A multimodal approach to identify wide-field ganglion cell types in macaque and human retina

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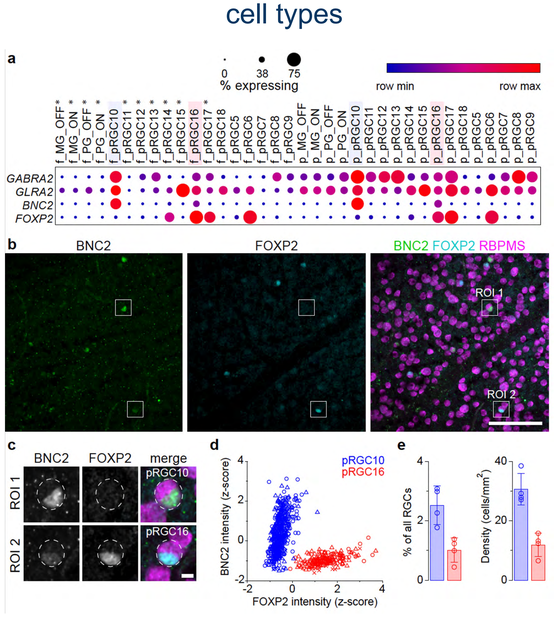
Introduction

Glaucomatous optic neuropathy is a leading cause of visual impairment and blindness that leads to progressive dysfunction and ultimately the death of retinal ganglion cells (RGC). There are at least 12 different human RGC types, each of which is thought to send a parallel representation of the visual scene to the brain (Yan et al., 2020). Of these types, the midget, parasol and small-bistratified RGCs are the most abundant and well characterized, together making up around 80% of the output from the optic nerve. However, the remaining wide-field RGCs remain poorly characterized, owing to their sparsity and the lack of tools to identify them. Understanding wide-field RGCs is important in the context of glaucoma for several reasons. Not only are wide-field RGCs expected to have less functional redundancy, but specific RGC types may also be more vulnerable to glaucomatous injury. Thus, functional tests tailored to stimulate wide-field RGC types may be more sensitive for detection of early glaucomatous damage (Landers et al 2003; Johnson et al 1994).

Here, we describe a multimodal approach, combining molecular, morphological and functional methods, to classify wide-field RGCs in non-human primate and human retina. Using this approach, we provide evidence for an ON-type direction selective ganglion cell in macaque retina, a wide-field RGC type that is involved in reflexive gaze stabilization in lower mammals but has hitherto remained elusive in primates. In lower mammals, ON-DSGCs provide the afferent input for the optokinetic reflex (OKR), a reflexive eye movement that serves to stabilize motion of the visual field on the retina. The OKR may hold promise for objective assessment of peripheral visual function in glaucoma (Doustkouhi et al., 2020; McDonald et al, 2022). Thus, understanding the retinal inputs to this pathway may be important for developing improved subjective tests for detection of glaucoma.

Results

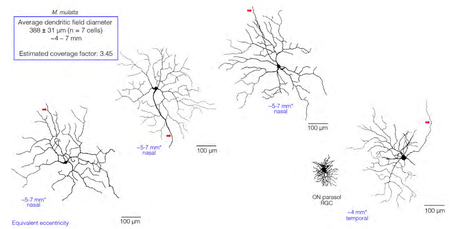
1 Molecular identification of wide-field retinal ganglion cell types

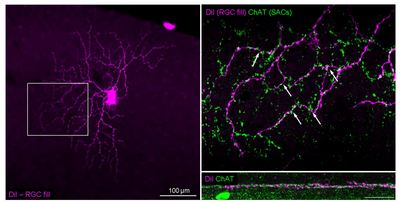


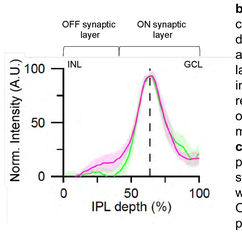
a. We mined an existing single-cell transcriptome from macaque retina (Peng et al., 2019) to identify a candidate direction selective RGC based on high foveal expression of inhibitory neurotransmitter receptors important for directional and speed tuning of these cells (*GABRA2, GLRA2*). The candidate cell type, RGC10, can be distinguished based on expression of *BNC2* and the absence of *FOXP2* expression. **b-c.** RGC10 cells (BNC2+/FOXP2-) can be identified in macaque retina with immunohistochemistry **e.** Summary showing the percentage and density of RGC10 (blue) and RGC16 (red) cells in macaque retina. Note that RGC10 cells are present at low density and comprise ~2.5% of all RGCs in peripheral nasal retina. Scale bar = 100 µm in b, = 5µm in c.

2 RGC10 cells have the morphology and stratification expected for an ON-DSGC

a. To reveal the morphology of RGC10 cells, we prelabeled macaque retinas with BNC2 and targeted cells for intracellular injection with the lipophilic tracer, DiI. The filled cells were monostratified wide-field RGCs with sparse dendritic arbors and some dendritic segments that reflected back towards the soma. The morphology of these cells is similar to the recursive monostratified RGCs described previously by Kim et al., 2022. Axons are indicated by red arrows.

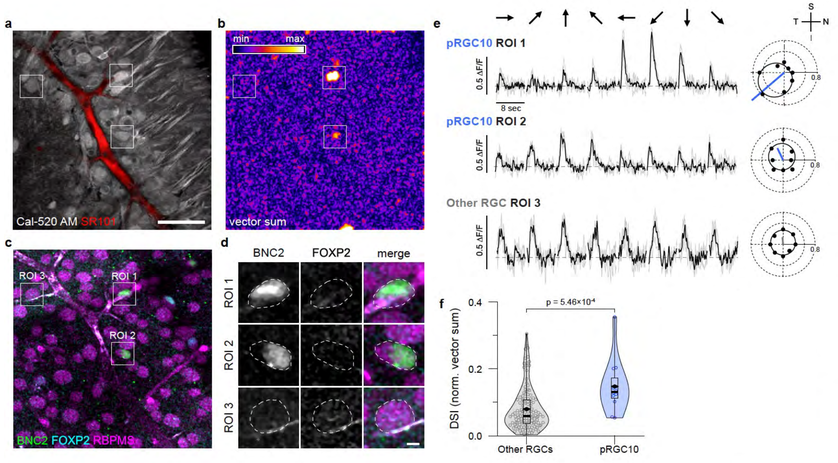






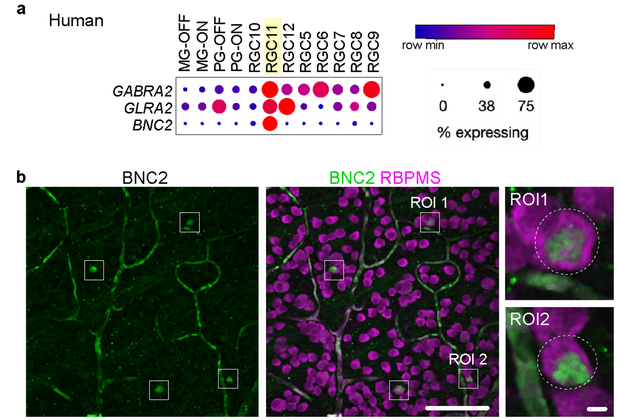
b. RGC10 dendrites (magenta) co-fasciculate with the dendrites of ON-starburst amacrine cells (SACs, green, labeled with ChAT), the inhibitory interneurons that are required for directional tuning of ON-DSGCs in other mammals (Vaney et al., 2012). **c.** Fluorescence intensity profiles showing co-stratification of RGC10 cells with ON-SAC dendrites in the ON sublamina of the inner plexiform layer.

3 RGC10 cells are functionally direction-selective



To determine whether RGC10 cells were direction-selective, we used two-photon calcium imaging to record light-evoked activity from neurons in the macaque ganglion cell layer. The stimulus was a drifting bar moving at 2.5 deg/s in 8 different directions as indicated above traces in **e**. **a.** A sample imaging field of the macaque ganglion cell layer showing the calcium indicator Cal520-AM and a vascular marker SR101. **b**. Heat map of the area shown in **a** highlighting pixels in the scan field with directionally tuned signals (vector sum mapping). **c.** Same area as in **a-b** immunolabeled for BNC2, FOXP2 and RBPMS to identify RGC10 cells after calcium imaging. **d.** ROIs showing examples of RGC10 (ROI 1 & 2) and another RGC type that showed no directional tuning (ROI 3). **e.** Delta F/F calcium responses for ROIs shown in a-d (left) and polar plots summarizing response tuning (right). **f.** Summary data showing a higher direction selectivity index (DSI, normalized vector sum) in RGC10 cells compared to other RGC types.

4 Immunolabeling of candidate ON-DSGCs in human retina



a. Prior studies have shown that RGC11 (highlighted) is the human homolog of the macaque RGC10 cell (Yan et al., 2020). By mining an existing dataset, we found human RGC11 expresses high levels of *GABRA2, GLRA2* and *BNC2* like the homolog in macaque retina. **b.** Immunostaning of RGC11 cells in human retina. These cells make up 0.88% of the total RGC population in the peripheral nasal human retina. Scale bar = 100 µm in b, = 5µm in c.

Conclusions

- RGC10 is an On-type RGC with morphology similar to the recursive monostratified RGCs described previously (Kim et al., 2022).
- RGC10 cells cofasciculate with ON-type starburst amacrine cells, the interneurons that provide asymmetric inhibitory input required for direction-selectivity in other mammals.
- By aligning data from calcium imaging experiments with *post-hoc* molecular identification, we show that RGC10 cells are functionally direction-selective.
- Our results provide evidence for a previously uncharacterized cell type in the primate retina and resolve long-standing debate as to whether direction selective RGCs are present in primates. More generally, our approach demonstrates the power of a combined molecular, morphological and functional approach to resolve the functions of uncharacterized RGC types.
- These results advance understanding of ganglion cell function in the normal human retina and thus provide a foundation for understanding functional changes in RGCs in the content of glaucoma.

Materials & Methods

Tissues: Eyes were obtained from adult rhesus macaques (*M. mulatta*) that were euthanized for unrelated experiments. Human donor eyes were obtained from the Lions Vision Gift (Portland, OR).

Two photon calcium imaging: Retinal pieces with choroid and pigment epithelium attached were maintained in carbogenated Ames' media and bolus loaded with the green fluorescent calcium indicator, Cal-520 AM. Recordings were made on a Scientifica multiphoton microscope and visual stimuli were delivered to the sample through a 20x/0.95 objective.

Morphological analysis: Fixed retinas were pre-labeled with antibodies for BNC2 and ChAT and BNC2+ cells were targeted for injection with the lipophilic tracer, DiI. Volumetric imaging of cell fills was performed on a Zeiss LSM 880 confocal microscope and analyzed in Image J using the SNT plugin.

Data & statistical analysis: Image based analysis was performed in Image J and functional analysis was performed in Igor Pro 9 (Wavemetrics) using custom routines. Statistical comparisons were made using Igor Pro 9.

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