# BRAIN FUNCTIONAL CONNECTIVITY IN CEREBRAL AMYLOID ANGIOPATHY, AN AGE-RELATED CEREBROVASCULAR DISEASE

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## BRAIN CONNECTIVITY AND AGING

There is growing evidence that in the aging process both the neural and the vascular aspects of brain function are affected. On the neural side, aging is associated with damage to the myelin sheaths and reduction in the total number of nerve fibers, resulting in a reduction in structural connectivity [1]. But connections in the brain are not only structural (*i.e.* synapses, axons, etc.); equally important are blood flow related (vascular) connections. This hypothesis arose after the first observation that blood flow increased and decreased simultaneously in the two motor regions in opposite brain hemispheres. We call regions such as these *functionally connected*. As we get older, so does our brain's vascular system: the elasticity of small blood vessels in the brain diminishes in some persons [2] and white matter lesions of presumed vascular origin increase [3]. These vascular changes affect blood flow to neurons and thus may reduce functional connectivity. The extent to which these vascular-related phenomena contribute to age-related reductions in structural or functional connectivity (and perhaps to cognitive decline) is not yet clear.

Cerebral Amyloid Angiopathy (CAA) is a common age-related disease characterized by deposition of amyloid beta protein in the small arteries of the brain. It affects in some form between 10% to 40% of elderly people and 80% or more of patients with Alzheimer's disease [4]. Amyloid beta is toxic to smooth muscle cells and causes degeneration of the vessel walls resulting in asymptomatic microbleeds (Fig.1a) and a greatly increased risk of hemorrhagic stroke. Recent human and animal studies suggest that CAA also decreases vascular reactivity to neuronal metabolic demands [5], and that CAA is associated with cognitive dysfunction, independent of the presence of hemorrhagic strokes or any coexisting Alzheimer pathology. It remains unproven if cognitive deficits in CAA are a direct consequence of the vascular dysfunction, or if it develops by other pathways.

**SUMMARY**

**We set out to investigate the difference in brain functional connectivity between healthy aging and persons with the age-related vascular disease cerebral amyloid angiopathy.**

## FUNCTIONAL CONNECTIVITY MRI

We used seed-based **functional connectivity magnetic resonance imaging** (fc-MRI) to assess the functional connection level of brains affected by CAA, to investigate the relationship between known vascular dysfunction and a hypothetical 'disconnection syndrome' which may be responsible for the associated cognitive impairments. We chose this approach because CAA is related to structural connectivity impairment in the form of white matter lesions (Fig.1b), which may affect functional networks, *i.e.* separate brain regions that work together to process information or perform a task [6].

Functional connectivity MRI is a promising new application of functional MRI (fMRI). In standard fMRI, active regions of the brain are identified by the so-called Blood Oxygenation Level-Dependent (BOLD) contrast. As a group of neurons increase their workload (for example your visual processing areas as you are reading this!), they use more oxygen. The small brain vessels then dilate and allow more fresh (oxygenated) blood to replace it. This increase in oxygenated blood causes changes in the MRI signal intensity, because the magnetic field properties of iron atoms in hemoglobin molecules are

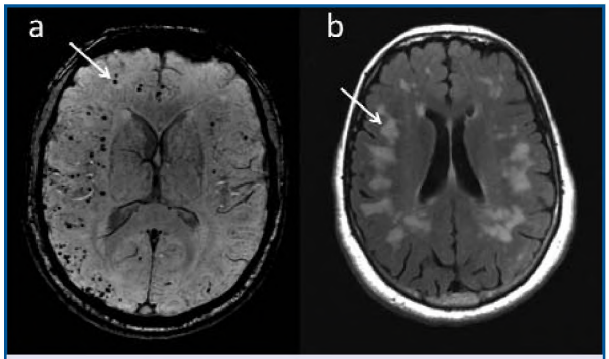


Fig 1. Cerebral Amyloid Angiopathy (CAA) is manifested on MRI images as microbleeds (a) or white matter lesions (b). Because structural white matter integrity - which is essential for connectivity - is known to be affected by CAA, we hypothesized that patients with CAA would also exhibit reduced functional connectivity.

dependent on whether the iron atom is bound to oxygen or not. In fc-MRI, spontaneous BOLD fluctuations at rest are mapped in the whole brain. Interestingly, there are separate regions in which the BOLD signal fluctuates in coherence, *i.e.* are functionally connected. Conserved functional networks have been identified by fc-MRI both during mental activity and during the resting state in normal individuals [7, 8].

Because posterior brain regions are most strongly affected by CAA [9], we chose the primary visual cortex (V1) as the 'seed' region, located in the occipital lobe, and mapped all brain regions that are functionally connected to it. To ensure precise functional location of V1, we ran a visual stimulus fMRI sequence before the fc-MRI sequence. We hypothesized that patients with CAA would exhibit reduced functional connectivity with V1 compared to similar-aged control subjects.

## METHODS

Nine CAA patients (age 72.4±9.4 y) and eleven age-matched healthy controls underwent fMRI during a visual stimulus and during a rest condition. Patients were free of hemorrhagic stroke in the occipital poles, and had normal or corrected-to-normal visual acuity and no visual field deficits. CAA patients had higher volumes of MRI white matter hyperintensities (median 40.9 mL, interquartile range 35.5-54.0 mL) compared to healthy controls (4.8 mL, 3.7-5.7 mL), p=0.002. Participants were recruited and gave informed consent in accordance with the guidelines of the Conjoint Health Research Ethics Board.

Visual stimulus fMRI consisted of four blocks of an 8-Hz contrast-reversing black/white checkerboard for 40 s alternating with 40 s of black cross fixation on a gray screen. To obtain the fc-MRI data, a separate resting-state fMRI sequence was acquired with subjects awake, eyes open, and continuous black cross fixation. Echo-planar images were acquired with standard parameters (TR/TE/FA = 2000ms 30ms/70°, 64x64 matrix, 24 cm FOV, 4 mm contiguous slices, 220 s in duration), and inversion-prepared, 3D gradient echo high-resolution images were also acquired for anatomical registration (TR/TE/TI/FA = 6.0 ms/2.4 ms/650 ms/8°, 256x256 matrix, 24 cm FOV, 1 mm slice thickness). All MRI data were acquired on a 3.0 T scanner using an 8-channel head coil (Signa VH/i, GE Healthcare, Waukesha, WI).

Data analysis was carried out using FSL (http://www.fmrib.ox.ac.uk/fsl). A mask was obtained for each subject's 50 most active voxels (2.8 $cm^3$) within the primary visual cortex. This masked region is schematically represented by the green volume in Fig.2. Whole-brain maps of the functional connectivity with this region were generated for each subject using the resting-state fMRI data. This was done by extracting the average time course of all voxels in the mask and using it in a whole-brain general linear model (GLM) analysis. Finally, a between-group connectivity analysis was performed using a mixed-effects GLM. Clusters corrected to $p<0.05$ were considered significant.

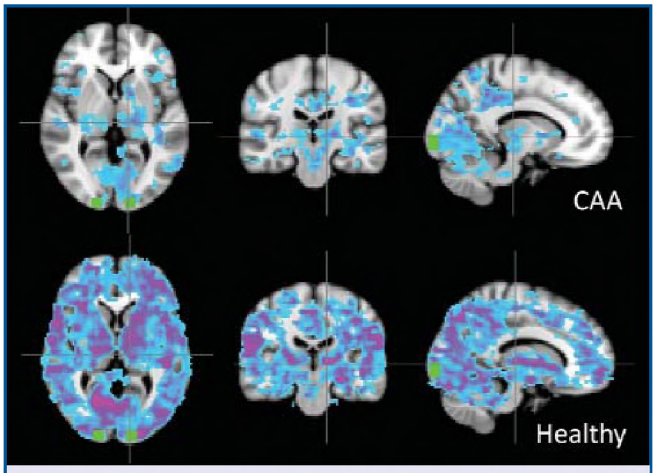


Fig 2. Functional connectivity with primary visual cortex (in green), for CAA patients (top) and controls (bottom). Color scale represents the strength of functional connectivity, expressed as statistical Z-scores ranging from 3.1 (cyan) to 5.0 (purple). Connectivity is weaker in CAA.

## RESULTS: BRAINS ARE 'DISCONNECTED'

Qualitatively, **whole-brain functional connectivity with V1 is weaker in CAA patients** (Fig.2, top) compared to healthy controls (Fig.2, bottom), particularly in regions farther from the seed.

Significant differences in functional connectivity were identified between the two groups in four brain regions (Fig. 3). Connectivity was significantly **reduced** in CAA patients bilaterally in the anterior section of the superior temporal cortices (Brodmann's areas 21 and 22), which are involved in language processing, and in the right putamen, which is associated with motor and executive function (Fig.3, top). Interestingly, connectivity was significantly **greater** in

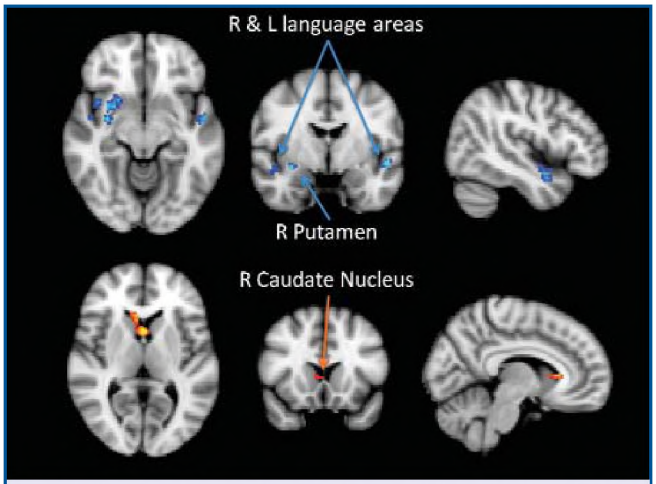


Fig 3 Regions where functional connectivity is significantly reduced (blue) or increased (red) in CAA patients compared to controls. Color scales represent Z-scores ranging from 2.3 to 3.0 (blue-to-light blue) and 2.3 to 2.6 (red-to-yellow).

CAA patients in the body of the right caudate nucleus (Fig.3, bottom), a region involved in learning, working memory, and language comprehension.

## CONCLUSIONS AND FUTURE DIRECTION

Our results suggest that functional connectivity with the primary visual cortex is affected by the presence of vascular amyloid in the brain. The connectivity differences with the right putamen and the right caudate may indicate that these structures are secondarily affected by CAA-related neurodegeneration, as suggested by previous studies that have shown that the basal ganglia may atrophy in neurodegenerative diseases, including Alzheimer's disease [11, 12]. Although it is possible that atrophy brings about functional disconnection, it does not explain our unexpected result of increased connectivity with the right caudate. One potential explanation for this increase is that the caudate nucleus could have been recruited as part of a compensatory network, thus increasing functional connectivity, as has been recently observed in cognitively impaired multiple sclerosis patients [13].

We speculate that the observed reduced connection between the primary visual cortex and the higher-level language processing areas may be a consequence of structural disconnection due to CAA-related white matter disease [6]. However, increased heterogeneity in vascular reactivity, due to CAA-related vascular dysfunction, could also potentially explain some of the observed disconnection in correlated fMRI signal fluctuations because the fMRI signal is dependent on local changes in blood flow. Functional disconnection may underlie some of the cognitive dysfunction that affects CAA patients; however, future larger studies will be needed to address this question. Future studies should also address the question of whether functional connectivity declines with aging, and whether cerebrovascular disease or altered cerebrovascular activity is a component of age-related decline. Further study of the impact of CAA and other age-related cerebrovascular syndromes on brain functional networks is warranted, and functional connectivity MRI will certainly prove to be a valuable tool to investigate these relationships.

## FUNDING

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