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Efficacy of the Hexpolar Configuration in Localizing the Activation of Retinal Ganglion Cells under Electrical Stimulation

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Abstract— **Retinal visual prostheses provide hope of restoring sight to patients suffering from retinal degeneration such as retinitis pigmentosa and age-related macular degeneration. Retinal prostheses are used to electrically stimulate residual neurons that are spared in these diseases, namely the retinal ganglion cells (RGCs), eliciting percepts of light termed 'phosphenes'. The elicitation of multiple phosphenes via an electrode array allows patterns to be produced, resulting in a rudimentary form of vision. For such patterns to be produced effectively, the prosthesis must generate well-defined phosphenes. To this end, the hexpolar configuration has been proposed as an alternative to the traditional monopolar or bipolar configurations. It utilizes six electrodes surrounding the stimulating electrode to serve as a combined return, or 'hex guard', purportedly localizing the activation to cells located within them. In this study, the efficacy of the hexpolar configuration in localizing activity was investigated by using patch-clamp electrophysiology to measure the activation thresholds of RGCs to electrical stimulation in isolated rabbit retina. Cells located outside the hex guard were found to have significantly higher relative hexpolar thresholds (>2 fold) as compared to cells located within the hex guard. This confirms the efficacy of the hexpolar configuration in localizing activity to within the hex guard. Furthermore, the effect of using cathodic-first versus anodic-first stimulation on hexpolar threshold and localization was investigated. No significant difference was observed between the two groups, in terms of lowering thresholds or improving localization.**

I. Introduction

The development of retinal visual prostheses by many groups around the world is providing hope for patients suffering from blinding diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD). These debilitating diseases lead to the loss of image forming vision due to degeneration of the photoreceptors. Fortunately, cells of the inner retina, chiefly the retinal ganglion cells (RGCs), remain functional and are capable of relaying information to the visual centers of the brain [1]. A number of recent studies [2-4] have shown that perception of light (phosphenes) can be elicited in human patients when the remaining RGCs are activated by electrical stimulation from an array of electrodes implanted on or near the retina. The use of an electrode array to stimulate populations of cells in the retina enables patterns of phosphenes to be generated, that may result in a rudimentary form of vision.

To achieve visual patterns using phosphenes, it is necessary to be able to control, as far as possible, the size of the active population of RGCs, such that the smaller the active population the higher the spatial resolution achievable. This involves limiting the RGCs activated to only those in the direct vicinity of the stimulating electrode(s). This may be achieved by strategic placement of the stimulating and return electrode(s) in particular configurations, thereby shaping and focusing the electric field produced by the stimulation.

Many groups utilize the monopolar configuration for electrical stimulation of retinal tissue [2, 5-9]. This configuration utilizes a larger electrode than the stimulating electrode to serve as a return, and is located far from the stimulating electrode. The resulting electric field is likely to spread across a large area, consequently activating a substantial zone of tissue around the stimulating electrode.

An alternative to the monopolar stimulation involves placing the return electrode in close proximity to the stimulating electrode, in what is known as bipolar configuration [6, 10, 11]. Modeling studies have shown that the placement of the return electrode – which is of comparable size to the stimulating electrode – near the stimulating electrode allows the electric field to be focused between the electrodes, limiting the spatial extent of cell activation [12, 13]. However, these studies also predict the stimulation of cells located underneath the return electrode, effectively increasing the area of retinal activation. Furthermore, modeling studies by Lovell et al. [14] show poor isolation between stimulation sites using this configuration, manifested as significant cross talk between stimulation channels when unbalanced currents are injected simultaneously.

The hexpolar configuration has been proposed as a method to localize the activation of retinal cells without the drawbacks of the bipolar configuration. It utilizes six adjacent electrodes surrounding the stimulating electrode that act as a combined return. The combined return surrounding the stimulating electrode focuses the electric field to the area within the hexagon, in close proximity to the stimulating electrode. Furthermore, the surrounding electrodes act as a 'guard', isolating the fields of the active electrodes from one another and subsequently reducing cross talk.

An *in-vivo* study by Wong et al. [15] demonstrated the localization of activity in the cat visual cortex in response to retinal stimulation using the hexpolar configuration.

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However, to date no studies have been conducted to assess the efficacy of the hexpolar configuration in localizing the activation of retinal cells *in-vitro*. This study investigates the spatial extent of activation of RGCs by electrical stimulation using the hexpolar configuration as compared to the monopolar configuration in isolated rabbit retina.

II. Methods

A. Tissue Preparation

All procedures were performed at the University of Western Sydney, and approved by the Animal Care and Ethics Committee of the University. New Zealand White Rabbits (2.5 – 3.0 kg) were anaesthetized by an intramuscular injection of ketamine (70 mg/kg) and xylazine (10 mg/kg). Isofluorane was administered via a mask for deeper anesthesia prior to enucleation. The eyes were enucleated, and the animal was immediately sacrificed via an overdose of sodium pentobarbital. Each eye was rinsed in ice-cold PBS for <2 s and placed in ice-cold Ames' medium bubbled with carbogen (95% O_2 and 5% CO_2). Each eye was hemisected and the vitreous and lens removed. The eyecups were then placed in an incubation chamber filled with bubbled Ames' medium at 35 °C for 1 hr. After incubation, the inferior retina – containing the visual streak – was cut into 5 x 5 mm sections using a razor blade, and a section transferred into the recording chamber. The retina was separated from the underlying choroid and sclera using a fine paintbrush, and was anchored onto the array using a tissue harp. The retina was oriented with the photoreceptors closest to the array and the RGCs facing up, allowing the retina to be electrically stimulated using a subretinal stimulation paradigm.

B. Recording Chamber and Multielectrode Array

The recording chamber consisted of a Multielectrode Array (MEA, Qwane Biosciences S.A., Lausanne, Switzerland) with a silicone rubber ring surrounding the electrodes to form a tissue chamber. The perfusion fluid was gravity-fed into the chamber and consisted of bubbled Ames' medium at 33-34 °C.

The MEA comprised 60 circular platinum electrodes, each with a diameter of 40 μm, arranged in an elongated hexagonal arrangement with interelectrode distances of 140 μm and 200 μm, as shown in Fig. 1.

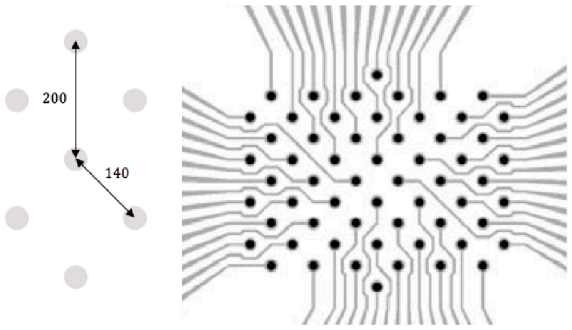

Figure 1. Right: hexagonal arrangement of electrodes in multielectrode array. Left: center-to-center interelectrode spacing (in μm).

C. Patch-Clamp Recordings

RGCs, identified by their size (10-20 μm), at different distances to the stimulating electrode were targeted for whole-cell patch-clamp recordings. A CCD camera (CA-152, Wooju ,Communications, Incheon, Korea) was connected to a fixed-stage upright microscope (BX51WI, Olympus, Tokyo, Japan) housing a 40x objective to view the preparation. A CCD camera (iXon^{EM}+ 897, Andor, Belfast, Northern Ireland) was used to capture images, which were viewed using MetaFluor (Molecular Devices, California, USA) imaging software.

Borosilicate glass micropipettes were fabricated with the aid of a glass puller (P-97, Sutter Instruments, California, USA). The tip of the micropipette had an impedance of 5-6 MΩ when filled with an internal solution of the following composition (in mM): 120 $KMeSO_4$, 10 KCl, 0.008 $CaCl_2$, 0.5 EGTA, 1 MgCl, 10 HEPES, 4 ATP-Na_2 and 0.5 GTP-Na_3. The solution was adjusted to a pH of 7.4 with KOH, and to 270-290 mOsm.

Amplification and data acquisition were achieved via an Axon MultiClamp 700b amplifier and a Digidata 1440 digitizer (both by Molecular Devices). Data were recorded using pClamp 10 software (Molecular Devices) at a sampling rate of 50 kHz and low-pass filtered at 3 kHz.

D. Electrical Stimulation

Electrical stimulation of the retina was achieved by connecting a stimulator (Multichannel Systems) to the electrodes of the array via a signal divider (Multichannel Systems), allowing each electrode to be addressed individually.

Ten constant current, cathodic-first, balanced, biphasic waveforms, with pulse-widths of 500 μs per phase, were injected at a rate of 1 Hz, in both the monopolar and the hex return configurations. Threshold values for each of the configurations were then measured, and the process was repeated for anodic-first stimuli. Threshold was taken as the minimum current amplitude required to elicit a spike in ≥50% of the stimulation events (5 or more of the 10 applied pulses).

E. Data Analysis

Statistical analyses were performed using Prism 5 (GraphPad, California, USA). In all cases, a statistical significance level of $p<0.05$ was adopted.

III. Results

A. Monopolar Stimulation

Threshold values for cells stimulated using the monopolar configuration are plotted in Fig. 2. The data show an increase in activation thresholds as the distance of the cell to the stimulating electrode increases, for both cathodic-first and anodic-first stimulation.

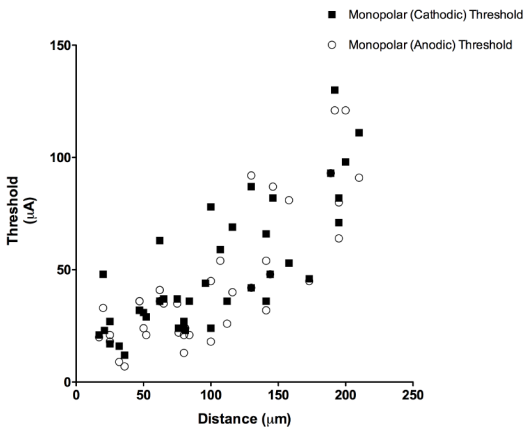

Figure 2. Activation thresholds for monopolar cathodic-first and anodic-first stimulation increase with the distance of the cell from the stimulating electrode.

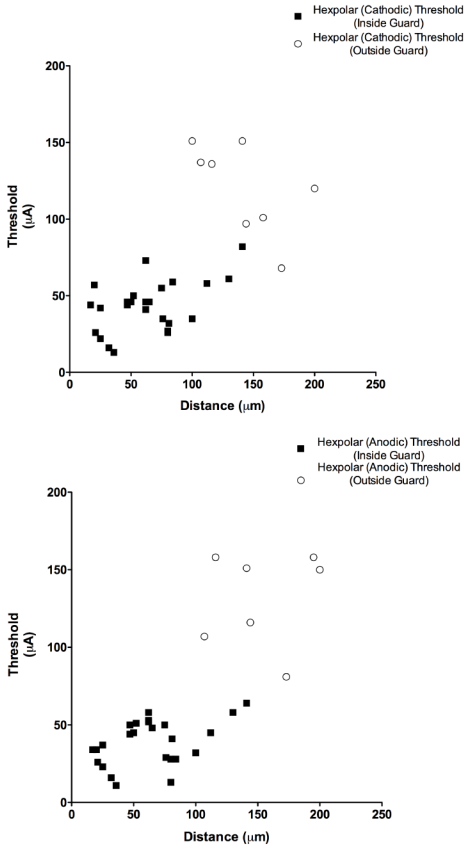

Figure 3. Activation thresholds for cells located inside and outside the hex guard increase with the distance of the cell from the stimulating electrode, for both cathodic-first and anodic-first stimulation.

B. Hexpolar Stimulation

Analysis of hexpolar thresholds was split into two categories; cells located within the hex guard, and cells located outside of the guard.

For cells located within the hex, activation thresholds were observed to increase with increasing cell distance from the stimulating electrode, as shown in Fig. 3. No significant difference was observed in threshold between hexpolar cathodic-first versus anodic-first stimulation (Wilcoxon matched-pairs signed rank test, $p=0.05$).

Many of the cells located outside the guard (8/16 cells for cathodic-first, and 10/16 cells for anodic-first stimulation) could not be activated using the hexpolar configuration at the maximum current output of the stimulator (160 μA). These cells were assigned a threshold of 160 μA, an underestimate of their true threshold, for statistical analysis.

A virtue of the elongated hex is that cells located within a distance of 98-140 μm from the stimulating electrode may be either inside or outside the hex guard. Eight of the RGCs were within that distance, four of which were located outside the hex guards and had significantly higher hexpolar thresholds than the four located within the hex guards, for both cathodic-first and anodic-first stimulation (inside/outside cathodic-first: n=8, 59.0±9.62/134±9.90, inside/outside anodic-first: n=8, 49.8±7.12/133±12.6, Mann-Whitney, $p<0.05$).

C. Hexpolar versus Monopolar Stimulation

For cells located within the hex guard, hexpolar thresholds were significantly higher than monopolar thresholds, for both cathodic-first and anodic-first stimulation (Wilcoxon matched-pairs signed rank test, $p<0.0001$).

For cells located outside the hex, hexpolar thresholds were significantly higher than monopolar thresholds, for both cathodic-first and anodic-first stimulation (Wilcoxon matched-pairs signed rank test, $p<0.0001$).

We calculated a relative hexpolar threshold. This value was taken as the difference between a cell's raw hexpolar threshold and its corresponding monopolar threshold. The relative hexpolar thresholds for cells located within the hex guard were then directly compared to that of the cells located outside the guard. These data show a significant difference between the data sets (Mann-Whitney, $p<0.0001$), with average relative threshold of cells outside the hex guard being >2 fold for those cells located within the hex guard (Fig. 4). No significant difference was observed between relative hexpolar thresholds for cathodic-first versus anodic-first stimulation (Wilcoxon matched-pairs signed rank test, $p>0.05$).

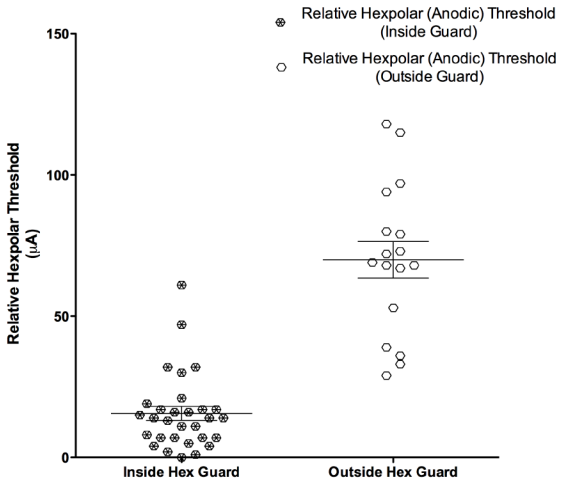

Figure 4. Relative anodic-first hexpolar thresholds for cells located within and outside the hex guard show a significant difference (Mann-Whitney, $p<0.0001$). Cells located outside the hex demonstrated higher relative hexpolar thresholds than those located within the hex guard.

IV. Discussion

A. Localization of Activity

The aim of this study was to assess the ability of the hexpolar configuration to provide better localization of RGC activity, compared to that of a monopolar configuration. As an indicator of the relative extent of spatial activation by each return configuration we measured the activation thresholds of RGCs to electrical stimulation at different distances from the stimulating electrode, within and outside the hex guard electrodes using both return configurations. The results showed that activation was indeed localized to cells located within the hex guard, as manifested by the significantly higher relative thresholds of activation for RGCs located outside the hex guard than those located within the guard. This result is in line with the findings by Wong et al. [15] on localization of activity in the feline cortex, in response to suprachoroidal retinal stimulation using the hexpolar configuration.

As noted earlier, many of the cells located outside the hex guard could not be stimulated using the hexpolar configuration, even with a stimulus amplitude set to the maximum output of the stimulator of 160 μA. It is important to note that these cells were indeed viable, and were able to be activated by the hexpolar configuration when using an adjacent stimulation electrode closer to the cell (the cell then being within the hex guard) as well as using the monopolar configuration. The hexpolar threshold value for these cells was set to 160 μA, which is an underestimate of their true hexpolar threshold. As a result, the difference in relative hexpolar thresholds presented here for cells located within the hex guard versus outside the guard is conservative estimate of the true difference in thresholds.

B. Cathodic-First versus Anodic-First Stimulation

Monopolar stimulation showed slightly but significantly higher thresholds for cathodic-first versus anodic-first stimulation, irrespective of the order in which they were presented. This finding is consistent with work by Jensen et al. [7] who showed higher thresholds for OFF RGCs using cathodic-first stimulation (but no significant difference for ON RGCs).

For cells located within the hex guard, there was no significant difference in cathodic-first versus anodic-first hexpolar thresholds, irrespective of the order in which they were presented. With regards to localization of activity, there was no significant difference observed between the relative hexpolar thresholds for cathodic-first versus anodic-first stimulation for cells located outside the hex guard. Therefore, combining these results, there seems to be no advantage, in terms of lower thresholds or increased localization, in using one stimulus polarity over the other when stimulating using the hexpolar configuration.

V. Conclusion

The aim of this study was to investigate the efficacy of the hexpolar configuration in localizing activity of RGCs, compared to the monopolar configuration. This study has shown that the hexpolar configuration can preferentially activate cells within the hex guard. Combined with results from *in-vivo* cortical recordings also conducted by our group [15], it has shown that the hexpolar configuration may prove valuable in cases where the stimulation of spatially distinct population of cells is required.

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