31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Direct Activation of Retinal Ganglion Cells with Subretinal Stimulation

D. Tsai, *Student Member, IEEE*, J. W. Morley, G. J. Suaning, *Member, IEEE*, N. H. Lovell, *Senior Member, IEEE*

Abstract— **Recent advances in the design and implementation of vision prostheses have made these devices a promising therapeutic option for restoring sight to blind patients in the near future. The success of vision prostheses in providing clinically useful vision, however, depends critically on our understanding of the retinal neural mechanisms evoked during electrical stimulation, and how these mechanisms can be controlled precisely to elicit the desired visual percept. We demonstrate here that subretinal stimulation can reliably elicit stimulus–locked short latency (≤ 2 ms) responses. To our knowledge, this is the first report of such responses using the subretinal paradigm. These responses could be readily distinguished from within the stimulus artifacts using cell–attached extracellular recording or whole–cell patch clamp. The thresholds for these short latency responses were determined for ON, OFF and ON–OFF type retinal ganglion cell classes across cathodic biphasic pulses of 0.1 – 5.0ms. No significant difference was found for the mean latency and the threshold for the different cell types over the pulse range tested.**

I. INTRODUCTION

Progress in recent years has made vision prostheses, devices that activate the retinal cells using artificial electrical stimuli, a promising therapeutic option for the profoundly vision–impaired. Indeed, a number of prototype devices have moved into the clinical trial phase [1]–[4]. Tests on blind human subjects have demonstrated that "bright spots", formally referred to as phosphenes, can be generated with electric pulses delivered via the implanted electrodes of a vision prosthesis. Although promising, percepts generated by devices to date have been rudimentary, and the psychophysical responses elicited in the patients were often unexpected [5], [6]. Thus one of the challenges in designing and implementing vision prostheses is understanding the retinal neural mechanisms elicited by electric impulses and how these mechanisms can be controlled artificially to provide clinically useful vision.

Several vision prosthesis designs have been proposed, including subretinal [7], [8], epiretinal [4], [9] and supra–choroidal [1], [10], [11]. These designs differ in the position of the stimulation electrodes relative to the retina. The subretinal approach places the electrodes between the photoreceptors and the retinal pigment epithelium. A number of investigators have previously reported on the neural responses with subretinal stimulation using frogs [12], chicken [13], rabbits [14]–[16] and mice [17], [18]. While charge balanced biphasic stimuli are considered to be the safest approach [19], one of these studies [14] used monophasic stimuli. Electrode size is a limiting factor for the perceived spatial resolution that vision prostheses can deliver. With the exception of [16], where 25 μm electrodes were used, all existing subretinal work used relatively large electrodes (≥ 50 μm). However, in the case of [16] they found the small platinum electrodes to be unreliable at eliciting responses when stimulus pulse widths below 1.0 ms were used. Most significantly, none of the previous subretinal stimulation studies provided conclusive evidence for the existence of short latency responses ($\leq$ 2 ms) elicited through direct activation of retinal ganglion cells (RGCs). In fact, the possibility of eliciting such responses has even been questioned [12].

In this study by using cell–attached extracellular recording and whole–cell patch clamp we demonstrate that electrodes with 25 μm diameter can reliably activate RGCs directly with the subretinal approach and that short latency stimulus-locked responses can be resolved from the stimulus artifact. We also report on the threshold for direct RGC activation using this configuration for pulse widths 0.1 – 5.0 ms.

II. METHODS

A. Flattened Whole–mount Retinal Preparation

All experimental procedures were approved by the UNSW Animal Care and Ethics Committee. New Zealand White rabbit (n = 12) was anesthetized with intra–muscular injection of ketamine (70 mg/kg) and xylazine (10 mg/kg). An eye was enucleated and hemisected 2 to 3 mm posterior to the ora serrata. The front portion and the vitreous were discarded. Three pieces of the inferior retina with the underlying sclera were dissected free and placed in an incubation chamber containing Ames' Medium (Sigma Aldrich) equilibrated to pH 7.4 with carbogen (95% O_2, 5% CO_2) with temperature at either 25°C or 31°C for 1 hour, then transferred to room temperature, and kept for up to 10 hours before recording.

Prior to recording the retinal ganglion cells were labeled by immersing a piece of the retina, with attached sclera, in Ames' Medium containing Azure B (1 mg/ml) [20], [21] for 45 seconds. The neural retina was then extracted and placed photoreceptor-side up in an imaging chamber perfused with Ames' Medium (pH 7.4, $34-35$ °C) at 5 ml per minute on an inverted microscope.

Tsai, Suaning and Lovell: Graduate School of Biomedical Engineering, University of New South Wales, Sydney, Australia. Morley: School of Medicine, University of Western Sydney, Penrith, Australia

The authors would like to thank Philip Byrnes–Preston for designing and expert assistance in implementing the neural stimulator.

This research is supported in part by funding from the National Health and Medical Research Council of Australia.

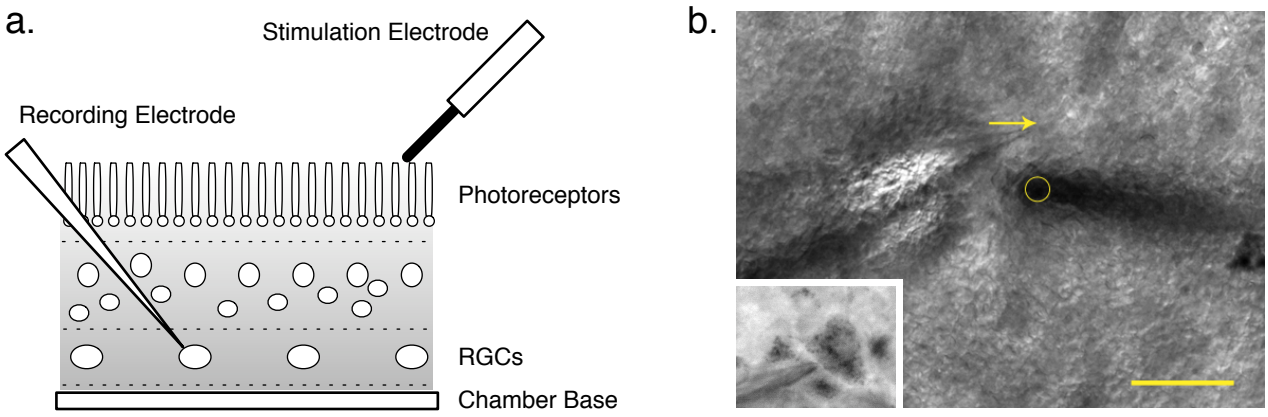

Fig. 1. The stimulation and recording setup. A. Schematic diagram for electrode placements. The stimulation electrode delivers pulses from the photoreceptor–side. B. Photograph of the retina during recording and stimulation, viewed from the RGC–side at 100X magnification. The arrow indicates cell location. The circle marks the contact position of the stimulation electrode at the photoreceptor side. The scale bar is 100 μm. Inset: 400X view of the cell and the attached recording electrode.

B. Delivery of Electrical Stimulation

The electrical stimuli consisted of cathodic–first constant current charge–balanced biphasic pulses without inter-pulse separation generated via a custom built neural stimulator. It is able to generate up to 200 μA of current with step resolution of 0.78 μA. The stimuli were delivered via an electrode fabricated from Pt–Ir wire with 25 μm diameter (A–M Systems) and placed 55 ± 10 μm from the cell soma under visual guidance. The stimulation ground electrode consisted of a large Pt wire loop placed in the perfusion bath. The stimulation pulse widths used in the study were (ms): 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0.

C. Recording of Retinal Ganglion Cell Responses

The responses of RGCs to electrical stimulation were recorded with a MultiClamp 700B patch clamp amplifier (Molecular Devices), under either cell–attached mode or whole–cell mode. Data were low–passed at the amplifier by an 8–pole 3 kHz filter and digitized at 10 kHz with Digidata 1440A and the pClamp 10 software (Molecular Devices). All recordings were carried out under mesopic lighting. The retinas were visualized with either Hoffman Modulation Contrast or Nomarski Differential Interference Contrast optics under near–IR illumination.

Recording electrodes were pulled from borosilicate capillary glass with a multi–stage puller (P–97, Sutter Instrument). When recording under whole–cell mode the electrodes were filled with a solution containing (mM): 116 $KMeSO_4$, 10 KCl, 0.5 EGTA, 1 $MgCl_2$, 10 HEPES, 4 $ATP - Na_2$ and 0.5 $GTP - Na_3$, adjusted to pH 7.2 with KOH. The electrodes had resistance of 3–6 MΩ with this solution.

The light response (ON, OFF, ON–OFF) of the recorded cells were determined by projecting a 250 μm spot of light over the cell body via the microscope's 10X objective.

Neural synapses were blocked pharmacologically as required with 250 μM of $CdCl_2$ added directly to the perfusion medium.

The threshold of electrical stimulation was defined as the current level required to elicit spikes in at least 10 of 20 consecutive trials (50%) with 1 second delay between trials to minimize potential long–lasting effects of repetitive stimuli [18]. Response latencies were calculated from the beginning of the electrical stimulation artifact to the peak of the cell's action potential. Latencies were considered long when > 2 ms, otherwise they were considered short. A schematic diagram of the recording and subretinal stimulation set up is shown in Figure 1a.

III. RESULTS

A. Subretinal Stimulation Can Activate RGCs Directly with Short Latency

Figure 1b shows a typical recording and stimulation arrangement. The retina was visualized from the ganglion cell side at 100x magnification. The figure shows the recording electrode approach the cell from the left. The arrow indicates the position of the cell. The circle marks the location of the stimulation electrode, approaching from the right, at the photoreceptor–side.

To determine the responses of RGCs to subretinal electrical stimulation we performed cell–attached extracellular recordings and whole–cell patch clamp recordings. RGCs could be activated directly with appropriate levels of stimulus strength, and importantly the stimulus artifacts did not mask the short–latency evoked spikes, a situation that would render them indistinguishable. Figure 2a shows ten superimposed cell–attached recordings of an OFF–type RGC upon stimulation with a 35 μA 0.1 ms pulse. The cathodic–first biphasic artifacts are apparent. A single action potential was evoked successfully following the electrical pulse, as characterized by the distinct downward hyperpolarizing component, in seven of the ten trials. Responses were also elicited with 5 ms pulses (Figure 2b) where a biphasic stimulus of 4 μA

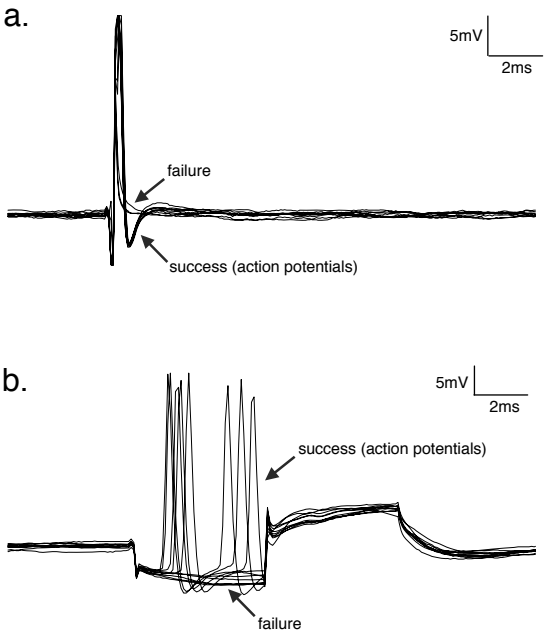

Fig. 2. Superimposed traces of short latency responses of an RGC to subretinal stimulation. A. 35 μA A 0.1ms pulse elicited a spike in 7 of 10 trials. B. 4 μA 5.0 ms pulses elicited spike(s) in 8 of 10 trials. In both cases the RGC was activated directly by the stimuli. These short latency responses are readily distinguished from the stimulus artifacts.

elicited a spike in eight of the ten trials. Additionally, three of the successful attempts also resulted in a second spike following the first. Similar to the 0.1 ms case of Figure 2a, action potentials here are easily distinguished from the stimulation artifacts.

These responses remained in the presence of the synaptic blocking agent $CdCl_2$ indicating they were not of presynaptic origin. Namely, the electrical stimuli activated the retinal ganglion cell directly, rather than through activation of cells in the outer retinal layers, which then subsequently synapsed onto the cell under study.

In summary, RGCs can be activated directly under the subretinal stimulation paradigm. Furthermore, cell–attached recording and whole–cell patch clamp can resolve the resulting short latency responses faithfully from within the stimulation artifacts.

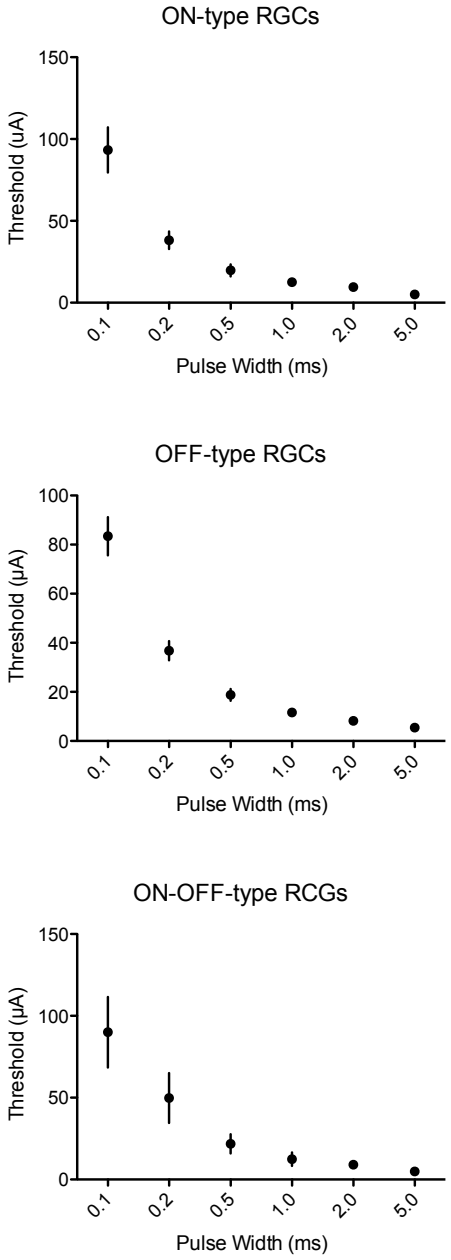

Fig. 3. Strength duration curve (mean $\pm$ SEM) for direct activation of 14 ON, 23 OFF and 5 ON–OFF type RGCs with subretinal stimulation.

B. Threshold and Latency of Direct RGC Activation

We determined the threshold for direct activation of RGCs through subretinal stimulation using biphasic pulses of widths 0.1 – 5.0ms. The strength duration curves for ON (n=14), OFF (n=23) and ON–OFF (n=5) cells are summarized in Figure 3. There is a tendency for ON cells to have slightly higher thresholds than OFF cells. However, there is no statistical significance for the threshold differences between the three cell types using two–way ANOVA (two–tail, P = 0.656).

The median response latency of direct activation using 0.1 ms biphasic pulses was found to be 0.999 ± 0.100 ms when tested on 7 cells, where all direct activation responses were verified pharmacologically with $CdCl_2$.

IV. Discussion

We have shown here that with appropriate stimulation strength 25 μm electrodes placed subretinally can reliably activate RGCs directly for all pulse widths tested (0.1 – 5.0ms). In addition, cell–attached recording or whole–cell patch clamp can discern the resulting short latency stimulus–locked spikes from the artifacts.

Previous works have not provided conclusive evidence for these responses during subretinal stimulations. The reason

for this is likely due to the recording technique used. With the exception of [12] and [13], tungsten or carbon fiber microelectrodes were used [14]–[18]. Notably the current findings parallel the development in epiretinal stimulation studies. Early reports in the area did not observe short latency responses (for example see [14], [22]). However, more recently Fried [23] demonstrated their existence during epiretinal stimulation using cell–attached recording and whole–cell patch clamp. Also, by using an artifact subtraction technique Sekirnjnak [24] have also been able to show these responses using multi–electrode array recordings of epiretinally stimulated mammalian retina.

Jensen [14] reported differences in threshold and latency of ON versus OFF cells. We have not found any systematic differences in the short latency responses reported here between the different cell types. In addition, no significant differences were apparent for the thresholds between cell types. This is in agreement with Sekirnjak's findings [24] involving direct activation of RGCs.

Thresholds were not systematically explored in [12] and [13]. Monophasic current pulses were used in [14] and anodic–first biphasic current pulses were used in [15] and [18]. The stimulation configurations used here are comparable to Shyu [16] and O'Hearn [17]. The thresholds for $1\ \mathrm{ms}$ pulses were reported in both. The values we found are slightly lower, but in general agreement with Shyu. They are, however, significantly lower than those of O'Hearn. This may be due to the larger electrodes used ($125\ \mu\mathrm{m}$) and possibly also experimental species (mice, wild–type).

Nevertheless care is warranted in comparing the thresholds reported herein to earlier works, where thresholds were determined on the basis of eliciting RGC spikes with latencies in the order of several to tens of milliseconds. Our unpublished observations suggest that these responses may appear concomitantly with short latency spikes (as shown in the present report) at the same threshold, or may appear at either lower or higher threshold than the short latency responses. Therefore we propose that RGC thresholds should always be reported in the context of response latency.

REFERENCES

[1] T. Fujikado, T. Morimoto, H. Kanda, S. Kusaka, K. Nakauchi, M. Ozawa, K. Matsushita, H. Sakaguchi, Y. Ikuno, M. Kamei, and Y. Tano, "Evaluation of phosphenes elicited by extraocular stimulation in normals and by suprachoroidal–transretinal stimulation in patients with retinitis pigmentosa," *Graefe's Arch Clin Exp Ophthalmol*, vol. 245, pp. 1411–1419, 2007.

[2] F. Gekeler, A. Messias, M. Ottinger, K. U. Bartz-Schmidt, and E. Zrenner, "Phosphenes electrically evoked with DTL electrodes: A study in patients with retinitis pigmentosa, glaucoma, and homonymous visual field loss and normal subjects," *Invest Ophthalmol Vis Sci*, vol. 47, pp. 4966–4974, 2006.

[3] J. F. Rizzo III, J. Wyatt, J. Loewenstein, S. Kelly, and D. Shire, "Methods and perceptual thresholds for short–term electrical stimulation of human retina with microelectrode arrays," *Invest Ophthalmol Vis Sci*, vol. 44, pp. 5355–5361, 2003.

[4] M. S. Humayun, E. de Juan Jr., J. D. Weiland, G. Dagnelie, S. Katona, R. Greenberg, and S. Suzuki, "Pattern electrical stimulation of the human retina," *Vision Res*, vol. 39, pp. 2569–2576, 1999.

[5] J. F. Rizzo III, R. J. Jensen, J. Loewenstein, and J. Wyatt, "Unexpectedly small percepts evoked by epi-retinal electrical stimulation in blind humans," in *Invest Ophthalmol Vis Sci (E–Abstract 4207)*, vol. 44, 2003.

[6] J. F. Rizzo III, J. Wyatt, J. Loewenstein, S. Kelly, and D. Shire, "Perceptual efficacy of electrical stimulation of human retina with a microelectrode array during short–term surgical trials," *Invest Ophthalmol Vis Sci*, vol. 44, pp. 5362–5369, 2003.

[7] J. Salzmann, O. P. Linderholm, J.-L. Guyomard, M. Paques, M. Simonutti, M. Lecchi, J. Sommerhalder, E. Dubus, M. Pelizzone, D. Bertrand, J. Sahel, P. Renaud, A. B. Safran, and S. Picaud, "Subretinal electrode implantation in the P23H rat for chronic stimulations," *Br J Ophthalmol*, vol. 90, no. 9, pp. 1183–1187, Sep 2006.

[8] M. Völker, K. Shinoda, H. Sachs, H. Gmeiner, T. Schwarz, K. Kohler, W. Inhoffen, K. U. Bartz-Schmidt, E. Zrenner, and F. Gekeler, "In vivo assessment of subretinally implanted microphotodiode arrays in cats by optical coherence tomography and fluorescein angiography," *Graefe's Arch Clin Exp Ophthalmo*, vol. 242, pp. 792–799, 2004.

[9] W. Liu, M. Sivaprakasam, P. R. Singh, R. Bashirullah, and G. Wang, "Electronic visual prosthesis," *Artificial Organs*, vol. 27, pp. 986–995, 2003.

[10] J. A. Zhou, S. J. Woo, S. I. Park, E. T. Kim, J. M. Seo, H. Chung, and S. J. Kim, "A suprachoroidal electrical retinal stimulator design for long–term animal experiments and in vivo assessment of its feasibility and biocompatibility in rabbits," *Journal of Biomedicine and Biotechnology*, vol. 2008, pp. 1–10, 2008.

[11] Y. T. Wong, S. C. Chen, J. M. Seo, J. W. Morley, N. H. Lovell, and G. J. Suaning, "Focal activation of the feline retina via a suprachoroidal electrode array," 2009, Vision Res, in press, doi: 10.1016/j.visres. 2009.02.018.

[12] L. Li, Y. Hayashida, and T. Yagi, "Temporal properties of retinal ganglion cell responses to local transretinal current stimuli in the frog retina," *Vision Res*, vol. 45, pp. 263–273, 2005.

[13] E. Stett, W. Barth, S. Weiss, H. Haemmerle, and E. Zrenner, "Electrical multisite stimulation of the isolated chicken retina," *Vision Res*, vol. 40, pp. 1785–1795, 2000.

[14] R. J. Jensen and J. F. Rizzo III, "Thresholds for activation of rabbit retinal ganglion cells with a subretinal electrode," *Exp Eye Res*, vol. 83, pp. 367–373, 2006.

[15] R. J. Jensen and J. F. Rizzo, "Responses of ganglion cells to repetitive electrical stimulation of the retina," *J Neural Eng*, vol. 4, pp. 1–6, 2007.

[16] J.-S. Shyu, M. Maia, J. D. Weiland, T. O'Hearn, S.-J. Chen, E. Margalit, S. Suzuki, and M. S. Humayun, "Electrical stimulation in isolated rabbit retina," *IEEE Trans Neural Syst Rehabil Eng*, vol. 14, pp. 290–298, 2006.

[17] T. M. O'Hearn, S. R. Sadda, J. D. Weiland, M. Maia, E. Margalit, and M. S. Humayun, "Electrical stimulation in normal and retinal degeneration (rd1) isolated mouse retina," *Vision Res*, vol. 46, pp. 3198–3204, 2006.

[18] R. J. Jensen and J. F. Rizzo III, "Activation of retinal ganglion cells in wild-type and rd1 mice through electrical stimulation of the retinal neural network," *Vision Res*, vol. 48, pp. 1562–1568, 2008.

[19] D. R. Merrill, M. Bikson, and J. G. R. Jefferys, "Electrical stimulation of excitable tissue: Design of efficacious and safe protocols," *J Neurosci Methods*, vol. 141, pp. 171–198, 2005.

[20] E. H. Hu, R. F. Dacheux, and S. A. Bloomfield, "A flattened retina-eyecup preparation suitable for electrophysiological studies of neurons visualized with trans-scleral infrared illumination," *J Neurosci Methods*, vol. 103, pp. 209–216, 2000.

[21] F. R. Amthor, K. T. Keyser, and N. A. Dmitrieva, "Effects of the destruction of starburst-cholinergic amacrine cells by the toxin AF64A on rabbit retinal directional selectivity," *Vis Neurosci*, vol. 19, pp. 495–509, 2002.

[22] R. J. Jensen and J. F. Rizzo III, "Responses of rabbit retinal ganglion cells to electrical stimulation with a subretinal electrode," in *Invest Ophthalmol Vis Sci (E–Abstract 5279)*, vol. 46, 2005.

[23] S. I. Fried, H. A. Hsueh, and F. S. Werblin, "A method for generating precise temporal patterns of retinal spiking using prosthetic stimulation," *J Neurophysiol*, vol. 95, pp. 970–978, 2006.

[24] C. Sekirnjak, P. Hottowy, A. Sher, W. Dabrowski, A. M. Litke, and E. J. Chichilnisky, "Electrical stimulation of mammalian retinal ganglion cells with multielectrode arrays," *J Neurophysiol*, vol. 95, pp. 3311–3327, 2006.