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Authors

Engstrom, Allison
Alitin, John
Kapoor, Arunima
et al.

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RESEARCH ARTICLE

Spontaneous cerebrovascular reactivity at rest in older adults with and without mild cognitive impairment and memory deficits

Allison C. Engstrom[1] | **John Paul M. Alitin**[2] | **Arunima Kapoor**[1] | **Shubir Dutt**[3] | **Trevor Lohman**[2] | **Isabel J. Sible**[4] | **Anisa J. Marshall**[4] | **Fatemah Shenasa**[1] | **Aimée Gaubert**[2] | **Farrah Ferrer**[2] | **Amy Nguyen**[2] | **David Robert Bradford**[5] | **Kathleen Rodgers**[5] | **Lorena Sordo**[6] | **Elizabeth Head**[6] | **Xingfeng Shao**[7] | **Danny J. J. Wang**[7] | **Daniel A. Nation**[2,8]

[1]Department of Psychological Science, University of California, Irvine, Irvine, California, USA

[2]Leonard Davis School of Gerontology, University of Southern California, Los Angeles, California, USA

[3]Memory and Aging Center, Weill Institute for Neurosciences, Department of Neurology, University of California, San Francisco, California, USA

[4]Department of Psychology, University of Southern California, Los Angeles, California, USA

[5]Center for Innovations in Brain Science, Department of Pharmacology, University of Arizona, Tucson, Arizona, USA

[6]Department of Pathology and Laboratory Medicine, University of California, Irvine, Irvine, California, USA

[7]Stevens Neuroimaging and Informatics Institute, University of Southern California, Los Angeles, California, USA

[8]Keck School of Medicine, University of Southern California, Los Angeles, California, USA

Correspondence
Daniel A. Nation, Leonard Davis School of Gerontology, University of Southern California, 3715 McClintock Ave, Los Angeles, CA 90089, USA.
Email: danation@usc.edu

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Abstract

INTRODUCTION: Older adults with mild cognitive impairment (MCI) exhibit deficits in cerebrovascular reactivity (CVR), suggesting CVR is a biomarker for vascular contributions to MCI. This study examined if spontaneous CVR is associated with MCI and memory impairment.

METHODS: One hundred sixty-one older adults free of dementia or major neurological/psychiatric disorders were recruited. Participants underwent clinical interviews, cognitive testing, venipuncture for Alzheimer's disease (AD) biomarkers, and brain magnetic resonance imaging. Spontaneous CVR was quantified during 5 minutes of rest. Respiratory gases analyzed through nasal cannula to quantify end-tidal carbon dioxide ($_{ET}CO_2$) levels were used to estimate CVR.

RESULTS: Whole brain CVR was negatively associated with age, but not MCI. Lower CVR in the parahippocampal gyrus (PHG) was found in participants with MCI and was linked to worse memory performance on memory tests. Results remained significant after adjusting for AD biomarkers and vascular risk factors.

DISCUSSION: Spontaneous CVR deficits in the PHG are observed in older adults with MCI and memory impairment, suggesting medial temporal microvascular dysfunction is observed in cognitive decline.

KEYWORDS
amnestic mild cognitive impairment, memory impairment, mild cognitive impairment, spontaneous cerebrovascular reactivity

Highlights

- Aging is associated with decline in whole brain spontaneous cerebrovascular reactivity (CVR).
- Older adults with mild cognitive impairment exhibit deficits in spontaneous CVR in the parahippocampal gyrus (PHG).
- Memory impairment is correlated with reduced spontaneous CVR in the PHG.

1 | BACKGROUND

It is increasingly recognized that vascular contributions to cognitive impairment and dementia (VCID) can be substantial and are often present both in those with and without concomitant neurodegenerative changes such as Alzheimer's disease (AD).[1–5] Continued efforts to develop and investigate structural imaging markers of large and small vessel diseases relevant to VCID have yielded new insights and tools for earlier detection.[6,7] Deficits in cerebral blood flow (CBF), blood–brain barrier (BBB) permeability, and cerebrovascular reactivity (CVR) have also been reported in older adults,[8–10] and patients with mild cognitive impairment (MCI) exhibit further CBF deficits that include the posterior cingulate and precuneus,[11,12] increased BBB permeability in the parahippocampal gyrus (PHG) and hippocampus,[10,13–15] and deficits in whole brain CVR to hypercapnia.[16] However, prior research on the relationship between CVR and MCI is mixed, with some studies suggesting CVR deficits are associated with MCI,[8–10] while others have reported no differences in CVR in patients with MCI compared to healthy controls,[17] or reporting deficits in CVR in AD but not MCI.[18]

The study of CVR is of particular interest as a potential marker of VCID given it can be reliably studied non-invasively using magnetic resonance imaging (MRI) and is specific to cerebrovascular function.[19,20] CVR responses can be studied as a measure of cerebrovascular function by inducing hypercapnia through gas inhalation or controlled breathing while simultaneously monitoring end-tidal CO_2 ($_{ET}CO_2$), and is commonly quantified as a percent change in CBF per unit change in $_{ET}CO_2$.[8,19] In contrast to changes in resting CBF that may reflect either changes in cerebrovascular function or neuronal metabolism or both, CVR responses to blood and cerebrospinal fluid (CSF) CO_2 levels are thought to be specific to cerebrovascular function.[19] Even relatively small changes of just a few mmHg in $_{ET}CO_2$ can lead to measurable increases in CBF,[19] for instance breath holds as short as 3 to 6 seconds can lead to increases in CBF.[21] As such, the study of vasodilatory CVR responses is of particular interest as an imaging marker of VCID that may capture early changes in microvascular function that may predate irreversible neuronal injury and cognitive impairment.[8,19]

Fewer studies have examined regional changes in CVR and their relationship to relevant cognitive domains such as memory. One of the few longitudinal studies of CVR reported a decline in CVR in older adults that is related to decline in cognitive functioning and memory, and that CVR decline occurs most rapidly in the temporal lobe.[22] This is consistent with studies showing hippocampal vascularization patterns are related to memory ability in older adults[23] and that the medial temporal lobe (MTL) may be selectively vulnerable to hypoperfusion.[24,25] Microvascular dysfunction in the BBB has also been related to early-stage cognitive impairment, independent of traditional vascular risk factors and AD biomarker changes.[10,13–15]

Although the majority of research to date has studied CVR responses to experimentally induced hypercapnia, there are also a number of factors that impact CBF at rest including heart rate, blood pressure, respiration effort, arterial O_2, resting position, and spontaneous fluctuations in blood and CSF CO_2 levels.[26,27] Spontaneous fluctuations in blood and CSF CO_2 levels at rest can be captured by combining either pseudocontinuous arterial spin labeling (pCASL) or blood oxygen level dependent (BOLD) signal changes together with capnographic monitoring and are diminished in older adults.[9,28] If spontaneous CVR responses capture microvascular changes contributing to cognitive decline, the relative simplicity and tolerability of this resting state approach would be of great value relative to administration of gas or guided breathing. To our knowledge, no studies to date have examined spontaneous CVR in a well-characterized sample of older adults with and without MCI. Based on prior studies of CVR responses to experimentally induced hypercapnia, we hypothesized that spontaneous CVR in global and medial temporal regions would be diminished with age and in older adults with MCI and memory deficits, independent of AD biomarker levels.

2 | METHODS

2.1 | Participants

A total of 161 older adult participants were recruited from the Los Angeles County and Orange County community and all procedures were conducted as part of the Vascular Senescence and Cognition (VaSC) Study at University of Southern California (USC) and University of California Irvine (UCI). Participants were recruited from the community through outreach events, mailing lists, word of mouth, online portals, and other modes of community outreach facilitated by the USC Leonard Davis School of Gerontology, and the UCI Alzheimer's Disease Research Center. All participants were independently living at the time of recruitment and were aged 55 to 89 years. Study exclusion criteria were a prior diagnosis of dementia, history of clinical stroke, family history of dominantly inherited neurodegenerative disorders, current neurological or major psychiatric disorders that may impact cognitive function, history of moderate-to-severe traumatic brain injury, current use of medications that may impair the central nervous system (i.e., anticholinergics, benzodiazepines, barbiturates, etc.), current organ failure or other uncontrolled systemic illness, and contraindications for brain MRI. Eligibility for the study was verified via clinical interview and review of current medications with both the participant and an informant study partner when available. Additionally, after MRI acquisition, each participant's T2-weighted scan was screened by a board-certified neuroradiologist for any incidental findings, including silent infarctions. None of the participants in the sample had evidence of any silent infarctions or other exclusionary abnormalities.

2.2 | Neuropsychological testing and MCI diagnosis

All participants underwent a clinical interview and comprehensive neuropsychological assessment by a trained technician or doctoral student under the supervision of a licensed clinical neuropsychologist (D.A.N.). The assessment included multiple tests of global cognition, memory, attention/executive function, and language. The Mattis Dementia Rating Scale was also used to screen participants for dementia.[29] Participants were classified as cognitively unimpaired (CU) if they did not meet published neuropsychological criteria for MCI.[30] Specifically, MCI was diagnosed when participants scored > 1 standard deviation (SD) below demographically referenced *z* scores on > 1 measure within a domain or > 2 measures across domains. The neuropsychological battery for diagnosis used three tests per domain of function. Participants with impairment on > 1 memory measure were considered amnestic MCI. Numerous studies have previously established the validity of this neuropsychological approach to MCI.[31–33] All neuropsychological testing and diagnostic assessments were conducted blinded to all clinical, biomarker, and imaging findings.

In addition, a memory composite was created by averaging the demographically corrected *z* scores from the memory tests, which included: Logical Memory 2 (LM2), Rey Auditory Verbal Learning Test Trial 7 (RAVLT7), and Rey Auditory Verbal Learning Test Recognition (RAVLT Recognition).

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed literature using traditional sources (e.g., PubMed). Cerebrovascular reactivity (CVR) to a hypercapnia challenge has been studied widely; however, few studies have focused on spontaneous CVR at rest, and none in older adults with memory impairment.
2. **Interpretation**: Our findings suggest that deficits in spontaneous CVR at rest in medial temporal regions is associated with cognitive decline in older adults, independent of Alzheimer's disease pathology and vascular risk factors. Findings also highlight the utility of studying CVR at rest, rather than to induced hypercapnia.
3. **Future directions**: Future longitudinal research is needed to determine the predictive nature of spontaneous CVR in the medial temporal regions, and the role it may play in neurogenerative disease.

2.3 | Neuroimaging and cerebral perfusion

All participants underwent a brain MRI on a 3T scanner (Siemens MAGNETOM Prisma System). The direction of acquisition for both sites was anterior to posterior. The Siemens MAGNETOM Prisma System at UCI used software version ve11e, while USC operated ve11b. The following sequences were examined for the current analysis: 3D T1-weighted anatomical scan for qualitative assessment of brain structures and abnormalities (scan parameters: repetition time [TR] = 2300 ms; echo time [TE] = 2.98 ms; inversion time [TI] = 900 ms; flip angle = 9 deg; field of view [FOV] = 256 mm; resolution = 1.0 × 1.0 × 1.2 mm3; scan time = 9 minutes), and 3D gradient and spin-echo (GRASE) pCASL for CBF quantification. The scan parameters were as follows for pCASL: TR = 5000 ms; TE at USC = 36.3 ms; TE at UCI = 37.46 ms; FOV = 240 mm; resolution = 2.5 × 2.5 × 3.4 mm3; slice thickness = 3.42 mm; number of slices = 24; labeling duration = 1517 ms; post-labeling delay = 2000 ms. There was a total of 32 acquisitions (1 M0 image + 1 dummy image + 30 alternating tag and control images), with a total scan time of 5 minutes 25 seconds, yielding 15 tag–control pair images.

The pCASL scans were pre-processed using the previously published method in ASLtbx pipeline,[34,35] implemented in SPM12 within MATLAB.[34,35] Pre-processing steps for pCASL scans included motion correction, co-registration to individual participants' structural T1-weighted image, spatial smoothing with a 6 mm full width at half-maximum Gaussian kernel, masked to remove the background, and tag–control subtraction resulting in 15 tag–control pairs for each

participant with values for absolute CBF (ml/100 g tissue/minute). All CBF images were thresholded < 10 or > 150 ml/100 g/minute to exclude CBF outside the expected physiological range of gray matter.[36,37] These CBF quantification maps are generated in T1 space. Then tag–control pairs were warped to 2 mm Monreal Neurological Institute (MNI) space and averaged to create mean CBF maps for each participant. Resulting mean CBF maps were visually inspected for quality and gross abnormalities (i.e., large signal dropout). Partial volume correction was performed by applying participant-specific gray matter masks derived from the gray matter tissue class segmentation of T1-weighted structural images.[38] Segmented gray matter maps were thresholded at 0.3,[39] binarized, warped into 2 mm MNI space, and multiplied by the mean CBF maps to ensure CBF was limited to gray matter.

Capnography indexed $_{ET}CO_2$ during MRI acquisition using an M3015A sidestream CO_2 extension module (Philips Medical Systems) connected to a nasal cannula into which participants breathed. To correct for sampling tubing latency, $_{ET}CO_2$ time series were shifted by a pre-calibrated duration of time (i.e., 10 seconds for the present study). This time shift was consistent across sites. For the baseline pCASL scan, $_{ET}CO_2$ was extracted from the raw time series in accordance with the breathing rate (i.e., at every expiration). Participants were provided with both verbal and visual instructions to "keep your eyes open and stay awake. Breathe as you normally do. Breathe only through your nose. Stay as still as possible." Participants were excluded from analysis if they failed to adhere to breathing instructions (e.g., lack of positive peaks in raw data—participant might be breathing through the mouth).

2.4 | CVR maps

Spontaneous CVR has been quantified variously in the literature.[26] For consistency with prior studies using our paradigm, CVR was conceptualized as the percent change in CBF per unit change in $_{ET}CO_2$.[19,40] This specific computational approach has been previously outlined.[8,41] In the present study, participants' individual CVR maps were generated using resting state CBF with simultaneous capnography with the following equation (1) computed in each voxel:

$$\text{CVR (\%CBF change/mmHg)} = \frac{100 \times (\text{CBF}_{\text{maximum}} - \text{CBF}_{\text{minimum}}) / \text{CBF}_{\text{minimum}}}{\text{EtCO2}_{\text{maximum}} - \text{EtCO2}_{\text{minimum}}} \quad (1)$$

Based on prior studies[13,16] and our hypothesized relations with MCI and memory impairment, we examined spontaneous CVR in the whole brain and in two medial temporal structures (hippocampus and PHG; Figure 1).

2.5 | Vascular risk factors

The participants' vascular risk factors (VRF) burden was evaluated through clinical interviews with the participant and a knowledgeable informant (when available) and included a history of cardiovascular disease (heart failure, angina, stent placement, coronary artery bypass graft, intermittent claudication), hypertension, hyperlipidemia, type 2 diabetes, smoking, atrial fibrillation, and transient ischemic attack or minor stroke. The total VRF burden was defined by the sum of these risk factors and then dichotomized into lower versus higher burden (0–1 vs. 2+ VRFs) based on prior studies.[13,42,43]

2.6 | Plasma AD biomarkers

Participants underwent venipuncture after an overnight fast, and blood plasma was separated by centrifugation and stored at −80°C until AD biomarker assays. Plasma phosphorylated tau (p-tau$_{181}$) and amyloid beta (Aβ_{40} and Aβ_{42}) concentrations were obtained using the digital immunoassay, single molecule array (Simoa) p-tau$_{181}$ Advantage v2.1 and Neurology 3-Plex A (N3PA) Advantage Kits, respectively, conducted in the same lab (E.H.).

2.7 | Apolipoprotein E genotyping

Blood samples were used to determine participant apolipoprotein E (*APOE* genotype, as previously described.[44] Briefly, genomic DNA was extracted using the PureLink Genomic DNA Mini Kit (Thermo). The isolated DNA concentration was determined using a NanoDrop One (Thermo). DNA was then stored at −80°C for long-term storage. Isolated DNA was first diluted to a concentration of 10 mg/μL. Polymerase chain reaction (PCR) was performed in a final volume of 25 μL containing 25 ng DNA, 0.5 μM of both forward and reverse primers (forward: ACGGCTGTCCAAGGAGCTG; reverse: CCCCGGCCTGGTACACTG), and 1× SYBR Green Master Mix (Qiagen) diluted in H_2O. For the amplification, a T100 Thermal Cycler (BioRad) was used with the following settings: 95°C for 10 minutes; 32 cycles of 94°C for 20 seconds, 64°C for 20 seconds, and 72°C for 40 seconds; followed by 72°C for 3 minutes. Fifteen microliters of the DNA PCR product was digested with Hhal-fast enzyme at 37°C for 15 minutes. The digested PCR product was added to a 3% agarose gel in 1× borax buffer for gel electrophoresis. The gel was run at 175 V for 25 minutes and visualized on ChemiDoc (BioRad) with a GelRed 10,000 × gel dye. *APOE* ε4 carrier status was defined as *APOE* ε4 carriers (at least one copy of the ε4 allele - *APOE* ε3/ε4 or *APOE* ε4/ε4) or *APOE* ε4 non-carriers (no copies of the ε4 allele).

2.8 | Data analysis

A total of 161 participants were studied and characterized by demographics, spontaneous whole brain CVR, and MCI data for statistical analysis (Table 1, Figure 2). Spontaneous CVR in the hippocampus and PHG was available on a subset of participants with usable data after exclusion of negative CVR values and outlier removal ($N = 150$). Spontaneous CVR values (whole brain, hippocampus, and PHG) were normally distributed. Outliers of whole brain, hippocampus, and PHG

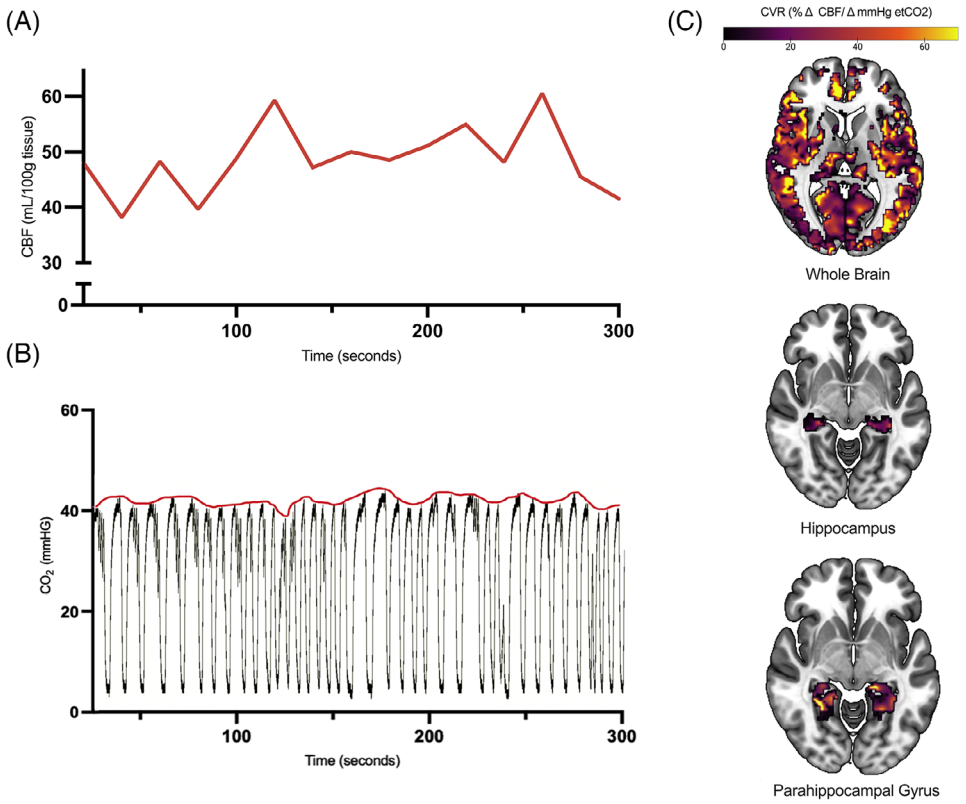

FIGURE 1 Spontaneous CVR mapping methodology. CVR was conceptualized as the percent change in CBF per unit change in $_{ET}CO_2$. Using participants continuous 4-D CBF maps obtained during a resting pCASL scan (A), percent change in CBF is divided by unit change in $_{ET}CO_2$, which is calculated from participants' continuous $_{ET}CO_2$ during the resting pCASL scan (B) to create a map of spontaneous CVR (C). Whole brain and regional mean CVR values were extracted from the spontaneous CVR map. CBF, cerebral blood flow; CVR, cerebrovascular reactivity; $_{ET}CO_2$, end-tidal carbon dioxide; pCASL, pseudocontinuous arterial spin labeling

spontaneous CVR were screened and removed if they were greater than ± 3 SD from the mean, with a total of two outliers removed.

All statistical analyses were conducted using SPSS (version 29.0.1.1.) and R (version 4.3.3). Linear regression analyses were conducted, with age as the predictor and spontaneous CVR as the outcome variable, to determine the relationship between spontaneous CVR and age, for whole brain, hippocampus, and PHG spontaneous CVR. The relationship between CBF and age was also evaluated to determine whether spontaneous CVR was indicative of microvascular change above what is evident in CBF. All neuropsychological test scores used in the diagnosis of MCI were demographically corrected for age, sex, and education; therefore, independent samples *t* tests were performed to determine the relationship between hippocampus and PHG spontaneous CVR in global cognitive impairment. An additional, independent means *t* test was conducted to evaluate the relationship between hippocampus and PHG spontaneous CVR and memory impairment by comparing hippocampal and PHG CVR in those with amnestic MCI and participants without cognitive impairment.

The relationship between spontaneous CVR in the hippocampus and the PHG was compared in participants with and without amnestic MCI (defined by impairment on at least two memory tests). Linear regression was performed with hippocampal and PHG spontaneous CVR and a memory composite. In addition, an independent means *t* test was performed to compare hippocampal and PHG spontaneous CVR in participants with memory composite *z* scores of < −1 to those with composite *z* scores of > −1. Last, to determine the relationship between individual memory tests (LM2, RAVLT7, and RAVLT Recognition) and hippocampal and PHG spontaneous CVR, linear regression was conducted using raw scores and controlling for age, sex, and education.

In addition, sensitivity analyses were conducted using multiple regression and analyses of covariance to determine whether the results are impacted by plasma AD biomarkers ($A\beta_{42/40}$ and p-tau181). Additionally, vascular risk factor burden was also included in these models for sensitivity analyses to determine whether the relationship between CVR and cognitive functioning is impacted by the vascular risk factor burden. *APOE* ε4 status was not included in the sensitivity analyses due to limited availability of data resulting in significantly reduced sample size (only 10 participants in the MCI group have *APOE* ε4 data).

Lastly, to account for multiple comparisons, false discovery rate (FDR) correction was performed.[38]

3 | RESULTS

A total of 161 older adults were included in the age analyses (mean age = 69.5, 65.2% female, mean years of education = 16.5). Linear regression revealed a significant negative association between age and

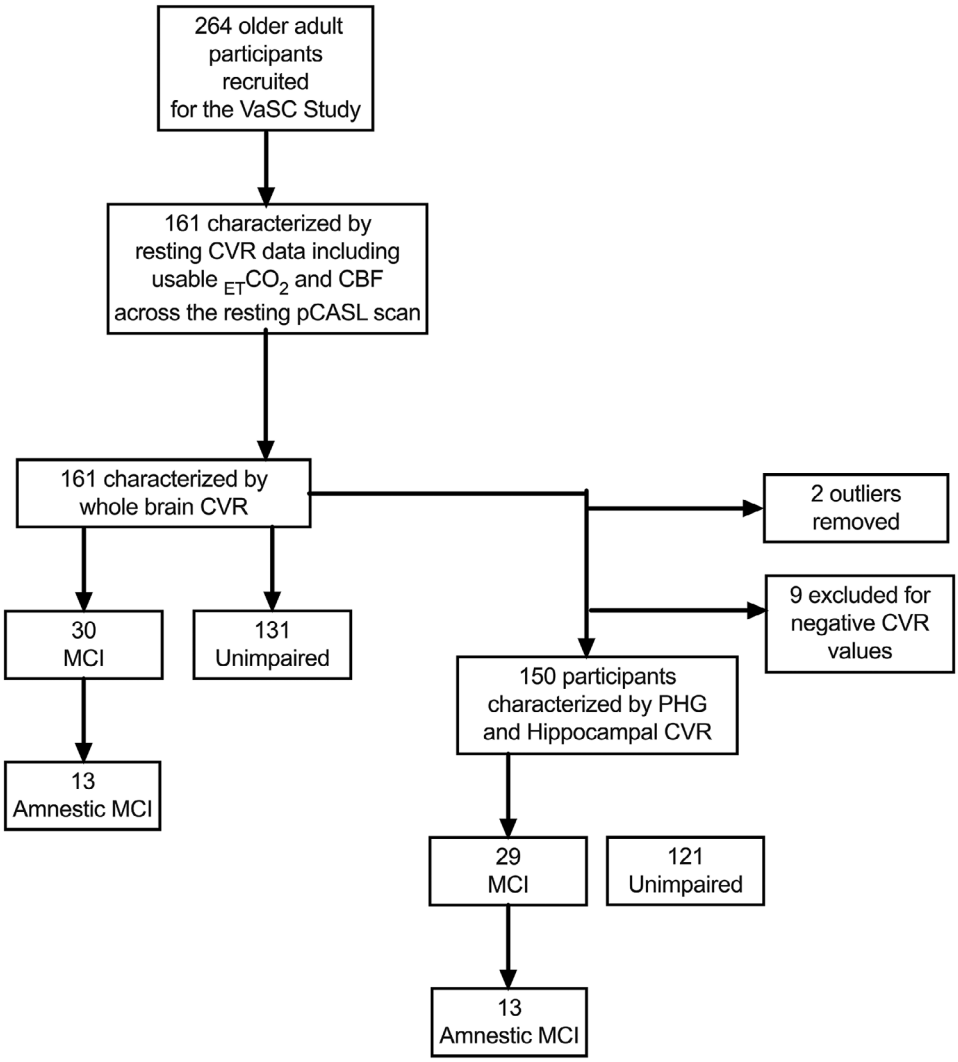

FIGURE 2 Participant flow diagram. CBF, cerebral blood flow; CVR, cerebrovascular reactivity; $_{ET}CO_2$, end-tidal carbon dioxide; MCI, mild cognitive impairment; pCASL, pseudocontinuous arterial spin labeling; PHG, parahippocampal gyrus; VaSC, Vascular Senescence and Cognition

whole brain spontaneous CVR (B = −0.41, 95% confidence interval [CI: −0.65, −0.17], $P = 0.00096$), and this relationship was not attenuated by adjustment for sex or MCI status. In a regression model with age and whole brain CBF, there was no significant association (B = 0.09, 95% CI [−0.05, 0.23], $P = 0.646$). PHG spontaneous CVR was also not significantly associated with age (B = −0.142, 95% CI [−0.52, 0.03], $P = 0.083$), and PHG CBF did not show a significant association with age (B = 0.109, 95% CI [−0.05, 0.23], $P = 0.186$; Figure 3). There was no significant relationship between hippocampal spontaneous CVR and age.

A total of 150 older adults were included in the MCI analyses, of which 129 were considering CU (mean age = 70.0, 67.9% female, mean years of education = 16.7) and 29 met criteria for MCI (mean age = 67.5, 53.3% female, mean years of education = 15.4). Spontaneous PHG CVR was significantly lower in participants with MCI (M = 14.27, SD = 9.28) than those who were CU (M = 20.24, SD = 13.87; $t[61.8] = 2.90$, 95% CI [1.93, 10.46], $P = 0.005$), but there was no difference in PHG CBF between MCI and CU (Figure 4A and B). Spontaneous PHG CVR was significantly lower in participants with amnestic MCI (M = 12.68 SD = 9.08) compared to those who were CU (M = 20.24, SD = 13.87; $[t[25.46] = 3.055$, 95% CI [2.60, 13.29], $P = 0.005$; Figure 4C). There was no significant relationship between hippocampal spontaneous CVR and MCI or amnestic MCI.

Spontaneous PHG CVR was significantly lower in participants with impaired (z score < −1) memory composite z scores (M = 14.50 SD = 10.09) compared to those with memory composite z scores > −1 (M = 20.72 SD = 14.13; $t[42.42] = 2.40$, 95% CI [0.98, 11.45], $P = 0.021$; Figure 4D). In linear regression, spontaneous PHG CVR was positively associated with memory composite z scores (B = 0.255, 95% CI [0.56, 3.12], $P = 0.005$; Table 2). Specifically, for every one unit change in spontaneous PHG CVR there is a predicted 0.03 unit change in memory composite z scores. There was no significant relationship between hippocampal spontaneous CVR and memory composite impairment.

Linear regression evaluating the relationship between spontaneous PHG CVR and raw scores on memory tests revealed a positive association between participant performance on delayed story recall (LM2) and spontaneous PHG CVR (B = 0.188, 95% CI [−0.01, 0.63], $P = 0.055$).

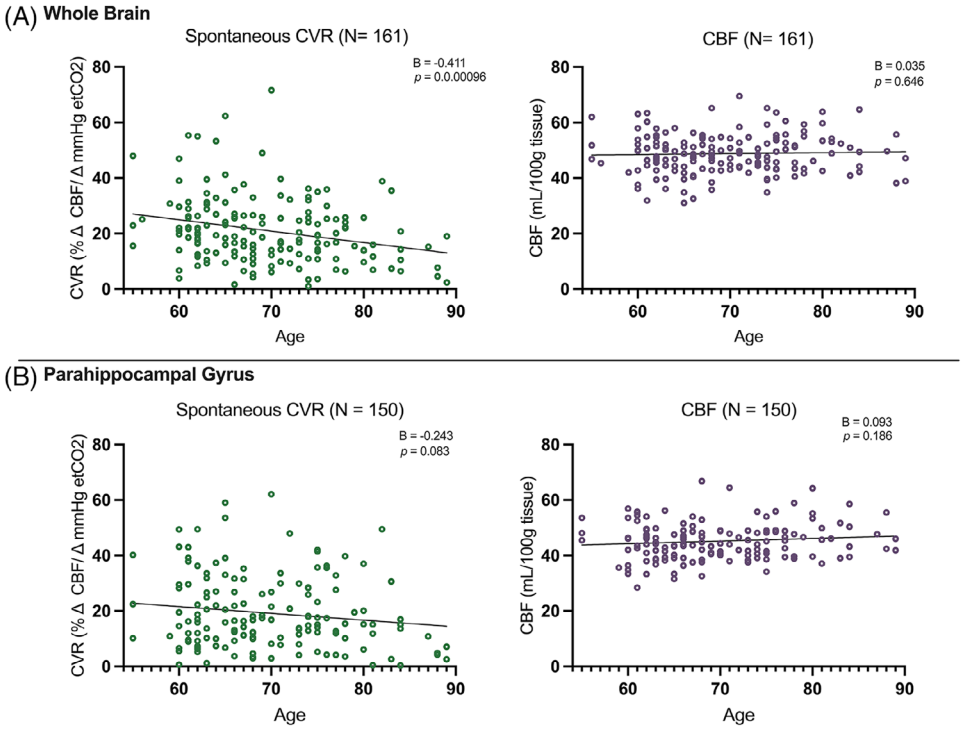

FIGURE 3 Regression plots of the relationship between age and spontaneous cerebrovascular reactivity (CVR) compared to that of age and cerebral blood flow (CBF) for (A) whole brain, and (B) parahippocampal gyrus (PHG). CVR, cerebrovascular reactivity; CBF, cerebral blood flow; $_{ET}CO_2$, end-tidal carbon dioxide

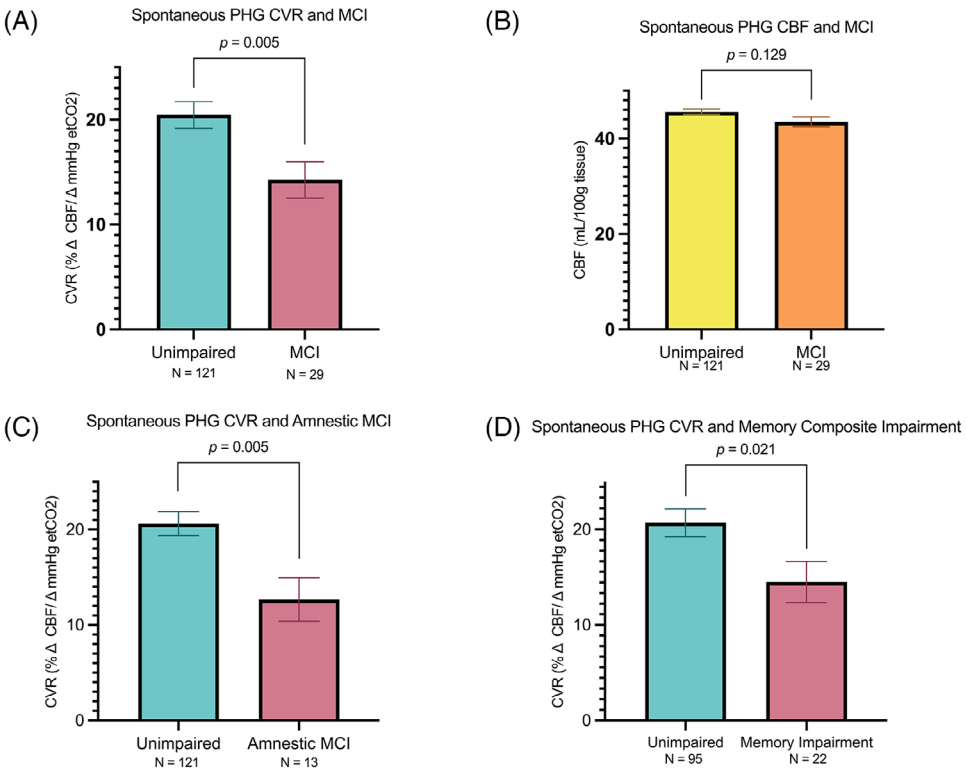

FIGURE 4 Spontaneous PHG CVR and cognitive impairment. A, Spontaneous CVR in the PHG in unimpaired participants compared to participants with MCI, and (B) CBF in the PHG in unimpaired participants compared to participants with MCI. C, Spontaneous CVR in the PHG in unimpaired participants compared to participants with amnestic MCI, and (D) Spontaneous PHG CVR in unimpaired participants compared to participants who had *z* scores < −1.0 on the memory composite. CBF, cerebral blood flow; CVR, cerebrovascular reactivity; $_{ET}CO_2$, end-tidal carbon dioxide; MCI, mild cognitive impairment; PHG, parahippocampal gyrus

TABLE 1 Participant demographics.

N = 161	M (SD) or *n* (%)
Age	69.5 (7.6)
Sex, female, *n* (%)	105 (65.2)
Education	16.5 (2.4)
Race, *n* (%)	
White	127 (78.9)
Black	4 (2.5)
American Indian or Alaska Native	2 (1.2)
Native Hawaiian or other Pacific Islander	1 (0.6)
Asian	23 (14.3)
Other	4 (2.5)
Ethnicity	
Hispanic or Latino	8 (5.0)
Non-Hispanic or Latino	153 (95.0)
Cognitive impairment, *n* (%)	
MCI	30 (18.6)
Amnestic MCI	16 (9.9)
Cognitively unimpaired	131 (81.4)
[a] *APOE ε4* **carriers (*n*, %)**	
ε3/ε4 or ε4/ε4 carriers	66 (47.1)
[b]**Plasma p-tau$_{181}$**	24.1 (12.0)
[c]**Plasma Aβ42$_{/40}$ ratio**	0.04 (0.02)
[d]**Vascular risk factors, *n* (%)**	
Hypertension	61 (37.9)
Hyperlipidemia	74 (46.3)
Diabetes	20 (12.4)
Smoking	56 (34.8)
Cardiovascular disease	17 (10.6)
Atrial fibrillation	8 (5.0)
Transient ischemic attack	3 (1.9)

Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; M, mean; MCI, mild cognitive impairment; N, sample size; p-tau, phosphorylated tau 181; SD, standard deviation.

[a]These data were available on a subset of *n* = 140 participants.

[b]These data were available on a subset of *n* = 114 participants.

[c]These data were available on a subset of *n* = 111 participants.

[d]Vascular risk factors reported are defined as the following: hypertension: current diagnosis of high blood pressure; hyperlipidemia: current diagnosis of high cholesterol and/or triglycerides; diabetes: current diagnosis of type I or type II diabetes; smoking: current use or history of regular use of cigarettes, cigars, or e-cigarettes; cardiovascular disease: current diagnosis of heart disease or other cardiovascular conditions impacting the heart and/or blood vessels; atrial Fibrillation: current diagnosis of an irregular heart rhythm; and transient ischemic attack: history of transient ischemic attack.

Specifically, for every one unit change in spontaneous PHG CVR there is a predicted 0.10 unit change in raw scores on delayed story recall. There was also a significant positive association between participant performance on delayed word list recall (RAVLT7) and spontaneous PHG CVR (B = 0.277, 95% CI [0.27, 1.69], *P* = 0.007). Specifically, for every one unit change in spontaneous PHG CVR there is a predicted 0.06 unit change in raw scores on delayed word recall. Last, there was also a significant positive association between participant performance on delayed word list recognition (RAVLT Recognition) and spontaneous PHG CVR (B = 0.182, 95% CI [0.02, 2.03], *P* = 0.046). Specifically, for every one unit change in spontaneous PHG CVR there is a predicted 0.03 unit change in raw scores on delayed word list recognition. All analyses were controlled for age, sex, and education (Table 2).

All primary analyses were included for FDR correction. All significant findings survived FDR correction except for the relationship between PHG spontaneous CVR and raw scores on RAVLT recognition (*q* = 0.46).

3.1 | Sensitivity analysis

Consistent results were observed in models controlling for (1) plasma A$\beta_{42/40}$ and (2) vascular risk factors, and in models controlling for (1) p-tau$_{181}$ and (2) vascular risk factors, with the exception of the relationship between PHG spontaneous CVR and memory composite impairment (Tables S1 and S2 in supporting information). In models with Aβ and vascular risk factors the relationship between PHG spontaneous CVR and memory composite impairment was attenuated to *P* = 0.08. Please note that due to plasma biomarker data availability, decreased sample sizes/participant loss is present in these sensitivity analyses (see Tables S1 and S2)

4 | DISCUSSION

The present study found that deficits in spontaneous PHG CVR at rest are associated with cognitive impairment, and in particular with amnestic cognitive impairment and deficits on multiple tests of delayed verbal memory. These findings are consistent with previously reported deficits in experimentally induced CVR related to cognitive impairment and memory decline, and further extend these findings to spontaneous CVR at rest.[16,22,45,46] Prior studies have noted microvascular dysfunction in the hippocampus and PHG in older adults with cognitive impairment, including vulnerability to hypoperfusion and BBB breakdown,[13,47] but few CVR studies have examined medial temporal regions related to memory impairment. Importantly, the study findings were largely unchanged after controlling for traditional vascular risk factors and plasma AD biomarkers, and were not accounted for by differences in resting CBF. Together, these findings suggest cerebrovascular dysfunction within medial temporal regions is observed during early-stage cognitive and memory impairment, independent of AD pathophysiologic change. Additional research is needed to determine whether spontaneous CVR at rest may be a valuable early-stage biomarker of vascular contributions to cognitive decline.

Although whole brain spontaneous CVR was correlated with age, there were no whole brain differences between MCI and CU participants. Conversely, PHG spontaneous CVR was not significantly correlated with age, but was decreased in MCI and memory impair-

TABLE 2 Breakdown of regression analysis results investigating the relationship between spontaneous PHG CVR and neuropsychological tests of memory including: a memory composite, RAVLT 7, RAVLT Recognition, and Logical Memory 2.

Model	R	ΔR^2	*Df*	*β*	*P* value
PHG CVR and memory composite	0.255	0.065	(1,116)	0.255	0.005*
PHG CVR and RAVLT trial 7	0.301	0.090	(4,113)		0.029*
RAVLT 7 raw score				0.277	0.007*
Age				–0.046	0.643
Sex				0.069	0.467
Education				0.082	0.403
PHG CVR and RAVLT recognition	0.226	0.051	(4, 134)		0.133
RAVLT recog raw score				0.182	0.046*
Age				–0.115	0.187
Sex				0.019	0.830
Education				0.062	0.498
PHG CVR and LM2	0.248	0.062	(4, 113)		0.124
LM2 raw score				0.188	0.055
Age				–0.110	0.256
Sex				0.010	0.915
Education				0.092	0.360

Note: The memory composite is an average of demographically corrected *z* scores on RAVLT trial 7, RAVLT Recognition, and Logical Memory 2.
Abbreviations: *β*, standardized regression coefficient; CVR, cerebrovascular reactivity; ΔR^2, squared semi-partial correlation coefficient or change in R^2; *df*, degrees of freedom; LM2, Logical Memory 2; PHG, parahippocampal gyrus; R, correlation coefficient; RAVLT, Rey Auditory Verbal Learning Test.

ment. Prior studies using experimentally induced CVR approaches have noted age effects both on whole brain and temporal lobe CVR to hypercapnia.[8,16] Further studies are needed, but the findings of the present study could suggest that deficits in spontaneous PHG CVR are indicative of pathological versus normal aging.

The potential value of spontaneous PHG CVR as a biomarker is in part due to its relative simplicity and tolerability compared to other methods requiring manipulation of blood and CSF CO_2 levels. Prior studies have evaluated spontaneous CVR using various methods, often focused on resting state BOLD fluctuations.[26,48–50] However, the ability of spontaneous CVR to capture adequate changes in $_{ET}CO_2$ at rest has been questioned.[51–53] Our approach focused on resting state pCASL, which has the advantage of being a straightforward measure of CBF, but has the disadvantage of low temporal resolution and signal-to-noise ratio. Nevertheless, the present study found that calculation of CVR with spontaneous CBF and $_{ET}CO_2$ changes at rest using standard methods did in fact provide useful information beyond CBF alone. This study also observed that CVR values at rest are much higher than during breath challenge due to much smaller resting state fluctuations in $_{ET}CO_2$ paired with larger CBF fluctuations. Additional studies using other modalities, including BOLD, and quantification methods will provide further insights into the relative advantages of different approaches to spontaneous regional CVR at rest.

Some limitations of this study include the cross-sectional design and relative lack of diversity in the sample, which may limit the generalizability of the results. The present study also only evaluated CBF and CVR in the gray matter as the current sequence was not optimized to capture white matter CBF. Additionally, due to the low temporal resolution of the pCASL images, we were not able to temporally shift $_{ET}CO_2$ data or adjust for blood transit time from the lung to the brain in our method of processing $_{ET}CO_2$. Moreover, in this study we were able to assess spontaneous CVR using pCASL. Given that participants in the study had no history of stroke, dementia, or other neurological condition, additional studies are needed to determine whether deficits in spontaneous CVR are associated with more severe cerebrovascular and neurodegenerative conditions. Longitudinal studies are also warranted to examine the predictive value of spontaneous CVR as a preclinical biomarker of cognitive decline.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest. Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request from the corresponding author, D.A.N., based on the conditions outlined by the Data Availability Policy and Statement.

CONSENT STATEMENT

The VaSC Study was approved by the USC (HS-14-00784) and UCI (HS-2019-5324) Institutional Review Boards, and all participants gave informed consent prior to participating in the study.

ORCID

Daniel A. Nation https://orcid.org/0000-0002-9123-0658

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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