Ganglion cell dysfunction in glaucoma

Yvonne Ou, Luca Della Santina, Felice Dunn
Department of Ophthalmology, University of California, San Francisco
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INTRODUCTION

Major gaps in glaucoma diagnosis and management are the lack of objective tools for functional measurements that permit earlier diagnosis and improved progression detection. Furthermore, the improved sensitivity of functional measurements would greatly benefit clinical trial endpoints in future neuroprotection and neuroregeneration studies that are independent of intraocular pressure (IOP)-based outcomes. In diagnosing early glaucoma, optic nerve imaging can identify changes that precede the development of visual field defects. However, in more advanced glaucoma, a "floor effect" is reached and this imaging is no longer effective at identifying disease progression. Another major clinical tool is visual field testing, which is a subjective test requiring patient cooperation and is not sensitive enough to identify early changes when RGC function is diminished but not yet irreversibly damaged. Indeed, it has been estimated that up to 35% of RGCs are lost prior to detectable visual field loss (Kerrigan-Baumrind et al., 2000; Medeiros et al., 2013). Therefore, ***major gaps*** in glaucoma management include the inability to a) identify early functional perturbations and b) monitor glaucoma progression in patients using an **objective** test that can identify **functional impairment of specific visual pathways** that are vulnerable early in the disease.

This pilot project seeks to exploit recent evidence generated in our laboratory that OFF ganglion cells are selectively vulnerable in glaucoma (Della Santina and Ou, 2016; Ou et al., 2016). Our *central hypothesis* is that OFF ganglion cells and the OFF pathway are damaged earlier and to a greater extent than ON ganglion cells and the ON pathway. The *objective* of this project is to determine the cell type and visual pathway specificity of RGC dysfunction in a mouse model of experimental glaucoma, with the ***overall goal of identifying novel ERG protocols that could be adopted in clinical practice to assess perturbations in the retinal function of glaucoma patients, especially when visual field defects are not yet detectable or their progression cannot be followed with sufficient sensitivity***.

DESIGN & METHODS

Laser-induced ocular hypertension (LIOH) model. We have modified a laser model that results in transient IOP elevation (Salinas-Navarro et al., 2009; Fu and Sretavan, 2010). This robust model is useful for studying acute changes in RGC structure and function and displays features seen in experimental glaucoma models, such as specific and sectorial damage to RGC somas and axons. Histology (Fu and Sretavan, 2010) and OCT imaging (Zhang et al., 2017) demonstrate that other retinal layers are not thinned. Briefly, laser photocoagulation using a diode laser of the limbal and episcleral veins is performed to transiently obstruct aqueous outflow (Ou et al., 2016; Zhang et al., 2017). Each animal (CD-1; age 2 mo.) received one treatment with the contralateral eye serving as control. IOP is transiently elevated with peak IOP occurring after 1 day and returning to baseline by 7 days (data not shown).

ERG recordings. 7, 14, and 30 days after IOP elevation, mice were dark adapted overnight prior to ERG recordings (Celeris, Diagnosys). Full field illumination of the eyes was achieved and three stimulus patterns were adopted to assess specific retinal processing pathways:
1) Brief scotopic flashes, generating the typical flash ERG response (a- and b-waves) ranging from 0.003 to 100 cd s/m^2.
2) Brief photopic flashes (1 to 100 cd s/m^2) delivered on a steady rod-saturating white background (30 cd/m^2). Photopic ERG responses will be recorded after 10 minute adaptation to background light.
3) Sinusoidal flicker stimuli with frequencies ranging from 0.5 to 30 Hz, to elicit periodic responses and evaluate the frequency-response profile of each animal.

Light response properties of specific RGC types. Using the patch clamp technique, we measured spontaneous and light-evoked currents and spiking activity from αON-sustained (αON-S), αOFF-sustained (αOFF-S), and αOFF-transient (αOFF-T) ganglion cells. Beginning in cell-attached mode, we quantified spike activity. Then we used whole-cell patch clamp to measure excitatory currents. We quantified spontaneous activity in the absence of light stimuli. We also quantified the spiking and excitatory current responses to a brief square-wave LED stimulus of varying intensities. We measured the responses from darkness to probe rod-driven pathways, as well as use a rod-adapting background and UV light flashes to saturate rods and stimulate the cones in ventral retina where UV opsin dominates. These flash families without and with the background were used to determine the average intensity-response relationships for each cell type.

ERG RECORDINGS

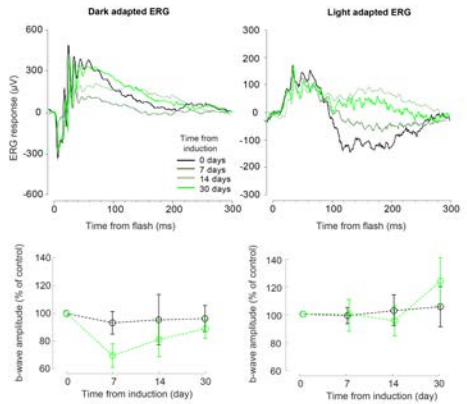

Figure 1. Scotopic but not photopic ERG responses are affected after IOP elevation.
In the upper panel, example tracings are shown for longitudinal scotopic (left) and photopic (right) ERG recordings at 0, 7, 14, and 30 days after IOP elevation (flash intensity 100 cd*s/m^2). In the lower left panel, the scotopic normalized b-wave amplitude is decreased 7 days after IOP elevation and gradually returns to baseline at 30 days (n=8). In the lower right panel, there is no change to the photopic normalized b-wave amplitude. Values are expressed as relative to the response amplitude in the same animal recorded before the procedure.

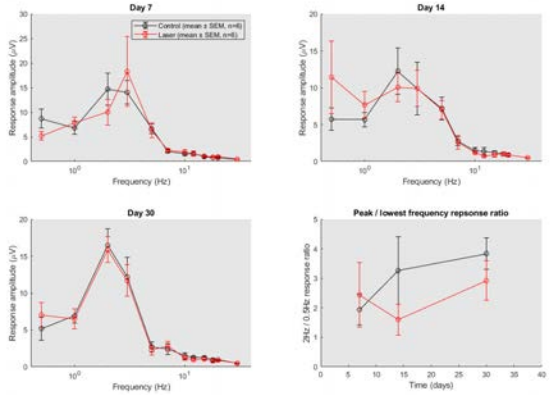

Figure 2. Frequency response curves to sinusoidal flicker stimuli at 7, 14, and 30 days after IOP elevation.
The frequency response curves to flicker stimuli (average intensity 30 cd/m^2, 100% contrast) at the various time points do not show any significant difference between the control and laser conditions. In the lower right panel, the ratio between the response at 2 Hz (peak response) vs. 0.5 Hz (lowest frequency tested) is plotted to examine any changes to bandpass properties.

SINGLE CELL RECORDINGS

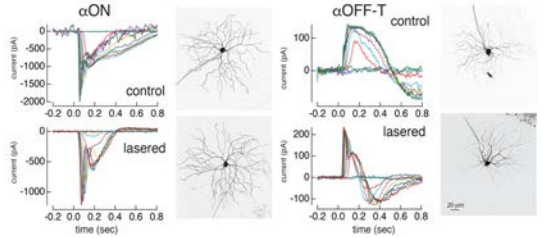

Figure 3. Light responses of αON-sustained and αOFF-transient RGCs 14 days after IOP elevation.
The tracings show responses to a flash family made up of 10 ms flashes doubling in intensity. The first flash strength is 0.015 Rh*/rod. Adjacent to the tracings are confocal images of the dye-filled RGCs; RGC type was confirmed with SMI-32 (not shown).

CONCLUSIONS & NEXT STEPS

- **Scotopic but not photopic ERG responses are transiently affected after IOP elevation.**
  - The scotopic normalized b-wave amplitude is decreased 7 days after IOP elevation and recovers by 30 days. In data not shown here, we also observe rewiring by rod bipolar cells onto αON-sustained RGCs. This suggests that there may be functional resilience within this circuit and we are continuing to explore this avenue.
- **The frequency response curves to sinusoidal flicker stimuli do not differ between control and IOP elevation conditions.**
  - We thus far see no evidence of differences in frequency response properties between the ON and OFF pathway. In both conditions the response profile of animals is a bandpass filter with peak frequency around 2 Hz.
- **Light responses of αON-sustained and αOFF-transient RGCs can be stably recorded.**
  - We are currently continuing single cell recordings and also exploring the spatiotemporal receptive field properties of these cells in response to IOP elevation.

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