Biomimetic Stimulation of Rat Retinal Ganglion Cells with the Neurotransmitter Glutamate

Corey M. Rountree, Samsoon Inayat, Laxman Saggere, and John B. Troy

Abstract— **Millions of people worldwide face partial or total vision loss due to inherited photoreceptor degenerative diseases, which currently have no cure. Retinal prostheses have been developed to restore vision by electrically stimulating surviving retinal neurons, but have low spatial resolution and nonselectively stimulate retinal ganglion cell (RGC) axons along with somata. We propose a biomimetic solution: using the neurotransmitter glutamate to chemically stimulate RGCs to avoid the disadvantages of electrical stimulation. Our results demonstrate that glutamate stimulation has a spatial resolution comparable to current-generation electrical prostheses, can stimulate RGC somata without stimulating axons, and can produce spatially differential responses in RGC subtypes. These results highlight the benefits of a neurotransmitter-based retinal prosthesis over current-generation electrical prostheses.**

I. INTRODUCTION

Millions of people around the world suffer from inherited photoreceptor degenerative diseases, such as retinitis pigmentosa and age-related macular degeneration, that result in the gradual loss of photoreceptor cells [1]. The loss of these cells leads to irreparable vision loss though other retinal neurons, such as retinal ganglion cells (RGCs), remain intact and functional even in late stages of photoreceptor degeneration [2], [3]. Several groups are developing retinal prostheses to electrically stimulate RGCs in the hope of restoring vision in patients [4], [5], [6], [7], [8]. While some of these devices have demonstrated promising results and progressed through clinical trials, there are serious concerns regarding the quality of vision that electrical stimulation provides [7]. First, the spatial resolution of electrical prostheses is limited due to electrical charge density limitations [9]. Current generation prostheses such as the Argus II have only been shown to restore vision to ~1.6-2.9 logMAR, well below the legal definition of blindness (1.0 logMAR) in many countries [7]. Second, electrical stimulation has been shown to nonselectively stimulate both RGC somata and axons, which limits spatial resolution [10].

We are developing a microfluidics-based retinal prosthesis to stimulate RGCs with the neurotransmitter glutamate in the hope of overcoming the disadvantages of electrical stimulation. A neurotransmitter-based retinal prosthesis offers several potential advantages over electrical prostheses. First, the spatial resolution of microfluidic injection ports could be higher than current electrical prostheses [11], [12], [13]. Second, neurotransmitter-based stimulation should selectively stimulate only RGC somata through dendritic fields as there are no known glutamate receptors on RGC axons. Despite these advantages, only a few groups have investigated neurotransmitter-based stimulation [14], [15], [16], [17].

Previous studies have focused on demonstrating the feasibility of glutamate stimulation but none have examined its effect on RGC somata and axons. In this study, we divided spikes recorded with a multielectrode array (MEA) system into somal and axonal units based on their waveform shapes. We then investigated how populations of RGC somata and axons responded to epiretinal injections of glutamate in terms of their spike rate responses, timing, spatial resolution, and the types of RGC stimulated.

II. METHODS

A. Animals and MEA recordings

Retinas were isolated from dark-adapted wild-type rats (26-30 days old) and placed RGC side down on a perforated MEA (Multichannel Systems, 60pMEA200/30iR-Ti-pr-T with 60 30 μm electrodes spaced 200 μm apart and a MEA1060 amplifier) to record RGC spikes [19]. All retinas were perfused with oxygenated (95% O_2, 5% CO_2) Ames medium at room temperature. A 570 nm LED was used to classify RGC subtypes based on full field flash responses.

B. Glutamate injections

Glutamate was injected into the retina through a glass pipette (~10 μm tip diameter) using a pneumatic microinjection system (PM-8, Harvard Apparatus). A motorized micromanipulator was used to guide the pipette through the perforations on the bottom surface of the MEA. Contact with the epiretinal surface was detected by measuring a change in the pipette impedance. The pipette was inserted approximately 20 μm inside the inner limiting membrane (near the ganglion cell/inner plexiform layer border) before beginning a series of 30-50 injection trials. Injection pressures ranging between 0-1 PSI, combined with an injection duration of 100 ms, resulted in injection volumes between 10-1000 pL per injection.

C. Data analysis

Spikes were sorted into distinct units using the commercial software Offline Sorter. Sorted units were separated into axonal and somal units using principal component analysis in Offline Sorter. The spike rate of individual axonal and somal units was calculated using Gaussian kernel density estimation and custom Matlab scripts. Responsive cells were those with amplitudes (see Figure 1A) greater than or equal to 5 Hz. The spatial distribution of glutamate-responsive units was computed using the relative distances and positions between electrodes with responsive units and the injection site. The

978-1-4244-7929-0/14/$26.00

locations of axonal and somal units were assumed to be at the same locations as their corresponding electrodes. At each point of the spatial distribution, the amplitude and width at half maximum were averaged to produce spatial distributions of response parameters. Mann-Whitney-U tests were used to compare different groups of data as most distributions were not normal. Statistical significance was determined using a P-value of 0.05.

A cross correlation analysis was also performed to detect groups of correlated axonal and somal units during both full field flash and glutamate stimulation. An average cross-correllelogram between two units was computed by averaging the difference between cross correlations of spike times during identical and different trials to remove stimulus bias. Pairs of units were deemed to be correlated if the maximum correllelogram value was above 1. The time lag of the maximum correllelogram value was used as the estimated time delay between correlated units.

III. RESULTS

A. Somal and axonal responses to glutamate injections

Epiretinal glutamate injections evoked responses in somal and axonal units with biphasic and triphasic spike shapes, respectively [18]. Fig. 1 shows representative somal (Fig. 1A) and axonal (Fig. 1C) responses to 30 trials of glutamate injections, as well as their spike shapes (Fig. 1B and D). These units were separated by 600 μm and both had robust responses to glutamate. As can be seen, the axonal response was delayed behind the somal response by approximately 100 ms. To better compare axonal and somal responses, the distributions of axonal and somal response amplitudes, widths at half maximum, latency, and spike amplitudes were examined. Somal responses were found to have significantly smaller spike rate amplitudes ($p < 0.001$), shorter latencies ($p < 0.001$), and wider widths ($p < 0.001$) than axonal responses.

B. Timing of RGC responses

Separation of correlated units into groups revealed linear paths that resemble axonal tracts. The direction of axonal tracts was determined using the relative time delays between correlated units. Fig. 2A shows a collection of correlated groups for a single injection trial at the green 'X'. As can be seen, axonal tracts run parallel to one another and in the same direction, presumably toward the optic disc. In every case, the direction and approximate time delays of axonal tracts for glutamate injections were identical to those from full field flash.

C. Somal responses are spatially localized

Glutamate injections produced spatially localized somal responses with a median distance from the injection site of 630 μm, corresponding to 2.1 logMAR (Fig. 2B-D). Responsive somal units ranged between 200-1500 μm from the injection site, representing 1.3-2.5 logMAR. Glutamate injections stimulated 82% of somal units at the site of injection in 29 sets of injections, with only 6 somal cells unresponsive to glutamate. In contrast, axonal responses were

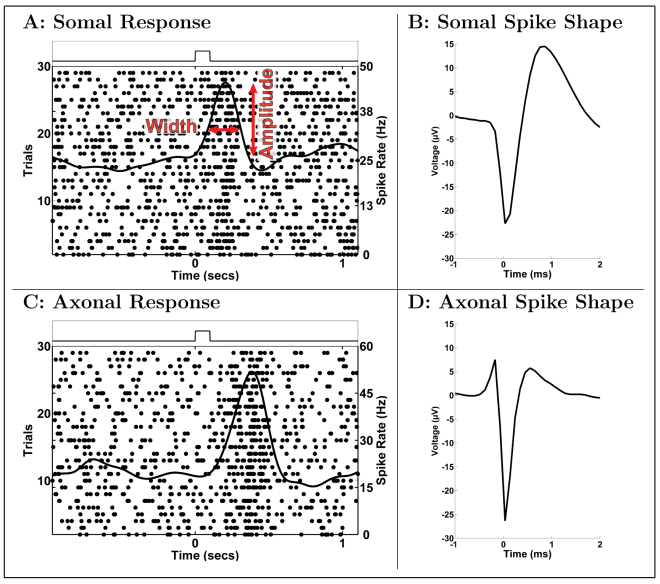

Fig. 1. The spiking activity and spike shapes of representative somal (A and B) and axonal (C and D) units in response to the same epiretinal glutamate injection. In plots A and C, each black dot is a spike with the left y-axis indicating the number of trials and the black line indicating the Gaussian kernel density estimation of this cell's spike rate in Hz. Both cells show a robust response to glutamate injection with a delay between somal and axonal responses of approximately 100 ms. Plots B and D show the characteristic biphasic and triphasic spike shapes of somal and axonal units, respectively, for the units from plots A and C. The amplitude and width of responses are indicated by red lines in plot A.

significantly farther from the injection site. No apparent relationships were identified between the injection volume and the spatial localization of responses.

D. Spatially differential responses in RGC subtypes

Separation of the somal glutamate-responsive spatial distribution into ON, OFF, and ON-OFF RGC subtypes revealed a spatially differential response to glutamate. Fig. 3A shows that ON RGCs were confined to near the injection site while OFF and ON-OFF RGCs were more widely distributed. Furthermore, ON RGCs displayed significantly larger amplitudes ($p < 0.001$) and widths at half maximum ($p < 0.001$) near the injection site (distance $\leq$ 200 μm) compared with OFF and ON-OFF RGCs over the same range.

IV. DISCUSSION

We have shown that the introduction of exogenous glutamate into a wild-type rat retina elicits responses from both somal and axonal units. Furthermore, we have demonstrated that axonal and somal responses are significantly different in terms of amplitude, width, and latency. The significantly shorter latencies of somal responses suggest selective stimulation of RGC somata or dendritic fields over axons. Our spatial distribution results compare very favorably with electrical prostheses such as the Argus II. The median somal logMAR of 2.1 falls well within the same visual acuities that the Argus II has exhibited. Some injections produced very spatially localized somal responses corresponding to a logMAR value of 1.3, which actually surpasses the reported values for the Argus II. While these are only estimates

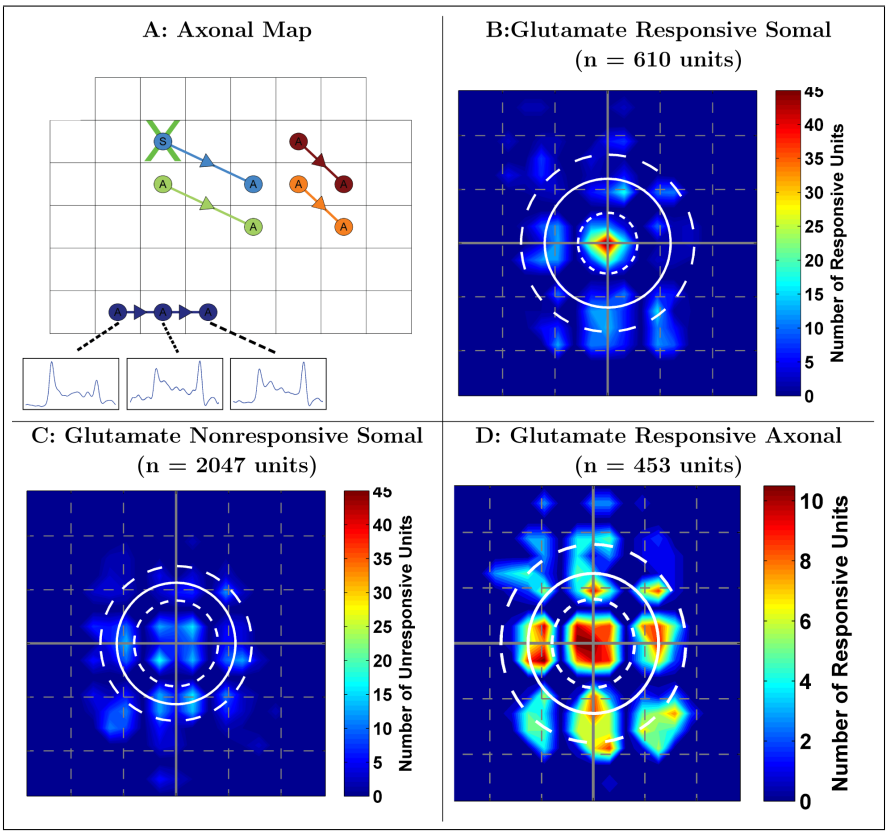

Fig. 2. Plot A shows the location of several groups of correlated units from a single set of identical glutamate injections at electrode 32 (green 'X'). The locations of somal and axonal units are indicated by circles with 'S' or 'A' while unique colors correspond to different groups. Some groups lack somal units, presumably due to the limited spatial resolution of the MEA. The arrows between units show the direction of increasing time delays between correlations. Each square is 200 μm in length. Most groups run parallel to one another and all groups show identical time delays for full field flash and glutamate stimulation. The insets at the bottom show representative full field flash responses for the indicated units, showing that they are similar. Plots B-D are spatial distributions for somal glutamate-responsive and nonresponsive units (B and C, respectively) and glutamate-responsive axonal units (D), showing the locations of all cells with respect to the injection site (center). Red regions indicate areas with a higher number of cells compared to blue regions. The white circles represent the lower (inner dotted line), median (solid line), and upper (outer dotted line) quartiles for distance. In general, glutamate injections elicited spatially localized somal responses, with few glutamate non-responsive somal recordings at the injection site. Axonal responses were more widely distributed. The grey gridlines are separated by 500 μm.

of visual acuity based on the spatial spread of glutamate responses, the general similarity between mammalian retinas outside of the fovea makes it a worthwhile comparison.

The presence of a spatially differential response in ON, OFF, and ON-OFF RGC subtypes was unexpected since RGCs possess only excitatory glutamate receptors. Our results indicate that ON RGCs are more spatially localized and possess larger response amplitudes and widths near the injection site compared with OFF and ON-OFF RGCs. This is likely due to the different locations at which ON, OFF, and ON-OFF RGC dendritic fields synapse with bipolar cells in the inner plexiform layer. Since we only inserted the pipette 20 μm into the retina, it should be closer to the ON RGC synapses than the OFF and ON-OFF ones. While we have not measured the injection profile of glutamate into retinal tissue, we know that dilution plays a large role since it is an aqueous environment. Hence, it is likely that ON RGC dendritic fields near the site of injection are presented with higher glutamate concentrations than OFF and ON-OFF dendritic fields since farther locations should receive a more dilute glutamate dose. The elevated dose of glutamate received by ON RGCs could be responsible for the larger numbers, amplitudes, and widths of ON RGCs near the injection site.

V. CONCLUSIONS

Our data indicate that epiretinal glutamate injections can provide biomimetic stimulation of RGCs. Injected glutamate can selectively stimulate RGC somata with a spatial localization similar to current electrical prostheses. Our future work will focus on: (1) further improving the spatial localization and (2) the development of a multiport microfluidic retinal prosthesis.

ACKNOWLEDGMENT

The work presented in the paper was supported by the National Science Foundation (NSF) EFRI program grant number 0938072. The contents of this paper are solely the responsibility of the authors and do not represent the official views of the NSF.

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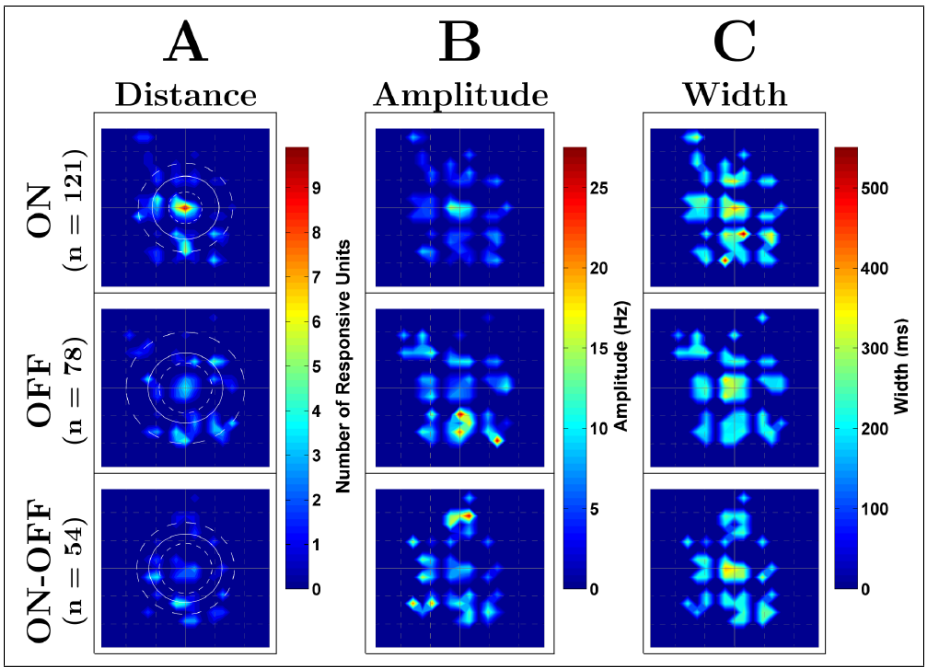

Fig. 3. Glutamate injections produced spatially differential responses in ON, OFF, and ON-OFF RGCs with somal spike shapes. Plot A shows the spatial distributions for these subtypes while plots B and C show the average amplitudes and widths at half maximum at each point of the spatial distribution. As in Fig. 2, the spatial maps show the locations of all cells with respect to the injection site (center), with warm colors indicating a higher number of responses than cool colors. In general, ON RGCs were more spatially localized and displayed significantly larger amplitudes and widths near the injection site (distance $\leq$ 200 μ) compared with OFF and ON-OFF RGCs. The grey gridlines are separated by 500 μm.

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