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Increased Cerebral Blood Flow with Increased Amyloid burden in the preclinical phase of Alzheimer's disease

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Running Title: Perfusion increases with amyloid in pre-AD

Increased Cerebral Blood Flow with Increased Amyloid burden in the preclinical phase of Alzheimer's disease

Background: Arterial spin labeling (ASL) is an emerging MRI technique for non-invasive measurement of cerebral blood flow (CBF) that has been used to show hemodynamic

changes in the brains of people with Alzheimer's disease (AD). CBF changes have been measured using PET across the AD spectrum, but ASL showed limited success in measuring CBF variations in the preclinical phase of AD, where Amyloid β ($A\beta$) plaques accumulate in the decades prior to symptom onset.

Purpose: The aim of this study is to investigate the relationship between CBF measured by multiphase-pseudo-continuous-ASL (MP-PCASL) and $A\beta$ burden as measured by ^{11}C -PiB PET imaging in a study of cognitively normal (CN) subjects aged over 65.

Study type: Cross-sectional

Population: Forty-six cognitively normal subjects including 33 with low levels of $A\beta$ burden and 13 with high levels of $A\beta$.

Field Strength/Sequence: 3 T/ 3D multiphase-pseudo-continuous-ASL (MP-PCASL)

Assessment: The MP-PCASL method was chosen because it has a high signal-to-noise ratio. Furthermore, the data were analyzed using an efficient processing pipeline consisting of motion correction, ASL motion correction imprecision removal, temporal and spatial filtering, and partial volume effect correction.

Statistical Tests: General Linear model

Results: In CN subjects positive for $A\beta$ burden ($n=13$) we observed a positive correlation between CBF and $A\beta$ burden in the hippocampus, amygdala, caudate ($p < 0.01$), frontal, temporal and insula ($p < 0.05$).

Data Conclusion: To the best of our knowledge, this is the first study using MP-PCASL in the study of AD, and the results suggest a potential compensatory hemodynamic mechanism that protects against pathology in the early stages of AD.

Keywords:

Perfusion, Multiphase PCASL, preclinical Alzheimer's disease, CBF, amyloid

ALZHEIMER'S DISEASE (AD) IS the most common cause of dementia (~70%) and is characterized by Amyloid β ($A\beta$) depositions and tau tangles pathologies.¹ So far, treatments targeting $A\beta$ protein have had very limited results for AD patients,^{2,3} and interest has shifted to the pre-clinical phase (asymptomatic phase with the presence of amyloid pathology as defined in ⁴). The pre-clinical phase may provide an opportunity for effective interventions. In the pre-clinical phase of AD, regional cerebral metabolism and blood flow are altered: metabolism as measured by glucose ⁵⁻⁸ and cerebral blood flow (CBF) when measured by positron emission tomography (PET) ⁹ or arterial spin labelling (ASL) MRI.¹⁰

Indeed, cerebral blood flow (CBF) as an indicator of brain dysfunction is a well-established biomarker of AD.¹¹ CBF varies across the AD spectrum from cognitively normal (CN), to mild cognitive impairment (MCI), to the clinical diagnosis of AD. Based on gold standard PET imaging, there are regions with increased ^{6,9} and decreased ⁸ activity in CN, increased ¹² and decreased ^{13,14} activity in patients with MCI, and decreased ^{15,16} regional activity in patients with AD.

Studies investigating brain function changes in the preclinical phase of AD in association with $A\beta$ are limited. In the preclinical phase of AD using PET imaging, a few studies have investigated the mutual effect of CBF and $A\beta$ deposition. Sojkova *et al.* investigated whether $A\beta$ status (+/-) is related to longitudinal CBF changes measured by ¹⁵O-H₂O-PET in 28 CN subjects, and found hyper-perfusion in the medial frontal, inferior frontal gyri, precuneus, inferior parietal lobule and postcentral gyrus and hypo-perfusion in anterior and middle

cingulate, supramarginal gyrus and thalamus.⁹ Johanson *et al.* examined the effect of A β burden and metabolism in a subset of 200 CN subjects, and found that relative to the A β -group, A β ⁺ and A β ⁱ (intermediate) participants had increased glucose metabolism measured by ¹⁸F-FDG-PET in the bilateral thalamus and bilateral superior temporal gyrus.⁶ Other recent studies found a positive correlation between cortical A β and metabolism measured by ¹⁸F-FDG-PET in CN in temporal, parietal and frontal regions,^{5,7} whereas another study showed a significant negative trend only in the medial temporal lobe.⁸

Due to the cost and radiation risk associated with PET imaging, ASL-MRI technique is becoming a popular alternative for measuring CBF. ASL is less expensive, non-invasive, relatively fast (less than 10 minutes) and, with the improvement in MRI hardware and software technology, can be performed on most MRI scanners with comparable results to ¹⁵O-H₂O for CBF quantification.^{17,18} However, there is a large variability in terms of sensitivity across the many ASL variants, namely pulsed-ASL (PASL), continuous-ASL (CASL), pseudo-continuous-ASL (PCASL), and multiphase-pseudo-continuous-ASL (MP-PCASL).¹⁹ Among these, PCASL and its variants were reported to outperform other methods (higher SNR and less labeling artifacts),^{20,21} and are currently the recommended ASL method by the ISMRM perfusion community and the European consortium for ASL in dementia.²²

In the preclinical phase of AD using ASL imaging, there is limited and contradicting evidence of CBF increase. Mattsson *et al.* reported that a higher cortical A β load measured by ¹⁸F-florbetapir PET imaging was associated with reduced CBF measured by PASL across

diagnostic groups (CN, MCI and AD) but found no significant difference between CN A β ⁺ and CN A β ⁻ groups.²³ In another study using ¹¹C-PiB PET and PCASL imaging techniques, a lower CBF was associated with higher A β load in CN and amnesic MCI.¹⁰

ASL techniques broadly suffer from low SNR and measurement instabilities,²⁴ which may contribute to the inconsistent findings across the dementia research. In this study, by taking advantage of MP-PCASL technique with GRASE readout and background suppression, higher SNR and lower spatial distortion could be achieved.^{20,25} Additional processing with a well-established ASL post-processing workflow also improves the precision of CBF measurements.²⁶

The aim of this study was to investigate the associations of CBF measured by MP-PCASL MRI and A β burden as measured by ¹¹C-PiB PET imaging in a cross-sectional design with the focus on CN participants.

Material and methods

Participants

The data used for this study were obtained from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study.²⁷ Written informed consent was obtained from participants in accordance with the Austin Health human research ethics committee. MCI participants met

the criteria for subjective and objective cognitive difficulties in the absence of manifest functional loss. AD participants met National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD.²⁸ CN older adults were recruited from advertisements and their cognitive health was confirmed by their performance within normal limits on the AIBL neuropsychological test battery and unremarkable medical history.²⁷

The cross-sectional data from 46 CN participants. The characteristics of the study population are summarized in **Table 1**.

MRI Acquisition

All MR images were acquired on a 3T Siemens Tim Trio scanner with a 12-channel head coil. All subject underwent anatomical T1-weighted (T1w) imaging and MP-PCASL with background suppression and 3D GRASE readout.²⁹ The T1w images were acquired using a standard 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence with $1 \times 1 \times 1$ mm³ resolution, TR/TE/TI = 1900/2.55/900 ms, flip angle 9°, field of view 256×256 , and 160 slices.

For the MP-PCASL labeling, 40 pairs of conventional PCASL (with RF offset phases of 0° for tag (T₀) and 180° for control(C₀)) were collected. Subsequently, 20 ASL pairs with +90° shift in phase offset (T₉₀ and C₉₀) relative to conventional PCASL were also acquired. For all datasets, the labeling plane was selected using an MR angiogram. For data acquisition, a 3D GRASE PCASL sequence with k-space sharing to improve brain coverage was used.²⁹ The

relevant imaging parameters were: TR/TE of 3750/56 ms (TR = 4150 ms was used for both scans from one subject, due to power deposition issues), labeling RF pulse flip angle of 28.5°, RF pulse width 650 μ s, with inter-pulse gap of 450 μ s (spacing between RF pulses of 1070 μ s), peak/average gradient of 8/1 mT/m, peak RF amplitude of 53 mG, labeling duration of 1584 ms, post-labeling delay of 1540 ms, and background suppression inversion-times (relative to imaging module) of 1800 ms and 520 ms. The images have an in-plane resolution of $4 \times 4 \text{ mm}^2$ and slice thickness of 6 mm, with a matrix size of $64 \times 51 \times 20$. A proton density (PD) image (often referred to as M0 or calibration image) with 8 repetitions and without labeling and background suppression was also acquired. This image was used for ASL quantification, as well as a reference for multi-modal image registration. The overall acquisition times for MP-PCASL was 10 minutes, and a static tissue suppression level of approximately 90% was achieved from the background suppression technique.

PET Imaging

Amyloid PET scans were obtained using the ^{11}C -PiB ligand on a Philips Allegro scanner. Image acquisition time was 20 minutes, starting 40 minutes after injection of the radiotracer. PET images were processed using an automated region of interest method.³⁰ Standardized uptake values (SUV) for neocortex were calculated within a mask comprised of frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions.³¹ A ratio of neocortical SUV to the cerebellar cortex SUV was defined as the SUV

ratio (SUVR) representing neocortical A β burden, and a value above 1.4 designated amyloid positivity.

Neuropsychological testing

Several neuropsychological tests were available to study cognitive performance across diagnostic groups. The neuropsychological examination as part of the AIBL study included the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT), Rey Complex Figure Test with 30-minutes delay (RCFT), Logical Memory II (LMII), Verbal Fluency Animals (VFA) and Letter (VFL). Two composite scores were calculated: a composite episodic memory score by averaging CVLT, LMII and RCFT z-score values, and a composite executive function score by averaging VFL and VFL z-scores.

T1w and ASL image post-processing

The T1w image was segmented into gray matter (GM), white matter (WM), cerebrospinal fluid (CSF) maps, and brain mask using the expectation maximization segmentation algorithm. T1w data were divided into 73 grey-matter (GM) regions by segmentation propagation using the Neuromorphometrics database (63 subjects, <http://www.neuromorphometrics.com/>). Subsequently, a rigid alignment between the T1w and M0-ASL calibration image was used to propagate the tissue maps and parcellated regions (a reliable match as 3D GRASE has no image distortion). These small sub-regions were then combined to form cerebral lobes.

CBF was computed using a previously published in-house processing pipeline, which comprised of motion correction, temporal and spatial denoising, partial volume correction and quantification, as previously detailed in.³³ Relative CBF (rCBF) maps were computed by intensity normalization using the medial segment of post-central gyrus (with a regression model where the reference region as the independent variable).³² From the parcellated GM regions, nine volumetric areas including the neocortex, frontal, parietal, temporal, insula, amygdala, hippocampus, caudate and posterior cingulate, were formed.

For the analysis, subjects with quantitative evaluation index (QEI) less than 0.4 for ASL image were excluded, as they were deemed to have low-quality ASL data.³³ However, the main results are also reported without any subject exclusion.

Statistical Analysis

The region of interest analysis was conducted using multiple general linear models to investigate the association of CBF with neocortical SUVR, controlling for age, gender, and APOE $\epsilon 4$ carrier status. All statistical work was conducted with R (version 3.2.4) and all hypothesis tests were 2-sided.

Results

Table 2 presents the association between neuropsychological test and diagnosis. The results show that there are no significant differences in cognitive performance between CN A β ⁻ and CN A β ⁺ subjects included in the study. Table 2 also shows no significant difference in terms of hippocampal atrophy between CN A β ⁻ and CN A β ⁺.

Figure 1 shows the overall distribution of CBF and amyloid for four age-matched subjects in CN A β ⁻ and CN A β ⁺ with rCBF values of 37.3 and 32.8, respectively. **Figure 2** compares CBF maps of two CN A β ⁺ subjects with a different level of neocortical A β (1.5 SUVR and 2.46 SUVR where the A β cut-off is 1.4) particularly in the hippocampus (overlayed) and anterior insula and frontal regions.

Figure 3 shows the overall association between rCBF and SUVR. While there were no significant differences in cognitive performance between CN A β ⁻ and CN A β ⁺, in the CN A β ⁺ group, a positive correlation between rCBF and A β burden was observed in the frontal (regression β coefficient=10.5 $\pm$ 4.1, p = 0.04), temporal (β =11.6 $\pm$ 4.1, p = 0.03), and hippocampus (β =14.9 $\pm$ 2.8, p = 0.002), amygdala (β =12.8 $\pm$ 3.5, p = 0.008), insula (β =11.9 $\pm$ 4.4, p = 0.03), caudate (β =16.4 $\pm$ 3.0, p = 0.001) but not in posterior cingulate (β =1.9 $\pm$ 2.8, p = 0.158).

To study the influence of having considered subject exclusion (when the ASL data quality was deemed to be too low, i.e. QEI<0.4), **Figure 4** shows the corresponding associations between CBF and SUVR without exclusion of any subjects. The excluded subjects included 2 CN A β ⁻ and 1 CN A β ⁺. The same qualitative trends as in **Figure 3** were observed; the same

statistically significant associations were found (albeit with lower significance), with the exception of the frontal lobe region, which did not reach significance when low-quality ASL data were included in the analysis.

Discussion

This study investigated the association of blood flow measured by MP-PCASL-MRI with amyloid- β burden assessed using ^{11}C -PiB-PET in the preclinical phase of AD, and found a positive correlation in the frontal, temporal, caudate and insular regions. We found that in the early stages of AD, in the absence of brain atrophy or cognitive decline, cerebral perfusion increases with increased A β deposition. This increased perfusion may play an important role in the preclinical staging of AD, particularly if those with significant amyloid and hypermetabolism are less likely to be classified as neurodegeneration positive.³⁴

To the best of our knowledge, this is the first study using MP-PCASL in the study of Alzheimer's disease. Among available ASL sequences (PASL, CASL and PCASL), PCASL sequence with background suppression and 3D GRASE readout has been nominated as the recommended implementation for the clinical use of ASL.²² In our study, we use an ASL variant known as MP-PCASL, which considers 4 phase offsets for tag and control acquisitions, and has been shown to substantially improve the reliability of the perfusion measurement by allowing to compensate for the labeling efficiency loss in PCASL.²⁶ The

ASL post-processing workflow further improves the reliability of CBF measurements²⁶ and we expect that the CBF maps used for this analysis have a higher sensitivity to subtle changes in the pre-clinical phase.

Our study extends previous ASL-CBF studies of AD by reproducing similar results as obtained with PET imaging (as the gold standard method) in the pre-clinical phase of AD. Unlike previous ASL studies, PET-based studies reported patterns of hypermetabolism in an elderly population with amyloid pathology in frontal,^{5,7} parietal and temporal,^{5,7} precuneus and postcentral,⁹ and bilateral thalamus regions.⁶ In the current study, we found a positive correlation between rCBF and A β burden in the frontal, temporal, hippocampus, amygdala, insula and caudate. Visual comparison of the CBF maps clearly shows the pattern of increased CBF among CN A β + subjects with different levels of amyloid burden (**Figure 2**). Among CN A β - older adults, there were no significant relationships between CBF and amyloid, likely due to the narrow dynamic range of amyloid level. Among A β + subjects, the positive association was directly linked to amyloid burden as no significant relationship with brain atrophy and cognitive scores could be identified.

Increased CBF might reflect increased brain function. An increase in regional CBF in the preclinical phase of AD when cognitive performance is preserved (as shown in **Table 2**), has been suggested as a compensatory response to the accumulation of A β pathology.³⁵ Other studies also found significant increased perfusion in non-demented elderly subjects at risk for AD, which was also interpreted as a compensatory mechanism leading to a need for extra

glucose and oxygen to support neuronal activity.^{36,37} Several functional MRI (fMRI) studies of CN A β ⁺ also reported increased neural activity during cognitive activity in comparison to young people or CN A β ⁻ older participants.^{38,39,34} The identified regions with a significant association between CBF and A β were also consistent with previous studies reporting the early sign of deterioration in the hippocampus and the posterior cingulate, and with disease progression more extensive in the temporal lobe.⁴⁰ Current results in **Figure 3** may suggest that the increased CBF makes the brain resilient to A β pathology in the preclinical phase of AD, and this increase is lost in the clinical presentation of the disease. However, longitudinal follow up scans to study subjects with low and high CBF in CN A β ⁺ are required. The absence of any associations between CBF and amyloid in the CN A β ⁻ subjects is more challenging to study due to the narrow dynamic range of amyloid values.

The main limitations of the current study are the small cohort size of 46 subjects comprised of 33 CN A β ⁻ and 13 CN A β ⁺, and the cross-sectional nature of the analysis. Furthermore, the current analysis did not control for diurnal variations, the level of caffeine consumption, or other factors that could affect CBF. However, controlling for these effects should (if they do have an effect at all) decrease the variability of the CBF measurement, and improve the significance of our findings.

Our cross-sectional study revealed an association between A β and CBF in CN A β ⁺ in the absence of any cognitive decline and atrophy (**Table 1**). At such an early stage, this finding

suggests that the brain is able to compensate for any stress caused by A β accumulation as evidenced by increased CBF. This may represent an ideal time for the administration of therapeutic interventions prior to irreversible neuro-degeneration while the brain is still able to compensate. However, the extent of this CBF compensation is unknown and further studies are needed to elucidate this phenomenon.

In conclusion, this study revealed a positive correlation between cerebral blood flow measured by multiphase PCASL-MRI and A β measured by PET in the hippocampus, amygdala, caudate, frontal, temporal and insula of asymptomatic subjects. This suggests a potential compensatory mechanism against AD pathology which possible to measure with MRI. However, these cross-sectional findings need to be replicated in a larger cohort and followed longitudinally.

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Table 1: Subject characteristics

	CN A β -	CN A β +
#N	33	13
Age (mean $\pm$ sd)	78 $\pm$ 7	80 $\pm$ 7
Sex (female/male)	19/14	6/7
MMSE (mean $\pm$ sd)	29.2 $\pm$ 1	28.6 $\pm$ 1
CDR	0 $\pm$ 0	0 $\pm$ 0
APOE ϵ 4 (+ / -)	4/29	5/8
NeoCortical SUVR	1.27 $\pm$ 0.1	1.88 $\pm$ 0.3*
NecCortical rCBF	21.5 $\pm$ 5.8	19.16 $\pm$ 5.1

* $p < 0.05$

CN = Cognitively Normal; A β = Amyloid β ; APOE ϵ 4 positive = presence of at least one ϵ 4 allele; SUVR = standardized uptake value ratio; Neocortical CBF is the relative CBF adjusted for age, gender and ApoE status

Table 2: Association of Cognitive Neuropsychological scores and hippocampal atrophy with subject demographics & diagnosis.

	Episodic Memory		Executive Function		Composite Score		Hippocampal Atrophy	
	β	P	β	P	β	P	β	P
Age	-0.029	0.16	0.004	0.85	0.004	0.85	-44.1	0.003**
Sex	-0.175	0.55	0.418	0.16	0.205	0.46	110.6	0.571
APOE: $\epsilon 4$	-0.152	0.70	0.99	0.80	0.103	0.79	-366.9	0.176
Diagnosis: CN+	-0.296	0.4	0.30	0.93	-0.202	0.57	338.6	0.167

β : regression beta coefficients, P : p-value

Increased Cerebral Blood Flow with Increased Amyloid burden in the preclinical phase of Alzheimer's disease

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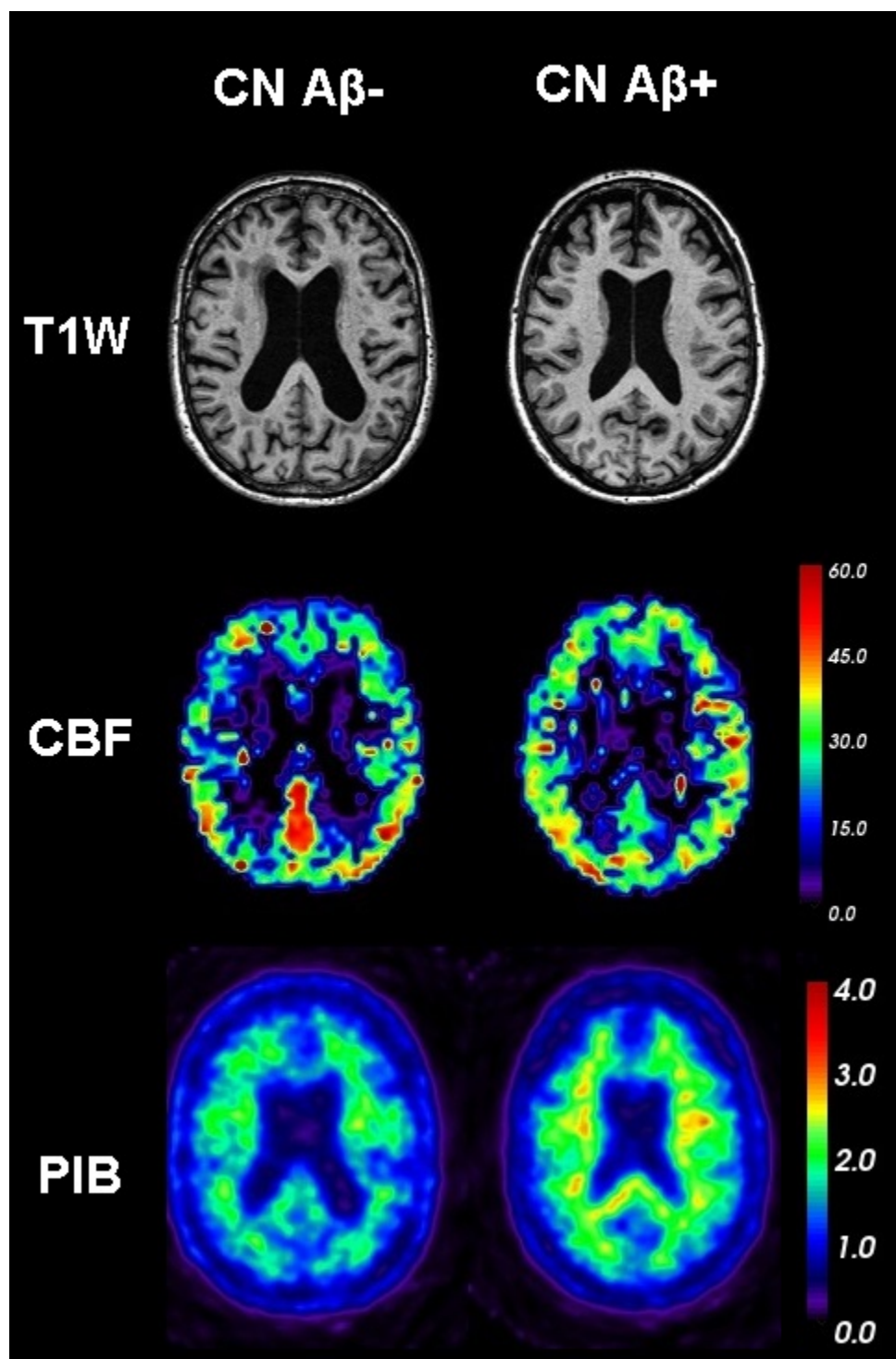
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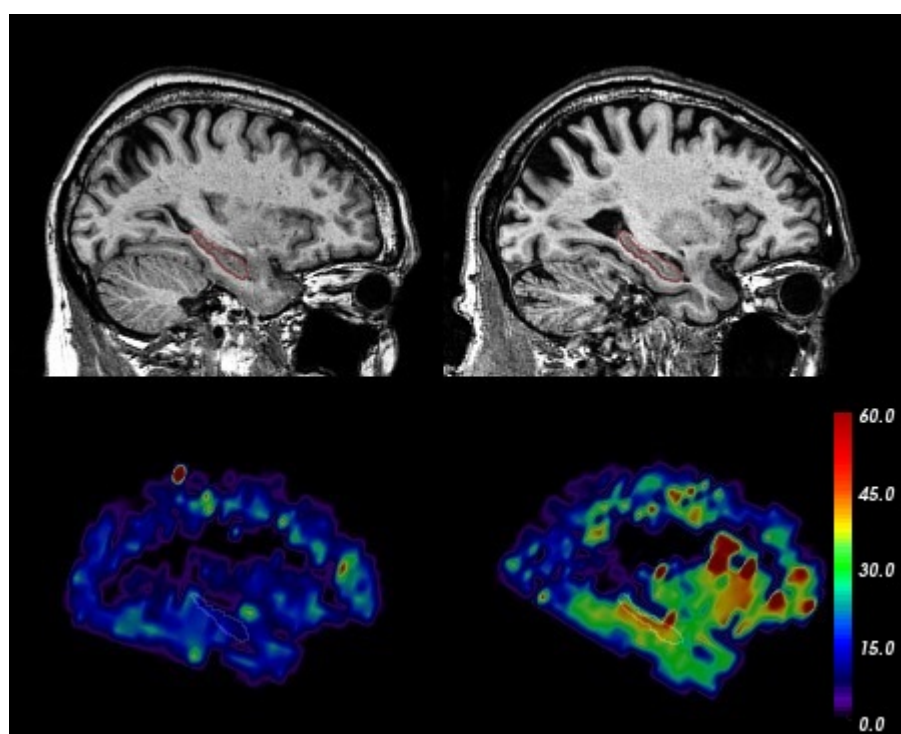
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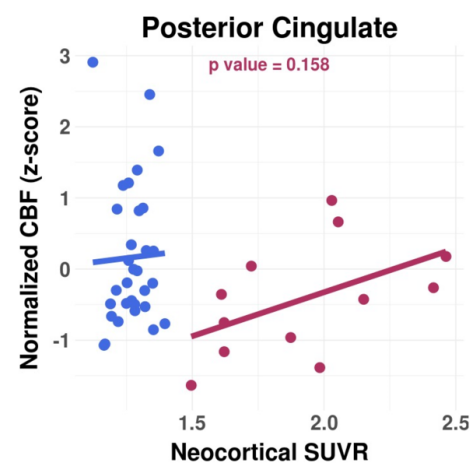
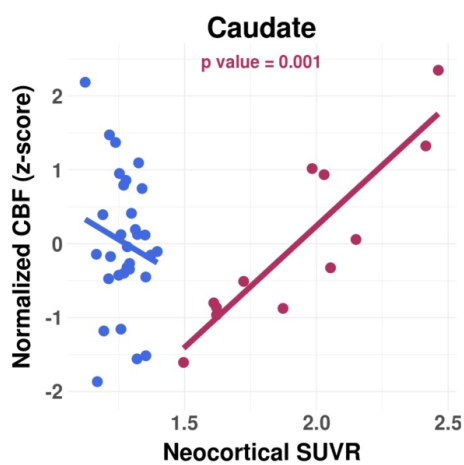
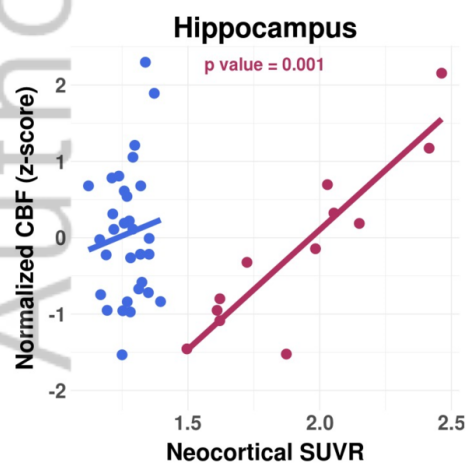
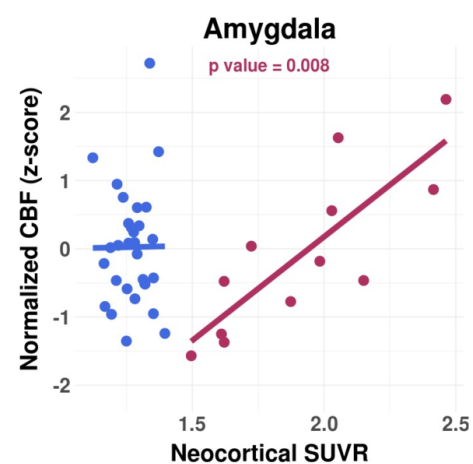
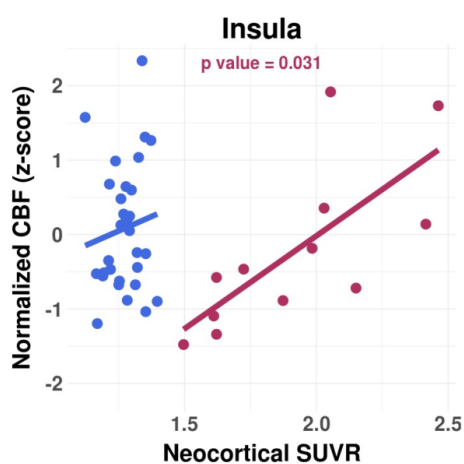
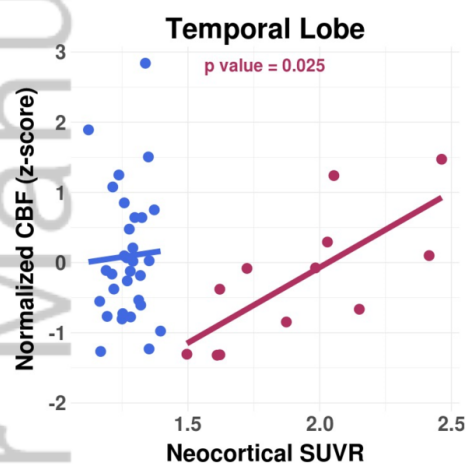
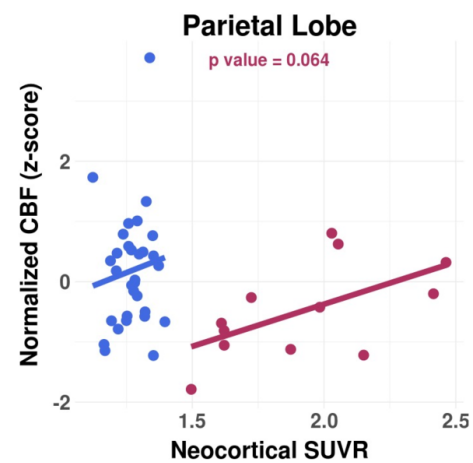
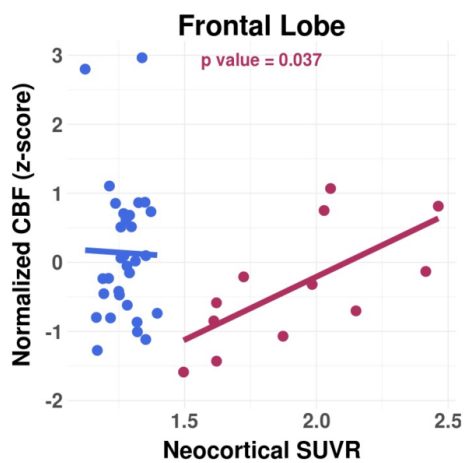
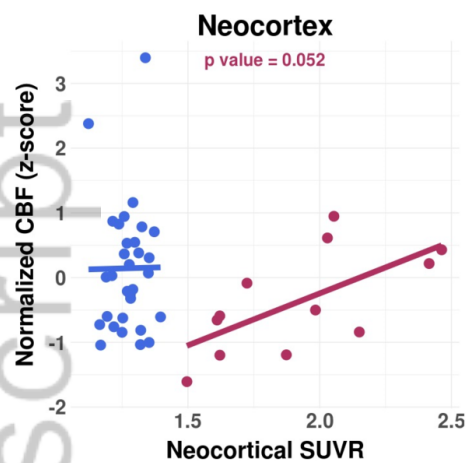
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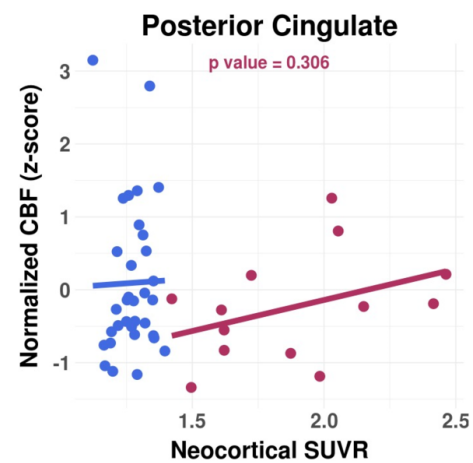
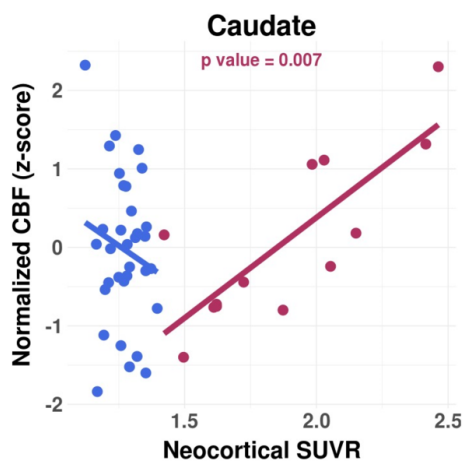
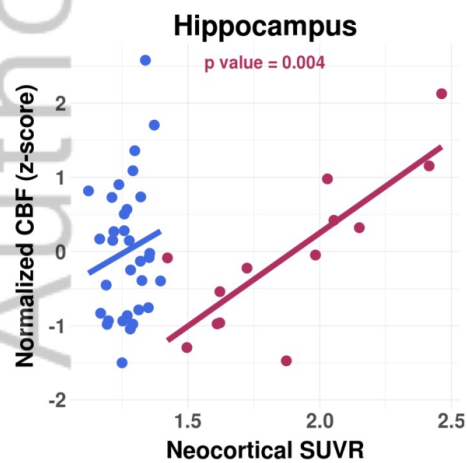
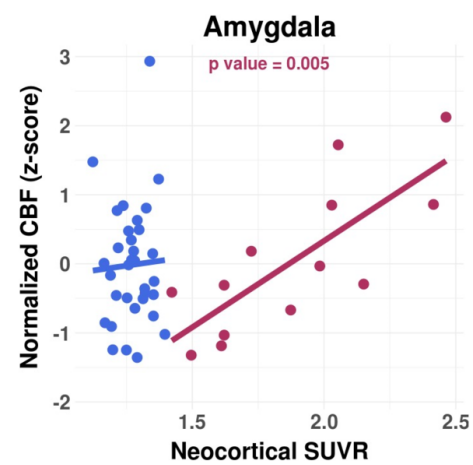
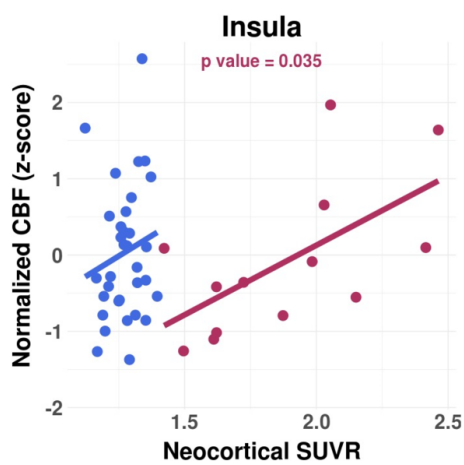
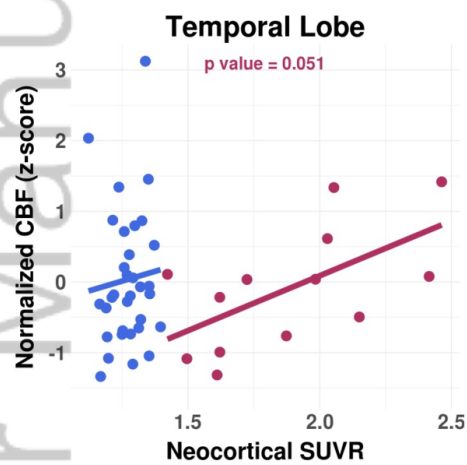
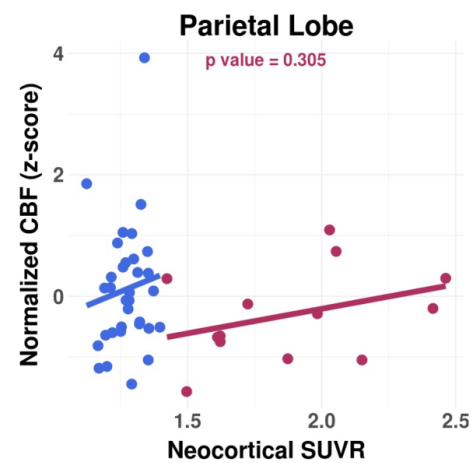
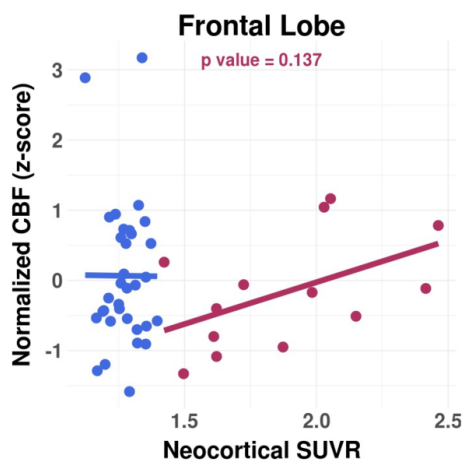
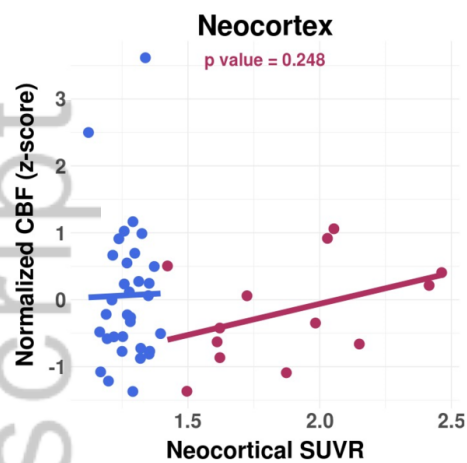
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