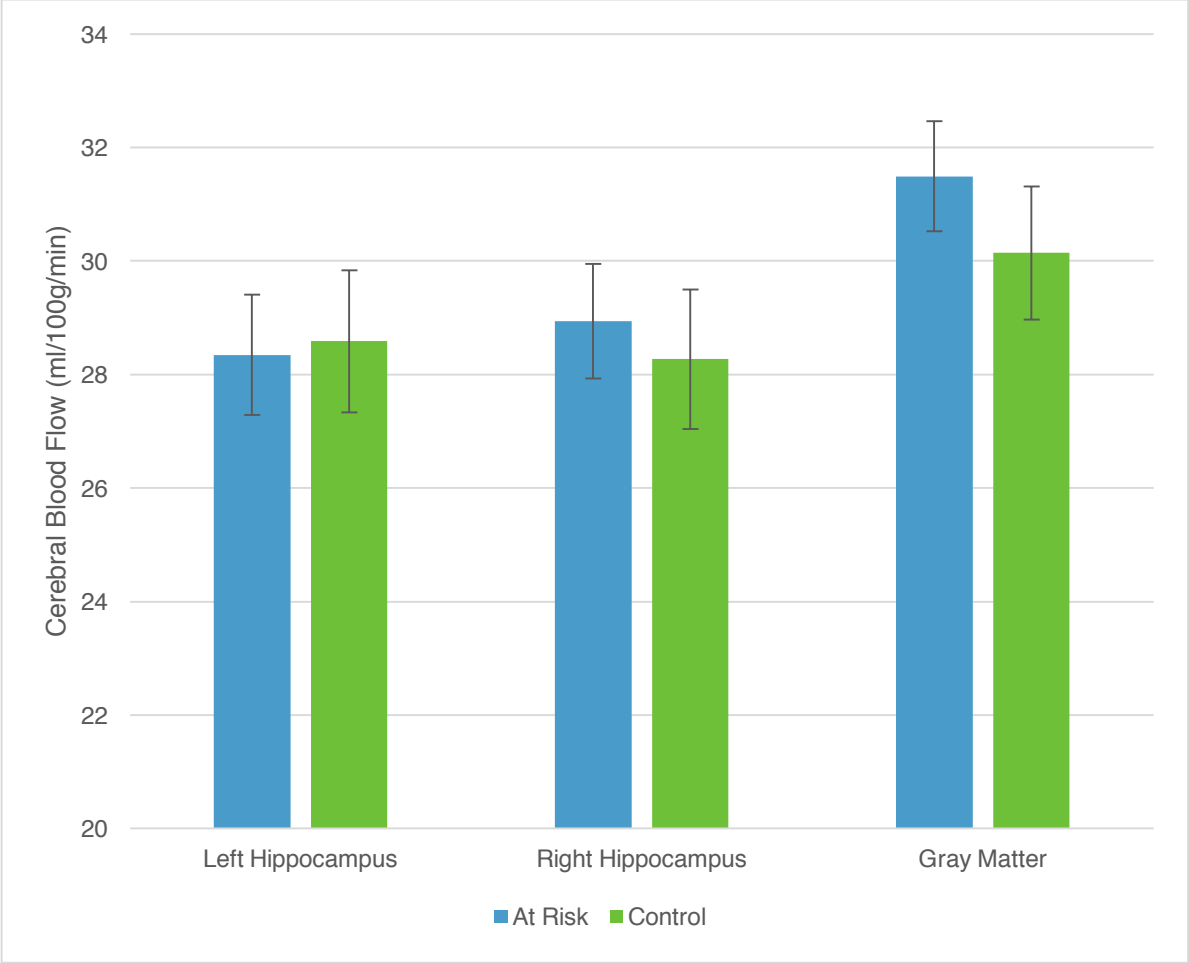

Figure 12: Cerebral blood flow results. No statistically significant differences were found in CBF between the at risk group and the control group in any region studied. Error bars are SEM

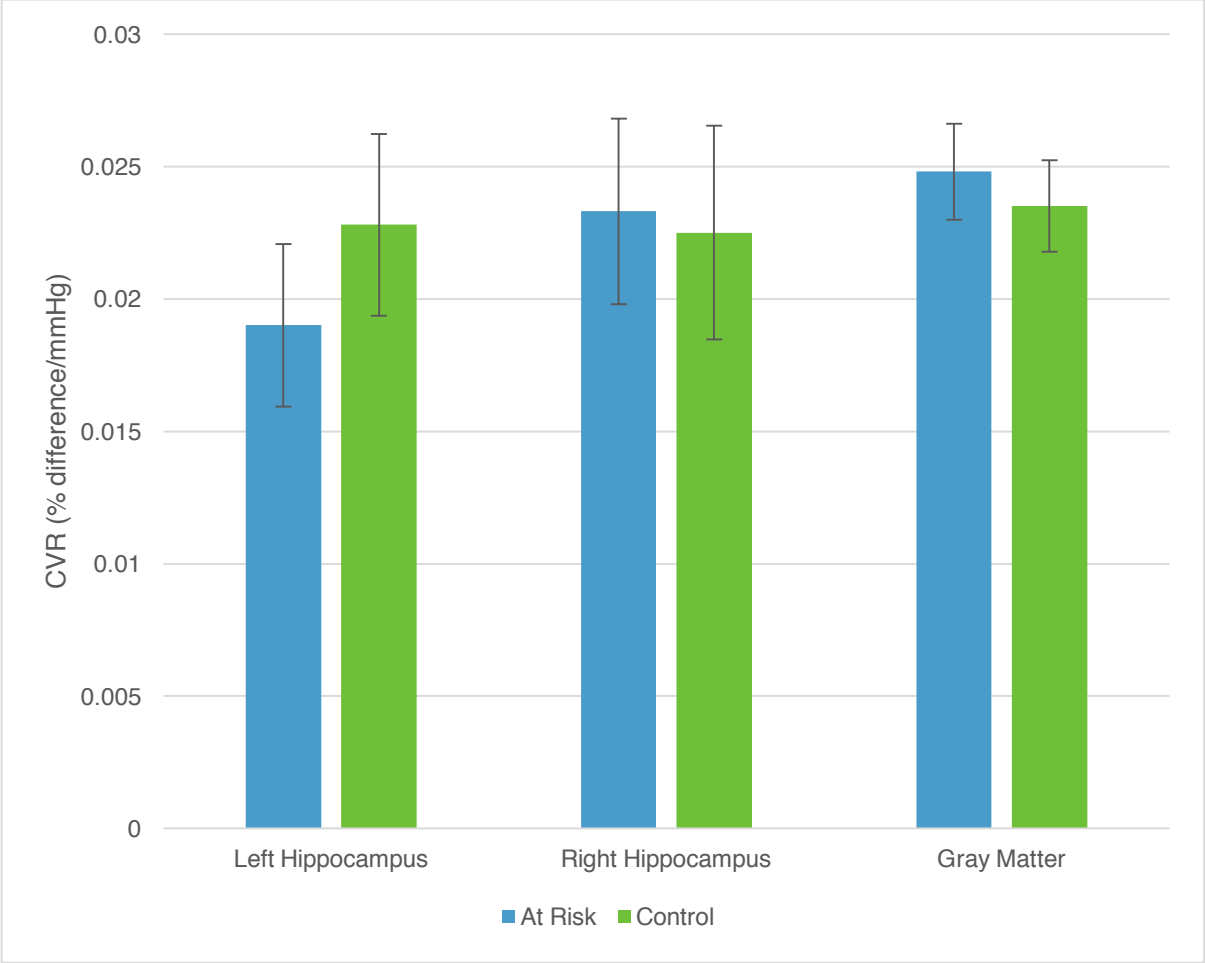

Figure 13: Cerebrovascular reactivity results. No statistically significant differences were found in CVR between the at risk group and the control group in any region studied. Error bars are SEM.

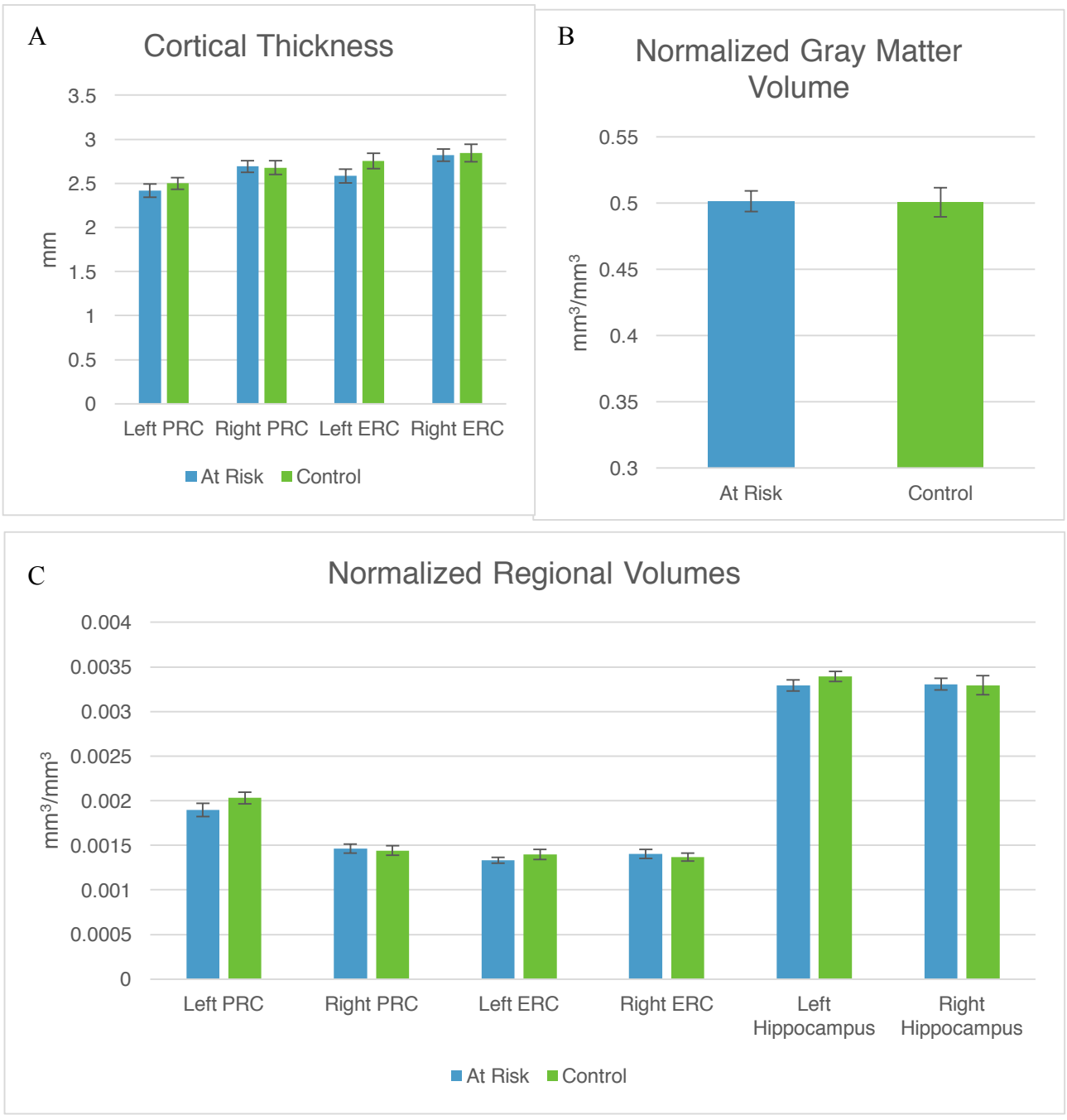

Figure 14: Volumetric results by family history. There were no between group differences in A. cortical thickness, B. Normalized gray matter volume, or C. Region of interest volumes in any region studied.

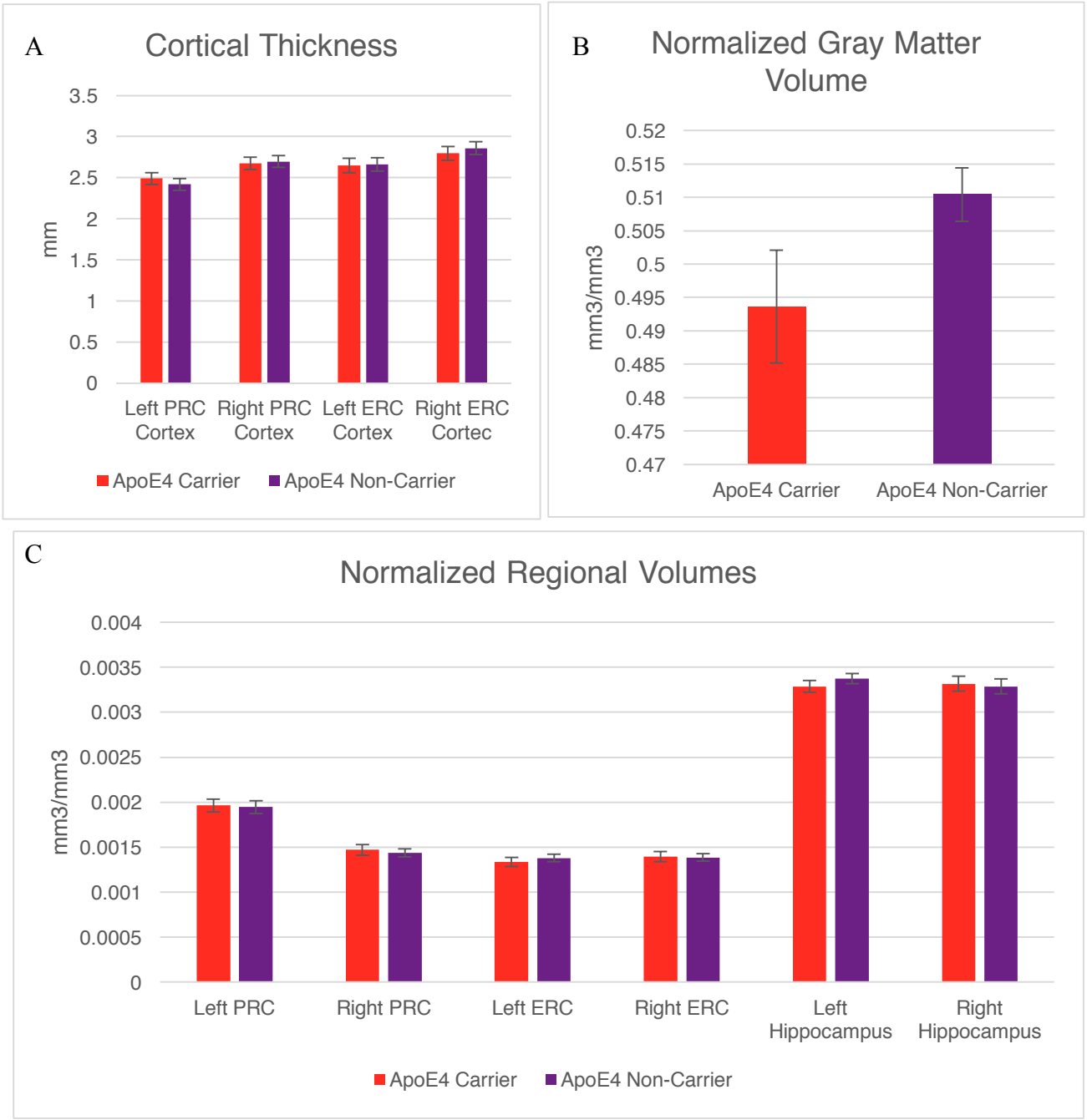

Figure 15: Volumetric results by ApoE4 Carrier Status. There were no between group differences in A. cortical thickness, B. Normalized gray matter volume, or C. Region of interest volumes in any region studied.

Discussion

The aim of this study was to determine if middle aged subjects who are at increased risk for developing AD in the future show any functional cerebrovascular differences. To determine this, arterial spin labeling in conjunction with a hypercapnic challenge was used to measure cerebral blood flow and cerebrovascular reactivity. We found that there were no significant differences between groups in either cerebral blood flow, or cerebrovascular reactivity.

Measuring cerebral blood flow has been described as a potentially useful method for identifying candidates for future AD trials who are currently in the preclinical phase of the disease[138]. Previous studies have indicated that subjects at risk for AD have higher resting CBF than controls[33,104,108], however we did not observe any differences between groups whether determining risk based on family history or ApoE4 carrier status. Wierenga et. al. have posited that CBF may be higher in subjects at risk for AD, but while AD advances CBF becomes lower than controls[139]. Weirenga's theory is based on work that has been done in younger adults (20s)[33] at risk due to ApoE4 carrier status, older adults (60s+)[140] at risk due to ApoE4 carrier status, and MCI and AD patients. One other study investigated CBF in middle aged adults (age 40-65 years) and found evidence of hypoperfusion in the right superior and middle frontal cortices of subjects with a family history of AD compared to controls. They did not find any significant differences between groups in the medial temporal lobes. Subjects in their 40s and 50s are at an age in which we expect that some AD pathology is present, but it isn't yet debilitating. It is possible that the 40s and 50s are the transition point between hyper- and hypoperfusion, which would make it difficult to detect any differences between groups. There may be real changes

taking place in the cerebrovasculature, but methods such as ASL are not sufficiently sensitive to identify these changes.

Another study that looked at hypoperfusion in healthy middle aged adults with and without a family history of AD found that there was only minimal hypoperfusion in the group with a family history, but when only the subjects with a maternal history of AD were analyzed there was a significant difference between groups[107]. We did not collect information on maternal vs. paternal history of AD in this group, but it is possible that if we had broken the data down in this way we would have found a significant result. If that is true, that raises the question of why maternal history in particular would be driving perfusion differences between groups, and is worth further study.

Cerebrovascular reactivity is an index by which the dilatory capacity of blood vessels can be measured. A deficit in CVR would indicate that blood vessels have difficulty expanding in response to a vasoactive stimulus such as hypercapnia. Previous studies have shown that CVR is diminished in animal models of AD, and AD patients[37,141,142]. In studies of cerebrovascular aging, CVR seems to decline earlier and at a greater rate than CBF[143], so we predicted that subjects at risk for AD would have decreased CVR compared to controls even if there were no detectible differences in CBF. We found that there were no differences between groups in any region in our measure of CVR when groups were divided based on either family history or ApoE4 carrier status. Arterial spin labeling has been shown to be a reliable method for the quantification of CVR and comparable with techniques that have used BOLD measures, particularly in the frontal and temporal lobes[144]. The average CVR maps that were generated individually and for each

group demonstrate a low signal to noise ratio, so it is possible that there is a difference in CVR between groups, but it is not detectible. Detection could be improved by further optimizing the pCASL sequence, or adjusting the background suppression pulses. BOLD imaging has a higher signal to noise ratio than pCASL, so repeating the experiment using BOLD may provide additional information.

If there truly are no differences between the at risk group and the control group in cerebrovascular function, this would lend additional support to neuroimaging studies that have used BOLD fMRI to assess neural activation and/or connectivity. BOLD fMRI relies on intact vascular function. There are thousands of published studies that have used BOLD fMRI as a marker of neural dysfunction, but changes in vasculature would limit the interpretability of those studies. The current study has not demonstrated any differences in vascular function between groups, meaning that fMRI studies in this population may not need to adjust for vascular insufficiencies and can be interpreted as neural changes.

As expected, no differences were observed in tissue volume or cortical thickness in any region that was studied when determining risk based on either family history or ApoE4 carrier status. Subjects are relatively young (40-50 years old) and we would not anticipate that they would be experiencing any AD-related tissue atrophy at this stage.

One of the major limitations to this study is that it is cross-sectional rather than longitudinal. We currently have no way of knowing if the at risk group will go on to develop AD in the future. If

we were able to revisit these subjects in 10-20 years to assess who developed AD and who didn't, it would give us a better idea of the changes that are taking place in preclinical AD.

Conclusions

Based on previous studies, we hypothesized that subjects would have increased cerebral blood flow and deceased cerebrovascular reactivity. We found that there were no significant differences between groups in either CBF or CVR. There were also no significant differences between groups in the volume or cortical thickness of any region studied.

CHAPTER V

CEREBROVASCULAR REACTIVITY, BUT NOT CEREBRAL BLOOD FLOW, CORRELATES WITH COGNITION IN SUBJECTS AT RISK FOR ALZHEIMER'S DISEASE

Introduction

In the earliest stages of AD, there are likely complex interactions occurring between different physiological processes. In order to better understand these interactions, it is useful to determine if any potential early biomarkers of the disease correlate with one other. The first aim of this study was to determine if four cognitive tests that relied on various structures of the medial temporal lobe (oddity detection, face/name/occupation memory, object/place memory, and pattern separation) were impaired in subjects at risk for AD. The second aim of this study was to determine if imaging markers of neurovascular function (cerebral blood flow and cerebrovascular reactivity) were either higher or lower in subjects at risk for AD, indicating early neurovascular impairments. Neurovascular function has been shown to correlate with cognitive function[35,37,40,145-147], therefore the third aim of this study was to determine if neurovascular function was correlated with cognitive function in these subjects using the specific cognitive tests and neuroimaging methodology described previously. We wanted to determine if the relationship between these factors differs in subjects at risk for AD compared to control subjects.

Identifying correlations between neuroimaging and cognition has been difficult in the past due to an inherent circularity in the analyses[148] or not controlling for underlying factors such as age or education[149]. Because our neuroimaging markers were vascular in nature and didn't involve any psychological manipulation, we did not feel that circular analyses would be an issue. In addition, we corrected for age in all of our correlations. Pearson correlations can be sensitive to outliers, so we carefully removed outliers from analyses.

We hypothesized that a higher rate of cerebral blood flow (CBF) and a lower rate of cerebrovascular reactivity (CVR) would be correlated with reduced performance in the cognitive tasks. As we presented in Chapter III, the cognitive tasks were designed to target the areas that are first impacted by AD pathology[49], therefore we anticipated that oddity detection (perirhinal cortex) would correlate most strongly with neurovascular function, followed by face/name/occupation memory (CA1), object/place memory (CA1), and pattern separation (DG/CA3).

Methods

Subjects

Methods for recruitment and screening, demographic data collection, neuropsychological data collection, and genetic data collection are described in Chapter II.

Cognitive Tests

All cognitive tests used in this analysis were described in Chapter III. To reduce the number of comparisons, only the variables that we considered most relevant for each cognitive test were included in correlational analyses. For the oddity detection task, accuracy and the within between

ratio in the four stimuli categories were included. For the face/name/occupation task, accuracy in face/name memory, accuracy in face/occupation memory, and total accuracy were included. For the object/place task accuracy, distance from target, and incorrect distance from the target were included. For pattern separation, accuracy, responding "old" to a lure stimulus (uncorrected pattern completion), responding "similar" to a lure stimulus (uncorrected pattern separation), bias-corrected pattern separation, and bias-corrected pattern completion were included.

Neurovascular Tests

Neurovascular tests were done as described in Chapter IV. Again, only the most relevant variables were included in correlational analyses. For both CBF and CVR, only the right and left hippocampi and total gray matter were included. If a correlation was found between a behavioral measure and total gray matter, a further analysis was done to determine if a particular brain lobe was driving the correlation. The CBF and CVR maps were registered to the Montreal Neurological Institute's 2 mm brain atlas using FSL FLIRT[117,118], and the brain was segmented into the four major brain lobes (frontal, occipital, parietal, and temporal) using a probabilistic atlas thresholded at 50%.

Statistical Tests

Data were inspected for outliers, and any outliers were removed from analyses. Outliers were defined as >1.5 times the interquartile range (IQR). A zero-order Pearson's correlation analysis was done correcting for age. Missing values were handled pairwise.

Because of the relatively small number of subjects we had compared to many clinical correlation studies, and the high number of comparisons we made, we felt that correcting for multiple comparisons may result in a high number of type II errors (false negatives). It has been suggested

that often in clinical research it may be more appropriate to report the statistical tests used and keep the multiple comparison problem in mind while interpreting the results, rather than performing a multiple comparison correction outright[150]. We chose to report results without performing this correction.

Results

At Risk Subjects

i. Oddity Detection and Neurovasculature

A significant positive correlation was found between the within/between ratio when viewing face stimuli, and CVR in total gray matter (r_s=0.46, p=0.03), which indicates that subjects with higher CVR performed better in this task. To better understand if a specific brain lobe was driving this correlation, gray matter was further segmented into the major brain lobes (frontal, occipital, parietal, and temporal) and it was found that there were no significant correlations between face within/between ratio and any specific brain lobe, although there was a trend toward a negative correlation between face within/between ratio and the occipital lobe (r_s=-0.392, p=0.058) and a positive correlation between face within/between ratio and the parietal lobe (r_s=0.381, p=0.067) A higher within/between ratio is indicative of a higher binding ability of complex features, so increased CVR in the parietal lobe may improve binding ability.

ii. Face/Name/Occupation Memory and Neurovasculature

A significant negative correlation was found between cerebrovascular reactivity in the left hippocampus and face/name memory accuracy (r_s=-0.45, p=0.03), which indicates that subjects

with higher CVR in the hippocampus perform worse on this task. No other significant relationships were found.

			Accuracy				Within-Between Ratio			
			Face	Object	Scene	Greeble	Face	Object	Scene	Greeble
Cerebral Blood Flow	Left Hippocampus	r_s	0.17	0.04	-0.01	-0.07	-0.24	-0.17	-0.08	-0.31
		p	0.38	0.86	0.98	0.74	0.20	0.37	0.69	0.09
		d.f.	27	28	28	27	28	27	28	28
	Right Hippocampus	r_s	0.07	0.05	-0.01	0.04	-0.15	-0.14	-0.01	-0.24
		p	0.72	0.80	0.95	0.84	0.43	0.47	0.97	0.21
		d.f.	27	28	28	27	28	27	28	28
	Gray Matter	r_s	0.08	0.21	0.10	0.20	0.09	0.12	0.09	-0.26
		p	0.69	0.28	0.61	0.30	0.66	0.56	0.65	0.18
		d.f.	26	27	27	26	27	26	27	27
Cerebrovascular Reactivity	Left Hippocampus	r_s	-0.04	0.08	-0.03	-0.21	-0.09	0.19	-0.23	-0.05
		p	0.87	0.71	0.90	0.34	0.66	0.39	0.27	0.80
		d.f.	22	23	23	22	23	22	23	23
	Right Hippocampus	r_s	-0.32	0.02	0.09	0.03	0.00	0.06	-0.10	-0.06
		p	0.10	0.94	0.65	0.90	0.99	0.76	0.60	0.76
		d.f.	25	26	26	25	26	25	26	26
	Gray Matter	r_s	-0.10	0.01	0.11	0.30	**0.46**	0.34	0.20	0.18
		p	0.67	0.96	0.60	0.17	**0.03***	0.11	0.36	0.40
		d.f.	21	22	22	21	**22**	21	22	22

Table 6: Correlations between CBF and CVR and behavioral measures from the oddity detection task in at risk subjects. A significant correlation was found between the within-between ratio when viewing face stimuli and gray matter CVR.

Brain Region			Face Within-Between Ratio
Cerebrovascular Reactivity	Frontal Lobe	r	0.211
		p	0.323
		d.f.	22
	Occipital Lobe	r	-0.392
		p	0.058
		d.f.	22
	Parietal Lobe	r	0.381
		p	0.067
		d.f.	22
	Temporal Lobe	r	0.248
		p	0.244
		d.f.	22

Table 7: Correlations CVR and Face Within-Between Ratios broken down by brain lobe. There were no significant correlations between CVR in any specific lobe and Face Within-Between Ratio, although there is a trend toward a correlation in the occipital lobe and parietal lobe.

			Total Accuracy	Name Accuracy	Occupation Accuracy
Cerebral Blood Flow	Left Hippocampus	r_s	-0.06	-0.04	-0.13
		p	0.76	0.85	0.50
		d.f.	28	28	29
	Right Hippocampus	r_s	0.02	0.00	-0.03
		p	0.91	0.99	0.87
		d.f.	28	28	29
	Gray Matter	r_s	-0.17	-0.25	-0.04
		p	0.37	0.19	0.83
		d.f.	27	27	28
Cerebrovascular Reactivity	Left Hippocampus	r_s	-0.36	**-0.45**	0.02
		p	0.08	**0.03***	0.91
		d.f.	22	**22**	23
	Right Hippocampus	r_s	0.00	-0.13	0.28
		p	0.99	0.51	0.14
		d.f.	25	25	26
	Gray Matter	r_s	-0.16	-0.24	0.14
		p	0.47	0.28	0.51
		d.f.	21	21	22

Table 8: Correlations between CBF and CVR and behavioral measures from the face/name/occupation memory task in at risk subjects. A significant correlation was found between face/name memory and CVR in the left hippocampus.

iii. Object/Place Memory and Neurovasculature

Several significant correlations were found between CVR and object/place memory. There was a negative correlation found between distance from target and right hippocampus CVR (r_s=-0.38, p=0.046) and distance from target and gray matter CVR (r_s=-0.46, p=0.02). There was also a positive correlation found between accuracy and right hippocampus CVR (r_s=0.4, p=0.04) and accuracy and gray matter CVR (r_s=0.51, p=0.01). These results indicate that, in general, higher CVR in the right hippocampus and gray matter results in a better performance on this task. To better understand if any specific brain lobe was driving the trend in gray matter, the brain was further segmented into the major brain lobes (frontal, occipital, parietal, and temporal) and it was found that there were no significant correlations between distance from target and any brain lobe. There were also no significant correlations found between accuracy and any brain lobe, although there was a trend toward a positive correlation between accuracy and CVR in the parietal lobe (r_s=0.403, p=0.051).

iv. Pattern Separation and Neurovasculature

No significant correlations were found between performance in the pattern separation task and any neurosvascular factor studied.

			Distance	Accuracy	Incorrect Distance
Cerebral Blood Flow	Left Hippocampus	r_s	0.17	-0.18	0.05
		p	0.36	0.35	0.79
		d.f.	28	28	25
	Right Hippocampus	r_s	0.11	-0.12	0.13
		p	0.55	0.55	0.52
		d.f.	28	28	25
	Gray Matter	r_s	-0.13	0.17	0.30
		p	0.51	0.38	0.13
		d.f.	27	27	24
Cerebrovascular Reactivity	Left Hippocampus	r_s	0.32	-0.24	0.23
		p	0.12	0.24	0.32
		d.f.	23	23	20
	Right Hippocampus	r_s	**-0.38**	**0.40**	0.12
		p	**0.046***	**0.04***	0.56
		d.f.	**26**	**26**	23
	Gray Matter	r_s	**-0.46**	**0.51**	0.29
		p	**0.02***	**0.01***	0.20
		d.f.	**22**	**22**	19

Table 9: Correlations between CBF and CVR and behavioral measures from the object/place memory task in at risk subjects. Significant correlations were found between distance and accuracy in the task and right hippocampus and gray matter CVR.

Brain Region			Distance	Accuracy
Cerebrovascular Reactivity	Frontal Lobe	r	-0.337	0.381
		p	0.107	0.66
		d.f.	22	22
	Occipital Lobe	r	-0.299	0.318
		p	0.155	0.13
		d.f.	22	22
	Parietal Lobe	r	-0.310	0.403
		p	0.141	0.051
		d.f.	22	22
	Temporal Lobe	r	-0.173	0.292
		p	0.418	0.166
		d.f.	22	22

Table 10: Correlations CVR and Object Place measures broken down by brain lobe. There were no significant correlations between CVR in any specific lobe and Object Place measures, although there is a trend toward a correlation between accuracy and CVR in the parietal lobe.

			Responses to Lures		Accuracy	Pattern Separation	Pattern Completion
			"Old"	"Similar"			
Cerebral Blood Flow	Left hippocampus	r_s	-0.20	0.17	0.06	0.10	-0.20
		p	0.28	0.38	0.75	0.59	0.28
		d.f.	29	29	29	29	29
	Right Hippocampus	r_s	-0.32	0.28	0.08	0.21	-0.32
		p	0.08	0.12	0.68	0.26	0.08
		d.f.	29	29	29	29	29
	Gray Matter	r_s	-0.22	0.21	0.25	0.15	-0.26
		p	0.25	0.28	0.19	0.44	0.17
		d.f.	28	28	28	28	28
Cerebrovascular Reactivity	Left Hippocampus	r_s	0.12	-0.14	0.15	-0.22	0.17
		p	0.56	0.50	0.47	0.30	0.42
		d.f.	23	23	23	23	23
	Right Hippocampus	r_s	0.07	0.03	0.21	0.02	0.12
		p	0.71	0.90	0.28	0.91	0.55
		d.f.	26	26	26	26	26
	Gray Matter	r_s	0.02	0.08	0.07	0.06	0.00
		p	0.92	0.71	0.76	0.80	1.00
		d.f.	22	22	22	22	22

Table 11: Correlations between CBF and CVR and behavioral measures from the pattern separation task in at risk subjects. No significant correlations were found.

Control Subjects

i. Oddity Detection and Neurovasculature

A significant positive correlation was found between accuracy when viewing face stimuli and gray matter CBF (r_s=0.63, p=0.01), indicating that higher CBF is associated with a better performance. To determine if one particular brain lobe was driving this trend, the brain was further segmented into the major brain lobes (frontal, occipital, parietal, and temporal). A significant positive correlation was found between face accuracy and CBF in the occipital lobe (r_s=0.64, p=0.019) and the temporal lobe (r_s=0.606, p=0.028) and a trend toward a positive correlation between face accuracy and CBF in the parietal lobe (r_s=0.524, p=0.066).

ii. Face/Name/Occupation Memory and Neurovasculature

No significant correlations were found between face/name/occupation memory and neurovascular function.

iii. Object/Place Memory and Neurovasculature

A significant negative correlation was found between gray matter CBF and distance from target (r_s=-0.53, p=0.02). There was also a trend toward a significant positive correlation between gray matter CBF and accuracy (r_s=0.45, p=0.06). Both of these results indicate that higher CBF in the gray matter results in a better performance on this task. To determine if one particular brain lobe was driving this trend, the brain was further segmented into the major brain lobes (frontal, occipital, parietal, and temporal). A strong negative correlation was found between the parietal lobe and distance from target (r_s=-0.689, p=0.009), and a negative correlation as found between

the frontal lobe and distance from target (r_s=-0.575, p=0.04). There was also a trend toward a negative correlation between the occipital lobe and distance from target (r_s=-0.521, p=0.068).

iv. Pattern Separation and Neurovasculature

No significant correlations were found between performance on the pattern separation task and neurovascular function.

			Accuracy				Within-Between Ratio			
			Face	Object	Scene	Greeble	Face	Object	Scene	Greeble
Cerebral Blood Flow	Left Hippocampus	r_s	0.29	0.14	0.08	0.31	0.11	0.24	0.14	0.21
		p	0.26	0.56	0.73	0.19	0.65	0.33	0.56	0.39
		d.f.	15	17	17	17	17	17	17	17
	Right Hippocampus	r_s	0.30	0.11	-0.08	0.18	0.20	0.29	0.05	0.18
		p	0.24	0.66	0.76	0.47	0.42	0.23	0.83	0.45
		d.f.	15	17	17	17	17	17	17	17
	Gray Matter	r_s	**0.63**	0.33	0.20	0.23	0.14	0.17	0.02	0.06
		p	**0.01***	0.16	0.41	0.35	0.57	0.48	0.93	0.81
		d.f.	**15**	17	17	17	17	17	17	17
Cerebrovascular Reactivity	Left Hippocampus	r_s	0.15	-0.03	0.19	0.13	-0.09	0.17	-0.17	-0.19
		p	0.56	0.91	0.46	0.61	0.73	0.51	0.51	0.44
		d.f.	15	16	16	16	16	16	16	16
	Right Hippocampus	r_s	0.13	0.14	0.35	0.19	-0.03	0.20	0.10	0.07
		p	0.62	0.58	0.15	0.43	0.91	0.42	0.69	0.77
		d.f.	15	17	17	17	17	17	17	17
	Gray Matter	r_s	0.03	0.17	0.41	0.24	0.28	0.27	0.39	0.29
		p	0.90	0.51	0.09	0.34	0.25	0.28	0.12	0.24
		d.f.	15	16	16	16	16	16	16	16

Table 12: Correlations between CBF and CVR and behavioral measures from the oddity detection task in control subjects. A significant correlation was found between accuracy when viewing face stimuli and cerebral blood flow in gray matter.

Brain Region			Face Accuracy
Cerebral Blood Flow	Frontal Lobe	r	0.446
		p	0.127
		d.f.	11
	Occipital Lobe	r	**0.64**
		p	**0.019**
		d.f.	**11**
	Parietal Lobe	r	0.524
		p	0.066
		d.f.	11
	Temporal Lobe	r	**0.606**
		p	**0.028**
		d.f.	**11**

Table 13: Correlations between CBF and accuracy when viewing face stimuli broken down by brain lobe. Significant correlations were found between CBF in the occipital lobe and the temporal lobe and accuracy when viewing face stimuli.

			Total Accuracy	Name Accuracy	Occupation Accuracy
Cerebral Blood Flow	Left Hippocampus	r_s	0.04	-0.11	0.31
		p	0.88	0.66	0.19
		d.f.	17	17	18
	Right Hippocampus	r_s	0.00	-0.14	0.25
		p	0.99	0.58	0.29
		d.f.	17	17	18
	Gray Matter	r_s	0.30	0.19	0.20
		p	0.22	0.43	0.42
		d.f.	17	17	17
Cerebrovascular Reactivity	Left Hippocampus	r_s	-0.11	-0.20	0.14
		p	0.66	0.43	0.58
		d.f.	16	16	16
	Right Hippocampus	r_s	-0.31	-0.37	0.12
		p	0.20	0.12	0.62
		d.f.	17	17	17
	Gray Matter	r_s	-0.25	-0.33	0.21
		p	0.32	0.19	0.40
		d.f.	16	16	16

Table 14: Correlations between CBF and CVR and behavioral measures from the face/name/occupation memory task. No significant correlations were found.

			Distance	Accuracy	Incorrect Distance
Cerebral Blood Flow	Left Hippocampus	r_s	-0.37	0.36	0.24
		p	0.13	0.14	0.39
		d.f.	16	16	13
	Right Hippocampus	r_s	-0.29	0.26	0.35
		p	0.24	0.30	0.20
		d.f.	16	16	13
	Gray Matter	r_s	**-0.53**	0.45	-0.09
		p	**0.02***	0.06	0.75
		d.f.	**16**	16	13
Cerebrovascular Reactivity	Left Hippocampus	r_s	0.12	-0.14	0.10
		p	0.65	0.59	0.73
		d.f.	15	15	12
	Right Hippocampus	r_s	0.20	-0.24	-0.24
		p	0.43	0.34	0.39
		d.f.	16	16	13
	Gray Matter	r_s	0.04	-0.10	-0.32
		p	0.89	0.71	0.27
		d.f.	15	15	12

Table 15: Correlations between CBF and CVR and behavioral measures from the object/place memory task. There was a significant correlation between distance from target and gray matter CBF.

Brain Region			Object place Distance
Cerebral Blood Flow	Frontal Lobe	r	**-0.575**
		p	**0.04**
		d.f.	**11**
	Occipital Lobe	r	-0.521
		p	0.068
		d.f.	11
	Parietal Lobe	r	**-0.689**
		p	**0.009**
		d.f.	**11**
	Temporal Lobe	r	-0.47
		p	0.105
		d.f.	11

Table 16: Correlations between Object Place Distance and CBF broken down by brain region. There is a significant correlation between object place distance and CBF in the frontal lobe and parietal lobe.

			Response to Lure		Accuracy	Pattern Separation	Pattern Completion
			"Old"	"Similar"			
Cerebral Blood Flow	Left Hippocampus	r_s	0.02	0.03	0.18	0.29	0.15
		p	0.93	0.92	0.45	0.22	0.53
		d.f.	18	18	17	18	18
	Right Hippocampus	r_s	0.10	-0.05	0.16	0.22	0.21
		p	0.69	0.83	0.52	0.35	0.38
		d.f.	18	18	17	18	18
	Gray Matter	r_s	0.15	0.16	0.12	0.13	0.16
		p	0.54	0.52	0.63	0.59	0.51
		d.f.	17	17	17	17	17
Cerebrovascular Reactivity	Left Hippocampus	r_s	0.00	0.12	-0.28	-0.05	0.09
		p	0.99	0.62	0.27	0.84	0.73
		d.f.	16	16	16	16	16
	Right Hippocampus	r_s	0.07	0.12	-0.22	-0.17	0.13
		p	0.77	0.62	0.36	0.49	0.60
		d.f.	17	17	17	17	17
	Gray Matter	r_s	-0.03	0.23	-0.14	-0.01	0.05
		p	0.90	0.36	0.58	0.97	0.84
		d.f.	16	16	16	16	16

Table 17: Correlations between CBF and CVR and behavioral measures from the pattern separation task. No significant correlations were found.

Discussion

After correcting for age, several correlations were found between cognition and neurovascular function.

At Risk Subjects

When only the subjects at risk for AD were analyzed, relationships between CVR and cognition were discovered. There was a significant positive correlation found between the within between ratio when viewing face stimuli and CVR in the gray matter. When the gray matter trend was further explored, there was not a specific brain region that was significantly driving this trend, although there was a trend toward a negative correlation between the occipital lobe and face within between ratio and a positive correlation between the parietal lobe and face within between ratio. Additionally, CVR in the left hippocampus was associated with decreased face/name memory. Finally, right hippocampus CVR and gray matter CVR was negatively correlated with distance to the target and positively correlated with accuracy in the object/place memory task. When the gray matter trend was analyzed further, there again was not a specific brain region that was significantly driving the trend, although there was a trend toward a correlation between accuracy and CVR in the parietal lobe. No significant correlations were found between CBF and any cognitive measure.

Previous studies have indicated that CVR declines more rapidly than CBF during aging[143], and our group hypothesized that CVR would be decreased in the at risk group compared to the control group. While we did not see a group difference in CVR (see Chapter IV), it is interesting that we found correlations between CVR and cognition in the at risk group.

The within-between ratio of the oddity detection task is an indirect method for determining how many features of a complex object a person can hold in his or her memory at a time. A higher number means that more features can be held. We found that the within between ratio for face stimuli is associated with increased CVR throughout the brain. The oddity detection task is reliant on the perirhinal cortex, but processing face stimuli also involves the complex interplay of several brain structures including the occipital face area and the fusiform face area[151,152]. To further determine if one specific brain region was driving this trend, the brain was further segmented into brain lobes. It was found that there was a positive correlation between CVR in the parietal lobe and face within between ratio. Surprisingly, there was a trend toward a negative correlation between face within between ratio and CVR in the occipital lobe.

Higher CVR in the right hippocampus correlated with better performance on the object/place memory task. The right hippocampus has been associated with spatial processing[153,154], so it would make sense that CVR in that specific brain region would correlate with a test of spatial memory. Further, higher CVR in total gray matter was associated with better performance on this task. When gray matter was divided into brain lobes, there were no significant correlations found between any specific brain lobe and performance in this task, meaning perhaps it is increased CVR in general and not in any specific cortical area that is driving increased accuracy in this task.

Interestingly, we found a negative correlation between face/name memory and left hippocampal CVR. Whereas the oddity detection task required complex visual processing of a face, the

face/name memory task required binding an image of a face to an arbitrary name in memory. The left hippocampus is associated with object naming[155] and verbal memory[153,156], and left anterior temporal lobe damage is associated with an inability to retrieve proper names[157]. During the encoding portion of this task subjects are shown a number of faces and names, and the retrieval portion is a forced-choice task between two possible names. Perhaps the increased CVR in the left hippocampus results in either inefficient encoding, or a false memory for the wrong name during retrieval.

If blood vessels are unable to dilate in response to a stimulus, brain tissue may become hypoperfused and mildly ischemic. Hippocampal subfields have been shown to be particularly sensitive to ischemic injury[158]. CVR is an index of vessel dilatory capacity, and these results indicate that a reduced dilatory capacity is correlated with decreased cognitive ability. We have no current way of knowing which subjects in our at risk group will go on to develop the disease, however one might predict that the subjects that currently have lower CVR and lower performance on the cognitive tasks may be at an even higher risk for AD.

Control Subjects

The control subjects demonstrated significant correlations between CBF and cognition, but no correlations between CVR and cognition. There was a positive correlation between gray matter CBF and face/name memory, a negative relationship between gray matter CBF and distance from the target in object/place memory, and a positive relationship between gray matter CBF and accuracy in object/place memory.

All of the significant correlations found in the control subjects indicate that gray matter CBF is associated with better performance in the cognitive test. This fits with other studies that have demonstrated a relationship between CBF and cognition[159,160]. When gray matter CBF was broken down by brain lobe, many of the correlations remained significant. Or approached significance, indicating that it may be a global increase in CBF that is driving increased accuracy in the cognitive tasks.

Higher CBF typically means better vascular function, and a higher availability of oxygen and glucose for neurons. It is logical that increased CBF would be associated with a better cognitive performance.

It is interesting that CBF correlates with cognition in control subjects, but not in at risk subjects. CBF has been shown to decline with healthy aging, however Wierenga et. al. have posited that there may be a biphasic curve to CBF in the years leading up to an AD diagnosis[139]. According to this theory, CBF first increases to levels higher than controls, then during advanced stages of the disease it declines. If there is a biphasic curve that would explain why at risk subjects do not have any significant correlations between CBF and cognition, as their CBF may be at different levels based on the stage of the disease process that they are in.

Conclusions

We found that CVR correlates with cognition in at risk subjects but not control subjects, while CBF correlates with cognition in control subjects but not at risk subjects. CBF and cognition have been shown to decline with age, but the relationship between CBF and AD and how it may

influence cognition is unclear. We believe that CVR may be a more powerful predictor of future decline than CBF.

CHAPTER VI

CONCLUSIONS AND FUTURE DIRECTIONS

Summary of Findings

Alzheimer's disease (AD) is the most common form of dementia, and it is a tremendous public health concern. One potential way to better understand AD is to identify the earliest possible changes that may occur in the beginning stages of pathogenesis. For this study we aimed to identify any differences already present in middle-aged adults at risk for Alzheimer's disease due to a first degree family history.

We first examined the performance of at risk subjects on cognitive tasks designed to target the areas of the brain first impacted by AD pathology. We found that there were differences between subject groups in the oddity detection and face/name memory tasks, which were designed to target the perirhinal and CA1 regions of the hippocampus, respectively. This demonstrates that even in cognitively normal and otherwise healthy subjects very subtle cognitive changes are potentially occurring and are able to be identified in our small sample of subjects.

Next, we sought to determine if there were any neurovascular or volumetric differences between at risk subjects and controls using novel neuroimaging techniques. We did not find any differences between groups, which indicates that there are either no changes in neurovasculature at the stage that we were studying, or that the specific sequences that we used were not sensitive enough to detect subtle differences.

Finally, we wished to determine if there were any correlations between cognition and neuroimaging, and how those relationships were different in the at risk group compared to the control group. We found that there were several cognitive measures in the at risk group that correlated with cerebrovascular reactivity, but none of the cognitive tests correlated with cerebral blood flow. In the control group, we found the opposite pattern. Cognitive tests correlated with cerebral blood flow, but not cerebrovascular reactivity.

Together, these results indicate that subjects at risk for AD have subtle but detectible cognitive changes compared to age-matched controls.

Future Directions

The biggest caveat to this study is its cross sectional nature. The best way to truly understand the results would be to follow the subjects used in this study longitudinally with repeated testing, and eventually to identify the subjects who develop AD. We also do not have knowledge of which subjects will develop AD in the future, and thus cannot interpret the predictability of these tasks.

Since we believe that the cognitive differences may be tau-driven, it would also be interesting to add a tau detection component, either using the newly developed PET ligands, through CSF testing. We could also add more testing to bring this study in line with the protocols used in other major longitudinal studies, such as the Alzheimer's Disease Neuroimaging Initiative (ADNI, protocol available at: http://www.adni-info.org/Scientists/ADNIStudyProcedures.html).

Although we found a significant difference between study groups in the cognitive testing, there are ways that we could improve the sensitivity of these tests and learn more about the

physiological processes underlying these differences. For the oddity detection task, the feature ambiguity of the visual stimuli could be varied across real-world objects and Greebles so that we can determine whether feature ambiguity or the novelty to the Greeble stimuli is the driver of the difference. The object/place memory test found no difference between the at risk and control groups, however this test could be made more ecologically valid by implementing it in a virtual reality system rather than on a two-dimensional screen. The results of the pattern separation task could be better understood by adding eye-tracking to determine if the trend in pattern completion was due to impaired encoding, or retrieval processes. It would also be interesting to determine if training in any of these cognitive tests would be protective against future decline.

We did not see any differences in structural or neurovascular imaging. We believe that this is a true finding and not a result of the study being underpowered. To confirm this, we performed a power analysis using the actual data from this study (Table 18), and found that the number of subjects required to achieve significance would be unreasonable for this type of study. There are constantly new advances being made in neuroimaging. Perhaps with a higher spatial resolution, or a higher signal to noise ratio the neurovascular measurements could be more sensitive and show a difference between subject groups. If there are truly no differences between groups in neurovascular function at this stage, that increases the validity of fMRI studies that attribute the BOLD response to changes purely to neural activation. An fMRI component could be added to the cognitive tests to determine if there are changes in the pattern of neural activation in subjects at risk for AD.

ROI	At Risk Mean	Control Mean	Average Standard Deviation	Required N	Effect Size
Left Hippocampus CVR	0.016	0.022	0.014	752	0.435
Right Hippocampus CVR	0.017	0.021	0.016	536	0.243
Gray Matter CVR	0.024	0.024	0.008	53166	0.024
Left Hippocampus CBF	28.34	28.59	5.97	19154	0.040
Right Hippocampus CBF	28.94	28.27	5.78	2334	0.116
Gray Matter CBF	31.49	30.14	5.53	532	0.244

Table 18: Power analysis of neurovascular imaging. Significance was set at 0.05 and power was set at 0.8.

The correlations that we found between neurovascular imaging markers and cognitive testing indicate that perhaps CBF may have a stronger influence on cognitive processes in normal aging, but CVR may have an influence on cognitive processes in the early stages of AD. To better understand these influences, it would be interesting to divide the at risk group into people with high CVR and people with low CVR and track them over time. Our hypothesis would be that those with lower CVR would be at an even higher risk for the development of AD. The correlative analysis was not corrected for multiple comparisons, because we believed that this might result in too many Type II errors (false negatives). If they had been corrected, the statistical tests would not have reached significance. Many studies that look at human data, particularly studies that combine physiology and cognition, have large cohorts of subjects to maximize statistical power. While the number of subjects used in this study were comparable to purely cognitive studies and purely imaging studies, we lacked to statistical power to thoroughly investigate the relationship between these two factors. In the future, an increase in the number of subjects would likely improve the interpretability of this result.

This study did not suffer from any issues in recruiting subjects, and many of the subjects that were in the study have expressed an interest in returning in the future for further studies. I believe that it would be feasible to accomplish many of the directions listed above.

Broad Implications

AD is the most common form of dementia, and it is becoming increasingly common as the population of the industrialized world ages. There are no treatments that have been shown to delay or prevent the onset of AD, and currently there is no cure. The best way to develop a treatment for AD would be to better understand the earliest physiological changes that are occurring in the disease.

Unfortunately, the earliest stages of AD are not well characterized. A figure developed by Jack et. al.[161] (figure 16A) is often used to show the field's best understanding of the timeline of early events in AD. The figure illustrates changes in brain structure, often measured volumetrically, preceding changes in memory or clinical function. We have updated the Jack figure (Figure 16B) to include our hypotheses for the order of events in early AD.

This study has demonstrated that changes in cognition are detectible prior to changes in brain structure. These cognitive changes correspond to the route of tau accumulation in the medial temporal lobe, indicating that they may be tau-driven.

While the figure did not originally include neurovascular imaging, we have also shown that cognitive changes are detectable prior to neurovascular changes using our methods. Since CVR correlates with several cognitive tests in the at risk group, perhaps CVR changes are occurring around the same time but our tests are not sensitive enough to detect this change at the group level.

CBF does not correlate with cognition in the at risk group. Based on the Weirenga model of CBF changes during AD, we believe that early in life people at risk for AD may have increased CBF, but in this age group CBF may be transitioning from being higher than controls to being lower than controls. This process is not well understood, and difficult to model, therefore we did not include it in our updated figure.

Altogether, this study has expanded our knowledge of the earliest physiological changes that occur in AD, and may provide a clearer picture of the order of events.

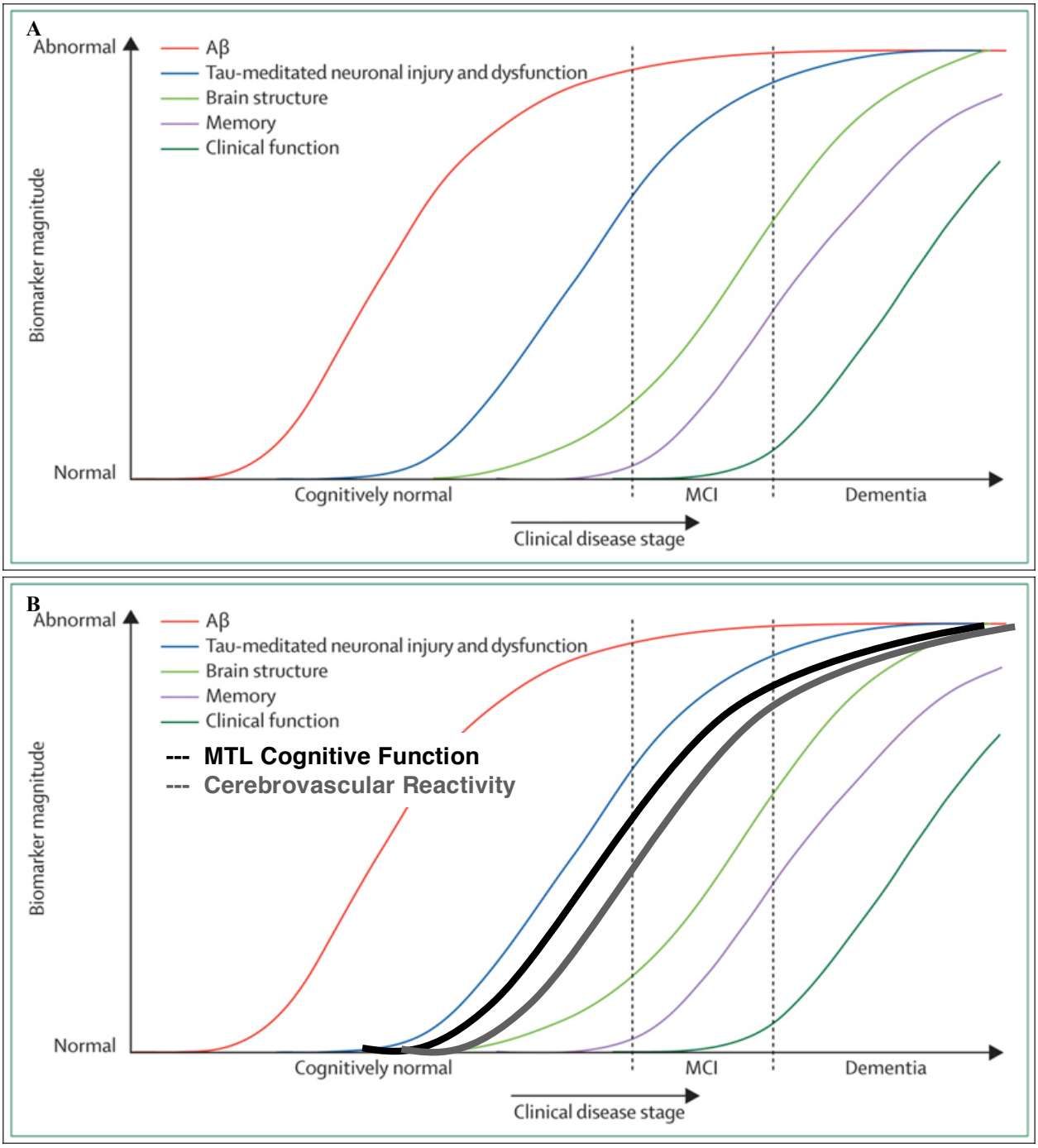

Figure 16: Modified timeline of symptom development during the course of AD. A: Original figure from the Jack 2010 paper. B: updated figure to reflect the results of this study. MTL specific cognitive function declines after tau, but before volume changes. CVR changes are likely occurring around the same time, and CBF changes occur after CVR changes but prior to volumetric changes.

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