Relationships between cerebrovascular reactivity, visual-evoked functional activity, and resting-state functional connectivity in the visual cortex and basal forebrain in glaucoma

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Abstract – Glaucoma is primarily considered an eye disease with widespread involvements of the brain. Yet, it remains unclear how cerebrovasculature is regulated in glaucoma and how different brain regions coordinate functionally across disease severity. To address these questions, we applied a novel whole-brain relative cerebrovascular reactivity (rCVR) mapping technique using resting-state functional magnetic resonance imaging (fMRI) without gas challenges to 38 glaucoma patients and 21 healthy subjects. The relationships between rCVR, visual-evoked fMRI response, and resting-state functional connectivity in glaucoma were then established. In the visual cortex, rCVR has a decreasing trend with glaucoma severity ($p<0.05$), and is coupled with visual-evoked response and functional connectivity in both hemispheres ($p<0.001$). Interestingly, rCVR in the basal forebrain (BF) has an increasing trend with glaucoma severity ($p<0.05$). The functional connectivity between right diagonal band of Broca (a sub-region of BF) and lateral visual cortex decreases with glaucoma ($p<0.05$), while such connectivity is inversely coupled with rCVR in the BF ($p<0.05$), but not the visual cortex. Overall, we demonstrate opposite trends of rCVR changes in the visual cortex and BF in glaucoma patients, suggestive of compensatory actions in vascular reserve between the two brain regions. The neurovascular coupling within the visual cortex appears deteriorated in glaucoma, whereas the association between BF-visual cortex functional connectivity and rCVR of BF indicates the functional and vascular involvements in glaucoma beyond the primary visual pathway.

Clinical Relevance – This study provides insights towards our understanding of glaucoma etiology in the brain, which may help guide better strategies for glaucoma detection and treatment.

I. INTRODUCTION

Cerebrovascular reactivity (CVR) is the response of cerebral blood vessels to vasoactive stimuli. CVR impairments often precede and contribute to neuropathology over time. Glaucoma can cause progressive vision loss and irreversible blindness, and is primarily considered an eye disease with widespread involvements of the brain. Doppler ultrasound studies showed CVR reduction in the visual cortex[1] and middle cerebral artery[2,3] in glaucoma patients. However, whether and how glaucoma could induce widespread CVR changes in other brain regions is yet to be elucidated, partly due to limited whole-brain CVR mapping techniques. Previous task-evoked functional magnetic resonance imaging (fMRI) and resting-state fMRI (rsfMRI) studies also demonstrated that visual cortex activity[4-6] and functional connectivity[7,8] are impaired in glaucoma, respectively. However, whether CVR is coupled with visual brain fMRI response and functional connectivity changes in glaucoma remains unclear.

Beyond the primary visual pathway, the basal forebrain (BF) has cholinergic projections to the visual cortex[9-12], and can play a role in visual perception, visual attention and cortical plasticity[13-18]. Since visual cortex may possess lower choline levels in glaucoma[5,19], we postulate that BF is involved in glaucoma[19]. While initial evidence indicated functional connectivity changes between BF and other brain regions in glaucoma[8], whether local changes occur in BF with glaucoma severity, and how functional brain connectivity relates to local changes in BF remain unexplored.

Whole-brain CVR mapping is typically performed using hypercapnic gas inhalation as a vasoactive challenge while collecting blood-oxygen-level-dependent (BOLD) fMRI images[20]. The required gas inhalation setup presents an obstacle for routine clinical use. Recently, relative CVR (rCVR) mapping is achieved using rsfMRI[21] without gas challenges, and has been shown to resemble hypercapnia-inhalation CVR mapping. This provides a task-free method to measure vascular reserve. In this study, we applied whole-brain rCVR mapping using rsfMRI to verify visual cortical vascular reserve changes in glaucoma patients across different severity. We then established the relationship between rCVR, visual function response, and functional connectivity in the visual cortex with glaucoma severity. Lastly, we explored rCVR and functional connectivity changes involving the BF.

II. MATERIALS AND METHODS

A. Study approval and clinical assessment

This study followed the tenets of the Declaration of Helsinki and was conducted in compliance with the Health Insurance Portability and Accountability Act. The institutional review

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board and ethics committee approved this study. Optical coherence tomography measurements [peripapillary retinal nerve fiber layer (RNFL) thickness, macular ganglion cell and inner plexiform layers (GCIPL) thickness, optic nerve head cup/disc ratio], and Humphrey visual field mean deviation (VF-MD) were obtained from clinical records.

B. MRI protocol

Normal controls (n = 21; 41 – 79 years old), early glaucoma patients (n = 19; 50 – 74 years old), and advanced glaucoma patients (n = 19; 48 – 84 years old) underwent anatomical MRI and rsfMRI. A subset of 9 normal controls, 13 early glaucoma patients, and 12 advanced glaucoma patients underwent fMRI with visual stimulation (vfMRI) using 8 Hz flickering checkerboard patterns to the upper or lower field of each subject[5]. Experiments were performed with a 3T Allegra scanner (Siemens, Germany) after obtaining informed written consent. Anatomical MRI was performed with T1-weighted MRI using a 3D magnetization prepared rapid acquisition gradient echo (MPRAGE) pulse sequence with echo time (TE)/inversion time (TI)/repetition time (TR) = 2.5/800/1400 ms, flip angle = 8°, field of view (FOV) = 25.6×25.6×17.6 cm^3, 256×256 matrix, and 176 contiguous 1-mm sagittal slices. rsfMRI images were collected using a single-shot echo-planar-imaging (EPI) pulse sequence with TE/TR = 26/2000 ms, FOV = 20.5×20.5 cm^2, 64×64 matrix, 38 contiguous 3-mm axial slices, and 240 volumes. vfMRI images were collected using the same single-shot EPI pulse sequence as rsfMRI except for 28 contiguous 3-mm axial slices, and 300 volumes.

C. Data analyses

The optic nerve and optic chiasm volumes were measured from T1-weighted images using ImageJ (imagej.nih.gov/ij/). Voxel-wise CVR index (α) is first calculated using a general linear model between normalized BOLD time series (ΔBOLD/BOLD) and the global signal time series (GS). Voxel-wise rCVR is then obtained by normalizing α by tissue signal intensity averaged across the whole brain (SI). These steps can be summarized as follows. Note the residuals term (β) was not used for analysis.

$$rCVR = \frac{\alpha}{SI} \text{ where } \alpha \text{ is obtained from } \frac{\Delta BOLD}{BOLD} = \alpha \cdot GS + \beta$$

rCVR maps were generated from the rsfMRI images with MriCloud (braingps.mricloud.org/). rCVR values were extracted using Montreal Neurological Institute (MNI) defined regions of interest (ROIs). vfMRI maps and % BOLD were generated and extracted using a combination of SPM subroutines (fil.ion.ucl.ac.uk/spm/) and in-house code. For functional connectivity, rsfMRI images were analyzed using the Functional Connectivity toolbox (web.conn-toolbox.org/) with default pre-processing pipeline. ROIs were defined in MNI space for both vfMRI and rsfMRI. Results are presented as mean ± standard error of mean (SEM). One-way ANOVA followed by Bonferroni's post hoc test and trend analysis were applied. For scatter plots, linear regression was applied.

III. RESULTS

A. Clinical ophthalmic assessments and volumetric MRI assessments in normal and glaucoma groups

When comparing clinical ophthalmic assessments (RNFL thickness, GCIPL thickness, optic nerve head cup/disc ratio,

and VF-MD) and volumetric MRI assessments (optic nerve volume and optic chiasm volume) between normal, early glaucoma and advanced glaucoma groups, RNFL thickness, GCIPL thickness, VF-MD, optic nerve volume, and optic chiasm volume show decreasing trends with severity (p < 0.001), while optic nerve head cup/disc ratio has an increasing trend (p < 0.001) (**Figure 1**). These characterize the anterior visual pathways and clinical visual function in the groups.
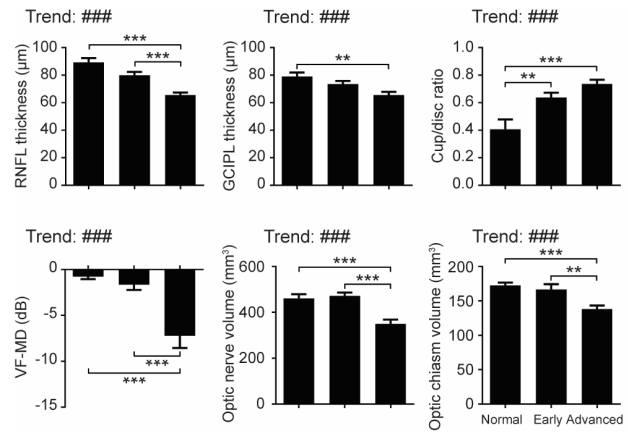

Figure 1. Clinical and anatomical MRI assessments characterize the anterior visual pathways among the normal control, early glaucoma, and advanced glaucoma groups. RNFL thickness, GCIPL thickness, VF-MD, optic nerve volume and optic chiasm volume have decreasing trends with glaucoma severity, while cup/disc ratio has an increasing trend. One-way ANOVA followed by Bonferroni's post-hoc tests (**p<0.01 and ***p<0.001) and trend analyses (###p<0.001) were applied.

B. rCVR decreases in the visual cortex and increases in the basal forebrain with glaucoma severity

Average rCVR maps were calculated for the groups (**Figure 2**). The average rCVR map from normal controls is consistent with a previous study[21]. rCVR in the bilateral, left, and right visual cortex have decreasing trends with severity (p < 0.05). No significant rCVR change was observed in the somatosensory cortex. Interestingly, rCVR in the right basal forebrain have an increasing trend with severity (p < 0.05).

C. rCVR, visual function response, and functional connectivity are coupled in the visual cortex in glaucoma

We further analyzed the rCVR, visual response and functional connectivity in the sub-regions of the visual cortex including the primary [Brodmann Area (BA) 17], secondary (BA 18), and tertiary (BA 19) visual cortices. rCVR in bilateral BA 17 has a decreasing trend with glaucoma severity (p < 0.05), but not BA 18 or BA 19 (**Figure 3A**). The visual responses in BA 17 were significantly lower in the advanced glaucoma patients compared to normal controls (p < 0.05) and early glaucoma patients (p < 0.01), but not BA 18 or BA 19 (**Figure 3A**). In addition, visual responses exhibit decreasing trends with glaucoma severity in bilateral BA 17 (p < 0.05) and BA 18 (p < 0.05), but not BA 19 (**Figure 3A**). Bilateral functional connectivity decreased with glaucoma severity in BA 18, but not BA 17 or BA 19 (**Figure 3A**). Using scatter plots (**Figure 3B**), our results demonstrate that rCVR, visual responses and functional connectivity are coupled in the visual cortex in glaucoma and healthy subjects (p < 0.001).
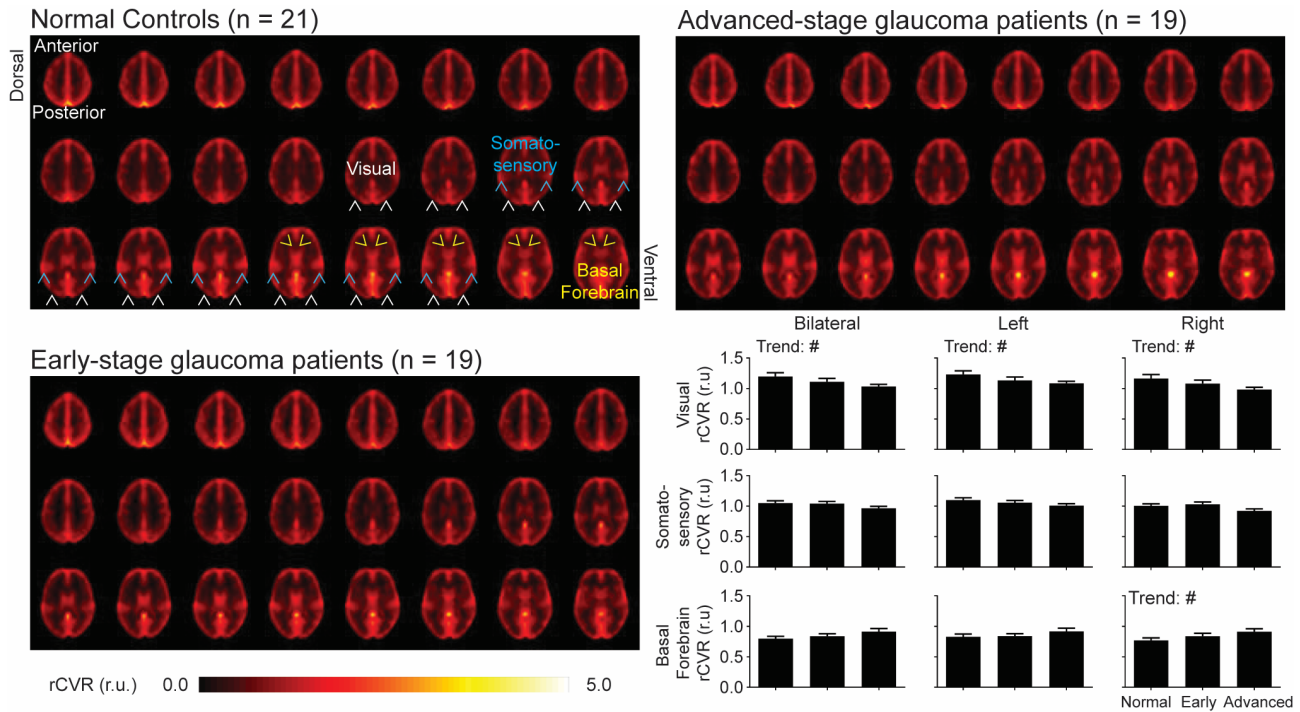
Figure 2. rCVR decreases in the visual cortex but increases in the right basal forebrain with glaucoma severity. Average rCVR maps were calculated for normal controls, early glaucoma patients, and advanced glaucoma patients. rCVR values were extracted from the visual cortex, somatosensory cortex and basal forebrain, and compared between the groups. rCVR in the bilateral, left and right visual cortex show decreasing trends with glaucoma severity. No significant rCVR change was observed in the somatosensory cortices. Interestingly, rCVR in the right basal forebrain shows an increasing trend with glaucoma severity. One-way ANOVA followed by Bonferroni's post-hoc tests (*$p < 0.05$) and trend analyses (#$p < 0.05$) were applied.

D. rCVR in the basal forebrain is associated with functional connectivity between basal forebrain and visual cortex

While BF and visual cortex exhibit an inverse relationship in rCVR across glaucoma and healthy subjects (**Figure 4A**), within sub-regions of the BF, functional connectivity between bilateral diagonal band of Broca (DB)[12] exhibited a decreasing trend ($p < 0.05$) with glaucoma severity, while no such trend was found between bilateral basal nucleus of Meynert[22] (BNM; **Figure 4B**). Comparing between BF sub-regions and the visual cortex, functional connectivity between right DB and left/ right lateral visual cortex decreases with glaucoma severity ($p < 0.05$; **Figure 4C**), but not between left DB and left/right lateral visual cortex or between BNM and lateral visual cortex with glaucoma severity. Furthermore, functional connectivity between right DB and left/right lateral visual cortex are inversely coupled with rCVR in BF, but not with rCVR in the visual cortex (**Figure 4D**).

IV. DISCUSSION

Despite recent *in vivo* imaging efforts in understanding widespread structural, metabolic, and functional brain changes in glaucoma[23,24], it remains controversial whether glaucoma involves vascular impairments in the visual system and beyond[25]. In this study, the observed rCVR reduction in the visual cortex with glaucoma severity (**Figure 2** and **Figure 3A**) corroborates with previous Doppler ultrasound studies showing CVR reduction in the visual cortex[1] and middle cerebral artery[2,3] in glaucoma patients upon hypercapnic gas challenges. Conventionally, CVR reflects the regulatory
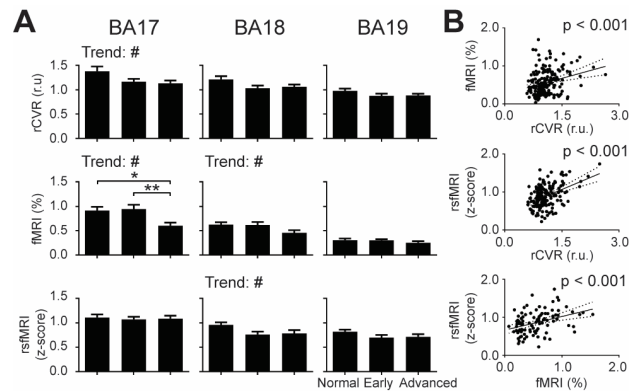
Figure 3. rCVR, visual response and functional connectivity in visual cortex are coupled. (A) rCVR (top) in bilateral BA 17 has a decreasing trend with glaucoma severity, but not BA 18 nor BA 19. Visual fMRI response (middle) in bilateral BA 17 was significantly lower in the advanced glaucoma, but not BA 18 nor BA 19. Decreasing trends in fMRI response with severity are observed in BA 17 and BA 18, but not BA 19. Bilateral functional connectivity (bottom) had a decreasing trend in BA 18, but not BA 17 nor BA 19. **(B)** rCVR, visual response and functional connectivity are coupled in glaucoma. Note that a data point on the scatter plot represents a sub-region of a subject. One-way ANOVA followed by Bonferroni's post-hoc tests (*$p <0.05$, and **$p<0.01$) and trend analyses (#$p<0.05$) were applied.

ability of the cerebrovasculature and is inferred by measuring the magnitude of the cerebral blood flow (CBF) response after applying a vasodilatory stimulus. Resting CBF in the primary visual cortex has been shown to be lower in glaucoma patients
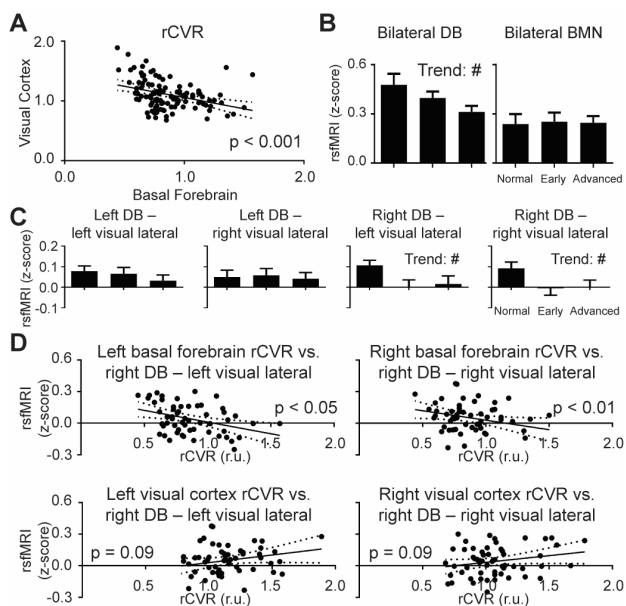
Figure 4. rCVR in the basal forebrain is inversely associated with both rCVR in the visual cortex and rsfMRI connectivity between basal forebrain and visual cortex. (A) An inverse relationship in rCVR between BF and visual cortex in healthy, early glaucoma, and advanced glaucoma subjects is observed. **(B)** rCVR in bilateral DB has a decreasing trend with glaucoma severity, but not BMN. **(C)** Early glaucoma patients have significantly lower rsfMRI connectivity between right DB and left/right lateral visual when compared to normal controls, but not between left DB and left/right lateral visual. **(D)** The rsfMRI connectivity between right DB and left/right lateral visual are inversely coupled with rCVR in the left/right BF (top), but not with rCVR in the left/right visual cortex (bottom).

and such reduction is correlated with the loss of visual function[26] and functional connectivity[27]. Our results show a significantly lower visual response in advanced glaucoma patients that is correlated with rCVR measurements across subjects (**Figure 3**). Taken together, these observations further solidify the presence of cerebrovascular deterioration in the visual cortex, supporting glaucoma as a manifestation of a degenerative disease involving neurovascular dysfunction.

Since visual cortex has lower choline levels in glaucoma[5,19], while acetylcholine deficits may lead to altered vascular physiology[28] such as vasomotor dysfunction and increased arterial stiffness, it is possible that the reduced rCVR in the visual cortex in glaucoma can be explained by its metabolic state with respect to cholinergic dysfunctions. In contrast, since BF (including DB[12]) is considered a major cholinergic output of the central nervous system[12,16], together with the reduced functional connectivity between BF and the visual cortex among other regions[8] in glaucoma, we speculate that the rCVR increase observed in the right BF of glaucoma patients may be explained by the accumulation of choline-containing compounds as a result of reduced BF projections to the cortex or enhanced visual attention needed to compensate for the same task performance in glaucoma. These suggest functional and neurovascular involvements of the BF in glaucoma. Future studies are warranted to elucidate the underlying mechanisms of these rCVR changes, and their association with brain-wide network disruption in glaucoma as demonstrated by structural and functional connectivity studies.

Cholinergic neurons from BF to the visual cortex[9-11] can act to coordinate cognitive operations, modulate visual processing and alter visual perception[13-18]. BF also has cholinergic projections to cortical microvessels[29] for regulating cerebral cortical blood flow. More recently, the BF is postulated to modulate intracranial pressure and intraocular pressure[19]. Perivascular cholinergic innervations from the BF to the cortex may also help modulate metabolic brain waste clearance via the cerebrospinal fluid[30], whereas the metabolic waste clearance system is recently suggested to be impaired in glaucoma[31]. Besides cholinergic neurons, BF also consists of glutamatergic and GABAergic neurons. However, there has been no direct evidence of the differential effects of these cell-type specific pathways on brain-wide circuit function, especially in the macroscopic and mesoscopic scales. In the future, investigating the causality of BF-visual cortex influences in glaucoma may improve our fundamental understanding of disease etiology and guide better strategies for glaucoma detection and treatment.

V. CONCLUSION

rCVR mapping using rsfMRI detected widespread brain changes in glaucoma patients without the need of gas challenges. rCVR is reduced in the visual cortex with glaucoma severity, and is associated with visual-evoked response and functional connectivity in the same regions. The rCVR and functional connectivity changes in the basal forebrain indicate the vascular and functional involvements in glaucoma beyond the primary visual pathways.

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