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# Microfluidic Neurotransmiter-based Neural Interfaces for Retinal Prosthesis

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***Abstract*—Natural inter-neuronal communication is mediated primarily via neurotransmitter-gated ion channels. While most of the methods for neural interfacing have been based upon electrical stimulation, neurotransmitter-based approaches for the spatially and temporally controlled delivery of neurotransmitters are relatively new. Methods of neurotransmitter stimulation retinal prosthesis may provide new ways to control neural excitation. Experimental results for retinal ganglion cell stimulation demonstrate the feasibility of a neurotransmitter-based retinal prosthesis.**

## I. Introduction

MANY groups have been working toward the development of retinal prosthesis sensory-substitution systems that can provide rudimentary vision to the blind. An effective retinal prosthesis would improve the lives of hundreds of thousands of patients with Retinitis Pigmentosa (RP) or millions of blind patients with advanced Age-Related Macular Degeneration (ARMD), depending on its effectiveness. Designs for these devices have been based upon the success of electrical stimulation in the cochlear implant. Prostheses based on electrical stimulation of the retina have been under development over the past two-decades. Vision, however is our greatest bandwidth sensory input, requiring a neural interface with high spatial and temporal resolution. Testing in acute human studies has demonstrated limited success in providing useful vision. Chronic human experiments have been limited to low-resolution devices, since large electrodes are required to handle the high currents required to stimulate degenerated retinal tissues. Small-diameter electrodes, required for a high-resolution prosthesis, are prone to failure due to high charge-densities that erode metals and stimulation voltages that often exceed those required to dissociate water. These facts make small-diameter electrodes more capable of inducing retinal tissue damage from free radicals that are toxic to the lipid membranes of neurons and glia. Further limiting the efficacy of current stimulation methods is the fact that electricity cannot selectively stimulate specific types of visual pathways (e.g. ON and OFF channels) within the visual system. Thus electricity cannot encode important sensory features used in normal central visual processing. Many of these limitations could be circumvented by using more naturalistic means of stimulating retinal ganglion cells (RGCs) for retinal prosthesis. Natural vision is encoded as neurotransmitter signals.

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## II. Methods

Flattened whole-mount retinas were prepared from 5-10 week old (106 – 365g) male Sprague Dawley rats. Animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). Eyes were stored before use in cold (4ºC), oxygenated Ames media, which was constantly aerated with 95%/5% $O_2/CO_2$. Eyecups were then transferred to the perfusion chamber on a fixed stage for recordings. Retinas were held in place with bridal veil, and constantly perfused (8 ml/min) with warm (34ºC), aerated (95%/5% $O_2/CO_2$) Ames media.

Retinal ganglion cell activity was recorded extracellularly with a Multiclamp 700B and signals were digitized at 10 kHz with a Digidata 1322 and pClamp 9 (Molecular Devices). Recording electrodes were filled with 2 M NaCl and had resistances ranging from 2 to 20 Mohm. Glass micropipettes for local drug application had tip openings between 1 and 2 μm, and were filled with solutions containing 400 μM to 10 mM L-glutamate dissolved in Ames media. Recording and drug pipettes were manipulated (Sutter MP285, ROE-200 controller) into the tissue under microscopic video monitoring (Olympus BX51 microscope, Cooke Sensicam QE camera, Camware video capture

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software). Images of electrode positions were captured for determination of distances between recording and drug application sites. Drugs were applied by pressure ejections controlled by a Neuro Phore BH-2 (Harvard Apparatus) pressure ejection module (PPM-2). In the first set of experiments, glutamate was applied at the surface of the retina just above the ganglion cell layer. In the second set of experiments, glutamate was applied with the tip of the electrode 50 μm below the retinal surface.

## III. Results

Figure one, below shows retinal ganglion cell (RGC) spike activation to a single puff of L-glutamate, applied extracellularly to the retinal ganglion cells of the rat. These data were obtained via pneumatic ejection of L-glutamate. The capacitance of the tubing is seen as the characteristic rise and decay of the pressure curve during ejection. RGC spike responses occur as bursts, similar to those of visual responses. Response latencies are highly reproducible.

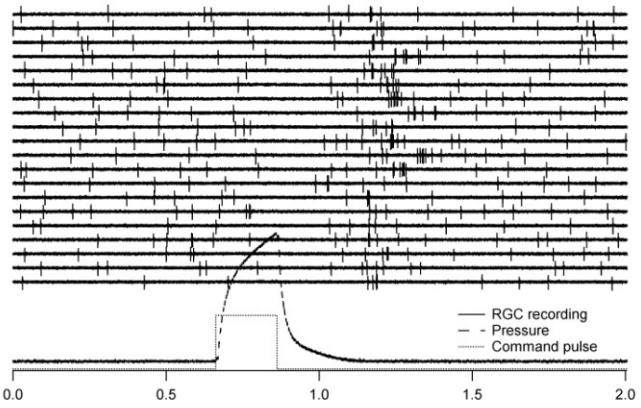


**Figure 1.) Retinal ganglion cell responses to extracellular application of 2mM L-glutamate. Note reproducible temporal response characteristics. (x-axis denotes Sec.)**

Figure two shows the amplitude and temporal RGC firing characteristics for a cell with high spontaneous firing. In this instance, the high sponaneous firing rate is initially suppressed by the local application of L-glutamate. Subsequently, a burst of RGC firing occurs. Multiple traces are shown which demonstrate the high degree of reproducibility of this phenomenon.

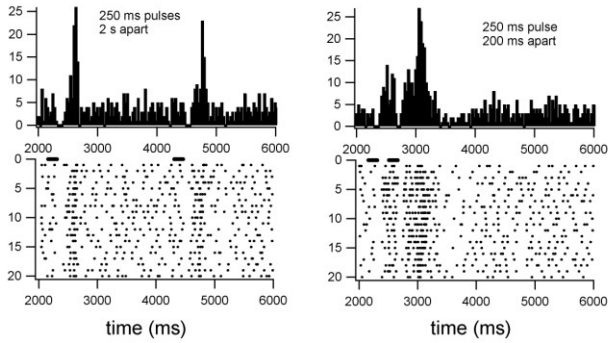


**Figure 2.) Temporal features of retinal ganglion cell responses to extracellular L-glutamate application. In these examples, the RGC had high spontaneous activity. Note that there is an initial supression of RGC firing prior to the event-related burst of action potentials.**

## IV. Discussion

Any neurotransmitter-based retinal prosthesis will need to mimic patterns of quantal excitation induced by visual stimulation. Freed determined that the just-maximal sustained RGC response to visual stimulation was induced by 3700 quanta of L-glutamate per second, among all synapses[1]. Studies of the number of L-glutamate molecules per synaptic vesicle report a range between 500 to 10,000 [2]. Thus, between $1.85 \times 10^6$ to $37 \times 10^6$ L-glutamate molecules per second would be required to induce a sustained RCG response. Freed and Sterling reported that there are approximately 550 bipolar synapses upon an ON alpha-RGC in the area centralis [3]. At $10^O$ eccentricity, the larger membrane surface area of ON alpha-RGCs causes them to have approximately 2200 bipolar cell synapses, since the density of bipolar cell synapses on the membrane is constant[1]. Based upon a synapse diameter of 200 nanometers[2] and a synaptic cleft of 20 nanometers, the volume of each synapse is approximately 2.5 attoliters [4]. Thus, the total synaptic volume for a single ON alpha-RGC ranges between 1.38 femptoliters near the area centralis and 5.5 femptoliters at $10^O$ eccentricity. Using the lowest molar quantity of L-glutamate needed for sustained RGC stimulation, combined with the largest total synaptic volume for an ON alpha RGC we arrive at a predicted minimum molar concentration of L-glutamate necessary for stimulation by a neurotransmitter-based retinal prosthesis of 0.55 millimolar L-glutamate. By taking the higher molar quantity of L-glutamate from the above computations, divided by the smallest total synaptic volume for an ON alpha-RGC, we predict that the upper concentration for L-glutamate required for sustained

stimulation is 11.1 millimolar. This range is consistent with our experimental findings for RGC stimulation via exogenous application of L-glutamate in normal Sprague-Dawley, RCS and S334-ter-4 rats.

The data in figure two show an initial suppression of RGC firing activity preceeding response bursting. This suggest that the retinal network is being stimulated. The suppression occurs with a shorter latency than the RGC bursting suggesting that perhaps the cells mediating this suppression are closer to the epiretinally-placed stimulation pipette than the RGC dendrites. Consequently, a plausible explanation for the response patterns observed may be that amacrine cells are being stimulated first, exerting an initial suppression upon RGC firing which is subsequently overcome as the puff of L-glutamate initiates burst activity either directly upon RGC's or indirectly via bipolar cell dendrites.

The issue of potential framerates for neurotransmitter-based retinal prostheses has been raised. The data in figure two show that separate responses were elicited with an interstimulus interval of 200 milliseconds. This would provide a framerate of 5 Hertz, which we believe is adequate for most low-vision needs. This is also limited by the pneumatic method of ejection used in these studies. The time require to charge and discharge the tubing capacitance slows the temporal response of our ejection system. This would be significantly improved if electro-osmotic flow were employed.

## V. References


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