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Markers of Cognitive Decline: An Integration of Functional and Structural Neuroimaging, APOE Genotyping, and Neuropsychological Testing

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Clinical Psychology

by

Chelsea Corinne Hays

Committee in charge:

University of California San Diego

Professor Christina E. Wierenga, Chair
Professor Mark W. Bondi
Professor Richard B. Buxton
Professor Zvinka Z. Zlatar

San Diego State University

Professor Paul E. Gilbert
Professor Jonathan L. Helm

2019

The Dissertation of Chelsea Corinne Hays is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California San Diego

San Diego State University

2019

DEDICATION

I dedicate my doctoral dissertation to my mother Rebekah, my father Steve, my sisters Tawnya and Molly, my brother Matthew, my brother-in-law Steve, my nephew Paris, and my brand-new baby niece Alita. Although the process of earning my Ph.D. placed vast geographic distance between us, the equally vast love I have experienced for and from my family has been my deepest inspiration and I could not have made this dream a reality without it. SEAHORSE!!!!

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VITA

2013	University of California San Diego B.S., Psychology Honors Thesis Title: *Follow the Liar: The Effects of Adult Lies on Children's Honesty*
2016	San Diego State University M.S., Clinical Psychology; Area of concentration: Neuropsychology Master Thesis Title: *Subjective Cognitive Decline Modifies the Relationship Between Cerebral Blood Flow and Memory Function in Cognitively Normal Older Adults.* Thesis chair: Christina E. Wierenga, Ph.D.
2018 – 2019	VA Boston Healthcare System Clinical Psychology Internship APA Accredited Affiliates: Harvard University, Boston University Major rotation: Neuropsychology; Minor rotation: Behavioral Medicine
2014 – 2019	San Diego State University/University of California San Diego Joint Doctoral Program in Clinical Psychology APA Accredited Ph.D., Clinical Psychology; Area of concentration: Neuropsychology Dissertation Title: *Markers of Cognitive Decline: An Integration of Functional & Structural Neuroimaging, APOE Genotyping, and Neuropsychological Testing*. Dissertation Chair: Christina E. Wierenga, Ph.D.

FELLOWSHIPS

2012 - 2017	National Science Foundation (NSF) Graduate Research Fellowship

PUBLICATIONS

Twamley, E. W., **Hays, C. C.,** Seewald, P. M., Orff, H. J., Depp, C. A., Olsen, D. C., & Jak, A. J. (2019). Neurocognition, psychiatric symptoms, and homelessness among Veterans with a history of traumatic brain injury. *Psychiatry Research, 271,* 167-170.

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ABSTRACT OF THE DISSERTATION

Markers of Cognitive Decline: An Integration of Functional and Structural Neuroimaging, APOE Genotyping, and Neuropsychological Testing

by

Chelsea Corinne Hays

Doctor of Philosophy in Clinical Psychology

University of California San Diego, 2019
San Diego State University, 2019

Professor Christina E. Wierenga, Chair

Subjective cognitive decline (SCD) and possession of the ε4 allele of the apolipoprotein E (APOE) gene are both associated with increased risk for age-related cognitive decline and Alzheimer's disease (AD). While mechanisms contributing to the increased risk associated with these factors are not well understood, evidence suggests that altered cerebral blood flow (CBF) may play a critical role. There is also evidence that altered CBF and brain structure among APOE ε4 carriers may *interact* to negatively impact cognition. The current studies utilized arterial spin labeling (ASL) magnetic resonance imaging (MRI) and high-resolution structural scans among cognitively normal older adults to determine the extent to which: 1) SCD modifies the effect of CBF on concurrent memory (Study 1), 2) APOE modifies effects of medial temporal lobe (MTL) CBF and brain structure (cortical thickness [CT], volume [Vo]) on concurrent memory (Study 2), and on 3) memory *change*

(Study 3). Study 1 results showed that those without SCD exhibited positive associations between memory and CBF within the posterior cingulate cortex, middle temporal gyrus, and inferior frontal gyrus, whereas those with SCD displayed negative associations within the posterior cingulate cortex, middle temporal gyrus, hippocampus, fusiform gyrus, and inferior frontal gyrus. Findings suggest that while higher CBF is supportive of memory function in those without SCD, it may no longer support memory in those reporting SCD, likely reflecting neurovascular dysregulation. Results from Studies 2 and 3 demonstrated that, for APOE ε4 carriers, the combination of *higher* CBF and lower CT in the entorhinal cortex was associated with worse concurrent memory, but *lower* CBF and lower CT in this same region was associated with greater memory decline. Findings suggest that APOE ε4 carriers experience neurovascular dysregulation and concomitant morphological alterations in the MTL that interact to negatively affect cognition even in the absence of overt clinical symptoms. Results support the presence of distinct multimodal neural signatures in the entorhinal cortex that may signal relative risk for cognitive decline among ε4 carriers, likely reflecting different stages of neurovascular compensation. More broadly, results add to accumulating evidence supporting the early role of vascular dysregulation in AD risk.

INTRODUCTION TO THE DISSERTATION

Age-related cognitive decline is heterogeneous and may occur with varying accompanying brain pathology. With progressive accumulation of neuropathology, subtle cognitive changes and early symptoms of dementia may eventually develop. Identifying risk factors and mechanisms of age-related cognitive decline during the earliest stages may provide a clearer understanding of the transition to mild cognitive impairment (MCI), thus enabling early personalized intervention. Subjective cognitive decline (SCD), or self-reported cognitive decline despite normal neuropsychological test performance, and possession of the ε4 allele of the apolipoprotein E (APOE) gene, are both associated with increased risk for cognitive decline and Alzheimer's disease (AD) (Belloy, Napolioni, & Greicius, 2019; Y. Sun, Yang, Lin, & Han, 2015). While mechanisms contributing to increased risk associated with these factors are not well understood, evidence suggests that altered cerebral blood flow (CBF) may play a critical role. CBF, or the rate of delivery of arterial blood to the capillary bed of a particular mass of tissue, is an indirect measure of neural function (Buxton, 2009) that has been implicated in both normal aging and AD-related cognitive decline (Bertsch et al., 2009; Hays, Zlatar, & Wierenga, 2016; Heo et al., 2010), demonstrating reliable correlations with cognitive performance across the lifespan (Bangen et al., 2012; Bertsch et al., 2009; Okonkwo et al., 2014; Wierenga et al., 2012) and predicting conversion to MCI and AD (Beason-Held et al., 2013; Chao et al., 2010). Exploring relationships among CBF and cognitive performance in individuals who possess these risk factors could provide critical insight into brain mechanisms contributing to cognitive decline and lead to the identification of vasoprotective treatments with the potential to delay or prevent the onset of age-related cognitive decline and/or AD.

Cognitively normal older adults with SCD are not only at greater risk for future cognitive decline, they also demonstrate an accelerated rate of decline (Reisberg, Shulman, Torossian, Leng, & Zhu, 2010) and are more likely to convert to MCI and AD, compared to those without SCD (Glodzik-Sobanska et al., 2007; Lista et al., 2015; Mitchell, Beaumont, Ferguson, Yadegarfar, & Stubbs, 2014; Y. Sun et al., 2015). Although our understanding of SCD is still in its infancy, some speculate that it represents an early stage of cognitive decline, with very subtle cognitive deterioration undetectable by current neuropsychological testing (Reisberg & Gauthier, 2008), while others posit that SCD results from early neuronal dysfunction together with compensatory mechanisms that preserve cognitive functions (Y. Sun et al., 2015). There is also emerging evidence of altered CBF among older adults reporting SCD. Specifically, older adults with SCD demonstrate both higher and lower

levels of regional CBF and metabolism in regions typically associated with memory function, aging, and AD-risk, compared to those without SCD (Hohman, Beason-Held, Lamar, & Resnick, 2011; Mosconi et al., 2008a; Scheef et al., 2012a; Wang et al., 2013). This small but growing body of literature suggests that SCD may confer risk for cognitive decline through changes in CBF. Yet, to our knowledge, no study has examined whether the relationship between CBF and cognition is altered in those with SCD.

Possession of the APOE ε4 allele represents the single greatest risk factor for AD, aside from age, increasing risk by 3-8 fold and lowering the age of disease onset in a dose dependent fashion (Coon et al., 2007; Corder et al., 1993; C.-C. Liu, Kanekiyo, Xu, & Bu, 2013; Sando et al., 2008). However, APOE ε4 carriers are not only at higher risk for AD, they show increased vulnerability to other neurodegenerative conditions that affect cognition (Tsuang et al., 2013) and appear to be at increased risk for age-related cognitive decline even in the absence of disease or disorder (Bretsky et al., 2003; Richard J. Caselli et al., 2009; Schiepers et al., 2012). The apparent broad sweeping effects of the APOE ε4 genotype on cognitive functioning is likely related to its role in a diverse range of biological processes, including glucose metabolism, mitochondrial function, synaptic function, neurogenesis, tau phosphorylation, neuronal atrophy, neuroinflammation, and amyloid-β metabolism and aggregation (Kanekiyo, Xu, & Bu, 2014; C.-C. Liu et al., 2013; Mahley & Rall, 2000). APOE ε4 carriers also demonstrate alterations in CBF across widespread medial temporal, frontal, and parietal regions (Tai et al., 2016; Wierenga, Hays, & Zlatar, 2014a). More specifically, APOE ε4 carriers tend to exhibit higher resting CBF than non-carriers in early adulthood and middle-age, but lower resting CBF in old age (Thambisetty, Beason-Held, An, Kraut, & Resnick, 2010; Wierenga et al., 2013). The biphasic nature of CBF among APOE ε4 carriers has been attributed to neurovascular compensation, with early increases reflecting attempts to compensate for the deleterious effects of APOE ε4 (e.g., impaired repair mechanisms, neurovascular disruption) and subsequent decreases reflecting a relative breakdown of this compensation (Dai et al., 2009; Hays, Zlatar, & Wierenga, 2016; Koizumi et al., 2018; Luckhaus et al., 2008; Ostergaard et al., 2013; Wierenga et al., 2012). Older adult APOE ε4 carriers also demonstrate alterations in structural integrity, with evidence of reduced cortical thickness and accelerated gray matter atrophy when compared to non-carriers, most notably in MTL regions (Cohen, Small, Lalonde, Friz, & Sunderland, 2001; den Heijer et al., 2002; Jak, Houston, Nagel, Corey-Bloom, & Bondi, 2007; Tohgi et al., 1997). Moreover, ε4 carriers who demonstrate APOE-related alterations in brain structure also exhibit cognitive deficits, compared to non-carriers (Honea, Vidoni, Harsha, & Burns, 2009; Lind et al.,

2006), suggesting that the APOE ε4 genotype may confer risk for cognitive decline through changes in brain structure, perhaps through its moderating role in myelination, brain plasticity, and repair functions (Zhong & Weisgraber, 2009). It has also been suggested that lower cortical reserve in carriers of the APOE ε4 allele may represent a neural endophenotype that increases susceptibility to neurodegeneration (Shaw et al., 2007). Therefore, rather than having direct effects on cognition, APOE ε4-related changes in brain structure might interact with concomitant alterations in CBF, exacerbating detrimental effects on cognition. Together, this converging evidence suggests that possession of the APOE ε4 allele may lead to concurrent alterations in MTL CBF and brain structure that might interact to negatively impact cognition. However, to our knowledge, no study has explored the interactions among these variables.

In order to bridge these gaps in the literature, the current studies used arterial spin labeling (ASL) magnetic resonance imaging (MRI) and a high-resolution structural scan among relatively large and well-characterized samples of cognitively normal older adults to determine the extent to which: 1) SCD (SCD+ vs. SCD-) modifies the independent effects of resting CBF on concurrent verbal memory performance (Study 1), 2) APOE genotype (ε4+ vs. ε4-) modifies independent and/or interactive effects of medial temporal resting CBF and brain structure (cortical thickness [CT], volume [Vo]) on concurrent verbal memory performance (Study 2), and 3) APOE genotype modifies independent and/or interactive effects of medial temporal resting CBF and brain structure (CT, Vo) on longitudinal *changes* in verbal memory performance (Study 3). We hypothesized that the association of resting CBF and memory would be moderated by SCD status (SCD+, SCD-), whereby those in the SCD+ group would display negative relationships between CBF and verbal memory performance in regions implicated in memory and the AD pathological process (e.g., medial temporal lobe, posterior cingulate, inferior frontal gyrus), whereas those in the SCD- group would display positive relationships. We also predicted that APOE genotype would modify the interactive effects of CBF and brain structure on memory, such that increased CBF (reflecting neurovascular compensation) and reduced CT and/or Vo in MTL regions (entorhinal cortex [EC], hippocampus [Hc]) would interact to predict worse concurrent memory performance and greater longitudinal declines in memory performance among APOE e4 carriers, but not among non-carriers. The exploration of these effects combined with the utilization of non-invasive and cutting-edge neuroimaging techniques among well-characterized samples of cognitively normal older adults may help elucidate the

mechanistic links between these risk factors (SCD/APOE ε4) and age-related cognitive decline, thus enabling early intervention strategies aimed at preventing or slowing age-related cognitive decline

Chapter 1: Study 1

Subjective Cognitive Decline Modifies the Relationship Between Cerebral Blood Flow and Memory Function in Cognitively Normal Older Adults.

Chelsea C. Hays[1,3], Zvinka Z. Zlatar[1,2], Laura Campbell[1], M.J. Meloy[1,2], & Christina E. Wierenga[1,2]

[1]VA San Diego Healthcare System, 3350 La Jolla Village Dr., San Diego, 92161

[2]Department of Psychiatry, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093

[3]SDSU/UC San Diego Joint Doctoral Program in Clinical Psychology, 6363 Alvarado Court, Suite 103, San Diego, CA 92120

Address for correspondence:

Christina E. Wierenga, Ph.D.

VA San Diego Healthcare System

3350 La Jolla Village Dr., MC 151B

San Diego, CA 92161

Phone: (858) 534-8047

Fax: (858) 642-1218

cwierenga@ucsd.edu

ABSTRACT

Objective: Subjective cognitive decline (SCD), or self-reported cognitive decline despite normal neuropsychological test performance, is a risk factor for objective cognitive decline and Alzheimer's disease (AD). While brain mechanisms contributing to SCD are not well defined, studies show associations with vascular risk factors and altered cerebral blood flow (CBF), raising the hypothesis that those with SCD might be experiencing vascular dysregulation, or a disruption in the normal relationship between CBF and cognition. We examined whether the association between CBF and verbal memory performance differs between those with SCD (SCD+) and those without SCD (SCD-).

Methods: Linear mixed effect models were employed to investigate whether the voxel-wise relationship between arterial spin labeling (ASL) MRI-measured CBF and verbal memory performance was modified by SCD among a group of 70 cognitively normal older adults (35 SCD+, 35 SCD-; mean age=72) matched on age, gender, and symptoms of depression.

Results: Results indicated that the SCD- group exhibited positive associations between verbal memory and CBF within the posterior cingulate cortex, middle temporal gyrus, and inferior frontal gyrus, whereas the SCD+ group displayed negative associations between verbal memory and CBF within the posterior cingulate cortex, middle temporal gyrus, hippocampus, fusiform gyrus, and inferior frontal gyrus.

Conclusions: Findings suggest that while higher CBF is supportive of memory function in those without SCD, higher CBF may no longer support memory function in those presenting with SCD, perhaps reflecting neurovascular dysregulation.

INTRODUCTION

Aging is associated with cognitive decline, yet less is known about the neurobiological basis of this change (Salthouse, 2011). Identifying risk factors and mechanisms of age-related cognitive decline is among the greatest challenges to improving the health of older adults. The brain relies on proper functioning of the vascular neural network for the maintenance of cognitive function. This network includes the neurovascular unit (endothelial cells, pericytes, glia and neurons) and the upstream arteries and arterioles that feed into the microcirculation of the brain (Lo & Rosenberg, 2009; Zhang et al., 2012). Research suggests that dysfunction within the vascular neural network can lead to neuronal injury and degeneration (Lo & Rosenberg, 2009; Zlokovic, 2010). Cerebral blood flow (CBF), or the rate of delivery of arterial blood to the capillary bed of a particular mass of tissue, is a functional measure of the vascular neural network and has been implicated in both normal aging and Alzheimer's disease (AD)-related cognitive decline (Bertsch et al., 2009; Hays et al., 2016; Heo et al., 2010). CBF has demonstrated reliable correlations with cognition across the lifespan in both normal and pathologic aging (Bangen et al., 2012; Bertsch et al., 2009; Okonkwo et al., 2014; Wierenga et al., 2012). Furthermore, CBF measurement has been shown to distinguish between normal controls and those with AD, identify those at risk for mild cognitive impairment (MCI) and AD, and predict conversion to MCI and AD, suggesting its usefulness as a preclinical marker of cognitive decline (Hays et al., 2016; Wierenga et al., 2014a).

Subjective cognitive decline (SCD), or self-reported cognitive decline despite normal neuropsychological test performance, has been identified as a risk factor for objective cognitive decline (Glodzik-Sobanska et al., 2007; Lista et al., 2015; Reisberg et al., 2010; Y. Sun et al., 2015; L. Wang et al., 2004). Although our understanding of SCD is in its infancy, some speculate that SCD represents an early stage of cognitive decline, with very subtle cognitive deterioration undetectable by current neuropsychological testing (Reisberg & Gauthier, 2008), while others suggest that SCD results from early neuronal dysfunction together with compensatory mechanisms that preserve cognitive functions (Y. Sun et al., 2015). There is also evidence that SCD is strongly related to depression and other affective symptoms, highlighting the importance of controlling and/or adjusting for psychiatric symptoms when investigating SCD in the context of cognitive decline (Schmand, Jonker, Geerlings, & Lindeboom, 1997; Y. Sun et al., 2015). Studies suggest that those with SCD are not only at a greater risk for future cognitive decline but that decline occurs at an accelerated rate, compared to those without SCD (Reisberg et al., 2010). Moreover, those with SCD are more likely to convert to

MCI and AD (Glodzik-Sobanska et al., 2007; Lista et al., 2015; Y. Sun et al., 2015), as demonstrated by findings from a recent meta-analysis indicating adults with SDC are twice as likely to develop dementia compared to those without SCD, with annual conversion rates of 2.3% in adults with SCD compared to 1% in those without SCD (Mitchell et al., 2014). Individuals with SCD also exhibit pathologic markers of AD, such as amyloid-beta and tau protein deposition (Barnes, Schneider, Boyle, Bienias, & Bennett, 2006), regional brain atrophy (Cherbuin, Sargent-Cox, Easteal, Sachdev, & Anstey, 2015; Stewart et al., 2011), and altered brain function (Y. Sun et al., 2015), with evidence of both higher and lower levels of regional cerebral perfusion and metabolism compared to those without SCD. For example, Hohman and colleagues found that high levels of cognitive complaints were associated with higher positron emission tomography (PET)-measured CBF in the insula, inferior parietal cortex, lingual gyrus, fusiform gyrus, and the cerebellum (Hohman et al., 2011). Similarly, another study showed that those with cognitive complaints demonstrated higher arterial spin labeling (ASL)-measured CBF along midline default mode network regions compared to those without cognitive complaints (Wang et al., 2013). Conversely, Mosconi and colleagues found that those with subjective memory complaints showed lower 2-[18F]fluoro-2-deoxy-D-glucose (FDG)-PET-measured glucose metabolism in parietotemporal and parahippocampal regions compared to those without memory complaints while another study found higher FDG-PET-measured glucose metabolism in the medial temporal lobe and lower glucose metabolism in the precuneus among a similar group of older adults with memory complaints (Mosconi et al., 2008b; Scheef et al., 2012b). Although cerebral perfusion and glucose metabolism are thought to be tightly linked (Roy & Sherrington, 1890; Verfaillie et al., 2015), it appears that SCD-related PET and ASL studies have produced conflicting results, likely due to differences in sample characteristics (e.g., operational definitions of SCD or normal control) or methodology (e.g., imaging modality limitations, statistical or experimental control of confounding variables). As such, it may be important to extend these prior studies using cutting-edge methodologies within well-characterized samples to further clarify the relationship between SCD and cerebral perfusion. Overall, current evidence suggests that those with SCD may exhibit altered CBF in regions typically associated with memory function, aging and AD-risk. This notion is consistent with the vascular theory of AD, which holds that vascular damage contributes to the development of AD. Notably, SCD has also been independently associated with vascular risk factors (Paradise, Glozier, Naismith, Davenport, & Hickie, 2011).

Taken together, evidence showing that SCD is associated with alterations in cerebral perfusion and future cognitive decline suggests that those with SCD may be experiencing vascular dysregulation, or a disruption in the normal relationship between CBF and cognition, yet no study to date has examined whether SCD modifies the relationship between CBF and cognition. The exploration of this moderating effect combined with the utilization of non-invasive neuroimaging techniques among a well-characterized sample of cognitively normal older adults might help elucidate the underlying mechanisms of SCD and enable early intervention strategies aimed at preventing cognitive decline and AD. The current study used ASL magnetic resonance imaging (MRI) to determine if the relationship between CBF and memory function was modified by SCD. Based on previous reports, we hypothesized that SCD status would have a direct association with CBF in areas associated with aging and AD-risk (hippocampus, parahippocampal gyrus, posterior cingulate, precuneus). We also hypothesized that the association of resting CBF and cognitive function (memory) would be moderated by SCD status (SCD+, SCD-), whereby those in the SCD+ group would display an inverse relationship, suggesting evidence of vascular dysregulation and supporting its role in cognitive decline and AD-risk.

METHODS

Participants

See **Chapter 1 Table 1** for participant demographic and cognitive characteristics. Participants were community-dwelling older adult volunteers between the ages of 65 and 88 enrolled in a longitudinal study of aging and/or other ongoing research studies at the VA San Diego Healthcare System (VASDHS) and the University of California San Diego (UCSD). Of the 80 participants with available data who met inclusion/exclusion criteria, 35 reported SCD (SCD+) and were matched to 35 controls who reported no SCD (SCD-), based on age, sex, and Geriatric Depression Scale (GDS) score using the nearest neighbor matching method (Stuart, 2010).

Normal cognitive function was determined based on a comprehensive neuropsychological test battery. Participants were excluded if they met the empirically-derived criteria for mild cognitive impairment developed by Jak and colleagues (Jak et al., 2009). Potential participants were also excluded if they had a history of dementia, severe head injury, uncontrolled hypertension, had a DSM-IV diagnosis of learning disability, attention deficit disorder, mood disorder, or substance abuse, or if they reported a significant level of depressive

symptoms on the 30-item GDS (i.e., GDS≥10). Participants were also excluded if they had contraindications to MRI scanning, or if they were taking prescription psychoactive medications. All data was collected in accordance with UCSD and VA institutional research standards for human research and the Helinski Declaration.

Testing Sequence

All participants completed a comprehensive neuropsychological battery to determine normal cognitive functioning. Self-report measures, including the Subjective Memory Rating Scale (SMRS), were administered directly after the completion of cognitive testing. All participants were tested in one of three similar testing suites at UCSD and most were tested in the morning hours, though specific times varied. Participants also underwent an fMRI scan at the same location within 90 days of completing cognitive testing.

Verbal Memory Composite

A verbal memory composite score was created using trials 1-5, short delay free-recall, and long delay free-recall raw scores from the California Verbal Learning Test 2 (CVLT-2), measuring word list learning, and the Logical Memory immediate and delayed recall subtests of the Wechsler Memory Scale-Revised (WMS-R), measuring story recall. These tests were selected based on results from a principal component analysis previously reported by our group on a similar sample of older adults (Wierenga et al., 2012). Verbal memory composite scores were derived by averaging the z-scores for each of the tests within the composite for the entire sample.

SCD assessment

The SMRS is a 5-item questionnaire asking: "In the past year, do you think: (1) Your ability to remember the names of people you have just met has changed 2) Your ability to remember the faces of people you have just met has changed (3) Your ability to remember the names of close friends or relatives has changed (4) Your ability to remember appointments correctly has changed? (5) Your ability to judge the passage of time and guessing the time of day without looking at a clock or the sun has changed." Response categories for each item were: 1=definitely improved, 2=slightly improved, 3=no change, 4=slightly deteriorated, and 5=definitely

deteriorated. SCD+ was operationally defined by normal cognitive function based on current neuropsychological performance and the presence of scores of "4=slightly deteriorated" and/or "5=definitely deteriorated" and the absence of any scores of "1=definitely improved" or "2=slightly improved". SCD- was operationally defined by normal cognitive function and the presence of scores of "1=definitely improved", "2=slightly improved", and/or "3=no change" and the absence of scores of "4=slightly deteriorated" or "5=definitely deteriorated." Therefore, those in the SCD- group reported only improvements and stability in memory, while those in the SCD+ group reported deterioration and no improvements. For ease of interpretation, total scores on the SMRS were centered around "3=no change" so that positive total scores represented subjective improvements and negative total scores represented subjective declines. Although this measure is focused on memory changes, we use the term "subjective cognitive decline" rather than "subjective memory decline" as is recommended by the Subjective Cognitive Decline Initiative working group (Jessen et al., 2014). Among a sample of 1,883 dementia-free older adults, the SMRS demonstrated a Cronbach alpha coefficient of 0.6. Exploratory factor analysis found one common factor with an Eigen value greater than 1 and factor loadings from 0.4 to 0.5 for the five items. Furthermore, the SMRS demonstrated adequate face validity among this same sample (L. Wang et al., 2004). In the current sample, the SMRS demonstrated a Cronbach alpha coefficient of 0.68.

Apolipoprotein E genotyping

Genotyping was performed by the ADCS Biomarker Core at UCSD using real time PCR Restriction Fragment Length Polymorphism analysis. Genomic DNA was collected from participants using buccal swab and extracted using Qiamp DNA blood mini kit (Qiagen) followed by PCR amplification (Wierenga, et al., 2012).

MRI acquisition

Imaging data were acquired on a GE Discovery MR750 3T whole-body system with a body transmit coil and an 8-channel receive-only head coil at the University of California San Diego Center for Functional MRI. The structural brain sequence consisted of a high-resolution T1-weighted Fast Spoiled Gradient Recall (3D FSPGR) scan: 172 1 mm contiguous sagittal slices, FOV=25cm, TR=8ms, TE=3.1ms, flip angle=12, T1=600ms, 256x192 matrix, Bandwidth=31.25kHZ, frequency direction=S-I, NEX=1, scan time=8 min 13 seconds. Resting CBF was acquired with the Multiphase Pseudocontinuous ASL (MPPCASL) sequence, which is optimized for

robust CBF quantification (Jung, et al., 2010): tagging duration=2sec, TI=3.6sec, TR=4.2sec, TE=minimum, reps=64, FOV=22x22 cm, 20 5mm axial slices with a single shot spiral acquisition, collecting 8 cycles where each cycle consists of 8 images acquired with unique phase offsets, acquisition time=4:46 minutes. A spiral scan with a long TR (4000ms) and short TE (3.4ms) was also acquired to obtain an estimate of the equilibrium magnetization of cerebral spinal fluid, which is used to convert the perfusion signal into calibrated CBF units (mL blood/100g tissue/min). Finally, a minimum contrast image was acquired to adjust for transmit and receive coil inhomogeneities. Two field map scans were also acquired and used for off-line field map correction to help correct for signal bunching and dropouts in the frontal/medial temporal lobes.

MRI pre-processing

Image processing was performed with Analysis of Functional NeuroImages (AFNI, afni.nimh.nih.gov) (Cox, 1996), FMRIB Software Library (FSL, Oxford, United Kingdom), and locally created Matlab scripts. Field map correction was applied to the ASL time series prior to co-registration to the middle time point to minimize the effects of participant motion. For each participant, a mean ASL image was formed from the average difference of the control and tag images using surround subtraction to create an uncorrected perfusion time series, and slice timing delays were accounted for, making the inversion time (TI2) slice specific (Liu and Wong, 2005). This mean ASL image was then converted to absolute units of CBF (mL/100 g tissue/min) using an estimate of the equilibrium magnetization of CSF as a reference signal (Chalela, et al., 2000). This procedure resulted in a calibrated perfusion value for each voxel. Skull stripping of the high-resolution T1-weighted image was performed using AFNI's 3dSkullStrip. Tissue segmentation was performed using FSL's Automated Segmentation Tool algorithm to define CSF, gray matter (GM) and white matter (WM) regions. The high-resolution T1-weighted image and partial volume segmentations were registered to ASL space, and partial volume segmentations were down-sampled to the resolution of the ASL data. To correct the CBF measures for partial volume effects and ensure that CBF values were not influenced by known decreased perfusion in white matter or increased volume of CSF (Parkes, et al., 2004), we used the method previously reported by Johnson and colleagues (Johnson, et al., 2005). These calculations assume that CSF has 0 CBF, and that CBF in GM is 2.5 times greater than that in WM. The following formula was used to compute partial volume corrected CBF signal intensities: CBFcorr=CBFuncorr/(GM+0.4*WM). CBFcorr and CBFuncorr are corrected and uncorrected

CBF values, respectively. GM and WM are gray matter and white matter partial volume fractions, respectively. Information from the high-resolution structural image and the FSL FAST was used to determine the tissue content of each perfusion voxel. A 4.0 mm full-width, half-maximum Gaussian filter was applied to the CBFcorr data. Voxels with negative intensities were replaced with zero (Brown, et al., 2003) and GM voxels were thresholded at 0.9 probability. CBFcorr data were registered to the MNI-152 atlas using FMRIB's Non-linear Image Registration Tool (FNIRT), part of FSL (http://fsl.fmrib.ox.ac.uk/fsl/) and resampled to a 3x3x3 mm resolution grid.

Statistical analyses

T-tests were used to compare groups on age, years of education, GDS score, SMRS score, whole brain resting CBF and cognitive variables. Chi-square tests were used to compare groups on sex, APOE status, and family history of dementia. A voxel-wise linear mixed-effects (LME) regression model was conducted in R with resting CBF as the dependent variable and with independent variables: 1) verbal memory composite score, 2) SCD status (SCD+ vs. SCD-), and 3) the interaction term between verbal memory composite score and SCD status. In addition to matching groups on age, GDS score, and sex, statistical analyses also adjusted for these same variables to further decrease the chance of confounding (Pearce, 2016). The LME yielded statistical maps displaying the brain regions for which there were significant main effects of SCD status on CBF and the interactive effects of SCD status and verbal memory composite on CBF. Significance was determined by applying cluster-size correction derived from Monte-Carlo simulations (via AFNI's 3dClustSim) to guard against false positives on data initially thresholded at a value of $p<0.025$ (uncorrected). Based on these simulations, it was determined that a cluster size of 28 contiguous voxels (756 mm^3) ensured an overall $p<0.025$ (corrected). To characterize the direction and magnitude of interactive and main effects, post-hoc regression analyses were carried out using the mean CBF extracted from each significant cluster. Post-hoc analyses were conducted in IBM SPSS Statistics, version 22 (bootstrapped with 1,000 samples) and were conducted only to characterize the significant LME interaction terms.

RESULTS

Groups did not differ significantly on age, APOE status, family history of dementia, years of education, sex, DRS score, GDS score, whole-brain resting CBF, the verbal memory composite score, nor on any of the cognitive tests that comprised the verbal memory composite score (see **Chapter 1 Table 1**).

Chapter 1 Table 1. Participant demographic and cognitive characteristics. Note: ApoE = Apolipoprotein E; DRS = Mattis Dementia Rating Scale; rCBF = Resting Cerebral Blood Flow; GDS = Geriatric depression scale; SMRS = Subjective memory rating scale; VM = Verbal Memory; WMS-R = Wechsler Memory Scale-Revised; LM = Logical Memory; CVLT-2 = California Verbal Learning Test-2; SD = Short Delay; LD = Long Delay; *df* = degrees of freedom for independent samples T-test. B represents the standardized coefficient.

	SCD- (N=35)		**SCD+ (N=35)**				
	Mean	**SD**	**Mean**	**SD**	***t or χ2***	***df***	***p***
Age	73	6.25	72.54	5.07	0.34	65.2	0.738
Gender (male/female)	13/22	--	11/24	--	0.25	68	0.802
GDS (30-item)	2.02	2.31	2.89	2.26	1.57	68	0.122
Education	16.57	2.44	16.2	2.27	0.66	68	0.512
ApoE genotype	12 ε4	34.3%	14 ε4	40%	0.35	1	0.624
Family history of dementia	15	42.9%	18	51.4%	0.52	1	0.632
DRS Total Score	140.46	3.29	140.60	2.83	0.20	68	0.846
Whole Brain rCBF	71.75	14.82	72.10	11.68	0.11	64.4	0.913
SMRS (transformed)	0.37	1.54	-1.64	.90	--	--	--
VM Composite Total Z-score	0.09	0.83	-0.09	0.83	0.93	68	0.354
VM Composite Raw Scores:							
WMS-R LM Immediate Recall	31.09	5.48	30.14	6.65	0.65	68	0.520
WMS-R LM Delayed Recall	28.06	6.70	27.60	7.20	0.28	68	0.784
CVLT-2 List 1–5 Total	51.40	10.46	49.05	10.00	0.96	68	0.342
CVLT-2 SD Free Recall	10.91	3.07	10.20	3.17	0.96	68	0.342
CVLT-2 LD Free Recall	11.74	3.09	11.00	2.96	1.03	68	0.317

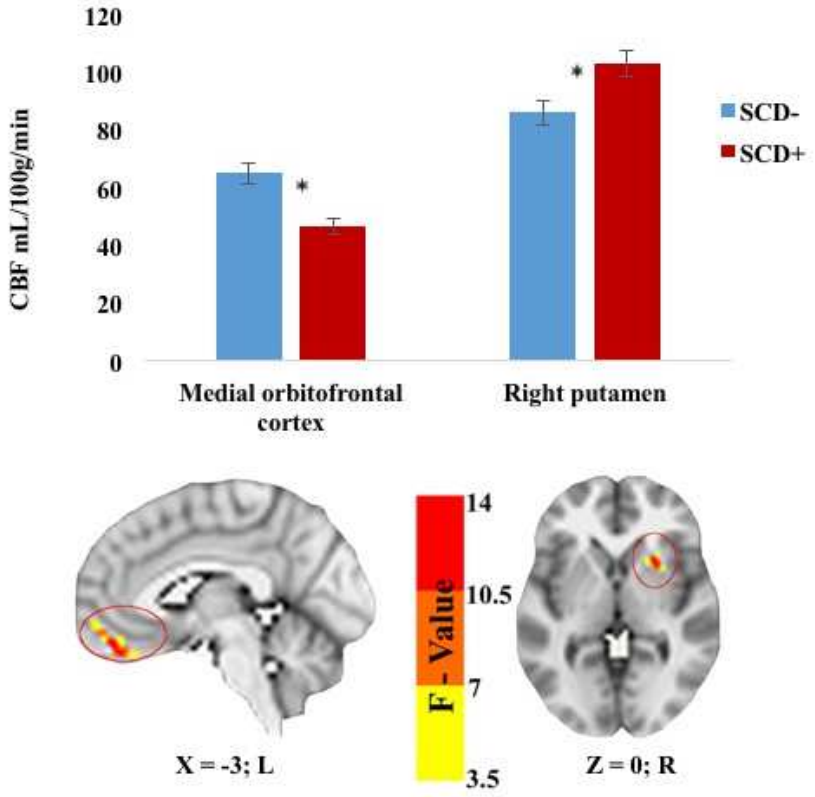
Main effect of SCD on CBF

Significant main effects of SCD on CBF were found in two clusters within the medial orbitofrontal cortex and the right putamen. Cluster locations with coordinates and corresponding Beta values by group are listed on **Chapter 1 Table 2**. To characterize the direction and magnitude of the main effects of SCD on CBF, mean CBF was extracted from the two main effect clusters, with evidence of both positive and negative associations between SCD status and CBF. Compared to the SCD- group, those in the SCD+ group demonstrated lower CBF in the medial orbitofrontal cortex and higher CBF in the right putamen (see **Chapter 1 Figure 1**).

Chapter 1 Table 2. Main effect of SCD status on CBF. Note: CBF = Cerebral blood flow; OFC = Orbital frontal cortex; Bi = Bilateral; R = Right; X, Y, and Z coordinates represent the peak F-value in MNI space. Beta values represent the standardized partial regression coefficients, with higher absolute values representing larger effect sizes.

SCD Status x CBF Cluster Locations

Mean Cerebral Blood Flow In:	Voxels	X	Y	Z	Max F-value	Beta	*p*
Bi OFC	55	3	-42	-24	13.4	-0.50	0.001
R Putamen	33	-24	-12	-3	13.2	0.36	0.002



Chapter 1 Figure 1. Main effects of SCD status on CBF. SCD= Subjective cognitive decline; CBF = Cerebral blood flow; L = left; R = right. * Denotes significance at p<0.025. Error bars represent standard error.

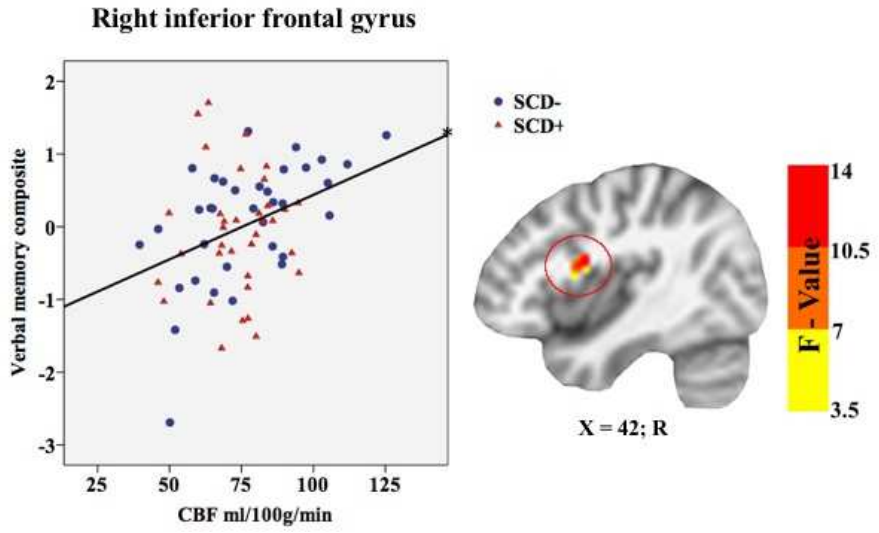
Main effect of memory function on CBF

A significant main effect of memory function on CBF was found in one cluster within the right inferior frontal gyrus. Cluster location with coordinates and corresponding Beta values by group are listed on **Chapter 1 Table 3**. To characterize the direction and magnitude of the main effect of memory function on CBF, mean CBF

was extracted from the main effect cluster, showing that memory function was positively associated with CBF in both groups (SCD+/-) within the right inferior frontal gyrus (see **Chapter 1 Figure 2**).

Chapter 1 Table 3. Main effect of verbal memory function on CBF. Note: CBF = Cerebral blood flow; R = Right; X, Y, and Z coordinates represent the peak F-value in MNI space; IFG = Inferior frontal gyrus. Beta value represents the standardized partial regression coefficient with higher absolute values representing larger effect sizes.

	Verbal Memory Composite x CBF Cluster Locations						
Mean Cerebral Blood Flow In:	**Voxels**	**X**	**Y**	**Z**	**Max F-value**	**Beta**	***p***
R IFG	39	-42	-3	18	15.7	0.30	0.016



Chapter 1 Figure 2. Main effect of memory function on CBF. SCD= Subjective cognitive decline; CBF = Cerebral blood flow; R = right. * Denotes significance at p<0.025.

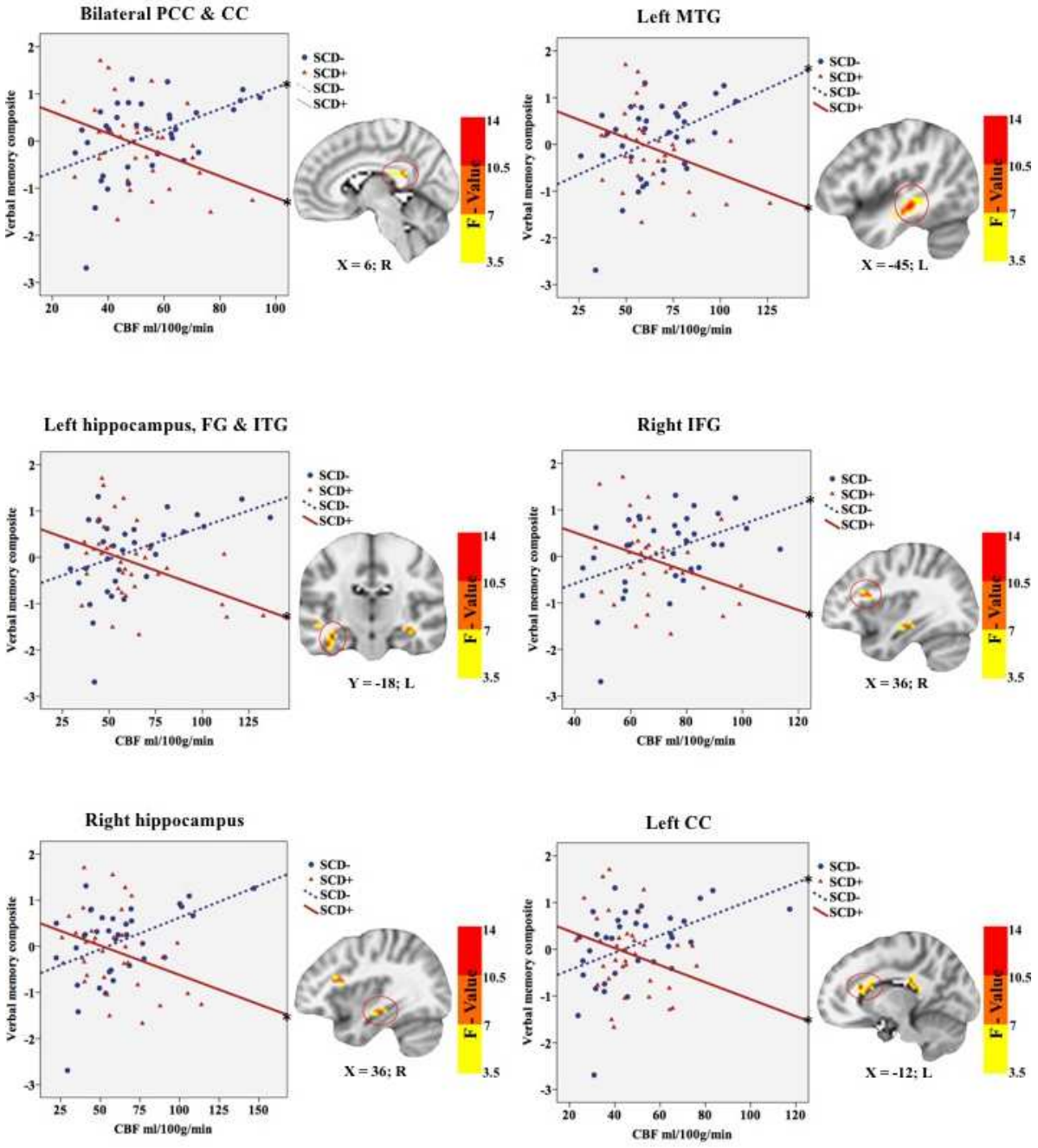
Interactive effects of SCD status and verbal memory on CBF

Significant interactions between SCD status and verbal memory composite scores on CBF were found in six clusters within the right hippocampus, inferior frontal gyrus, left middle temporal gyrus, left hippocampus and fusiform gyrus and inferior temporal gyrus, body of the corpus callosum, and bilateral posterior cingulate cortex and splenium of the corpus callosum. Cluster locations with coordinates and corresponding Beta values by group are listed on **Chapter 1 Table 4.** To characterize the direction and magnitude of the interaction effects mean CBF was extracted from the six significant clusters, demonstrating a consistent pattern of positive

associations between CBF and verbal memory functions for those in the SCD- group, and negative associations between CBF and verbal memory functions for those in the SCD+ group (see **Chapter 1 Figure 3)**. Higher CBF was associated with better verbal memory function for those in the SCD- group within bilateral posterior cingulate cortices, left middle temporal gyrus, right inferior temporal gyrus, and the corpus callosum. In contrast, higher CBF was associated with worse verbal memory performance within the left middle temporal gyrus, bilateral hippocampi, bilateral posterior cingulate cortices, left fusiform gyrus, left inferior temporal gyrus, and right inferior frontal gyrus in the SCD+ group (see **Chapter 1 Figure 3**).

Chapter 1 Table 4. Interactive effects of SCD status and verbal memory on CBF. Note: CBF = Cerebral blood flow; B = Bilateral; L = Left; R = Right; X, Y, and Z coordinates represent the peak F-value in MNI space; PCC = Posterior cingulate cortex; CC = Corpus callosum; MTG = Middle temporal gyrus; Hc = Hippocampus; FG = Fusiform Gyrus; ITG = Inferior Temporal Gyrus; IFG = Inferior Frontal Gyrus. Beta values represent the standardized partial regression coefficients, with higher absolute values representing larger effect sizes.

	SCD status x Verbal Memory Composite Cluster Locations					**SCD-**		**SCD+**	
Mean Cerebral Blood Flow In:	**Voxels**	**X**	**Y**	**Z**	**Max F-value**	**Beta**	***p***	**Beta**	***p***
B PCC & CC	92	-18	-45	12	13.3	0.27	0.020	-0.33	0.006
L MTG	63	-45	-27	-9	11.9	0.21	0.035	-0.38	0.001
L Hc, FG & ITG	36	-36	-15	-18	12.6	0.14	0.237	-0.38	0.004
R IFG	30	30	18	18	10.3	0.30	0.007	-0.27	0.013
R Hc	28	27	-15	-12	9.4	0.22	0.077	-0.30	0.003
L CC	28	-12	24	18	11.9	0.29	0.014	-0.25	0.004



Chapter 1 Figure 3. Interactive effects of SCD status and verbal memory on CBF. SCD = Subjective cognitive decline; CBF = Cerebral blood flow; PCC = Posterior cingulate cortex; CC = Corpus callosum; MTG = Middle temporal gyrus; FG = Fusiform Gyrus; ITG = Inferior temporal gyrus; IFG = Inferior frontal gyrus; L = left; R = right. * Denotes significance at $p<0.025$.

DISCUSSION

Results showed that older adults with SCD demonstrated lower CBF in the orbitofrontal cortex and higher CBF in the putamen, compared to those without SCD. Furthermore, our sample of cognitively normal older adults demonstrated an overall positive association between memory function and CBF that was modified by SCD, such that those presenting without SCD showed positive associations between memory functions and CBF within frontal, temporal, and parietal regions, whereas those presenting with SCD showed negative associations between memory function and CBF within frontal, temporal, and parietal regions. Our results showing that those with SCD demonstrate both higher and lower regional CBF, compared to those without SCD, are consistent with other SCD-related perfusion studies, further supporting the notion that regionally specific perfusion differences exist between these groups in areas that have been implicated in normal aging and AD-risk (Dai et al., 2009; Hays et al., 2016; Meltzer et al., 2000).

This is the first study, to our knowledge, to show that SCD modifies the relationship between voxel-wise CBF and memory function. These findings suggest that the normal beneficial effects of higher CBF on cognition may be disrupted among those with SCD, as higher CBF no longer appears to support better cognitive functioning in this group within regions associated with normal aging and AD-risk (Frederiksen, 2013; Grasby et al., 1993; Hays et al., 2016; Wierenga et al., 2014a). Although the underlying mechanisms associated with the observed differences between our groups are still unknown, other SCD-related studies showing higher activation, perfusion, and nodal efficiency among those with SCD in similar regions, have suggested that compensatory mechanisms could explain these differences (Erk et al., 2011; Z. Sun, 2015; Z. Wang, 2014). Similarly, the observance of higher CBF among those at risk for cognitive decline or AD has been interpreted as recruitment of early compensatory mechanisms, thought to reflect efforts to maintain adequate brain oxygenation in the face of vascular aging or damage. The notion of CBF-related compensation among those at risk for AD supports the vascular theory of AD, which posits that vascular damage plays a role in the pathogenesis of AD (Wierenga, Hays, & Zlatar, 2014b). This theory is further supported by longitudinal and cross-sectional studies showing that those in the preclinical phases of AD tend to show more areas of hyperperfusion early in the disease process, followed by more areas of hypoperfusion, thought to represent increasing heterogeneity of capillary flow patterns with disease progression (Ostergaard et al., 2013; Wierenga et al., 2014a). Taken together, this evidence suggests that the modifying effect of SCD on the relationship between CBF and cognition may reflect vascular

dysregulation among those with SCD, and that the perfusion alterations observed in this group may reflect attempts to compensate for these vascular changes.

The results of this current study are similar to those of a recent study of AD-risk, which showed that APOE status modifies the relationship between CBF and cognition, such that those carrying the e4 allele, a known risk factor for AD, showed inverse relationships between CBF and memory function, while non-carriers displayed positive relationships (Zlatar et al., 2016), adding to an accumulation of evidence suggesting that SCD may reflect an early preclinical stage of AD (Glodzik-Sobanska et al., 2007; Z. Sun, 2015). We found no differences in APOE status or family history of dementia between those with and without SCD in our sample, indicating that SCD might represent an independent risk factor for cognitive decline. The fact that group differences in the relationship between CBF and cognition were found despite normal cognitive function lends further support to the notion that SCD+ is distinct from normal aging (i.e., SCD-). It is also possible that current neuropsychological testing is not sensitive to the early subtle cognitive decline associated with SCD (Z. Sun, 2015). Results suggest that vascular dysregulation is occurring in those with SCD, even in the absence of clinical symptoms, further supporting its role as a preclinical marker of risk for cognitive decline.

The accurate assessment of SCD is a significant challenge facing the field. To address the lack of standardized and well-validated assessment tools for SCD, the Subjective Cognitive Decline Initiative (SCD-I) Working Group was established in 2014 with the goal of developing a conceptual framework and research criteria for SCD (Jessen et al., 2014). They recommended "well-constructed, easy-to-administer items with adequate reliability across diverse samples of older adults" (Rabin et al., 2015). Given that such a measure has yet to be developed or validated, the working group offered preliminary recommendations for instrument selection: 1. Select measures with appropriate demographic characterization. 2. Select measures with adequate content coverage for the target population. 3. Consider issues of psychometric adequacy (Rabin et al., 2015). Despite the psychometric limitations of the SMRS, its use represents an improvement over much of the SCD literature, which consists of studies utilizing only one or two questions to determine SCD. Furthermore, the five questions on the SMRS, which ask about changes in memory for names, faces, appointments and the ability to judge time over the last year, provide better clinical insight to specific subjective experiences of cognitive decline over a specified period, compared to questions which ask about cognitive difficulties in general without

reference to decline over time. Future SCD-related research would benefit from the development and widespread use of a more comprehensive consensus measure of SCD that has been shown to be valid and reliable.

In conclusion, this evidence supports the notion that those with SCD may be experiencing dysregulation within the vascular neural network. Elucidating the early vascular changes that accompany risk for cognitive decline and AD could lead to the identification of vasoprotective treatments with the potential to delay or prevent the onset of cognitive decline and AD. Although future research is needed to determine whether vascular dysregulation in those with SCD reflects normal or pathologic processes, the current results support SCD as a valid construct to detect those who might be at risk for cognitive decline and AD. Integrating SCD with other known markers of cognitive decline and AD could lead to earlier and more accurate identification of those who would likely benefit from treatments aimed at preventing cognitive decline.

Strengths and limitations

As mentioned above, this study was limited by the challenge faced by all studies of SCD, namely that of accurate assessment of subjective cognitive decline. We chose to use the SMRS because it offers several advantages over other available measures. However, the SMRS consists of only five self-report questions, from which we wholly defined our groups. Moreover, most of the questions on the SMRS are memory-related, reducing the likelihood of capturing those with SCD in non-memory cognitive domains, and the Cronbach alpha coefficient of 0.68 is not particularly high, although it is still within the range of acceptability for reliability (Loewenthal, 2001). Nevertheless, the proportion of participants classified as having SCD was in line with prevalence rates in other studies (Jonker, Geerlings, & Schmand, 2000; Mitchell, 2008). It is also possible that the timing of SCD assessment in this study may have influenced the way in which participants responded to this questionnaire, as we administered the SMRS directly after objective cognitive testing. However, it is important to note that the SMRS asks about changes in specific abilities (memory for names, faces, appointments and the ability to judge time) that were not tested within the cognitive battery. Furthermore, the SMRS asks participants to report changes in these specific abilities over the last year (rather than changes over weeks or months), further reducing the likelihood that the objective cognitive testing influenced responding on this questionnaire. Nonetheless, future studies should consider evaluating SCD and objective cognitive functioning on separate days. Overall, these limitations further highlight the need for a comprehensive consensus measure of SCD,

together with standardized administration instructions, including recommendations for the sequencing of SCD assessment in relation to objective cognitive testing.

Despite a well-characterized and relatively large sample, the use of a cross-sectional design restricted our ability to draw causal conclusions and limited our ability to determine whether the effects of SCD on CBF and the relationship between CBF and cognition represent normal or pathologic processes. It is also important to note that our sample had relatively high levels of education, and although this demographic factor was not associated with SCD status, its limited range may reduce the generalizability of these findings. Although groups (SCD+/SCD-) did not differ significantly in the mean time interval between cognitive testing and fMRI scanning (23 and 17 days, respectively), decreasing the time interval between cognitive testing and fMRI may improve accuracy of brain-behavior associations. Although only linear relationships were explored in the current study, future studies should consider exploring non-linear relationships between CBF and cognitive performance. Future investigations should also include longitudinal designs with larger, more diverse samples to replicate and extend the current findings. Furthermore, the inclusion of additional markers of AD, such as CSF biomarkers would help better characterize those with SCD.

The strengths of the current study include the use of non-invasive ASL MRI to measure CBF, the availability of several cognitive test performances to characterize cognitive status, and the use of voxel-wise linear mixed effects models which allowed us to examine the associations between SCD, verbal memory performance, and CBF across the entire brain. Despite these strengths, ASL methods are limited by low spatial resolution, transit time effects, and low signal-to-noise ratio (SNR) in deep white matter. Lastly, the current study represents an improvement over other studies of SCD, in our inclusion of a well-characterized, well-controlled sample of older adults. Many other studies of SCD have failed to exclude or control for depressive symptoms and other psychiatric disorders, making it difficult to determine whether observed results were due to SCD. The current study matched participants on age, sex, and GDS, in addition to statistically correcting for these same variables within the analyses.

Conclusions

This study found that the relationship between CBF and cognition is disrupted in cognitively normal older adults with SCD, compared to those without SCD. Whereas higher CBF supports verbal memory functions

in those without SCD, it appears that higher CBF is no longer supportive of verbal memory function among those with SCD. The current findings suggest that those with SCD may be experiencing vascular dysregulation and support SCD as a marker of risk for cognitive decline and AD. Future longitudinal studies should examine changes in perfusion and cognition over time among those with SCD to determine whether the moderating effect of SCD on the relationship between CBF and cognition represents normal age-related or pathologic processes and to further characterize the role of SCD in the trajectory from normal to pathologic aging.

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Chapter 2: Study 2

APOE Modifies the Interactive Effects of Entorhinal Cerebral Blood Flow and Cortical Thickness on Memory Function in Cognitively Normal Older Adults

Chelsea C. Hays[a,d], Zvinka Z. Zlatar[b, d], M.J. Meloy[a,b], Mark W. Bondi[a,b,d.],

Paul E. Gilbert [d,e], Thomas Liu[c], Jonathan L. Helm[d,e], & Christina E. Wierenga[a,b,d]

[a]VA San Diego Healthcare System, 3350 La Jolla Village Dr., San Diego, 92161

[b]Department of Psychiatry, UC San Diego, 9500 Gilman Dr., La Jolla, CA 92093

[c]Department of Radiology, UC San Diego, 9500 Gilman Dr., La Jolla, CA 92093

[d] SDSU/UC San Diego Joint Doctoral Program in Clinical Psychology, 6363 Alvarado Court, Suite 103, San Diego, CA 92120

[e]Department of Psychology, San Diego State University, 5500 Campanile Drive

San Diego, CA 92182

Address for correspondence:

Christina E. Wierenga, Ph.D.

VA San Diego Healthcare System

3350 La Jolla Village Dr., MC 151B

San Diego, CA 92161

Phone: (858) 534-8047

Fax: (858) 642-1218

cwierenga@ucsd.edu

ABSTRACT

Objective: The ε4 allele of the apolipoprotein E (APOE) gene increases risk for cognitive decline in normal and pathologic aging. However, precisely *how* APOE ε4 exerts its negative impact on cognition is poorly understood. The present study aimed to determine whether APOE genotype (ε4+ vs. ε4-) modifies direct and/or interactive effects of medial temporal lobe (MTL) resting cerebral blood flow (CBF) and brain structure (cortical thickness [CT], volume [Vo]) on verbal memory performance.

Methods: Multiple linear regression models were employed to investigate relationships between APOE genotype, arterial spin labeling MRI-measured CBF and FreeSurfer-based CT and Vo in four MTL regions of interest (left and right entorhinal cortex and hippocampus), and verbal memory performance among a sample of 117 cognitively healthy older adults (41 ε4+, 78 ε4-) between the ages of 64 and 89 (mean age=73).

Results: Results indicated that APOE genotype modified the interactive effects of CBF and CT on memory in the left entorhinal cortex, such that the relationship between entorhinal CBF and memory was negative (*lower* CBF was associated with better memory) in non-carriers with higher entorhinal CT, positive (*higher* CBF was associated with better memory) in non-carriers with lower entorhinal CT, and negative (higher CBF was associated with *worse* memory) in ε4 carriers with lower entorhinal CT.

Conclusions: Findings suggest that older adult APOE ε4 carriers may experience vascular dysregulation and concomitant morphological changes in the medial temporal lobe that interact to negatively affect memory prior to the onset of overt clinical symptoms, providing potential insight into the mechanistic link between APOE ε4 and detriments in cognition. Moreover, findings suggest a distinct multimodal neural signature in ε4-carriers (*higher* CBF and *lower* CT in the entorhinal cortex) that could aid in the identification of candidates for future clinical trials aimed at preventing or slowing cognitive decline. Differential findings with respect to ε4-carriers and non-carriers are discussed in the context of neurovascular compensation.

INTRODUCTION

With a rapidly growing older population, identifying risk factors and mechanisms of age-related cognitive decline represents one of the greatest challenges to improving the health and overall independence of older adults. The ε4 allele of the apolipoprotein E gene (APOE) confers risk for pathologic and normal age-related cognitive decline (Bretsky et al., 2003; Richard J. Caselli et al., 2009; Schiepers et al., 2012; Tsuang et al., 2013). Precisely *how* APOE ε4 exerts its negative impacts on cognition is still poorly understood, but is likely related to its role in a diverse range of biological processes including glucose metabolism, mitochondrial function, synaptic function, neurogenesis, tau phosphorylation, neuronal atrophy, neuroinflammation, and amyloid-β (Aβ) metabolism and aggregation (Kanekiyo et al., 2014; C.-C. Liu et al., 2013; Mahley & Rall, 2000). Interestingly, APOE ε4's effects on cognition appear largely dependent on age, with young ε4 carriers equal to or outperforming non-carriers on a wide range of cognitive abilities (Jochemsen, Muller, van der Graaf, & Geerlings, 2012; Marchant, King, Tabet, & Rusted, 2010; Mondadori et al., 2007; Rusted et al., 2013), but older ε4 carriers demonstrating worse cognitive performance (Adamson et al., 2010; De Blasi et al., 2009; Honea et al., 2009; Kukolja, Thiel, Eggermann, Zerres, & Fink, 2010; Tuminello & Han, 2011) and accelerated age-related cognitive decline, most notably in episodic memory (Bretsky et al., 2003; R. J. Caselli et al., 2004; Richard J. Caselli et al., 2009; Hayden et al., 2009; C.-C. Liu et al., 2013; Schiepers et al., 2012; Whitehair et al., 2010). Identifying and characterizing APOE-related brain changes could offer insight into the mechanistic link between APOE ε4 genotype and cognitive decline.

APOE ε4 carriers demonstrate alterations in cerebral blood flow (CBF), or the rate of delivery of arterial blood to the capillary bed in a volume of tissue. CBF is an indirect measure of neural function (Buxton, 2009) that has been implicated in both normal aging and AD-related cognitive decline (Bertsch et al., 2009; Hays et al., 2016; Heo et al., 2010), demonstrating reliable correlations with cognitive performance across the lifespan (Bangen et al., 2012; Bertsch et al., 2009; Okonkwo et al., 2014; Wierenga et al., 2012). APOE ε4 carriers demonstrate altered resting CBF across widespread medial temporal, frontal, and parietal regions (Tai et al., 2016; Wierenga et al., 2014a) and the temporal staging of CBF alteration appears to resemble APOE-related changes in cognition, with ε4 carriers exhibiting higher resting CBF than non-carriers in early adulthood and middle-age, but lower resting CBF in old age (Thambisetty et al., 2010; Wierenga et al., 2013). It has been suggested that higher brain perfusion among ε4 carriers reflects cerebrovascular compensation for the deleterious

effects of APOE ε4 (e.g., impaired repair mechanisms, neurovascular disruption) and that lower brain perfusion reflects a breakdown of these compensatory mechanisms (Dai et al., 2009; Hays et al., 2016; Koizumi et al., 2018; Luckhaus et al., 2008; Wierenga et al., 2012). Notably, cross-sectional investigations of cerebral perfusion in cognitively normal older adult ε4 carriers have produced mixed results, with reports of both increased (Bangen et al., 2009; Thambisetty et al., 2010) and decreased CBF (Filippini et al., 2011; Wierenga et al., 2013; Zlatar et al., 2016), relative to non-carriers. Similarly, findings from studies exploring associations between APOE-related CBF alteration and cognition have also been mixed, with reports of both positive and negative relationships between CBF and cognition in older adult ε4 carriers (Bangen et al., 2012; Wierenga et al., 2012; Zlatar et al., 2016). To the extent that some of these discordant findings are due to differences in sample characteristics (e.g., definitions of cognitively normal, middle age versus older adults) or methodology (e.g., imaging modality limitations, statistical or experimental control of confounding variables, regions of interest versus voxel wise analysis, differing methods of partial volume correction), it is important to extend these prior studies using cutting-edge methodologies within large, well-characterized samples to further clarify associations between APOE, CBF, and cognition.

APOE genotype is also associated with alterations in brain morphology. More specifically, ε4 carriers demonstrate reduced cortical thickness in youth relative to non-carriers (Alexander et al., 2012; Ringman, Pope, & Salamon, 2010; Shaw et al., 2007) and accelerated gray matter atrophy in old age, most notably in medial temporal lobe (MTL) regions (Cohen et al., 2001; den Heijer et al., 2002; Jak et al., 2007; Tohgi et al., 1997). Moreover, ε4 carriers who demonstrate APOE-related alterations in brain structure also exhibit cognitive deficits, compared to non-carriers (Honea et al., 2009; Lind et al., 2006), suggesting that APOE ε4 genotype might confer risk for cognitive decline through changes in brain structure, perhaps through its moderating role in myelination, brain plasticity, and repair functions (Zhong & Weisgraber, 2009). However, it is difficult to reconcile findings of reduced structural integrity in young ε4 carriers with evidence of increased cognitive performance in this same group. Thus, it has been suggested that lower cortical reserve in young carriers of the ε4 allele may represent a neural endophenotype that increases susceptibility to neurodegeneration later in life (Shaw et al., 2007). Therefore, rather than having direct effects on cognition, APOE ε4-related changes in brain structure may interact with concomitant alterations in CBF, exacerbating detrimental effects on cognition. If

correct, this could help explain findings of both positive and negative associations between CBF and cognition in ε4-carriers, as this relationship could vary as a function of structural integrity.

Together, this evidence suggests that cognitively normal APOE ε4 carriers experience alterations in CBF, brain structure, and memory, compared to non-carriers. Moreover, APOE-related alterations in MTL CBF and brain structure might interact to negatively impact memory performance in cognitively normal older adult carriers of the ε4 allele. No study, to our knowledge, has explored the interactions among these variables. In order to bridge this gap in the literature, the current study used arterial spin labeling (ASL) magnetic resonance imaging (MRI) and a high-resolution structural scan among a relatively large and well-characterized sample of cognitively normal older adults to determine whether APOE genotype (ε4+ vs. ε4-) modifies independent and/or interactive effects of medial temporal resting CBF and brain structure (cortical thickness [CT], volume [Vo]) on verbal episodic memory performance. We predicted that APOE genotype would modify the interactive effects of CBF and brain structure on memory, such that increased CBF (reflecting neurovascular compensation) and reduced CT and/or Vo in MTL regions (entorhinal cortex [EC], hippocampus [Hc]) would interact to predict worse memory performance in APOE e4 carriers, but not in non-carriers. Exploratory analyses also investigated these same relationships in frontal brain regions implicated in memory encoding and retrieval (caudal anterior cingulate cortex [cACC], rostral middle frontal cortex [rMFC]). Such findings may help elucidate the underlying mechanisms of APOE ε4 effects on cognition and enable early intervention strategies aimed at preventing age-related cognitive decline.

METHODS

Participants

See **Chapter 2 Table 1** for participant demographic and cognitive characteristics. Participants were community-dwelling older adult volunteers enrolled in a longitudinal study of normal aging at the VA San Diego Healthcare System (VASDHS). A total of 117 cognitively normal participants between the ages of 64 and 89 (mean age = 73.3, SD = 6.2) with available data were included in the current analyses. Forty-one participants were carriers of the APOE ε4 allele (ε3/ε4 = 37, ε4/ε4 = 4) and 76 were non-carriers (ε3/ε3 = 66, ε3/ε2 = 10). All participants were administered a full neuropsychological battery and an MRI scan (mean time interval between neuropsychological testing and MRI scan = 51 days). Normal cognitive function was determined based on a

comprehensive neuropsychological test battery. Participants were excluded if performance on more than one measure within a cognitive domain was more than one standard deviation below age-appropriate norms, consistent with the empirically-derived criteria for diagnosis of mild cognitive impairment (MCI) developed by Jak and colleagues (Jak et al., 2009), or if overall performance on the Dementia Rating Scale (DRS) was more than 1 standard deviation below age-appropriate norms (see **Chapter 2 Table A1** in the Appendix for specific cognitive tests, domains, and normative data; this resulted in the removal of 22 participants). Potential participants were also excluded if they had a history of severe head injury, uncontrolled hypertension, or a DSM-IV Axis I diagnosis of learning disability, attention deficit disorder, mood disorder, or substance abuse. In addition, participants were excluded if they had contraindications to MRI scanning such as ferrous implants or a pacemaker. All participants provided written informed consent prior to enrollment and data were collected in accordance with all ethical standards as stipulated by the UCSD and VASDHS institutional review board-approved procedures.

Chapter 2 Table 1. Participant demographic, cognitive, and brain characteristics. Note: APOE= Apolipoprotein E; SD= Standard deviation; t or $\chi 2$= either t-statistic or $\chi 2$; df= degrees of freedom; DRS= Mattis Dementia Rating Scale; WMS= Wechsler Memory Scale; LM= Logical Memory; SS= Scaled score; CVLT= California Verbal Learning Test; SD= Short delay; LD= Long delay; DKEFS= Delis-Kaplan Executive Function System; df= Degrees of freedom; + tests that were included in the memory composite; *significance at $p<0.05$; **significance at $p< 0.01$; *** significance at $p< 0.001$.

	APOE ε4- (*N*=76)		**APOE ε4+ (*N*=41)**				
	Mean	**SD**	**Mean**	**SD**	***t* or $\chi 2$**	***df***	***p-value***
Age (years)	72.98	6.03	74.02	6.62	0.83	115	0.406
Gender (male/female)	26/50	--	13/28	--	0.08	1	0.784
Education (years)	16.52	2.27	15.90	2.2	1.44	115	0.151
NP and MRI time interval (days)	51.09	70.6	53.29	73.3	0.15	114	0.874
Stroke Risk %	9.78	6.77	9.78	6.84	0.00	114	0.999
Systolic Blood Pressure	130.2	15.9	124.5	11.6	2.20	112	0.029*
Diastolic Blood Pressure	76.97	8.51	71.66	9.23	2.97	109	0.004**
DRS Total Score	140.95	2.82	140.66	3.03	0.52	115	0.608
Memory Composite	0.148	0.79	-0.275	0.89	2.53	115	0.013*
WMS-R LM Immediate Recall+	31.92	6.27	28.63	5.83	2.77	115	0.007**
WMS-R LM Delayed Recall+	29.12	7.13	25.78	7.45	2.38	115	0.019*
CVLT-II List 1–5 total+	51.59	9.49	46.41	11.09	2.65	115	0.009**
CVLT-II SD Free Recall+	10.56	3.05	9.78	3.1	1.32	115	0.189
CVLT-II LD Free Recall+	11.57	2.89	10.41	3.09	2.03	115	0.045*
DKEFS CW Inhibition	58.55	10.78	67.68	14.29	3.87	114	<0.001***
DKEFS CW Inhibition Switch	64.27	14.98	72.90	21.92	2.50	113	0.014*
Trail Making Test-A	32.56	9.16	32.39	10.26	0.09	114	0.927
Trail Making Test-B	76.80	28.84	81.46	27.21	0.84	114	0.398
DKEFS Letter Fluency	46.12	13.83	49.12	12.13	1.16	115	0.245
WISC-R Block Design	47.07	7.93	45.22	6.901	1.25	114	0.213

Chapter 2 Table 1. Participant demographic, cognitive, and brain characteristics, Continued.

R Hippocampal CBF	47.6	11.9	50.0	9.98	1.09	105	0.306
R Hippocampal Vo	3719	548	3670	474	0.47	115	0.633
L Hippocampal CBF	49.37	12.6	52.31	10.6	1.20	104	0.232
L Hippocampal Vo	3616	469	3529	492	0.93	114	0.350
R Entorhinal CBF	45.22	16.7	44.33	20.5	0.23	96	0.815
R Entorhinal CT	3.39	0.38	3.40	0.36	0.19	115	0.847
L Entorhinal CBF	45.69	13.6	39.55	15.8	2.01	94	0.047*
L Entorhinal CT	3.20	0.32	3.21	0.34	0.08	114	0.934
R Caudal Anterior Cingulate CBF	54.8	13.6	57.88	11.1	1.16	102	0.246
R Caudal Anterior Cingulate CT	2.67	0.29	2.70	0.32	0.62	115	0.530
L Caudal Anterior Cingulate CBF	55.31	15.7	58.53	11.9	1.08	104	0.282
L Caudal Anterior Cingulate CT	2.49	0.25	2.63	0.36	2.53	115	0.012*
R Rostral Middle Frontal CBF	61.11	14.7	62.78	10.1	0.61	106	0.541
R Rostral Middle Frontal CT	2.18	0.09	2.21	0.13	1.14	115	0.256
L Rostral Middle Frontal CBF	63.69	14.9	64.48	10.8	0.28	106	0.779
L Rostral Middle Frontal CT	2.25	0.13	2.22	0.14	1.11	115	0.266

Verbal Memory Composite

All participants were administered a full neuropsychological battery. A verbal memory composite score was created using trials 1-5, short delay free-recall, and long delay free-recall raw scores from the California Verbal Learning Test – Second Edition (CVLT-II), measuring word list recall, and the Logical Memory immediate and delayed recall subtests of the Wechsler Memory Scale-Revised (WMS-R), measuring story recall. These tests were selected based on results from a principal component analysis previously reported by our group on a similar sample of older adults (Wierenga et al., 2012). Verbal memory composite scores were derived by averaging the *z*-scores for each of the tests within the composite for the entire sample.

Apolipoprotein E genotyping

Genotyping was performed by the ADCS Biomarker Core at UCSD using real time PCR Restriction Fragment Length Polymorphism analysis. Genomic DNA was collected from participants using buccal swab and extracted using Qiamp DNA blood mini kit (Qiagen) followed by PCR amplification (Wierenga, et al., 2012). Those with at least one ε4 allele (i.e., ε2/ε4, ε3/ε4, ε4/ε4) were classified as APOE e4 carriers (ε4+) and those without an ε4 allele (i.e., ε2/ε2, ε3/ε3, ε3/ε3) were classified as non-carriers (ε4-). Given that the APOE ε2 allele is thought to have protective effects (Suri, Heise, Trachtenberg, & Mackay, 2013), we ran all analyses including and excluding ε2 carriers and results did not differ. Therefore, results from the entire sample are presented. An exact test of Hardy-Weinberg Equilibrium was not significant (p= 0.443), suggesting that the distribution of

APOE genotype in the sample included in this manuscript does not differ significantly from the expected distribution in the general population.

MRI acquisition

Imaging data were acquired on a GE Discovery MR750 3T whole body system with a body transmit coil and an 8-channel receive-only head coil at the University of California San Diego Center for Functional MRI. The structural brain sequence consisted of a high-resolution T1-weighted Fast Spoiled Gradient Recall (3D FSPGR) scan: 172 1 mm contiguous sagittal slices, FOV = 25 cm, TR = 8 ms, TE = 3.1 ms, flip angle = 12, T1 = 600 ms, 256 x 192 matrix, Bandwidth = 31.25 kHz, frequency direction = S-I, NEX = 1, scan time = 8 min and 13 seconds. Resting CBF was acquired with the Multiphase Pseudocontinuous Arterial Spin Labeling (MPPCASL) sequence, which is optimized for robust CBF quantification (Jung, Wong, & Liu, 2010): tagging duration = 2 sec, post-labeling delay = 1.6 sec, TR = 4.2 sec, TE = 3 ms, reps = 64, FOV = 22 x 22 cm, 20 5 mm axial slices with a single shot spiral acquisition, collecting 8 cycles where each cycle consists of 8 images acquired with unique phase offsets, acquisition time = 4:46 minutes. A spiral scan with a long TR (4000 ms) and short TE (3.4 ms) was also acquired to obtain an estimate of the equilibrium magnetization of cerebral spinal fluid, which is used to convert the perfusion signal into calibrated CBF units (mL blood/100g tissue/min). Finally, a minimum contrast image was acquired to adjust for transmit and receive coil inhomogeneities. Two field map scans were also acquired and used for off-line field map correction to help correct for signal bunching and dropouts in the frontal/medial temporal lobes.

MRI pre-processing

CBF

ASL image processing was performed with Analysis of Functional NeuroImages (AFNI, afni.nimh.nih.gov)(Cox, 1996), FMRIB Software Library (FSL, Oxford, United Kingdom), and locally created Matlab scripts. Field map correction was applied to the ASL time series prior to co-registration to the middle time point to minimize the effects of participant motion. For each participant, a mean ASL difference image was formed from the average difference of the control and tag images and slice timing delays were accounted for in order to make the post-labeling delay time slice specific (T. T. Liu & Wong, 2005). This mean ASL image was

then converted to absolute units of CBF (mL/100g tissue/min) using an estimate of the equilibrium magnetization of CSF as a reference signal (Chalela et al., 2000). This procedure resulted in a calibrated perfusion value for each voxel. Skull stripping of the high-resolution T1-weighted image was performed using AFNI's 3dSkullStrip. Tissue segmentation was performed using FSL's Automated Segmentation Tool (FAST) algorithm (http://fsl.fmrib.ox.ac.uk/fsl/) to define cerebrospinal fluid (CSF), gray matter (GM) and white matter (WM) regions. The high-resolution T1-weighted image and partial volume segmentations were registered to ASL space, and the CBF data were resampled to the same resolution as the T1-weighted image. The partial volume estimates were used to perform partial volume correction of the high-resolution CBF data using a linear regression approach with kernel size of 3 (Asllani et al., 2008; Zhao et al., 2017) as implemented by the ASL_file function in the BASIL toolset of FSL (Chappell, Groves, Whitcher, & Woolrich, 2009). The partial volume corrected gray matter CBF data were then resampled back to their native resolution and registered to FreeSurfer space. CBF values were extracted from each anatomically defined FreeSurfer ROI (see below for FreeSurfer methods). Quality assurance of ASL data was performed prior to analysis using outlier detection, inspection of CBF histograms, and visual checks of the CBF maps, with removal of values for regions with poor CBF map coverage. This resulted in removal of 4% of the data.

CT and Vo

Cortical thickness and volume analysis were performed using FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu), with the technical details of these procedures described in prior publications (Dale, Fischl, & Sereno, 1999; Dale & Sereno, 1993; B. Fischl & Dale, 2000; B. Fischl, Liu, & Dale, 2001; B. Fischl, Sereno, Tootell, & Dale, 1999; Bruce Fischl et al., 2002; Bruce Fischl, Salat, et al., 2004; Bruce Fischl, van der Kouwe, et al., 2004; Reuter, Rosas, & Fischl, 2010; Reuter, Schmansky, Rosas, & Fischl, 2012). Cortical thickness was extracted from the left and right EC, cACC, and rMFC and volume was extracted from the left and right Hc. A quality assurance protocol was performed before analysis using the ENIGMA guidelines (http://enigma.usc.edu/protocols/imaging-protocols) and included visual checks of the cortical segmentations and region-by-region removal of values for segmentations found to be incorrect. Histograms of all regions' values were also computed for visual inspection. This resulted in removal of 1% of the data.

Statistical analyses

t-tests were used to compare groups on age, years of education, continuous vascular risk factors, brain variables (CBF, CT/Vo), and cognitive variables. $\chi 2$-tests were used to compare groups on sex and categorical vascular risk factors. CBF and CT/Vo were extracted from FreeSurfer-derived regions of interest (i.e., CT of the EC, cACC, and rMFC; Vo of the Hc) and multiple linear regression models were employed in R with the memory composite score as the dependent variable and APOE genotype and brain variables (CBF, CT/Vo) as independent variables. The following 3-way interactions were explored: 1) APOE genotype x EC CBF x EC CT, and 2) APOE genotype x Hc CBF x Hc Vo. A Bonferroni correction was applied to correct for multiple comparisons, and correlations were only considered significant at $p<0.0125$. Exploratory analyses also examined: 1) APOE genotype x cACC CBF x cACC CT, and 2) APOE genotype x rMFC CBF x rMFC CT. All analyses statistically adjusted for the effects of age, sex, and education. Due to observed group differences, all analyses also adjusted for blood pressure (systolic blood pressure), and executive functioning performance (DKEFS color-word interference). Analyses including measures of Hc Vo also adjusted for the effects of total intracranial volume. Correlations between CBF and CT by region of interest (e.g., right EC CBF and right EC CT) were also explored, with no significant or marginal correlations found by group (ε4-, ε4+) or when collapsing across the whole sample (all *p*s > 0.2). Non-multicollinearity between all independent variables was confirmed by application of the multicollinearity index VIF (all VIF<2), linearity was confirmed by residuals versus fits plots, normality was confirmed by Q-Q plots, non-heteroskedasticity was confirmed by scale location plots, and no influential cases were identified through examinations of residuals versus leverage plots. Complex models with three-way interactions were compared to simpler models including only two-way interactions using ANOVA. Only the three-way interaction model in the left EC was significantly better at capturing data ($F = 11.143$, $p = 0.001$). Results from all three-way interactions are presented within the manuscript to allow for comparison and observation of trends.

RESULTS

Group differences in demographic and assessment variables

Groups (ε4+, ε4-) did not differ significantly on age, sex, or years of education, nor did they differ significantly in the time interval between neuropsychological testing and neuroimaging (all *p*s > 0.05; see

Chapter 2 Table 1). With regard to cardiovascular health, groups did not differ significantly on stroke risk percent based on the Framingham Stroke Risk Profile, nor did they differ on any of the variables that comprise this measure (i.e., history of smoking, cardiovascular disease, diabetes, stroke, atrial fibrillation, left ventricular hypertrophy, antihypertensive therapy), aside from significant differences in systolic and diastolic blood pressure ($t = 2.20$, $p = 0.029$; $t = 2.97$, $p = 0.004$; respectively; see **Chapter 2 Table 1**). With regard to cognition, APOE ε4 carriers demonstrated worse performance on the memory composite than did non-carriers ($t = 2.53$, $p = 0.013$). They also performed worse on four of the five subtests that were used to create the memory composite (i.e., CVLT-II trials 1-5, long delay free recall; WMS Logical Memory Immediate and Delayed Recall; see **Chapter 2 Table 1**). With regard to tests in other cognitive domains, ε4 carriers performed worse on two subtests of the DKEFS Color Word Interference test (i.e., DKEFS Color Word Interference Inhibition and Inhibition Switching; all ps < 0.01; see **Chapter 2 Table 1**), measuring cognitive flexibility and inhibitory control. No other group differences in cognitive performance were found (all ps > 0.05). With regard to measures of brain structure and function, APOE ε4 carriers exhibited lower left EC CBF than did non-carriers ($t = 2.01$, $p = 0.047$, see **Chapter 2 Table 1**). No other significant group differences in resting CBF were found in brain regions of interest (i.e., Hc, cACC, rMFC; all ps > .05). APOE ε4 carriers demonstrated greater left cACC CT than did non-carriers ($t = 2.53$, $p = 0.012$; see **Chapter 2 Table 1**). No other significant group differences in CT were found in brain regions of interest (i.e., EC, Hc, rMFC).

Effects of CBF, CT/Vo, and APOE on memory performance

A significant three-way interaction was found in the left EC ($t = 3.34$, $p = 0.001$; see **Chapter 2 Table 2**), such that the two-way interaction of left EC CBF and left EC CT on memory, differs by APOE genotype (see **Chapter 2 Figure 1**). More specifically, the relationship between entorhinal CBF and memory was negative (*lower* CBF was associated with better memory) in non-carriers with higher EC CT (≥ 2.3SD), positive (*higher* CBF was associated with better memory) in non-carriers with lower EC CT (≤ -1.2SD), and negative (higher CBF was associated with *worse* memory) in ε4 carriers with lower EC CT (≤ -0.62SD). There was no significant three-way interaction found in the right EC ($t = 1.05$, $p = 0.296$; see **Chapter 2 Table 2**); however, results in the right EC showed a trend toward the same three-way interaction (including directionality) seen in the left EC (see

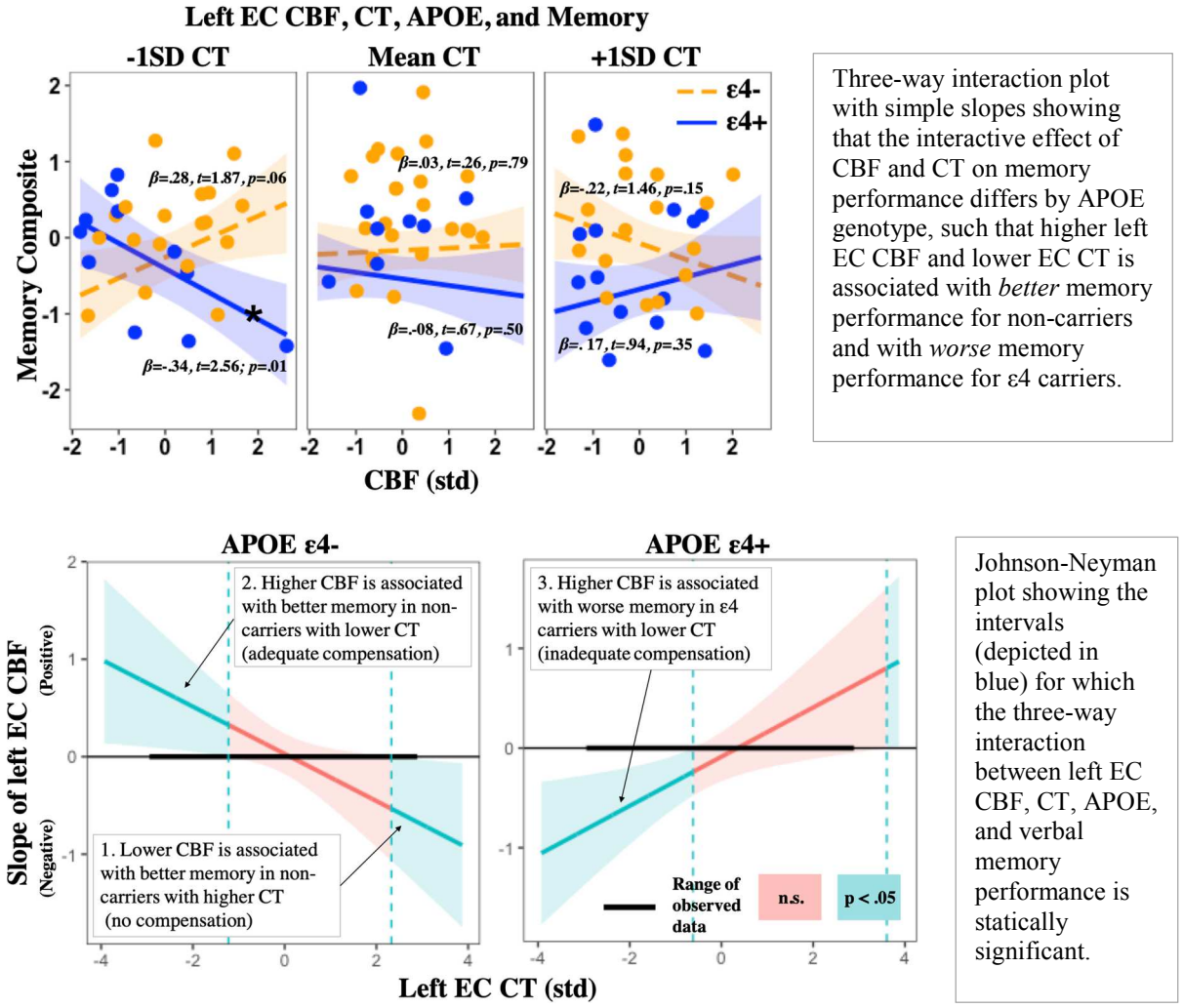
Chapter 2 Figure A1 in the Appendix). No significant three-way interactions were found in the left or right Hc (all *p*s >.0125, see **Chapter 2 Table 2** and **Chapter 2 Figures A2 & A3** in the Appendix); however, after removing the non-significant three-way interaction from the model, there was a significant effect of APOE genotype and right Hc CBF on memory ($t = 2.10$, $p = 0.04$; *uncorrected* for multiple comparisons), such that increased CBF in the right Hc was associated with worse verbal memory performance in ε4 carriers but not in non-carriers. Of note, although not statistically significant, results in the right Hc also showed a trend toward the same three-way interaction (including directionality) seen in the left EC (see **Chapter 2 Figure A2a and A2b** in the Appendix). Exploratory analysis of frontal regions (i.e., cACC, rMFC) found no significant three-way interactions (all *p*s > 0.05; see **Chapter 2 Table A2** in the Appendix). However, in the right cACC there was a significant two-way interaction of CT and APOE on memory ($t = 3.65$, $p = 0.031$), such that increased CT in the right cACC was associated with worse verbal memory performance in ε4 carriers but not in non-carriers (see **Chapter 2 Figure S4** in the Appendix**).**

Chapter 2 Table 2. Effects of CBF, CT/Vo, and APOE, on Memory Performance. Note: Only variables of interest are included in table; All continuous independent variables were standardized; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; Vo= Volume; EC= Entorhinal cortex; Hc= Hippocampus; R= Right; L = Left; β= Standardized regression coefficient; CI= confidence interval; bs= Bootstrapped with 5000 replications; t= t-statistic; p= p=value. The 95% confidence interval of the coefficient was derived using the bootstrap bias-adjusted and accelerated bootstrap interval; *Denotes significance at p<0.05; **Denotes significance at p<0.01

Independent Variable	***β***	***95% CI (bs)***	***s.e. (bs)***	***t***	***p-value***
APOE	-0.375	(-0.749, -0.019)	0.167	2.176	0.032*
Left EC CT	0.092	(-0.093, 0.280)	0.184	0.935	0.352
Left EC CBF	0.029	(-0.183, 0.197)	0.092	0.282	0.778
APOE*Left EC CT	-0.228	(-0.566, 0.084)	0.165	1.345	0.182
APOE*Left EC CBF	-0.115	(-0.482, 0.176)	0.156	0.705	0.482
Left EC CT*Left EC CBF	-0.241	(-0.423, -0.050)	0.092	2.302	0.023*
APOE*Left EC CT*Left EC CBF	0.488	(0.220, 0.761)	0.148	3.338	0.001**
APOE	-0.416	(-0.793, -0.064)	0.182	2.426	0.017*
Right EC CT	-0.039	(-0.219, 0.149)	0.092	0.409	0.683
Right EC CBF	0.038	(-0.166, 0.277)	0.114	0.357	0.722
APOE*Right EC CT	0.085	(-0.303, 0.467)	0.195	0.454	0.650
APOE*Right EC CBF	-0.266	(-0.590, 0.064)	0.168	1.557	0.123
Right EC CT*Right EC CBF	-0.117	(-0.346, 0.145)	0.124	0.970	0.334
APOE*Right EC CT*Right EC CBF	0.169	(-0.270, 0.526)	0.206	1.050	0.296
APOE	-0.303	(-0.645, 0.062)	0.178	1.821	0.071
Left Hc Vo	-0.007	(-0.196, 0.171)	0.094	0.071	0.943
Left Hc CBF	0.024	(-0.172, 0.200)	0.094	0.260	0.795
APOE*Left Hc Vo	0.011	(-0.330, 0.371)	0.178	0.070	0.943
APOE*Left Hc CBF	-0.231	(-0.711, 0.158)	0.218	1.280	0.203
Left Hc Vo*Left Hc CBF	0.037	(-0.151, 0.214)	0.092	0.384	0.702
APOE*Left Hc Vo*Left Hc CBF	0.076	(-0.354, 0.552)	0.228	0.405	0.686

Chapter 2 Table 2. Effects of CBF, CT/Vo, and APOE, on Memory Performance, Continued.

APOE	-0.290	(-0.786, 0.002)	0.182	1.717	0.089
Right Hc Vo	-0.088	(-0.097, 0.268)	0.102	0.904	0.368
Right Hc CBF	0.079	(-0.101, 0.263)	0.092	0.864	0.390
APOE*Right Hc Vo	0.049	(-0.307, 0.415)	0.180	0.270	0.787
APOE*Right Hc CBF	-0.343	(-0.797, 0.032)	0.205	1.844	0.068
Right HC Vo*Right Hc CBF	-0.152	(-0.369, 0.018)	0.096	1.784	0.077
APOE*Right Hc Vo*Right Hc CBF	0.220	(-0.154, 0.669)	0.214	1.432	0.155



Chapter 2 Figure 1. Three-way interaction of left EC CBF, CT, and APOE, on memory. 1) Lower EC CBF is associated with better memory in non-carriers with higher EC CT (≥ 2.3SD), perhaps reflecting neural efficiency and no current need for compensation; **2)** Higher EC CBF is associated with better memory in non-carriers with lower EC CT (≤ -1.2SD), perhaps reflecting compensatory increases in CBF that are supportive of current memory function; **3)** Higher EC CBF is associated with worse memory performance in ε4 carriers with lower EC CT (≤ -.62SD), perhaps reflecting maximally invoked compensatory increases in CBF that are not fully supportive of current memory function. Note: EC= Entorhinal cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; *Denotes simple slope significance at p<0.05

DISCUSSION

Results showed that APOE genotype modified the interactive effects of MTL CBF and CT on cognitive performance among a sample of cognitively normal older adults. More specifically, relationships between left EC CBF and memory were negative in ε4 carriers with lower CT, positive in non-carriers with lower CT, and negative in non-carriers with higher CT. Although not statistically significant, there were trends toward this same three-way interaction in the left EC and the right Hc. Interestingly, these results may help explain mixed findings in the literature with regard to relationships between CBF and cognition (findings of both positive and negative associations), as this relationship in the MTL appears to vary as a function of both CT and APOE genotype. Moreover, APOE genotype modified the direct effects of CBF and CT on cognitive performance, such that higher CBF in the right Hc and higher CT in the right cACC independently predicted worse verbal memory performance in ε4 carriers, but not in non-carriers. APOE ε4 carriers also exhibited worse verbal memory performance, worse executive functioning performance, lower left EC CBF, and higher right cACC CT. These findings are consistent with other APOE-related aging studies, supporting the notion that regionally specific perfusion and brain structure differences exist between ε4 carriers and non-carriers in areas that have been implicated in normal aging and AD-risk and that these differences are associated with worse cognitive function (Bangen et al., 2012; Dai et al., 2009; Hays et al., 2016; Meltzer et al., 2000; Wierenga et al., 2012; Zlatar et al., 2016). However, this is the first study, to our knowledge, to show that APOE genotype modifies the interactive relationship between MTL CBF and CT on cognitive function, providing a more complete accounting of the relationships among these variables.

The complex three-way interaction of left EC CBF, CT, and APOE on verbal memory performance may be appreciated in the context of regional compensatory brain activity, reflecting efforts to maintain adequate brain oxygenation in the face of vascular aging and/or neuropathological damage. For example, the observed negative relationship between EC CBF and cognition (*lower* CBF being associated with better memory) in non-carriers with higher CT may reflect neural efficiency, such that non-carriers with higher cortical reserve have no need to invoke compensatory increases in CBF. In contrast, the observed positive relationship between EC CBF and cognition (*higher* CBF being associated with better memory) in non-carriers with lower CT may reflect successful cerebrovascular compensation, such that non-carriers with lower cortical reserve are invoking compensatory increases in CBF that are adequately supporting current memory function. In this same context,

the negative relationship between EC CBF and cognition (higher CBF being associated with *worse* memory) in ε4 carriers with lower CT may reflect a relative breakdown of cerebrovascular compensation, such that compensatory increases in CBF among ε4 carriers with lower cortical reserve are maximally invoked and not fully supportive of current memory function (see the Johnson-Neyman plot in Figure 1 for a visual depiction), though more research is needed to determine if they adequately support memory function over *time* (e.g., memory stability). Overall, these findings are largely consistent with the Capillary Dysfunction Hypothesis of Alzheimer's disease, which posits that ε4 carriers experience increased heterogeneity of capillary blood flow, which reduces the amount of oxygen that can diffuse into tissue. This reduction in oxygen diffusion necessitates a compensatory increase in CBF to maintain adequate brain oxygenation (Østergaard et al., 2013). However, progressive increases in this heterogeneity of flow is thought to result in low tissue oxygen tension, a state which paradoxically benefits (due to increased blood-tissue oxygen concentration gradients) from *suppression* of CBF (Jespersen & Østergaard, 2012; Østergaard et al., 2013). Although essential for the maintenance of oxygen availability, this compensatory reduction in cerebral perfusion may ultimately lead to oxidative stress, activation of inflammatory pathways, and increased amyloid levels in the brain (Ostergaard et al., 2013). Taken together, this suggests that cognitively normal ε4 carriers with *higher* EC CBF and *lower* EC CT may be at elevated risk for cognitive decline due to inadequate/maximally invoked cerebrovascular compensatory mechanisms. Moreover, non-carriers showing this same compensatory pattern (higher CBF and lower CT) may also be at risk for cognitive decline, as this may signal some degree of vascular aging and/or or neuropathological damage. Of note, though not supported by the current cross-sectional analysis, it is also possible that ε4 carriers with lower cortical reserve who do *not* show compensatory increases in EC CBF at baseline might be at even greater risk for future cognitive decline, though longitudinal studies are needed to test this hypothesis.

More broadly, findings of an interaction between CBF, CT, APOE, and memory performance in the MTL in cognitively normal adults supports the hypothesis that the APOE ε4 isoform confers risk for cognitive decline through altered MTL perfusion, and that concomitant altered cortical morphology in the same region may further exacerbate the negative effects of CBF alteration on cognition. The concept of APOE-related alterations in MTL CBF (rather than MTL CT) being more tightly linked to cognitive function is supported by our observation of direct effects of CBF on cognitive performance in ε4 carriers in other MTL regions (i.e., right hippocampus), but no direct effects of CT on cognition in MTL regions. Moreover, the localization of the

interaction between APOE, CBF and CT on verbal memory performance in the left entorhinal cortex (and a trend toward this interaction in the right) suggests the presence of AD-related neuropathological processes, rather than an exacerbation or speeding up of normal aging processes, as the EC is one of the first regions to be affected by AD pathology (Braak & Braak, 1991), but is relatively spared in normal aging (Fjell et al., 2009; Good et al., 2001; Raz et al., 2005; Raz, Rodrigue, & Haacke, 2007). With regard to lateralization, the current finding of a significant three-way interaction in the left, but not the right, EC may be related to statistical power, given similar observable trends in the right EC. However, prior studies have shown that ε4 carriers have lower left than right EC CT (Donix et al., 2013), suggesting that the left EC may be more vulnerable to APOE-related neuropathological damage and thus may interact with CBF and cognitive function earlier on, when compared to the right EC. Similarly, the finding of a significant two-way interaction of CBF and APOE in the right, but not the left Hc may also reflect a lack of statistical power and is consistent with reports of greater atrophy in right hemisphere structures (including the hippocampus) in ε4 carriers compared to non-carriers (Tang, Holland, Dale, & Miller, 2015).

Exploratory analyses of prefrontal regions and memory function revealed a rather unexpected finding: *higher* right cACC CT among ε4 carriers compared to non-carriers was associated with worse verbal memory performance. Although counterintuitive, given that most studies report widespread cortical thinning in ε4 carriers, this finding may be better understood in the context of the normal aging cortical morphology literature. Against the backdrop of widespread age-related cortical thinning and volume reduction, there is accumulating evidence suggesting that there may be isolated areas of age-related cortical thickening in the prefrontal cortex, including the ACC (Abe et al., 2008; Fjell et al., 2009; Salat et al., 2004). As such, it is possible that the APOE ε4 isoform may lead to an exaggeration or speeding up of normal age-related morphological changes in the ACC. This concept is supported by recent studies showing that ε4 carriers do indeed exhibit higher CT and/or volume in prefrontal regions (including the ACC) compared to non-carriers (Dowell et al., 2016; Espeseth et al., 2008, 2012). The current results are similar to those reported on an event related potential study of attention and cortical thickness by APOE status. This 2012 study found that APOE ε4-related thickening of prefrontal cortical regions was associated with worse selective attention. Our results extend these prior findings to other cognitive domains, showing that increased thickness of prefrontal regions (i.e., cACC) in ε4 carriers is also associated with worse verbal memory performance. The negative association between CT and cognition also lends support to the

concept of APOE-related thickening of the prefrontal cortex as part of a dysfunctional process associated with the ε4 allele, perhaps due to impaired repair mechanisms (Sundstrom et al., 2004; Teter et al., 2002).

Conclusion

In conclusion, our findings support the notion that APOE ε4 carriers may be experiencing vascular dysregulation and concomitant morphological changes in the MTL that interact to negatively affect cognition prior to the onset of overt clinical symptoms, providing potential insight into the mechanistic link between APOE ε4 and detriments in cognition. Although future longitudinal research is needed to determine whether these relationships also predict future cognitive decline, the current observed pattern of *higher* CBF and *lower* CT in the EC predicting worse memory performance suggests a novel multimodal neural signature with the potential to detect ε4 carriers who are at elevated risk for cognitive impairment. Such early detection could inform candidate selection and study design for future clinical trials. On a broader scale, the current results add to accumulating evidence supporting the vascular theory of AD and could lead to the identification of vasoprotective treatments with the potential to delay or prevent the onset of age-related cognitive decline and/or AD.

Strengths and limitations

Limitations of the current study include the use of a cross-sectional design, which restricted our ability to draw causal conclusions and limited our ability to determine whether the effects of APOE on CBF and CT, and cognition represent normal aging or pathologic processes. It is also important to note that our sample had relatively high levels of education, and although this demographic factor was not associated with APOE genotype, its limited range may reduce the generalizability of these findings. Although groups (ε4+/ ε4-) did not differ significantly in the average time interval between cognitive testing and fMRI scanning (53 and 51 days, respectively), decreasing the time interval between cognitive testing and MRI may improve accuracy of brain-behavior associations. Moreover, our sample of 117 only included 41 APOE ε4 carriers, which may have limited our ability to detect statistically significant three-way interactions in other regions. Future investigations should include longitudinal designs with larger, more diverse samples to replicate and extend the current findings.

Furthermore, the inclusion of additional markers of AD, such as CSF biomarkers may help better characterize APOE ε4 carriers.

The strengths of the current study include the use of non-invasive ASL MRI to measure partial volume corrected CBF, and the use of a high-resolution structural MRI scan to examine CT and Vo. Furthermore, use of FreeSurfer offers advantages over traditional voxel-based morphometry methods, as it also allows for examination of the components of volume separately (thickness and surface area), as it has been found that these two do not necessarily track with one another. Moreover, the extraction of CBF from FreeSurfer-derived brain regions represents a strength, allowing us to directly investigate CBF (and brain structure) in regions that are defined by each individual's anatomy, rather than atlas-defined regions which are less sensitive to individual anatomical differences because they require that data are first aligned and warped to a generic anatomic template. Lastly, the current study benefited from the inclusion of a well-controlled and well-characterized sample of cognitive normal older adults, which included the use of several cognitive test performances to characterize cognitive status.

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Chapter 3: Study 3

Interaction of APOE, Cerebral Blood Flow, and Cortical Thickness in the Entorhinal Cortex Predicts Memory Decline

Chelsea C. Hays[a,d], Zvinka Z. Zlatar[b], M.J. Meloy[a,b], Mark W. Bondi[a,b,d],

Paul E. Gilbert [d,e] , Thomas Liu[c], Jonathan L. Helm[d,e], & Christina E. Wierenga[a,b,d]

[a]VA San Diego Healthcare System, 3350 La Jolla Village Dr., San Diego, 92161

[b]Department of Psychiatry, UC San Diego, 9500 Gilman Dr., La Jolla, CA 92093

[c]Department of Radiology, UC San Diego, 9500 Gilman Dr., La Jolla, CA 92093

[d] SDSU/UCSD Joint Doctoral Program in Clinical Psychology, 6363 Alvarado Ct, Suite 103, San Diego, CA 92120

[e]Department of Psychology, San Diego State University, 5500 Campanile Drive

San Diego, CA 92182

Address for correspondence:

Christina E. Wierenga, Ph.D.

VA San Diego Healthcare System

3350 La Jolla Village Dr., MC 151B

San Diego, CA 92161

Phone: (858) 534-8047

Fax: (858) 642-1218

cwierenga@ucsd.edu

ABSTRACT

The ε4 allele of the apolipoprotein E gene (APOE), a risk factor for cognitive decline, is associated with alterations in medial temporal lobe (MTL) structure and function, yet little research has been dedicated to understanding how these alterations might *interact* to negatively impact cognition. To bridge this gap, the present study employed linear regression models to determine the extent to which APOE genotype (ε4+, ε4-) modifies interactive effects of baseline arterial spin labeling MRI-measured CBF and FreeSurfer-derived CT/Vo in two MTL regions of interest (entorhinal cortex, hippocampus) on regression-based memory change in 98 older adults who were cognitively normal at baseline. Baseline entorhinal CBF was positively associated with memory change, but only among ε4 carriers with lower entorhinal CT. Similarly, baseline entorhinal CT was positively associated with memory change, but only among ε4 carriers with lower entorhinal CBF. Findings suggest that APOE ε4 carriers may experience vascular dysregulation and concomitant morphological alterations in the MTL that interact to negatively affect cognition prior to the onset of overt clinical symptoms. Results also suggest the presence of distinct multimodal neural signatures in the entorhinal cortex that may signal relative risk for decline among this group, likely reflecting different stages of cerebrovascular compensation.

INTRODUCTION

The ε4 allele of the apolipoprotein E (APOE) gene increases risk for pathologic age-related cognitive decline (Bretsky et al., 2003; Richard J. Caselli et al., 2009; Schiepers et al., 2012; Tsuang et al., 2013), but the mechanistic link between APOE ε4 and cognitive decline is still poorly understood. APOE-related alterations in cognition have been consistently reported, with older adult ε4 carriers demonstrating worse cognitive performance (Adamson et al., 2010; De Blasi et al., 2009; Honea, Vidoni, Harsha, & Burns, 2009; Kukolja, Thiel, Eggermann, Zerres, & Fink, 2010; Tuminello & Han, 2011) and accelerated cognitive decline, most notably in episodic memory ability (Bretsky et al., 2003; R. J. Caselli et al., 2004; Richard J. Caselli et al., 2009; Hayden et al., 2009; C.-C. Liu, Kanekiyo, Xu, & Bu, 2013; Schiepers et al., 2012; Whitehair et al., 2010). There is also converging evidence of APOE-related alterations in brain function and structure in medial temporal lobe (MTL) regions (Burggren et al., 2008; Cohen, Small, Lalonde, Friz, & Sunderland, 2001; den Heijer et al., 2002; Hays, Zlatar, & Wierenga, 2016; Jak, Houston, Nagel, Corey-Bloom, & Bondi, 2007). However, very little research has focused on understanding how alterations in MTL structure and function might *interact* to negatively impact cognition among APOE ε4 carriers. Successful attempts to characterize these complex interactions could lead to identification of earlier and more reliable markers of incipient cognitive decline or treatments with the potential to delay, or even prevent, age-related changes in cognition.

Older adult APOE ε4 carriers demonstrate reduced cortical thickness and accelerated gray matter atrophy in MTL regions when compared to non-carriers, most notably in the entorhinal cortex and hippocampus (Burggren et al., 2008; Cohen et al., 2001; den Heijer et al., 2002; Donix et al., 2013, 2010; Jak et al., 2007; Tohgi et al., 1997). Moreover, reduced structural integrity in these brain regions is linked to detriments in cognition and conversion to MCI and AD (Honea et al., 2009; Lind et al., 2006; Pacheco, Goh, Kraut, Ferrucci, & Resnick, 2015; Soldan et al., 2015), suggesting that the ε4 allele may exert negative impacts on cognition, at least in part, through altered MTL structure. APOE ε4 carriers also demonstrate alterations in cerebral blood flow (CBF), or the rate of delivery of arterial blood to the capillary bed in a volume of tissue, across widespread medial temporal, frontal, and parietal regions (Tai et al., 2016; Wierenga, Hays, & Zlatar, 2014). More specifically, ε4 carriers tend to exhibit higher resting CBF than non-carriers in early adulthood and middle-age, but lower resting CBF in old age (Thambisetty, Beason-Held, An, Kraut, & Resnick, 2010; Wierenga et al., 2013). The biphasic nature of CBF among ε4 carriers has been attributed to cerebrovascular compensation, with

early increases reflecting attempts to compensate for the deleterious effects of APOE ε4 (e.g., impaired repair mechanisms, neurovascular disruption) and subsequent decreases reflecting a relative breakdown of this compensation (Dai et al., 2009; Hays et al., 2016; Koizumi et al., 2018; Luckhaus et al., 2008; Ostergaard et al., 2013; Wierenga et al., 2012). Notably, cross-sectional investigations of cerebral perfusion in cognitively normal older adult ε4 carriers have produced mixed results, with reports of both increased and decreased CBF, relative to non-carriers (Bangen et al., 2009; Filippini et al., 2011, 2011; Thambisetty et al., 2010; Wierenga et al., 2013; Zlatar et al., 2016), and both positive and negative associations between CBF and cognition (Bangen et al., 2012; Wierenga et al., 2012; Zlatar et al., 2016). Although mixed findings with regard to links between CBF, APOE, and cognition may be partially accounted for by differences in sample characteristics or methodology, recent evidence by our group (Hays et al., Manuscript Submitted for Publication) suggests another possibility; namely, that these inconsistencies may reflect a failure to account for underlying structural integrity within the same anatomical region. We found evidence of an interactive effect of MTL CBF, structural integrity, and APOE on cognition among a sample of cognitively normal older adults, whereby the relationship between entorhinal CBF and memory performance varied as a function of APOE genotype and entorhinal cortical thickness. More specifically, observed relationships between entorhinal CBF and memory performance were negative in ε4 carriers with lower entorhinal cortical thickness, positive in non-carriers with lower entorhinal cortical thickness, and negative in non-carriers with higher entorhinal cortical thickness. We propose that differential relationships among these variables can be understood in the context of cerebrovascular compensation, with compensatory increases in CBF being invoked among individuals with lower cortical reserve in the same region, and differences in the direction of the relationship between CBF and cognition representing different points along a trajectory of compensation. For example, the observed positive relationship between entorhinal CBF and cognition (higher CBF associated with *better* memory) among non-carriers with lower entorhinal cortical reserve may reflect successful cerebrovascular compensation, whereas the negative relationship between entorhinal CBF and cognition (higher CBF associated with *worse* memory) among ε4 carriers with lower entorhinal cortical reserve may reflect a relative breakdown of cerebrovascular compensation, such that compensatory increases in CBF are maximally invoked and not supportive of concurrent memory function. Taken together, these data suggest that ε4 carriers with a combined pattern of higher CBF and lower CT in MTL regions might be at elevated risk for future cognitive decline. However, it remains unclear whether this pattern also predicts future

cognitive decline. For example, although relative hyperperfusion among this group does not appear supportive of concurrent memory function, it might be supportive of memory function over *time* (e.g., greater memory stability). Exploring links between APOE-related alterations in MTL structure and function and their impacts on longitudinal *changes* in cognition represents a critical next step toward improving our understanding of the mechanistic link between APOE and future cognitive decline.

Together, previous evidence suggests that relationships between MTL CBF and concurrent memory performance vary by APOE genotype and underlying structural integrity. However, to our knowledge, no study has explored the interactive effects of these variables as they relate to longitudinal changes in memory performance. In order to bridge this gap in the literature, the current study used arterial spin labeling (ASL) magnetic resonance imaging (MRI) and a high-resolution structural scan among a well-characterized sample of cognitively normal older adults with serial cognitive data to determine the extent to which APOE genotype (ε4+ vs. ε4-) modifies interactive effects of baseline MTL resting CBF and brain structure (cortical thickness [CT], volume [Vo]) on longitudinal changes in verbal episodic memory performance. Based on our cross-sectional findings, we predicted that APOE genotype would modify the interactive effects of baseline CBF and brain structure on memory change, such that *higher* CBF (reflecting maximally invoked compensatory response) and lower CT/Vo in MTL regions (entorhinal cortex [EC], hippocampus [Hc]) at baseline would be associated with greater memory decline among e4 carriers, but not among non-carriers.

METHODS

Participants

See **Chapter 3 Table 1** for participant demographic and cognitive characteristics. Participants were community-dwelling older adult volunteers enrolled in a longitudinal study of aging at the VA San Diego Healthcare System (VASDHS). A total of 98 cognitively normal participants between the ages of 64 and 89 (mean age = 72.8, SD = 6.0) with available data were included in the current analyses. Thirty-one participants were carriers of the APOE e4 allele (ε3/ε4 = 28, ε4/ε4 = 3) and 67 were non-carriers (ε3/ε3 = 58, ε3/ε2 = 9). All participants were administered a full neuropsychological battery and an MRI scan at baseline (mean time interval between neuropsychological testing and MRI scan = 16 days) and received follow-up neuropsychological testing at one-year intervals (time interval between baseline neuropsychological testing and most recent follow-up:

mean = 2.5 years, min = 0.9, max = 5.5 years). To ensure all participants had normal cognitive function, participants were excluded if performance on more than one measure within a cognitive domain was more than one standard deviation below age-appropriate norms, consistent with the empirically-derived criteria for diagnosis of MCI developed by Jak and colleagues (Jak et al., 2009), or if overall performance on the Dementia Rating Scale (DRS) was more than 1 standard deviation below age-appropriate norms (see **Chapter 3 Table A1** in the Appendix for specific cognitive tests, domains, and normative data). Potential participants were excluded if they had a dementia or MCI diagnosis, a history of severe head injury, major vascular event, uncontrolled hypertension, or a DSM-IV Axis I diagnosis of learning disability, attention deficit disorder, mood disorder, or substance abuse. In addition, participants were excluded if they had contraindications to MRI scanning such as ferrous implants or a pacemaker. All participants provided written informed consent prior to enrollment and data were collected in accordance with all ethical standards as stipulated by the UC San Diego and VA San Diego Healthcare System institutional review board-approved procedures.

Cognition

Standardized change scores were used to estimate neuropsychological change in individuals and account for practice effect and regression toward the mean (Cysique et al., 2009; Heaton et al., 2001). To develop the mean scaled score regression change score (MSR-CS), the first step consisted of defining a reference group for which no neuropsychological change is expected beyond practice effect: we used a sample of 77 cognitively normal older adults who were also assessed serially at one-year intervals. The demographic characteristics of the reference group were as follows: mean age of 73.3 years (SD = 8.0); with a mean level of education of 16.3 years (SD = 2.1); 36% were men. These demographic characteristics were not statistically different from the target sample. All subjects in the reference group were cognitively normal (using the same criteria described above for the target sample) at baseline and remained cognitively normal at all follow-up timepoints. The second step of the MSR-CS development was as follows: the reference group yielded a set of regression equations (see **Chapter 3 Table A2** in the Appendix) from baseline to first follow-up (mean time between baseline to first follow-up = 1.3 years, SD = 0.77), statistically adjusting for age, sex, education, and the follow-up interval, which was then used to derive predicted follow-up scores in the target sample. Subtracting the predicted follow-up score from individual's observed follow-up score and dividing that result by the standard

deviation of the residuals from the reference group regression models provided a standard score of cognitive change. This score was then used as a continuous variable; the MSR-CS (i.e., significant neuropsychological decline as: MSR-CS ≤-1.04 based on a two-tailed 70% confidence interval, MSR-CS ≤-1.28 based on a two-tailed 80% confidence interval, MSR-CS ≤-1.645 based on a two-tailed 90% confidence interval). The methods above were applied to the following cognitive test scores: trials 1-5, short delay free-recall, and long delay free-recall raw scores from the California Verbal Learning Test – Second Edition (CVLT-II), measuring word list recall, and the Logical Memory immediate and delayed recall subtests of the Wechsler Memory Scale-Revised (WMS-R), measuring story recall. These tests were selected based on results from a principal component analysis previously reported by our group on a similar sample of older adults (Wierenga et al., 2012). A verbal memory change score composite (Memory-CS-Composite) was calculated by averaging the MSR-CS's for the five verbal memory tests. A baseline verbal memory composite score was derived by averaging the *z*-scores for each of the tests within the composite for the entire sample.

Apolipoprotein E genotyping

Genotyping was performed by the ADCS Biomarker Core at UCSD using real time PCR Restriction Fragment Length Polymorphism analysis. Genomic DNA was collected from participants using buccal swab and extracted using Qiamp DNA blood mini kit (Qiagen) followed by PCR amplification (Wierenga, et al., 2012). Those with at least one ε4 allele (i.e., ε2/ε4, ε3/ε4, ε4/ε4) were classified as APOE e4 carriers (ε4+) and those without an ε4 allele (i.e., ε2/ε2, ε2/ε3, ε3/ε3) were classified as non-carriers (ε4-). Given that the APOE ε2 allele is thought to have protective effects (Suri, Heise, Trachtenberg, & Mackay, 2013), all analyses were run including and excluding ε2 carriers from the ε4+ group and the pattern of results was similar. Therefore, results from the entire sample are presented. An exact test of Hardy-Weinberg Equilibrium was not significant (p= 0.62), suggesting that the distribution of APOE genotype in the sample included in this manuscript does not differ significantly from the expected distribution in the general population.

MRI acquisition

Imaging data were acquired on a GE Discovery MR750 3T whole body system with a body transmit coil and an 8-channel receive-only head coil at the University of California San Diego Center for Functional MRI. The structural brain sequence consisted of a high-resolution T1-weighted Fast Spoiled Gradient Recall (3D FSPGR) scan: 172 1 mm contiguous sagittal slices, FOV = 25 cm, TR = 8 ms, TE = 3.1 ms, flip angle = 12, T1 = 600 ms, 256 x 192 matrix, Bandwidth = 31.25 kHz, frequency direction = S-I, NEX = 1, scan time = 8 min and 13 sec. Resting CBF was acquired with the Multiphase Pseudocontinuous Arterial Spin Labeling (MPPCASL) sequence, which is optimized for robust CBF quantification (Jung, Wong, & Liu, 2010): tagging duration = 2 sec, post-labeling delay = 1.6 sec, TR = 4.2 sec, TE = 3 ms, reps = 64, FOV = 22 x 22 cm, 20 5 mm axial slices with a single shot spiral acquisition, collecting 8 cycles where each cycle consists of 8 images acquired with unique phase offsets, acquisition time = 4:46 minutes. A CSF calibration scan was also obtained using a spiral readout with TR = 4 sec and TE = 3.4 ms and comprised nine 90◦ excitation pulses which were turned off for the first eight repetitions to generate PD-weighted contrast (scan time: 36 sec) to obtain an estimate of the equilibrium magnetization of cerebral spinal fluid, which is used to convert the perfusion signal into calibrated CBF units (mL blood/100g tissue/min). Finally, a minimum contrast image was acquired to adjust for transmit and receive coil inhomogeneities. Two field map scans were also acquired and used for off-line field map correction to help correct for signal bunching and dropouts in the frontal/medial temporal lobes.

MRI pre-processing

CBF

ASL image processing was performed with Analysis of Functional NeuroImages (AFNI, afni.nimh.nih.gov)(Cox, 1996), FMRIB Software Library (FSL, Oxford, United Kingdom), and locally created Matlab scripts. Field map correction was applied to the ASL time series prior to co-registration to the middle time point to minimize the effects of participant motion. For each participant, a mean ASL difference image was formed from the average difference of the control and tag images and slice timing delays were accounted for in order to make the post-labeling delay time slice specific (T. T. Liu & Wong, 2005). This mean ASL image was then converted to absolute units of CBF (mL/100g tissue/min) using an estimate of the equilibrium magnetization of CSF as a reference signal (Chalela et al., 2000). This procedure resulted in a calibrated perfusion value for each voxel. Skull stripping of the high-resolution T1-weighted image was performed using

AFNI's 3dSkullStrip. Tissue segmentation was performed using FSL's Automated Segmentation Tool (FAST) algorithm (http://fsl.fmrib.ox.ac.uk/fsl/) to define cerebrospinal fluid (CSF), gray matter (GM) and white matter (WM) regions. The high-resolution T1-weighted image and partial volume segmentations were registered to ASL space, and the CBF data were resampled to the same resolution as the T1-weighted image. The partial volume estimates were used to perform partial volume correction of the high-resolution CBF data using a linear regression approach with kernel size of 3 (Asllani et al., 2008; Zhao et al., 2017) as implemented by the ASL file function in the BASIL toolset of FSL (Chappell, Groves, Whitcher, & Woolrich, 2009). The partial volume corrected gray matter CBF data were then resampled back to their native resolution and registered to FreeSurfer space. Quality assurance of ASL data was performed prior to analysis using outlier detection, inspection of CBF histograms, and visual checks of the CBF maps, with removal of values for regions with poor CBF map coverage. This resulted in removal of 5% of the data. CBF values were extracted from each anatomically defined FreeSurfer ROI (see below for FreeSurfer methods). Because we did not aim to explore laterality and there were significant and robust correlations between hemispheres (all $r > 0.57$, $p < 0.0001$), we averaged CBF values across left and right hemispheres. Average EC and Hc CBF were used in subsequent analyses.

CT and Vo

Cortical thickness and volume analysis were performed using FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu), with the technical details of these procedures described in prior publications (Dale, Fischl, & Sereno, 1999; Dale & Sereno, 1993; B. Fischl & Dale, 2000; B. Fischl, Liu, & Dale, 2001; B. Fischl, Sereno, Tootell, & Dale, 1999; Bruce Fischl et al., 2002; Bruce Fischl, Salat, et al., 2004; Bruce Fischl, van der Kouwe, et al., 2004; Reuter, Rosas, & Fischl, 2010; Reuter, Schmansky, Rosas, & Fischl, 2012). Cortical thickness was extracted from the left and right EC and volume was extracted from the left and right Hc (measures of thickness are not available for subcortical brain structures). A quality assurance protocol was performed before analysis using the ENIGMA guidelines (http://enigma.usc.edu/protocols/imaging-protocols) and included visual checks of the cortical segmentations and region-by-region removal of values for segmentations found to be incorrect. Histograms of all regions' values were also computed for visual inspection. Because we did not aim to explore laterality and there were significant and robust correlations between

hemispheres (all r > 0.62, p < 0.0001), we averaged CT values across left and right hemispheres. Average EC CT and average Hc Vo were used in subsequent analyses.

Statistical analyses

t-tests were used to compare groups on age, years of education, brain variables (CBF, CT/Vo), and cognitive variables. A $\chi 2$-test was used to compare groups on sex. CBF and CT/Vo were extracted from FreeSurfer-derived regions of interest (i.e., CT of the EC, Vo of the Hc) and multiple linear regression models were employed in R with the Memory-CS-Composite as the dependent variable and APOE genotype and brain variables (CBF, CT/Vo) as independent variables. The following 3-way interactions were explored: 1) APOE genotype x EC CBF x EC CT, and 2) APOE genotype x Hc CBF x Hc Vo. A Bonferroni correction was applied to correct for the two comparisons, and coefficients were only considered significant at $p<0.025$. All analyses statistically adjusted for the effects of age, sex, education, and the cognitive follow-up interval (time between baseline cognitive testing and the most recent follow-up cognitive testing). Analyses including measures of Hc Vo also adjusted for the effects of total intracranial volume. Non-multicollinearity between independent variables was confirmed by application of the multicollinearity index VIF (all VIF<2), linearity was confirmed by residuals versus fits plots, normality was confirmed by Q-Q plots, non-heteroskedasticity was confirmed by scale location plots, and no influential cases were identified through examinations of residuals versus leverage plots.

RESULTS

Group differences in demographic and assessment variables

Groups (ε4+, ε4-) did not differ significantly on age, sex, or years of education, nor did they differ significantly in the time interval between baseline neuropsychological testing and neuroimaging or the cognitive follow-up interval (all *p*s > 0.05; see **Chapter 3 Table 1**). APOE ε4 carriers demonstrated worse performance on the baseline memory composite than did non-carriers ($t = 2.53$, $p = 0.013$). They also performed worse on four of the five subtests that were used to create the baseline memory composite (i.e., CVLT-II trials 1-5, long delay free recall; WMS Logical Memory Immediate and Delayed Recall; see **Chapter 3 Table 1**). Groups (ε4+, ε4-) did not differ significantly on the Memory-CS-Composite, but ε4 carriers demonstrated less decline on one of the

five subtests (CVLT-II trials 1-5; $t = 2.24$, $p = 0.026$, see **Chapter 3 Table 1**). No other significant group differences in cognitive performance were found (all ps > 0.05). No group differences in resting CBF or CT/Vo were found in brain regions of interest (i.e., Hc, EC; all ps > .05; see **Chapter 3 Table 1**).

APOE, CBF, and CT/Vo on memory change

A significant three-way interaction was found in the EC (β= -.59, t= 2.64, p= 0.009; see **Chapter 3 Table 2**), whereby there was a positive relationship between EC CBF and memory change, but only among ε4 carriers with lower baseline EC CT. Similarly, there was a positive relationship between EC CT and memory change, but only among ε4 carriers with lower baseline EC CBF (see **Chapter 3 Figures 1a, 1b, and 1c**). Within the Hc, there was a significant two-way interaction of Hc Vo and APOE on memory change (β= -.51, t= 2.41, p= .017; see **Chapter 3 Table 2**), such that Hc Vo was positively associated with memory change, but only among ε4 carriers. Although not statistically significant (β= -.22, t= 1.08, p= .282), there was also a trend toward the same three-way interaction (including directionality) seen in the EC (see **Chapter 3 Figure A1** in the Appendix).

Post hoc analyses

Aspects of the current longitudinal results differ from our previously reported cross-sectional study from which the current sample was selected (Hays et al., Manuscript Submitted for Publication). Cross-sectionally, we found that *higher* (rather than lower) CBF in the context of lower CT among ε4 carriers was associated with worse baseline memory performance. As such, we thought it important to confirm that the prior cross-sectional findings were replicable in this smaller subset of participants and that differential findings indeed reflect differences in the dependent variable (e.g., baseline memory performance versus longitudinal change in memory performance), rather than sampling error or minor methodological differences (i.e., reduced sample size, averaging across left and right hemispheres). Post hoc analyses of the current sample with baseline memory as the dependent variable largely replicated the prior three-way interaction in the EC (including directionality), such that independent associations between EC CBF and CT on baseline memory performance were dependent on APOE genotype and baseline brain measures (β=.51, t= 2.86, p= 0.005; see **Chapter 3 Table A3** in the Appendix). With regard to ε4 carriers, *higher* CBF and lower CT was associated with worse baseline memory

performance (see **Chapter 3 Figure A2** for a side by side comparison of results with regard to baseline memory versus longitudinal memory change).

Chapter 3 Table 1. Participant demographic, cognitive, and brain characteristics. Note: APOE= Apolipoprotein E; NP= Neuropsychological testing; DRS= Mattis Dementia Rating Scale; WMS= Wechsler Memory Scale; LM= Logical Memory; SS= Scaled score; CVLT= California Verbal Learning Test; SD= Short delay; LD= Long delay; MSR-CS= Mean scaled score regression change score; df= Degrees of freedom; *Denotes significance at p<0.05; **Denotes significance at p< 0.01. Raw scores are presented unless denoted otherwise.

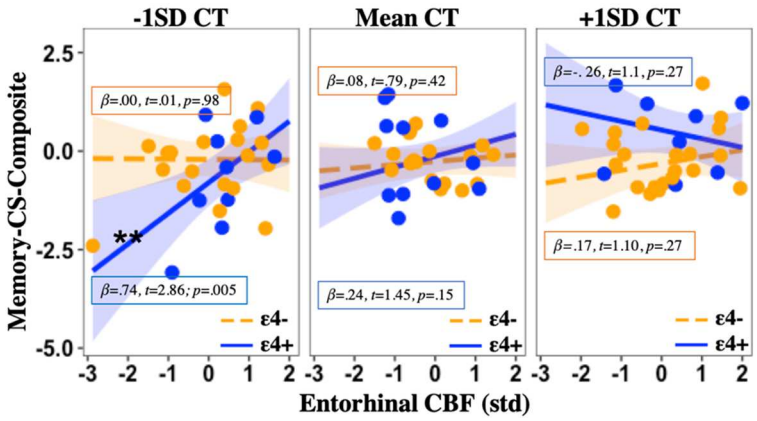
	APOE ε4- (*N*=67)		**APOE ε4+ (*N*=31)**				
Variable	***Mean***	***SD***	***Mean***	***SD***	***t or χ2***	***df***	***p***
Age (years)	72.94	6.36	72.58	5.96	0.27	96	0.786
Sex (male/female)	23/44	--	13/18	--	0.53	1	0.467
Education (years)	16.62	2.27	16.00	2.22	1.28	96	0.203
FSRP Stroke Risk Percent	9.65	6.71	9.83	7.74	0.11	95	0.909
NP and MRI time interval (days)	22.94	84.5	1.58	94.3	1.12	96	0.264
NP Baseline and follow-up interval (years)	2.43	1.12	2.67	1.06	1.00	96	0.319
Baseline DRS Total Score	141	2.76	141	2.85	0.00	96	0.999
Baseline Memory Composite	0.150	0.80	-0.325	0.85	2.66	96	0.009**
Baseline WMS-R LM Immediate Recall	31.55	6.38	28.00	5.62	2.66	96	0.009**
Baseline WMS-R LM Delayed Recall	28.64	7.23	25.00	7.46	2.29	96	0.023*
Baseline CVLT-II List 1–5 total	51.22	9.65	45.38	10.4	2.71	96	0.007**
Baseline CVLT-II SD Free Recall	10.34	3.06	9.35	2.90	1.51	96	0.134
Baseline CVLT-II LD Free Recall	11.40	2.86	10.12	3.10	1.99	96	0.048*
MSR-CS Memory Composite	-0.249	0.75	-0.193	1.21	0.27	96	0.780
MSR-CS WMS-R LM Immediate Recall	-0.128	1.10	-0.390	1.62	0.93	96	0.349
MSR-CS WMS-R LM Delayed Recall	-0.454	1.26	-0.821	1.54	1.24	96	0.216
MSR-CS CVLT-II List 1–5 total	-0.685	0.93	-0.195	1.12	2.24	95	0.026*
MSR-CS CVLT-II SD Free Recall	-0.051	1.07	0.402	1.37	1.77	95	0.078
MSR-CS CVLT-II LD Free Recall	0.026	1.16	0.040	1.60	0.04	95	0.960
Entorhinal CBF	43.32	15.3	43.4	14.7	0.01	85	0.987
Entorhinal CT	3.31	0.32	3.27	0.31	0.60	96	0.549
Hippocampal CBF	47.79	11.9	50.78	9.90	1.16	90	0.248
Hippocampal Vo	3698	451	3653	482	0.44	96	0.656

Chapter 3 Table 2. Effects of CBF, CT/Vo, and APOE, on memory change. Note: Only variables of interest are included; All continuous independent variables were standardized; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; Vo= Volume; β= Standardized regression coefficient; t= t-statistic; p= p=value; *Denotes significance at p<0.025; **Denotes significance at p<0.01

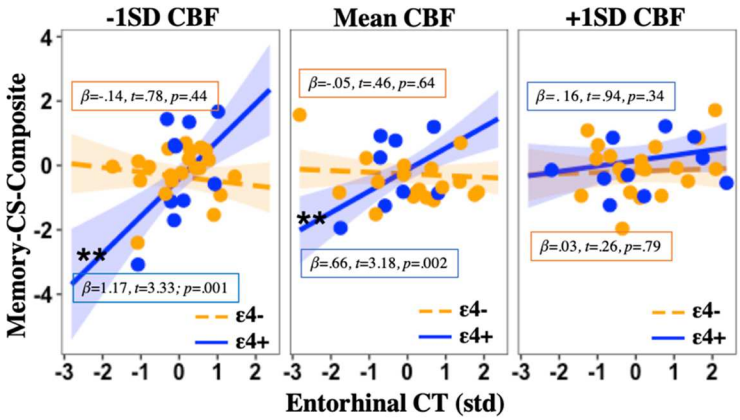
Independent Variable	***β***	***s.e.***	***t***	***p-value***
APOE	0.132	0.206	0.643	0.522
Entorhinal CT	-0.052	0.112	-0.468	0.640
Entorhinal CBF	0.082	0.112	0.734	0.465
APOE*Entorhinal CT	0.720	0.233	3.09	0.002**
APOE*Entorhinal CBF	0.197	0.204	0.967	0.336
Entorhinal CT*Entorhinal CBF	0.090	0.122	0.738	0.462
APOE*Entorhinal CT*Entorhinal CBF	-0.590	0.223	-2.647	0.009**

Chapter 3 Table 2. Effects of CBF, CT/Vo, and APOE, on memory change, Continued.

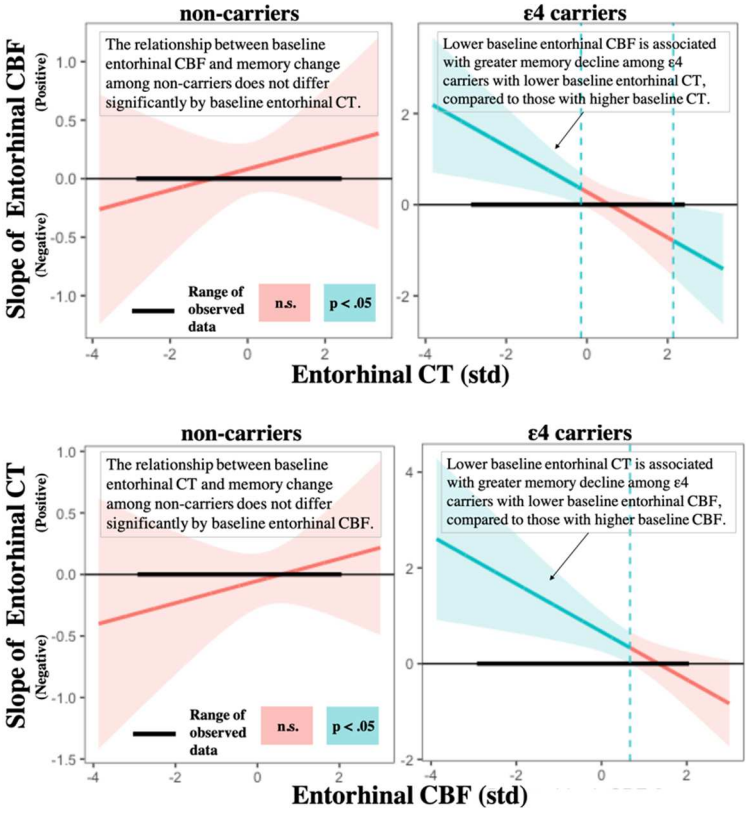
APOE	0.024	0.213	0.114	0.909
Hippocampal Vo	-0.027	0.012	-0.218	0.828
Hippocampal CBF	0.033	0.119	0.279	0.781
APOE*Hippocampal Vo	0.519	0.215	2.417	0.017*
APOE*Hippocampal CBF	0.055	0.228	0.243	0.808
Hc Vo*Hippocampal CBF	-0.001	0.119	-0.014	0.988
APOE*Hippocampal Vo*Hippocampal CBF	-0.222	0.205	-1.082	0.282



Chapter 3 Figure 1a. **Effects of entorhinal CT, CBF, and APOE on memory change.** Three-way interaction plot with simple slopes (mean ±1SD) showing that the relationship between baseline entorhinal CBF and memory change differs significantly by APOE genotype and CT, such that lower baseline entorhinal CBF is associated with greater memory decline, but only among ε4 carriers with lower baseline entorhinal CT, with higher CT appearing relatively protective against future cognitive decline. Note: EC= Entorhinal cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; **Denotes simple slope significance at p<0.01



Chapter 3 Figure 1b. Effects of entorhinal CT, CBF, and APOE on memory change. Three-way interaction plot with simple slopes (mean ±1SD) showing that the relationship between baseline entorhinal CT and memory change differs significantly by APOE genotype and CBF, such that lower baseline entorhinal CT is associated with greater memory decline, but only among ε4 carriers with average or lower baseline entorhinal CBF, with higher CBF appearing protective against future cognitive decline. Note: EC= Entorhinal cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; **Denotes simple slope significance at p<0.01



Chapter 3 Figure 1c. **Effects of entorhinal CT, CBF, and APOE on memory change.** Johnson-Neyman plots showing the intervals (depicted in blue) for which the three-way interaction between EC CBF, CT, APOE, and memory change is statically significant. Lower baseline entorhinal CBF is associated with greater memory decline, but only among ε4 carriers with lower (≤ -.14SD) baseline entorhinal CT. Similarly, lower baseline CT is associated with greater cognitive decline, but only among ε4 carriers with lower (≤ .63SD) baseline entorhinal CBF. These findings suggest that, among ε4 carriers, the combination of *lower* CT and *lower* CBF in the entorhinal cortex is associated with future cognitive decline, whereas having higher levels of at least one of these brain measures (CBF or CT) at baseline is relatively protective against future cognitive decline. Note: EC= Entorhinal cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; **Denotes simple slope significance at p<0.01

DISCUSSION

Results showed that lower baseline CBF is associated with greater memory decline, while higher baseline CBF is associated with greater memory stability, but only for ε4 carriers who show lower baseline CT in this same anatomical region. Similarly, baseline levels of EC CT are positively associated with future memory change, but only for carriers who show lower baseline EC CBF. These findings suggest that ε4 carriers who demonstrate lower CBF and lower CT in the EC at baseline are at elevated risk for future cognitive decline, whereas carriers who show higher levels of at least one of these brain indicators may be relatively protected

against future cognitive decline. The localization of this interaction within the EC is highly suggestive of AD-related neuropathological processes, rather than exacerbation or acceleration of normal aging processes, as the EC is one of the first regions to be affected by AD pathology (Braak & Braak, 1991), but is relatively spared in normal aging (Fjell et al., 2009; Good et al., 2001; Raz et al., 2005; Raz, Rodrigue, & Haacke, 2007). Although not statistically significant, there was also a trend toward this same interaction in the Hc, suggesting that these effects may extend to MTL regions more broadly. To our knowledge, this is the first study to demonstrate an interactive effect of CBF, CT, and APOE genotype on cognitive decline.

The current results extend our prior cross-sectional findings to show that interactions among MTL CBF, CT, and APOE also predict longitudinal changes in memory performance. Notably, the longitudinal results differ from our cross-sectional findings (Hays et al., Manuscript Submitted for Publication) in an important way; the longitudinal results showed that the combination of *lower* CBF and lower CT among APOE ε4 carriers was associated with worse memory function over time (memory decline), whereas the cross-sectional results showed that the combination of *higher* CBF and lower CT was associated with worse memory function at baseline. Of note, post hoc analyses within the current sample replicated these prior cross-sectional findings (see Figure S1 for a comparison of results [baseline memory versus longitudinal change in memory as dependent variable]). Together, these data suggest that higher baseline EC CBF among this group, although not supportive of memory function at baseline, has beneficial effects on memory function over time (e.g., greater memory stability), whereas lower baseline EC CBF has detrimental effects on memory function over time (e.g., greater memory decline). These findings can likely be appreciated in the context of cerebrovascular compensation, whereby ε4 carriers with lower entorhinal CT are engaged in a process of cerebrovascular compensation, with relative increases in CBF reflecting attempts to maintain adequate brain oxygenation in the face of vascular aging and/or neuropathological damage (e.g., *higher* CBF and lower CT being associated with worse baseline memory) and relative reductions in CBF reflecting a breakdown of compensation (e.g., *lower* CBF and lower CT associated with greater memory decline). Within this framework, the current results suggest that different stages of cerebrovascular compensation are indeed detectable in cognitively normal ε4 carriers with lower CT and may hold predictive utility with regard to cognitive trajectories. This notion is largely consistent with the Capillary Dysfunction Hypothesis of Alzheimer's disease (Jespersen & Østergaard, 2012; Ostergaard et al., 2013), which posits that ε4 carriers experience increased heterogeneity of capillary blood flow, which reduces the amount of

oxygen that can diffuse into tissue. This reduction in oxygen diffusion necessitates a compensatory increase in CBF to maintain adequate brain oxygenation. However, progressive increases in this heterogeneity of flow is thought to result in low tissue oxygen tension, a state which paradoxically benefits (due to increased blood-tissue oxygen concentration gradients) from *suppression* of CBF. Although essential for the maintenance of oxygen availability, this compensatory reduction in cerebral perfusion may ultimately lead to oxidative stress, activation of inflammatory pathways, and increased amyloid levels in the brain. In this context, the observed pattern of lower EC CBF and CT among ε4 carriers being associated with future cognitive decline appears to reflect the negative downstream effects of vascular dysfunction, with lower CBF reflecting suppression of perfusion and lower CT reflecting subsequent neurodegeneration due to the damaging effects of this suppression (e.g., oxidative stress, neuroinflammation, amyloid aggregation). Alternatively, lower CT among this group could represent long-standing APOE-related alterations in brain structure that are independent of capillary dysfunction and/or alterations in CBF but may confer additive risk for cognitive decline by way of lower cortical reserve. Future longitudinal studies with serial neuroimaging are needed to test these different hypotheses.

Taken together, the current results suggest that cognitively normal ε4 carriers who demonstrate a combined pattern of lower CBF and lower CT in the EC may be at elevated risk for future memory decline, whereas those who demonstrate higher levels of at least one of these measures may be relatively protected against decline. It should be noted, however, that the average follow-up interval in the current study was only 2.5 years. As such, ε4 carriers showing lower levels of either CBF or CT in the EC may still be at increased risk for cognitive decline beyond several years, relative to those with higher levels of both brain measures. This may be particularly true for ε4 carriers showing a combination of higher CBF and lower CT in the entorhinal cortex, as this pattern suggests the presence of cerebrovascular compensatory mechanisms that, although appearing supportive of memory function over several years, are likely to breakdown over time (e.g., increased CBF followed later by suppressed CBF). Interestingly, this suggests that there are distinct multimodal neural signatures in the entorhinal cortex that may be sensitive to relative risk for future cognitive decline among cognitively normal ε4 carriers (e.g., high risk= lower CBF and lower CT; moderate risk= higher CBF and lower CT; low risk= higher CBF and higher CT), reflecting different stages of cerebrovascular compensation. Another question that remains unanswered is whether the interaction of longitudinal *changes* in MTL CBF and CT might provide even earlier and more reliable detection of risk for cognitive decline among ε4 carriers, as longitudinal

changes in perfusion may capture more accurate information about where ε4 carries lie on this trajectory of compensation (e.g., longitudinal increases reflecting early compensation, longitudinal decreases reflecting breakdown of compensation) when compared to cross-sectional measures.

Strengths and limitations

Limitations of the current study include the use of a cross-sectional brain measures (CBF, CT/Vo) and an average cognitive follow-up interval of only 2.5 years, which restricted our ability to draw causal conclusions and limited our ability to determine whether the effects of CBF and CT on cognition among ε4 carriers represents normal aging or pathologic processes. Our sample was also characterized by relatively high levels of education, and although this demographic factor was not associated with APOE genotype, its limited range may reduce the generalizability of these findings. It is also important to note that we did not observe a significant decline in the MSR-CS Memory Composite in either group, nor did groups differ significantly on this measure. Although somewhat unexpected, these finding may suggest that a longer follow-up window is necessary to detect widespread decline in the current sample, which was highly educated and relatively healthy (no major vascular events, no brain injuries, no uncontrolled hypertension, no major mental health diagnoses, cognitively normal at baseline). Although groups did not differ significantly in the average time interval between cognitive testing and MRI scanning, decreasing the time interval between cognitive testing and MRI may improve accuracy of brain-behavior associations. Moreover, our sample of 98 included only 31 APOE ε4 carriers, which may have limited our ability to detect a statistically significant three-way interaction in the Hc. Future investigations should include longitudinal designs with larger, more diverse samples to replicate and extend the current finding. Future studies should also investigate whether interactive effects of CBF and CT/Vo on cognition extend to regions outside the MTL. Lastly, investigation of interactions between longitudinal *changes* in CBF and CT on cognitive decline, and the inclusion of additional markers of AD, such as CSF biomarkers may help better characterize APOE ε4 carriers.

Strengths of the current study include the use of non-invasive ASL MRI to measure partial volume corrected CBF, and the use of a high-resolution structural MRI scan to examine regional CT and Vo. Furthermore, use of FreeSurfer offers advantages over traditional voxel-based morphometry methods, as it also allows for examination of the components of volume separately (thickness and surface area), providing a direct

index of cortical morphology that is less susceptible to variations in individual positioning (Kim et al., 2005) and allowing for sub-voxel precision with thickness values being assigned to individual vertices rather than voxels (B. Fischl & Dale, 2000). Moreover, the extraction of CBF from FreeSurfer-derived brain regions allowed us to directly investigate CBF (and brain structure) in regions that are defined by each individual's anatomy, rather than atlas-defined regions which are less sensitive to individual anatomical differences because they require that data are first aligned and warped to a generic anatomic template. Another strength includes the use of longitudinal cognitive data and standardized regression-based change scores to account for practice effect and regression toward the mean. Lastly, the current study benefited from the inclusion of a well-controlled and well-characterized sample of cognitive normal older adults, which included the use of several cognitive test performances to characterize cognitive status.

Conclusions

The current findings indicate that older adult APOE ε4 carriers experience vascular dysregulation and concomitant morphological alteration in the MTL that interact with one another to negatively affect cognition prior to the onset of overt clinical symptoms, providing insight into the mechanistic link between APOE ε4 and cognitive decline. Results also suggest the presence of distinct multimodal neural signatures in the entorhinal cortex that may signal relative risk for cognitive decline among this group (e.g., high risk= lower CBF and lower CT; moderate risk= higher CBF and lower CT; low risk= higher CBF and higher CT), likely reflecting different stages of cerebrovascular compensation. Such early detection could inform candidate selection and study design for future clinical trials. On a broader scale, the current results add to accumulating evidence supporting the early role of vascular dysregulation in AD (de la Torre, 2010; Iadecola, 2004; Iturria-Medina et al., 2016; Zlokovic, 2011) and could lead to the identification of vasoprotective treatments with the potential to delay or prevent the onset of age-related cognitive decline and/or AD.

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DISCUSSION OF THE DISSERTATION

Our cross-sectional investigation of SCD, CBF, and memory (Study 1) is the first, to our knowledge, to show that SCD modifies the association between voxel-wise CBF and memory function among cognitively normal older adults. More specifically, results showed that higher CBF was associated with worse memory function in those with SCD but with better memory function in those without SCD. These findings suggest that the normal beneficial effects of higher CBF on cognition may be disrupted among those with SCD, as higher CBF no longer appears to support better cognitive functioning in this group within regions associated with normal aging and AD-risk (Frederiksen, 2013; Grasby et al., 1993; Hays et al., 2016; Wierenga et al., 2014a). The modifying role of SCD on the relationship between CBF and cognition can likely be explained by neurovascular dysregulation among those with SCD, reflecting attempts to compensate for age-related vascular or neuropathological changes. We found no differences in APOE status or family history of dementia between those with and without SCD in our sample, indicating that SCD might represent an independent risk factor for cognitive decline. The fact that group differences in the relationship between CBF and cognition were found despite normal cognitive function lends further support to the notion that SCD+ is distinct from normal aging (i.e., SCD-). Overall, results from this study suggest that vascular dysregulation may be occurring in those with SCD, even in the absence of clinical symptoms, further supporting its role as a preclinical marker of risk for cognitive decline.

Our cross-sectional study of APOE, CBF, CT/Vo, and memory (Study 2) appears to be the first to demonstrate that APOE genotype modifies the interactive effects of MTL CBF and CT on cognitive performance among a sample of cognitively normal older adults, such that the relationship between entorhinal CBF and memory performance was negative (*lower* CBF was associated with better memory) in non-carriers with higher entorhinal CT, positive (*higher* CBF was associated with better memory) in non-carriers with lower entorhinal CT, and negative (higher CBF was associated with *worse* memory) in APOE ε4 carriers with lower entorhinal CT. These findings suggest that older adult APOE ε4 carriers experience vascular dysregulation and concomitant morphological changes in the medial temporal lobe that interact to negatively affect memory function even in the absence of overt clinical symptoms, providing critical insight into the mechanistic link between APOE ε4 and detriments in cognition. Moreover, findings suggest a distinct multimodal neural signature in APOE ε4-carriers

(*higher* CBF and *lower* CT in the entorhinal cortex) that could aid in the identification of candidates for future clinical trials aimed at preventing or slowing cognitive decline.

Our longitudinal investigation of APOE, baseline CBF, baseline CT/Vo, and memory *change* (Study 3) is the first, to our knowledge, to show that APOE genotype modifies the interactive effects of MTL CBF and CT on cognitive performance among a sample of cognitively normal older adults, whereby there was a positive relationship between entorhinal CBF and memory change (*lower* CBF was associated with greater memory decline, *higher* CBF associated with greater memory stability), but only among APOE ε4 carriers who also showed lower entorhinal CT. These relationships were not evident in non-carriers. This suggests that APOE ε4 carriers who demonstrate a combination of lower CBF and lower CT in the EC at baseline are at elevated risk for future cognitive decline, whereas carriers who show higher levels of at least one of these brain indicators at baseline may be relatively protected against future cognitive decline. These results extend our cross-sectional findings to show that interactions among MTL CBF, CT, and APOE also predict longitudinal *changes* in memory performance.

Notably, the longitudinal results differ from our cross-sectional findings in an important way; the longitudinal results showed that the combination of *lower* CBF and lower CT among APOE ε4 carriers was associated with worse memory function over time (memory decline), whereas the cross-sectional results showed that the combination of *higher* CBF and lower CT was associated with worse memory function at baseline. These differences can likely be appreciated in the context of cerebrovascular compensation, whereby APOE ε4 carriers with lower entorhinal CT are engaged in a process of cerebrovascular compensation, with relative increases in CBF reflecting attempts to maintain adequate brain oxygenation in the face of vascular aging and/or neuropathological damage (e.g., *higher* CBF and lower CT being associated with worse baseline memory) and relative reductions in CBF reflecting a breakdown of compensation (e.g., *lower* CBF and lower CT associated with greater memory decline). This notion is largely consistent with the Capillary Dysfunction Hypothesis of Alzheimer's disease, which posits that APOE ε4 carriers experience increasing heterogeneity of capillary blood flow that leads to early compensatory CBF increases, followed later by reductions in response to increased heterogeneity of capillary blood flow, which eventually leads to oxidative stress, activation of inflammatory pathways, and neurodegeneration (Ostergaard et al., 2013). Within this theoretical framework, the current results

suggest that different stages of cerebrovascular compensation are indeed detectable in cognitively normal APOE ε4 carriers with lower CT and may hold predictive utility with regard to cognitive trajectories.

Strengths and limitations

Limitations of the current studies include the use of a cross-sectional brain measures (CBF, CT/Vo) and an average cognitive follow-up interval of only 2.5 years, all of which restricted our ability to draw causal conclusions and limited our ability to determine whether the effects of CBF and CT on cognition among SCD and/or APOE ε4 carriers represents normal aging or pathologic processes. It is also important to note that all three samples had relatively high levels of education, and although this demographic factor was not associated with SCD status or APOE genotype, its limited range may reduce the generalizability of these findings. Our study of SCD (Study 1), was also limited by the challenge faced by all studies of SCD, namely that of accurate assessment of subjective cognitive decline. It is also important to note that while our two studies of APOE (Studies 2 and 3) had relatively large sample sizes overall, they included relatively low numbers of APOE ε4 carriers (n= 41, n= 31, respectively), which may have limited our ability to detect a statistically significant three-way interactions in other MTL regions (e.g., hippocampus). Future investigations should include longitudinal designs with larger, more diverse samples to replicate and extend the current findings. Furthermore, investigation of interactions between longitudinal *changes* in CBF and CT on cognitive decline, and the inclusion of additional markers of AD, such as CSF biomarkers may help better characterize SCD and APOE ε4 carriers.

Strengths of the current studies include the use of non-invasive ASL MRI to measure partial volume corrected CBF, and the use of a high-resolution structural MRI scan to examine CT and Vo. Furthermore, use of FreeSurfer offers advantages over traditional voxel-based morphometry methods, as it also allows for examination of the components of volume separately (thickness and surface area), providing a direct index of cortical morphology that is less susceptible to variations in individual positioning (Kim et al., 2005) and allowing for sub-voxel precision with thickness values being assigned to individual vertices rather than voxels (B. Fischl & Dale, 2000). Moreover, the extraction of CBF from FreeSurfer-derived brain regions in our studies of APOE (studies 2 and 3) allowed us to directly investigate CBF (and brain structure) in regions that are defined by each individual's anatomy, rather than atlas-defined regions which are less sensitive to individual anatomical differences because they require that data are first aligned and warped to a generic anatomic template. Another

strength includes the use of longitudinal cognitive data and standardized regression-based change scores to account for practice effect and regression toward the mean. Lastly, the current studies all benefited from the inclusion of well-controlled and well-characterized samples of cognitive normal older adults, which included the use of several cognitive test performances to characterize cognitive status.

Conclusions

SCD findings indicate that older adults whom report SCD experience vascular dysregulation prior to the onset of overt clinical symptoms, suggesting that SCD may confer risk for cognitive change through alterations in CBF, further supporting its role as a preclinical marker of risk for future cognitive decline. APOE findings indicate that older adult APOE ε4 carriers experience vascular dysregulation and concomitant morphological alteration in the MTL that interact with one another to negatively affect cognition prior to the onset of overt clinical symptoms, providing critical insight into the mechanistic link between APOE ε4 and cognitive decline. Results also suggest the presence of distinct multimodal neural signatures in the entorhinal cortex that may signal relative risk for cognitive decline among this group (e.g., high risk= lower CBF and lower CT; moderate risk= higher CBF and lower CT; low risk= higher CBF and higher CT), likely reflecting different stages of cerebrovascular compensation. Such early detection, whether in SCD or APOE ε4, could inform candidate selection and study design for future clinical trials. On a broader scale, results from the current studies add to accumulating evidence supporting the early role of vascular dysregulation in AD (de la Torre, 2010; Iadecola, 2004; Iturria-Medina et al., 2016; Zlokovic, 2011) and could lead to the identification of vasoprotective treatments with the potential to delay or prevent the onset of age-related cognitive decline and/or AD.

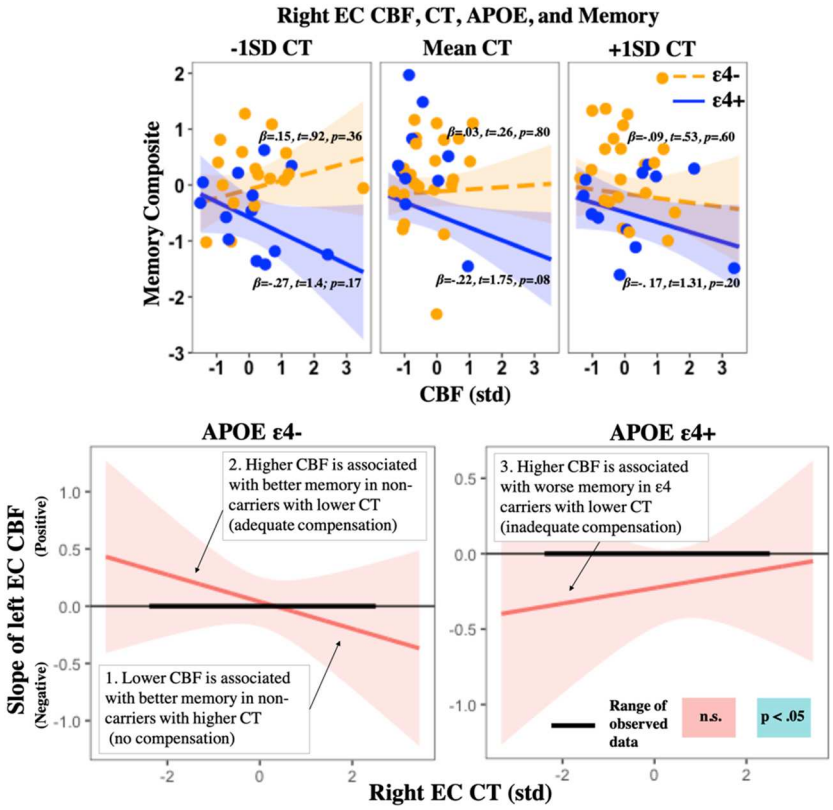
APPENDIX

Chapter 2 Table A1. Cognitive tests, domains, and normative data. Note: DRS= Mattis Dementia Rating Scale; WMS-R= Wechsler Memory Scale Revised; CVLT= California Verbal Learning Test; WCST= Wisconsin Card Sorting Test; DKEFS= Delis-Kaplan Executive Function System; CW= Color-Word; WAIS-R= Wechsler Adult Intelligence Scale Revised; MINT= Multilingual Naming Test

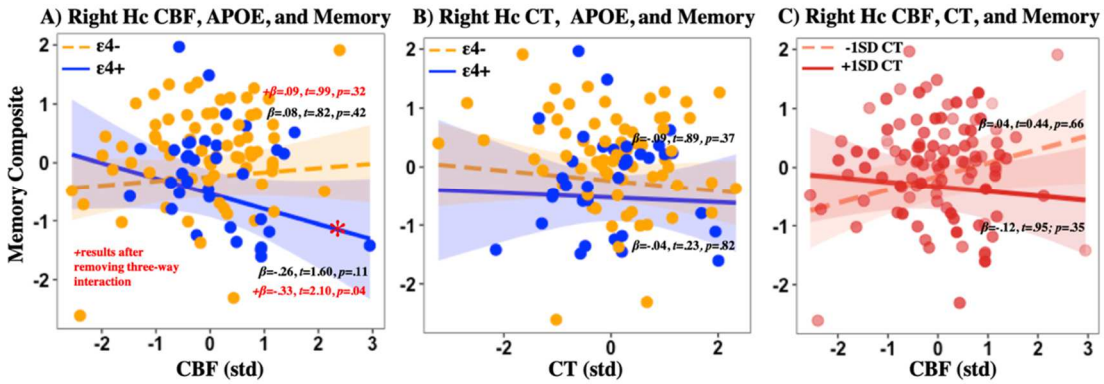
Test	*Cognitive Domain*	*Normative Data*
DRS	Global Cognition	(Mattis, 1988)
WMS-R Logical Memory	Memory	(Ivnik et al., 1992)
WMS-R Visual Reproduction Recall	Memory	(Ivnik et al., 1992)
CVLT-II	Memory	(Delis, Kramer, & Ober, 2000)
WCST-48 card version	Executive Functioning	(Lineweaver, Bond, Thomas, & Salmon, 1999)
DKEFS Trail Making	Executive Functioning	(Delis, Kaplan, & Kramer, 2001)
DKEFS CW Interference	Executive Functioning	(Delis et al., 2001)
DKEFS Letter Fluency	Language	(Delis et al., 2001)
DKEFS Category Fluency	Language	(Delis et al., 2001)
Boston Naming Test	Language	(Heaton & Psychological Assessment Resources, 2004)
MiNT	Language	(Ivanova, Salmon, & Gollan, 2013)
DKEFS Trails Visual Scanning	Visuospatial Functioning	(Delis et al., 2001)
WMS-R Visual Reproduction Copy	Visuospatial Functioning	(Ivnik et al., 1992)
Clock Drawing Test	Visuospatial Functioning	N/A
DKEFS CW Interference Word Reading	Attention/Processing Speed	(Delis et al., 2001)
WAIS-R Digit Symbol	Attention/Processing Speed	(Ivnik et al., 1992)
WAIS-R Digit Span	Attention/Processing Speed	(Ivnik et al., 1992)

Chapter 2 Table A2. cACC CBF, CT, and APOE on memory. Note: Only variables of interest are included in table; All continuous independent variables were standardized; cACC= Caudal anterior cingulate cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; L= Left; R= Right; β= Standardized regression coefficient; CI= confidence interval; bs= Bootstrapped with 5000 replications; t= t-statistic; p= p=value. The 95% confidence interval of the coefficient was derived using the bootstrap bias-adjusted and accelerated bootstrap interval; *Denotes significance at $p<0.05$

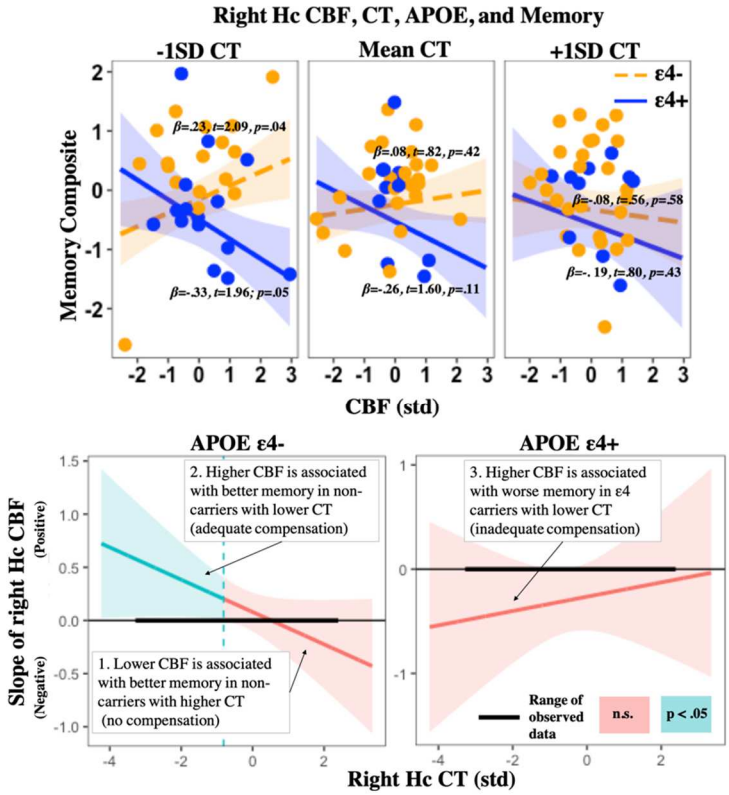
Independent Variable	*β*	*95% CI (bs)*	*s.e. (bs)*	*t*	*p*
APOE	0.371	(-0.762, 0.016)	0.201	2.136	0.035*
R cACC CT	-0.236	(-0.168, 0.393)	0.141	2.039	0.044*
R cACC CBF	-0.088	(-0.211, 0.292)	0.126	0.461	0.646
APOE* R cACC CT	0.369	(-0.739, -0.019)	0.184	3.655	0.031*
APOE* R cACC CBF	0.118	(-0.558, 0.299)	0.220	2.312	0.586
R cACC CT* R cACC CBF	-0.062	(-0.560, 0.103)	0.166	1.443	0.599
APOE* R cACC CT* R cACC CBF	0.041	(-0.468, 0.338)	0.207	2.178	0.804
APOE	-0.305	(-0.786, 0.002)	0.193	1.745	0.084
L cACC CT	-0.089	(-0.294, 0.101)	0.098	0.884	0.378
Right EC CBF	-0.003	(-0.235, 0.218)	0.114	0.032	0.974
APOE* L cACC CT	-0.017	(-0.330, 0.345)	0.175	0.110	0.912
APOE* L cACC CBF	-0.149	(-0.668, 0.230)	0.220	0.713	0.477
L cACC CT* L cACC CBF	-0.112	(-0.322, 0.086)	0.104	1.153	0.251
APOE* L cACC CT* L cACC CBF	0.130	(-0.288, 0.612)	0.237	0.647	0.519



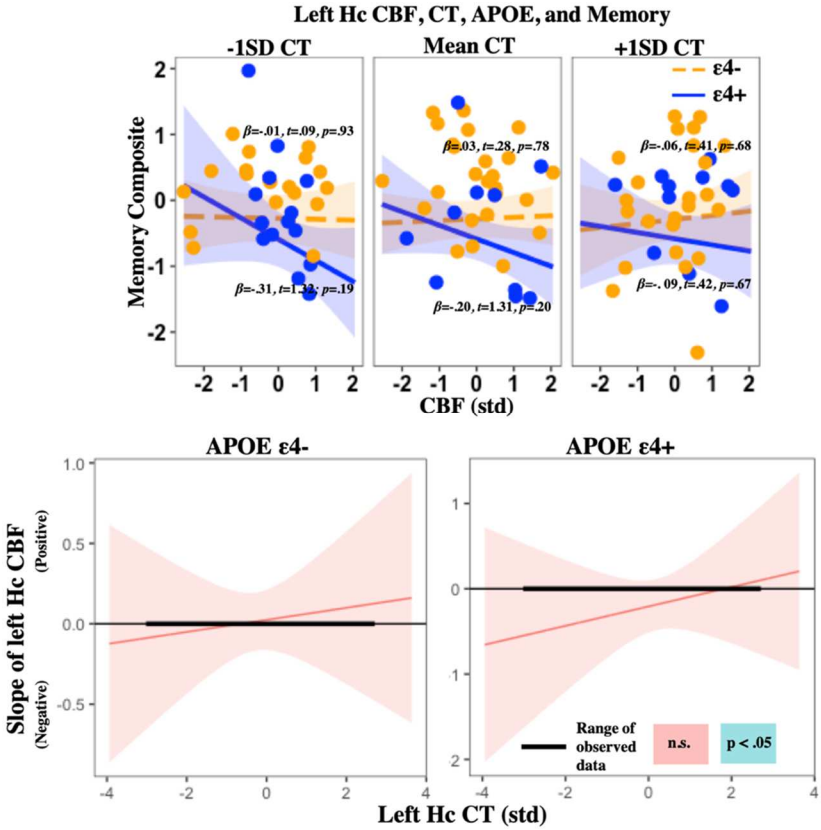
Chapter 2 Figure A1. Right EC CBF, CT, and APOE on memory. Three-way interaction and Johnson-Neyman plots showing that although *not* statistically significant: **1.** Lower right EC CBF is trending toward better memory in non-carriers with higher right EC CT, perhaps reflecting neural efficiency and no current need for compensation; **2.** Higher right EC CBF is trending toward better memory in non-carriers with lower right EC CT, perhaps reflecting compensatory increases in CBF that are fully supportive of current memory function; **3.** Higher right EC CBF is trending toward worse memory performance in ε4 carriers with lower right EC CT, perhaps reflecting maximally invoked compensatory increases in CBF that are not fully supportive of current memory function. Note: EC= Entorhinal cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; *Denotes simple slope significance at p<0.05.



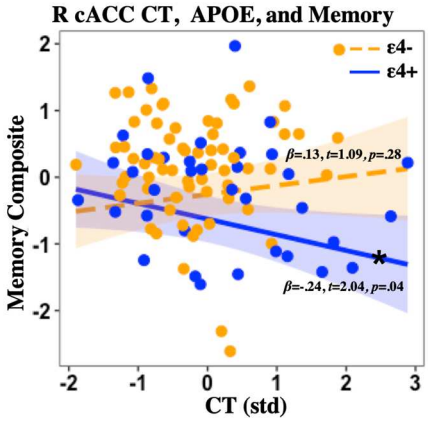
Chapter 2 Figure A2a. Right EC CBF, CT, and APOE on memory. Two-way interaction plots in the right Hc: A) Lower right Hc CBF is associated with worse memory in ε4 carriers but not non-carriers, B) The relationship between right Hc CT and memory performance does not differ between ε4 carriers and non-carriers, C) The relationship between right Hc CBF and memory performance does not differ by high and low CT.



Chapter 2 Figure A2b. Right EC CBF, CT, and APOE on memory. Three-way Interaction and Johnson-Neyman plots showing that although *not* statistically significant: **1.** Lower right Hc CBF is trending toward better memory in non-carriers with higher right Hc Vo, perhaps reflecting neural efficiency and no current need for compensation; **2.** Higher right Hc CBF is associated with better memory in non-carriers with lower right Hc Vo (≤ -.81SD), perhaps reflecting compensatory increases in CBF that are supportive of memory function; **3.** Higher right Hc CBF is trending toward worse memory performance in ε4 carriers with lower right Hc Vo, perhaps reflecting maximally invoked compensatory increases in CBF that are not fully supportive of current memory function.



Chapter 2 Figure A3. Left Hc CBF, Vo, and APOE on memory. Three-way interaction and Johnson-Neyman plots demonstrating that there is *not* a significant three-way interaction of left Hc CBF, CT, APOE, and memory performance. More specifically, the relationship between left Hc CBF, CT, and memory performance does not differ by APOE genotype. Note: Hc= Hippocampus; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; Vo= Volume; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; *Denotes simple slope significance at p<0.05.



Chapter 2 Figure A4. cACC CBF and APOE on memory. Two-way interaction plot showing a significant interactive effect of cACC CT and APOE on memory performance, such that higher CT in the right cACC is associated with *worse* memory performance in ε4 carriers but not in non-carriers. Note: cACC= Caudal anterior cingulate cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; std= Z-score standardization; p= p-value; *Denotes simple slope significance at p<0.05.

Chapter 3 Table A1. Cognitive tests, domains, and normative data. Note: DRS= Mattis Dementia Rating Scale; WMS-R= Wechsler Memory Scale Revised; CVLT= California Verbal Learning Test; WCST= Wisconsin Card Sorting Test; DKEFS= Delis-Kaplan Executive Function System; CW= Color-Word; WAIS-R= Wechsler Adult Intelligence Scale Revised; MINT= Multilingual Naming Test

Test	*Cognitive Domain*	*Normative Data*
DRS	Global Cognition	(Mattis, 1988)
WMS-R Logical Memory	Memory	(Ivnik et al., 1992)
WMS-R Visual Reproduction Recall	Memory	(Ivnik et al., 1992)
CVLT-II	Memory	(Delis, Kramer, & Ober, 2000)
WCST-48 card version	Executive Functioning	(Lineweaver, Bond, Thomas, & Salmon, 1999)
DKEFS Trail Making	Executive Functioning	(Delis, Kaplan, & Kramer, 2001)
DKEFS CW Interference	Executive Functioning	(Delis et al., 2001)
DKEFS Letter Fluency	Language	(Delis et al., 2001)
DKEFS Category Fluency	Language	(Delis et al., 2001)
Boston Naming Test	Language	(Heaton & Psychological Assessment Resources, 2004)
MiNT	Language	(Ivanova, Salmon, & Gollan, 2013)
DKEFS Trails Visual Scanning	Visuospatial Functioning	(Delis et al., 2001)
WMS-R Visual Reproduction Copy	Visuospatial Functioning	(Ivnik et al., 1992)
Clock Drawing Test	Visuospatial Functioning	N/A
DKEFS CW Interference Word Reading	Attention/Processing Speed	(Delis et al., 2001)
WAIS-R Digit Symbol	Attention/Processing Speed	(Ivnik et al., 1992)
WAIS-R Digit Span	Attention/Processing Speed	(Ivnik et al., 1992)

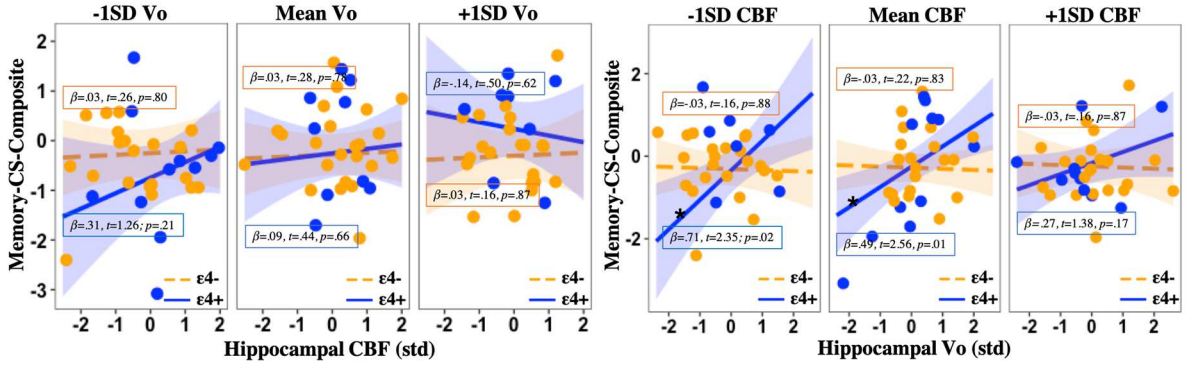
Chapter 3 Table A2. Regression equations for predicting follow-up scores on CVLT-II and WMS-R Logical Memory variables. Note: CVLT-II = California Verbal Learning Test- second edition; WMS-R= Wechsler Memory Scale- Revised; SEE = standard error of the estimate; a= Constant; b = Unstandardized *b* weight for time 1 index score; c=Unstandardized *b* weight for age; d= Unstandardized *b* weight for education; e= Unstandardized *b* weight for gender; f= Unstandardized *b* weight for retest interval; Age is in years; Education is in years; Gender is coded as 0 for female and 1 for male; Retest interval is in years

CVLT-II variable	R^2	*SEE*	*a*	*b*	*c*	*d*	*e*	*f*
Total 1-5	.39	8.55	10.04	.73	-.02	.27	.88	1.14
Short delay free recall	.42	2.45	8.18	.63	-.07	.14	-.38	-.17
Long delay free recall	.42	2.19	4.40	.54	-.03	.25	.35	-.38

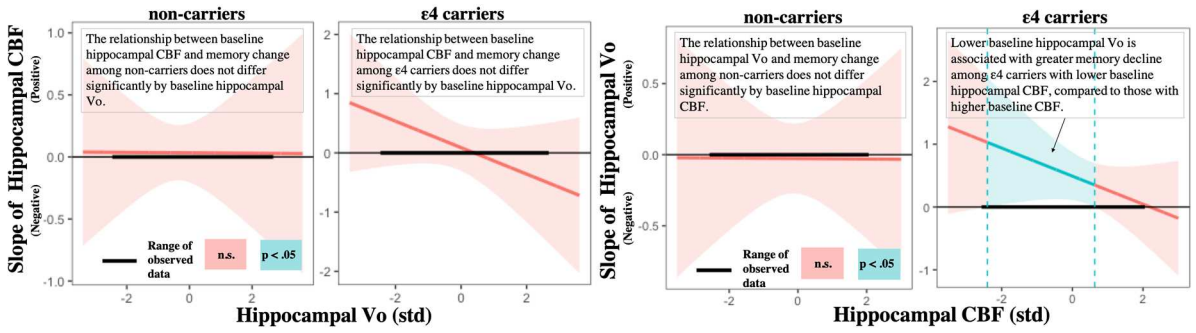
WMS-R Logical Memory variable	R^2	*SEE*	*a*	*b*	*c*	*d*	*e*	*f*
Logical Memory I	.40	4.53	10.68	.48	-.03	.49	-.91	1.11
Logical Memory II	.41	4.56	11.59	.49	-.09	.46	-.33	1.04

Chapter 3 Table A3. Post hoc analysis of EC CBF, CT/Vo, and APOE, on baseline memory. Note: Only variables of interest are included in table; All continuous independent variables were standardized; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; Vo= Volume; β= Standardized regression coefficient; t= t-statistic; p= p=value; +Denotes marginal significance at p<0.10; *Denotes significance at p<0.05; **Denotes significance at p<0.01

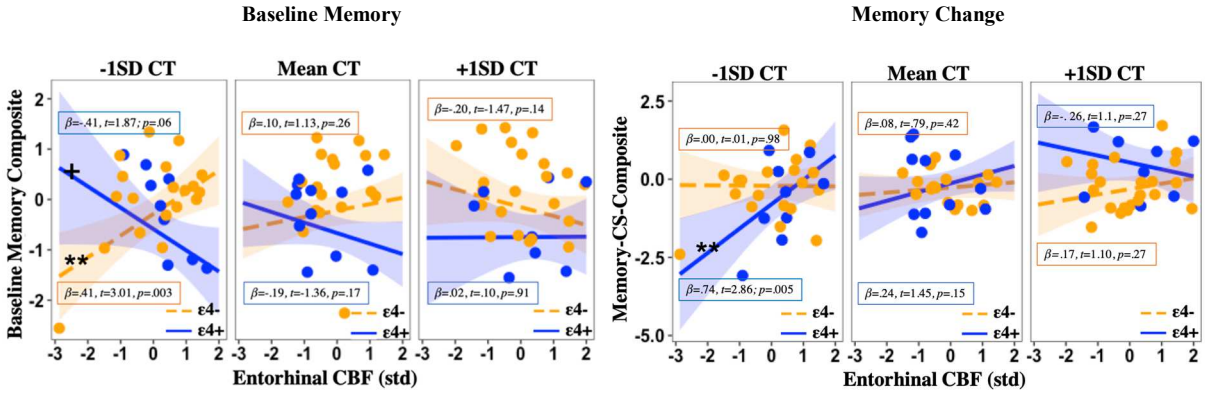
Independent Variable	*β*	*s.e.*	*t*	*p-value*
APOE	-0.448	0.168	-2.666	0.009**
Entorhinal CT	0.067	0.095	0.708	0.481
Entorhinal CBF	0.128	0.094	1.352	0.180
APOE*Entorhinal CT	-0.147	0.198	-0.743	0.460
APOE*Entorhinal CBF	-0.337	0.170	-1.983	0.050+
Entorhinal CT*Entorhinal CBF	-0.304	0.098	-3.101	0.002**
APOE*Entorhinal CT*Entorhinal CBF	0.518	0.181	2.864	0.005**



Chapter 3 Figure A1a. Effects of hippocampal Vo, CBF, and APOE, on memory change. Three-way interaction plot with simple slopes (mean ±1SD) showing a trend toward a relationship between baseline hippocampal CBF and memory change that differs significantly by APOE genotype and Vo, such that lower baseline hippocampal CBF is associated with greater memory decline, but only among ε4 carriers with lower baseline hippocampal Vo, with higher Vo appearing relatively protective against future cognitive decline. Similarly, the relationship between baseline hippocampal Vo and memory change differs significantly by APOE genotype and shows a trend toward differing by CBF, such that lower baseline hippocampal Vo is associated with greater memory decline, but only among ε4 carriers with average or lower baseline hippocampal CBF, with higher CBF appearing relatively protective against future cognitive decline. Note: Hc= Hippocampus; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; Vo=volume; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; *Denotes simple slope significance at p<0.01



Chapter 3 Figure A1b. Effects of hippocampal Vo, CBF, and APOE, on memory change. Johnson-Neyman plots showing the intervals (depicted in blue) for which the three-way interaction between Hc CBF, CT, APOE, and memory change is statically significant. Lower baseline hippocampal Vo is associated with greater memory decline, but only among ε4 carriers, and there is a trend toward this relationship being stronger among ε4 carriers with lower (≤ .63SD) baseline hippocampal CBF. Similarly, although not statistically significant, there is a trend toward lower baseline hippocampal CBF being associated with greater memory decline, but only among ε4 carriers with lower (≤ -.14SD) baseline hippocampal Vo These findings suggest that, among ε4 carriers, the combination of *lower* CBF and *lower* CT in the entorhinal cortex is associated with future cognitive decline, whereas having higher levels of at least one of these brain measures (CBF or Vo) at baseline may be relatively protective against future cognitive decline.



Chapter 3 Figure A2. Differential effects of CBF, CT, and APOE on baseline memory and memory change. Three-way interaction plots with simple slopes (mean ±1SD) showing that the combination of *higher* CBF and lower CT among ε4 carriers is associated with worse memory performance at baseline, but with greater memory stability over time. In contrast, the combination of *lower* CBF and lower CT at baseline among ε4 carriers is associated with greater memory decline over time. These differential findings suggest that compensatory increases in CBF at baseline among ε4 carriers with lower cortical reserve (although associated with worse baseline memory) are supportive of memory function over time, whereas relative reductions in CBF among this same group are associated with memory decline, likely reflecting an absence or breakdown of cerebrovascular compensatory mechanisms. Note: EC= Entorhinal cortex; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CT= Cortical thickness; std= Z-score standardization; SD= standard deviation; t= t-statistic; p= p-value; n.s.= not significant; +Denotes marginal simple slope significance at p<0.10; **Denotes simple slope significance at p<0.01

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