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Development of a Chemical Retinal Prosthesis: Stimulation of Rat Retina with Glutamate

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Abstract— **Retinal degenerative diseases cause partial or total blindness and affect millions of people worldwide, yet currently have no treatment. Retinal prostheses using electrical stimulation are being developed but face significant problems moving forward. Here we propose using chemical stimulation, via the neurotransmitter glutamate, to modulate retinal ganglion cell (RGC) spike rates. Our results demonstrate that it is feasible to stimulate RGCs in an explanted retina using focal ejections of glutamate from either subretinal or epiretinal sides. Preliminary evidence suggests we are primarily activating RGCs as opposed to bipolar cells. This is an important first step in the development of a chemical retinal prosthesis based on microelectromechanical systems (MEMS) technology.**

I. INTRODUCTION

Inherited retinal degenerative diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration, lead to partial or total blindness and affect millions of people worldwide [1]. These diseases result in the progressive loss of the photoreceptor layer in the retina, though the inner retinal layers survive and retain a degree of functionality [2], [3]. There are currently no treatments to cure or prevent retinal degenerative diseases, but several groups have developed retinal prostheses in the hopes of restoring vision [4]–[8]. Current generation retinal prostheses use electrical stimulation to stimulate the surviving retinal layers: the retinal ganglion cell (RGC) or bipolar cell layers. Early clinical results have revealed that retinal prostheses can (1) be relatively well tolerated when implanted and (2) provide rudimentary vision in patients [9]–[11]. Despite ongoing efforts to improve these devices, there appear to be two major problems with electrical stimulation. First, the spatial resolution is limited by both the electrical charge density limitations and current spread from the microelectrodes. The combination of these two factors imposes a physical limit of 150 μm to the maximum possible spatial resolution in order to safely achieve neuronal stimulation, though most devices have poorer resolution [12]. Second, electrical stimulation is nonselective, and thus, simultaneously activates cells of both the ON and OFF pathways, canceling the retina's differential output signal. Electrical stimulation thus negates a basic design feature of the natural retina.

We propose that a neurotransmitter-based retinal prosthesis using microfluidics could potentially overcome the weaknesses of electrical stimulation. First, the spatial resolution of fluid ejection ports in a microfluidic device can be significantly higher than the currently practicable resolution of the microelectrodes in the existing electrical prostheses [13]–[16]. Second, chemical stimulation of ON and OFF bipolar cells with the native neurotransmitter glutamate would, in principle, allow differential stimulation of the ON and OFF pathways, providing a more naturalistic percept compared to electrical prostheses. Glutamate stimulation is not without flaws however as excessive glutamate at high concentrations has been shown to induce excitotoxicity and cell death [17], [18].

Other groups have proposed using glutamate to biomimetically stimulate the retina, though only one has performed studies using retinal tissue [19]–[21]. One group has shown success with an epiretinal application of glutamate in wild type and retinal degenerated rats, recording transient modulations in RGC spike firing rates via single cell recordings [22]. In our study, we chose to first examine the feasibility of chemical stimulation from the subretinal side while recording RGC spikes with a multielectrode array (MEA) system. Subretinal stimulation offers a direct pathway for glutamate to activate the ON and OFF bipolar cells in the outer plexiform layer. Once we established the feasibility of subretinal glutamate stimulation, we investigated whether we were primarily stimulating bipolar cells or RGCs directly. Finally, we explored the effects of epiretinal chemical stimulation, again using the MEA to record RGC spikes.

II. METHODS

A. Animals

Wild type rats of either sex (~24-30 days old) were obtained from commercial suppliers and subject to 1 hour of dark adaptation before experiments. Following dark adaptation, animals were euthanized via CO_2 and cervical dislocation prior to isolation of the retina [23]. This study was conducted within the guidelines outlined by the National Research Council's 'Guide for the Care and Use of

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Laboratory Animals', and all protocols were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago.

B. MEA Recordings and Data Analysis

The MEA recordings were performed using a perforated MEA or pMEA (Multichannel Systems, 60pMEA200/30iR-Ti-pr-T with MEA1060 amplifier) with electrode diameters of 30 µm, electrode spacing of 200 µm, and various port diameters. Isolated retinas were placed flat onto the MEA surface with the RGC side contacting the electrodes. Retinas were perfused with oxygenated Ames medium at room temperature. A mesh grid and harp slice was used to flatten the retina onto the MEA.

A green LED source was used to produce full field stimulation of the retina to compare its light responses against glutamate responses. Spontaneous responses to a low light level were also recorded as a control. Glutamate ejections were accomplished using a pressure-based microinjection system (PM-8, Harvard Apparatus) to eject glutamate from a glass pipette (3-5 µm tip diameter) maneuvered by a motorized micromanipulator system (MPC-200, Sutter Instruments, Novato, CA). Glutamate ejections were targeted at electrodes that were recording high RGC spike rates and light responses. All glutamate ejections used a glutamate concentration of 1 mM. The contact of the pipette tip with the tissue was detected by sensing the change in pipette impedance using a patch clamp amplifier (Axon Instruments).

Spikes were assigned to individual RGCs using commercial software (Offline Sorter, Plexon Inc) via principal component clustering. The resulting timestamps were used to generate both raster plots and peristimulus Gaussian kernel density estimates (to estimate the spike firing rate) with custom Matlab (Mathworks Inc.) scripts.

C. Subretinal Glutamate Stimulation

The pipette was positioned above electrodes with which spikes from target cells were recorded and lowered into the retina to a depth of 20 µm. Glutamate was ejected using a variety of pressures and durations to elicit an RGC response. Each experiment consisted of 50 trials each of full field flash, spontaneous, and glutamate stimulations in sequence. Responses to subretinal glutamate stimulation were compared with visually-evoked responses to the full field flash.

D. Determining the Target of Subretinal Glutamate Stimulation

The primary target for subretinal glutamate stimulation was assessed by using the glutamate analog 2-amino-4-phophobutyric acid (APB), which is a specific agonist for the mGluR6 receptor found on ON bipolar cells [24]–[26]. Application of APB should only disrupt glutamate stimulation of ON RGCs if we are primarily stimulating the bipolar cell layer. ON RGCs were identified using full field flash and subjected to glutamate stimulation using the settings derived from the study described in C above. The retina was then incubated with a combination of Ames medium and 100 µM APB for 10 min to block the ON light responses [24]. After verifying that ON light responses were abolished, another set of glutamate ejections was conducted. The change, if any, in spike rate between the two sets of glutamate stimuli was used to determine the primary target of glutamate stimulation.

E. Epiretinal Glutamate Stimulation

For these experiments, the pipette tip was inserted into the retina through perforations in the pMEA near a target electrode from the bottom side . The tip was advanced to an epiretinal depth of 70 µm for ejections. Glutamate was delivered with an ejection time of 100 ms at various pressures to provoke a response. The effectiveness of epiretinal glutamate stimulation was also compared to full field flash responses.

III. Results

A. Subretinal Glutamate Stimulation

Subretinal glutamate ejections evoked repeatable, transient increases in RGC spike firing rates (Figure 1). Repeatable glutamate responses were obtained with glutamate boluses of 15, 30, and 45 nL per injection using a pressure of 138 kPa (20 PSI) and ejection durations of 100, 200, and 300 ms, respectively. Responses were temporally, but not spatially, localized with responses recorded as far as 1200 µm from the ejection site (Figure 1, Plot 4). While there were a few

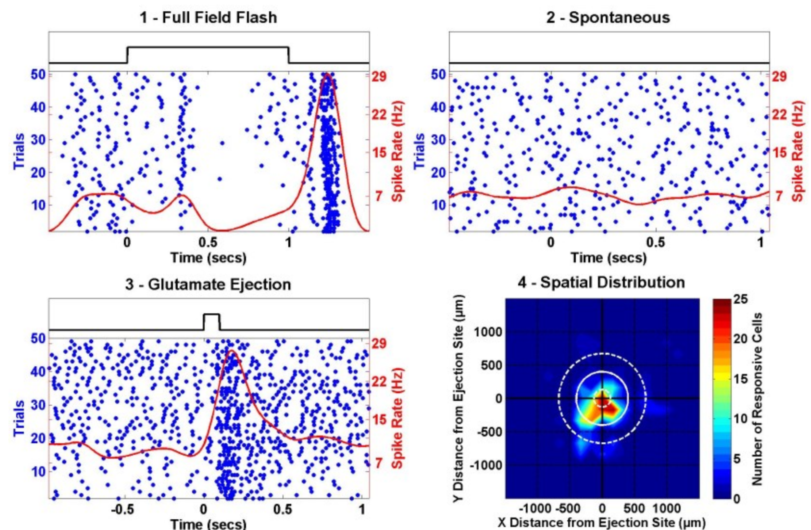

Figure 1. The spiking activity of a representative RGC at the site of subretinal ejection. Each blue dot is a spike with the left y-axis indicating number of trials and the red line the Gaussian kernel density estimation, with right y-axis indicating the spike rate in Hz. Plot 1 is a response of the cell to full field flash, indicating that the cell is an OFF RGC. The square wave above the plot shows the light ON period. Plot 2 is spontaneous activity recorded after the full field flash and shows the baseline activity for this cell. Plot 3 is the response of the neuron to glutamate pulses, showing a transient increase in spike rate. Corresponding observations were made for cells recorded on other electrodes. Plot 4 shows the spatial distribution of all responses to subretinal glutamate ejections. Red regions indicate areas with a higher number of responses compared to blue regions. The solid and dashed white circles indicate the mean (400 µm) and standard deviations (270 µm) for distance, respectively.

isolated cases of transitory inhibition, the only definite, repeatable responses were excitatory.

B. Determining the Target of Subretinal Glutamate Stimulation

Application of APB was found to completely abolish ON RGC light responses, but only inhibited glutamate responses for the initial ejections (Figure 2).

C. Epiretinal Glutamate Stimulation

Epiretinal glutamate ejections also produced repeatable, transient excitation in RGCs (Figure 3). These responses were elicited by small boluses of less than 12 nL per injection, using pressures ranging from 34-103 kPa (5-15 PSI) and a constant ejection duration of 100 ms. While approximately half of the responses were localized within a 300 μm radius, a few responses were noted as far as 1.2 mm from the ejection site (Figure 3, Plot 4). As with the subretinal ejections, the vast majority of responses were excitatory, with few inhibitory responses observed.

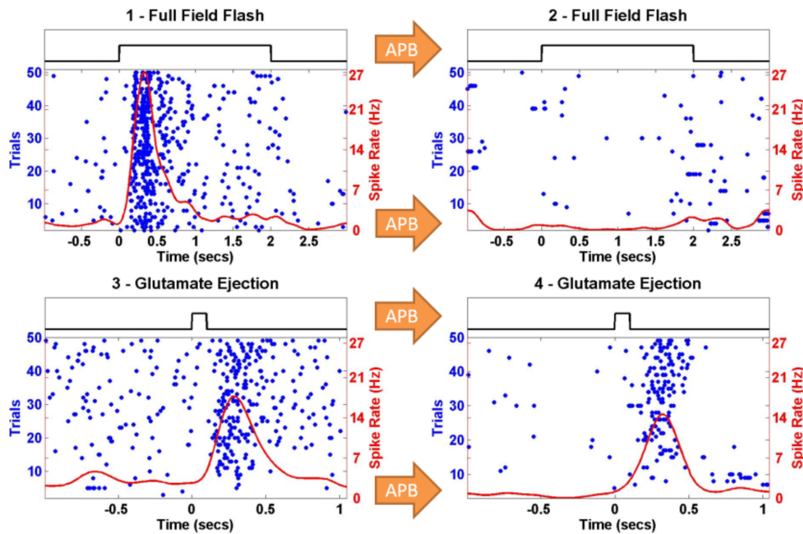

Figure 2. Stimulation of an ON RGC through subretinal ejection. Blue dots are spikes with the left y-axis indicating number of trials, the red line the Gaussian kernel density estimation, and the right y-axis spike rate in Hz. Plot 1 is a response of the cell to full field flash before APB, showing that the cell is an ON RGC. Square wave above the plot shows the light ON period. Plot 2 is the same cell's response to full field flash following APB, showing that it has competely abolished the light response. Plot 3 is the same cell's robust response to glutamate before APB. Plot 4 is the same cell's glutamate response following APB and shows it is still responsive to glutamate, though it appears to be inhibited in the initial trials.

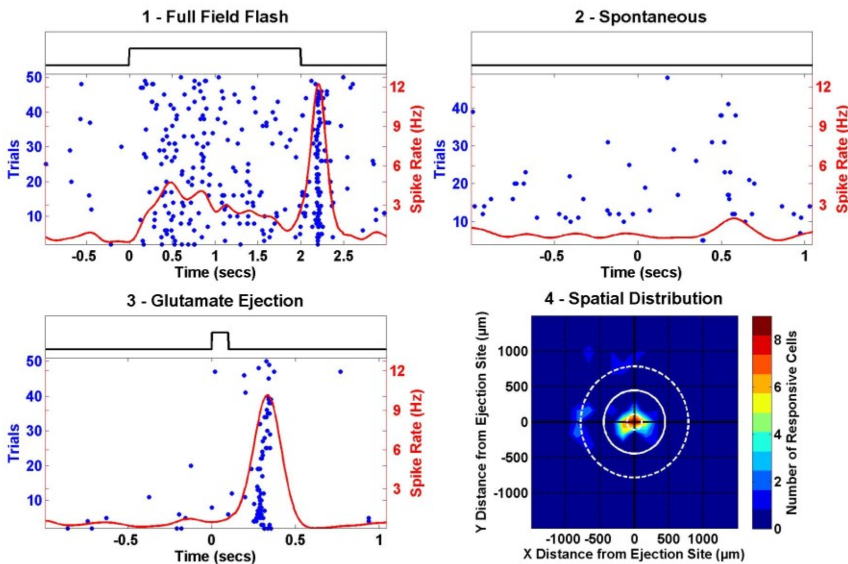

Figure 3. Activity of an RGC 630 μm from the site of epiretinal ejection. Blue dots are spikes with the left y-axis showing number of trials, the red line the Gaussian kernel density estimation, and right y-axis spike rate in Hz. Plot 1 is a response of the cell to full field flash,demonstrating that the cell is an OFF RGC. The square wave above the plot shows the light ON period. Plot 2 is spontaneous activity recorded after the full field flash, showing low baseline activity. Plot 3 is the response to glutamate pulses, showing a transient increase in spike rate. Corresponding observations were made for cells recorded on other electrodes. Plot 4 shows the spatial distribution of all responses to epiretinal glutamate ejections. Red regions indicate areas with a higher number of responses compared to blue regions. The solid and dashed white circles indicate the mean (450 μm) and standard deviations (340 μm) for distance, respectively.

IV. Discussion

We have demonstrated that both subretinal and epiretinal applications of glutamate can modulate RGC spike firing rates in an explanted rat retina. Evidence for differential stimulation of the OFF and ON pathways was inconclusive, as glutamate responses were inhibited in some trials, but not others (Figure 2). In general, we observed that subretinal stimulation requires a larger bolus of glutamate compared with epiretinal stimulation. We believe this is due to the pipette tip's closer proximity to RGCs in the epiretinal configuration than in the subretinal configuration. Both methods of stimulation produced responses far from the ejection site, though the spatial resolution is comparable to current electrical prostheses [4]–[7]. We also observed very little change in full field flash responses following glutamate ejections in either the subretinal or epiretinal configurations. This suggests that our application of exogenous glutamate is not producing an excitotoxic effect in the short term, though the retina was constantly perfused with fresh media.

Our results compare favorably with the recent work by Iezzi et al. in the case of epiretinal glutamate stimulation [22]. Both works have demonstrated transient modulation of RGCs with epiretinal applications of glutamate, although our use of an MEA has allowed us to observe the spread of glutamate in a large population of cells more easily. Data from our MEA recordings indicate that, like Iezzi et al., most responsive cells were located relatively close to the ejection site, but we also stimulated cells

farther away as well. The limitations of single cell recording in the Iezzi et al. study could explain this discrepancy. We were not able to achieve transient suppression of RGC spiking rates however except in isolated cases. This could be attributed to our lower glutamate concentration (1 mM vs 2-5mM), which could have also resulted in different RGC responses. Our results with subretinal glutamate stimulation are novel and warrant further investigation.

Together, our data indicate that it is feasible to chemically stimulate the retina from both the subretinal and epiretinal sides. These results suggest that a retinal prosthesis using MEMS technology could be designed to modulate RGC spiking rates. Future work will focus on two goals: (1) achieving differential stimulation of the ON and OFF pathways and (2) the development of a MEMS device. The first goal will build on our work from these experiments to find the correct approach to reliably stimulate bipolar cells. The second goal will use these results to design and fabricate a microfluidic device to apply glutamate to an explanted rat retina and study the feasibility of a benchtop retinal prosthesis. A successful prototype will represent a new stimulation paradigm that could be used with other neurotransmitters and in other types of neuroprostheses.

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