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Patterned Optical Activation of Retinal Ganglion Cells

Nairouz Farah*, Inna Reutsky* and Shy Shoham, *Member, IEEE*

Abstract— **Neuroprosthetic retinal interfaces depend upon the ability to bypass the damaged photoreceptor layer and directly activate populations of retinal ganglion cells (RGCs). To date, the preferred approach to this task largely relies on electrode array implants. We are currently pursuing two alternative methods for light-based direct activation of the RGCs. The first method is based on applying caged glutamate over the retina and uncaging it locally to obtain RGC excitation. The second method is to artificially cause RGCs to express Channelrhodopsin II (ChR2), a light-gated cation channel. In addition to being non-contact, optical techniques lend themselves relatively easily to a variety of technologies for achieving patterned stimulation with high temporal and spatial resolution. Using the Texas Instruments Digital Light Processing (DLP - DMD) technology, we have developed an optical stimulation system capable of controlled, large-scale, flexible stimulation of the retinal tissue with high temporal accuracy. In preliminary studies, we are performing patterned photo-stimulation experiments using samples of caged fluorescent probes and in rat retinas that were virally transfected with ChR2.**

I. Introduction

Some of the more common causes of blindness are degenerative diseases of the *outer* retina, like Age-related Macular Degeneration (AMD) and Retinitis Pigmentosa (RP), globally afflicting approximately 25-30 million [1] and 1.5 million individuals [2], respectively. Diseases of the outer retina result in photoreceptor loss, while the inner retinal neurons and in particular the retinal ganglion cells (RGCs) and their optic nerve projections remain largely functional [3]-[5]. Current approaches towards artificial stimulation of retinal ganglion cells largely rely on the development of micro-fabricated electrode array implants which will either lie on the epi-retinal (over the retina) or sub-retinal surfaces [1].

While promising, this technology may suffer from several fundamental drawbacks including the long term stability of the interface between electrode arrays and the very fragile retinal tissue and the challenge of creating interfaces with many thousands of independent channels in an attempt to approach highly functional vision. Alternative, non-contact, approaches could rely on techniques for direct light-based activation of the RGCs which can potentially cause reduced tissue damage. Recently, two different photo-stimulation technologies for light-based neuron activation have been demonstrated to achieve millisecond-precise activity control. These methods are based on uncaging caged glutamate using rapid UV laser deflection [6], and on Channelrhodopsin-2 (ChR2), a directly light-gated cation-selective ion channel that depolarizes neurons after absorbing blue photons [7]-[8].

Glutamate uncaging and ChR2 can both be considered as possible candidates for the development of a retina interface. First, Glutamate is the principal excitatory neurotransmitter in the retina, and retinal tissue appears to be highly tolerant to chronic intravitreal infusion of caged glutamate [9]. Second, mouse retinas have recently been shown to stably express ChR2 channels *in vivo* for periods exceeding a year following viral transfection [10], and ChR2- transfected blind rd1/rd1 transgenic mice subjected to full-field light flashes had normal-like visual evoked potentials (VEPs).

Combining these photo-stimulation methods with spatial light modulation technologies (micro-displays) can potentially allow a relatively simple route towards restoration of high-acuity vision. In this paper, we describe a micro-display based system adapted for patterned photo-stimulation. This system provides high spatial and temporal resolution, and is being applied to study retinal ganglion cell responses to patterned photo-stimulation in isolated retinas.

II. Methods

A. Projector-based System for Patterned Stimulation

To activate individual cells with high spatial and temporal resolution we are using a projection system based on a Texas Instruments DLP-DMD chip [11]. A commercial DLP based projector (A+K Astrobeam 530s) has been customized to incorporate two different light sources. The projector's original lamp was removed, and for glutamate uncaging studies, a UV lamp output (EFOS ultracure 100ss) is introduced into the projector's optical pathway via a designated port. For ChR2 activation studies our system uses a strong blue LED or a metal-halide lamp (EXFO X-cite) filtered at the suitable wavelengths (centered around ~470 nm)[8]. The output light is collected through a lateral port of

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The authors are with the Neural Interface Engineering Laboratory, Faculty of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa, Israel (phone: +972-4-8294125; fax: +972-4-8294599; e-mail: sshoham@bm.technion.ac.il).

an inverted microscope (Nikon TE2000U) and is focused through a 10x objective onto a ~3 mm^2 square area on the intact retina, allowing direct activation of the ganglion cell layer in various spatio-temporal resolution

B. Patterned Uncaging

A solution containing 0.1% CMNB caged fluorescein (Molecular Probes – Invitrogen) and 1% Bovine Serum Albumin (BSA) was placed on a microscope coverslip, heated to dry and then placed upside-down on a glass slide to create dry samples of caged fluorescein [12]. Sample preparation was performed under red illumination conditions in order to avoid spontaneous uncaging. UV patterns were then projected onto the coverslip through a 10X or 40X objectives.

C. ChR2 Transfection

Viral vectors are currently the most efficient way to induce stable expression of genes in non-dividing CNS neurons. Studies suggest that adeno-associated viruses (AAV) are the most suitable candidates for prolonged expression of genes of interest in a retina [13],[14].

To transfect RGCs with ChR2, we injected the AAV2-CAG-Chop2/GFP-WPRE-BGH-polyA expression vector (GeneDetect Ltd.) introduced by Bi *et al*.[10] into the vitreous of 15 adult rat eyes (a single eye per rat) near the back of the eye cup. RGCs are thus the main cells exposed to the virus and they are the main cells expected to be transfected. Different volumes of AAV vector were injected in order to assess the optimal concentration. Transfected rats are of the same age, and the evaluation of ChR2 expression (by measuring GFP fluorescence) was taken at different time points 3-8 weeks post-injection.

All 15 retinas in our sample exhibited some degree of ChR2 expression, and in 9 retinas the area of bright fluorescence (indicating successful ChR2 transfection) was 1mm^2 or larger. 3 of the transfected retinas exhibited gene expression covering the entire retinal surface (Fig. 1a,b). Cross sections show ChR2 expression across all retinal cell layers, including RGCs (Fig. 1c). This suggests that the virus introduced by Bi. *et al*. [10] for transfection in mice is also suitable for obtaining stable (at least 2 months) expression in rats.

D. Multi-electrode Array (MEA) Recording and Data Analysis

The retinas are dissected under IR illumination to prevent photoreceptor bleaching, and then mounted on MEA electrode arrays (Multi Channel Systems, GMBH) to allow recording the light evoked single unit responses of the transfected RGCs. The data is analyzed by spike sorting methods that detect and classify neural action potentials.

III. RESULTS

Our patterned-photostimulation system is capable of creating Blue or UV patterns with a high spatial (several microns) and temporal (4-5 miliseconds) resolution. Since Glutamate uncaging cannot be directly visualized, we tested our ability to create arbitrary UV patterns by uncaging caged fluorescein dextran in dry samples (without the presence of diffusion). A sparse UV pattern was projected onto a dry sample and subsequently imaged through a fluorescence microscope (Fig. 2). This pattern illustrates that the effective uncaging resolution is on the order of 5 microns (smaller than single rat RGCs –see fig. 1).

In early experiments, we tested whether we can obtain light-driven activity in a population of ChR2 expressing RGCs. Fig. 3 shows MEA recordings from a transfected retina, subjected to full-field flashes. The responses contain both normal on-off responses and characteristic sustained ChR2 responses. Our current experiments are focused on measuring and characterizing responses to patterned photo-stimuli.

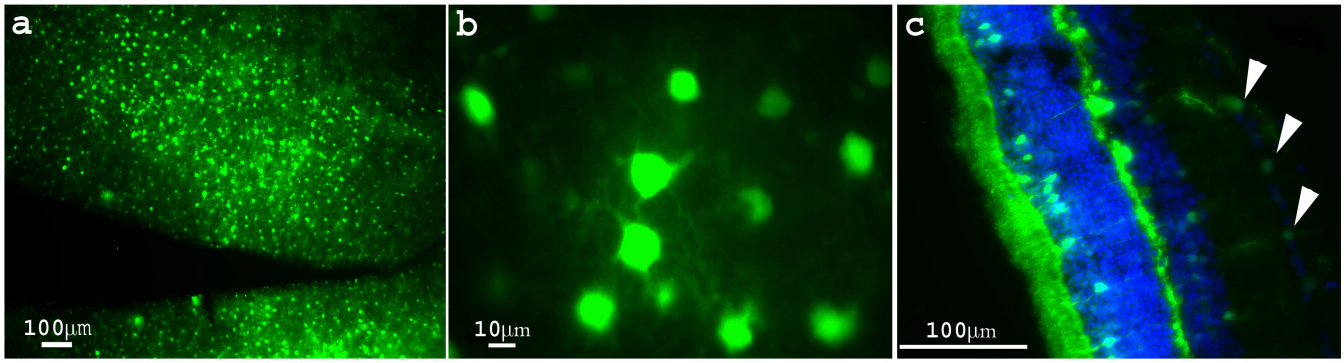

Fig. 1. **ChR2 expression in rat retinas. (a)** Transfected RGCs in flat mount of the rat retina. **(b)** Close-up of transfected RGCs. **(d)** Cross sections of the transfected retina. White arrows indicate the transfected RGCs. Green – GFP (ChR2 expressing cells), Blue – DAPI (nuclei staining)

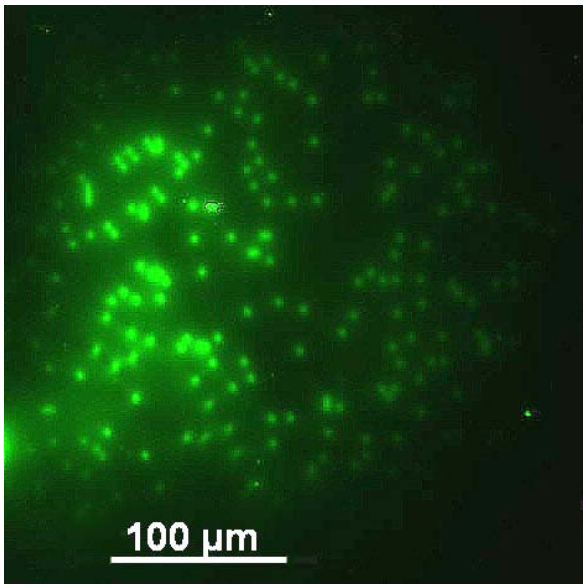

Fig. 2. **Fluorescein Uncaging.** Sparse fluorescence pattern observed after a UV pattern was projected onto a caged fluorescein-treated coverslip (uncaging using a 40x objective).

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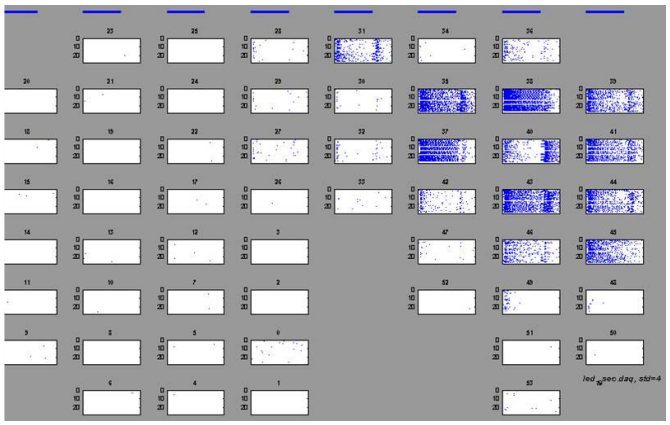

Fig. 3 **Multiple single-unit responses to photostimulation of a ChR2-transfected retina**. Transfected RGCs were present near top right part of array. Top bars indicate duration of flash, and successive raster lines correspond to different responses.

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