Original Paper

The Spatial Extent of Epiretinal Electrical Stimulation in the Healthy Mouse Retina

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Key Words

Electrical response function • Hysteresis • Retinal implant • Bionic vision • Electrical point spread function

Abstract

Background/Aims: Retinal prostheses use electrical stimulation to restore functional vision to patients blinded by retinitis pigmentosa. A key detail is the spatial pattern of ganglion cells activated by stimulation. Therefore, we characterized the spatial extent of network-mediated electrical activation of retinal ganglion cells (RGCs) in the epiretinal monopolar electrode configuration. **Methods:** Healthy mouse RGC activities were recorded with a micro-electrode array (MEA). The stimuli consisted of monophasic rectangular cathodic voltage pulses and cycling full-field light flashes. **Results:** Voltage tuning curves exhibited significant hysteresis, reflecting adaptation to electrical stimulation on the time scale of seconds. Responses decreased from 0 to 300 μm , and were also dependent on the strength of stimulation. Applying the Rayleigh criterion to the half-width at half-maximum of the electrical point spread function suggests a visual acuity limit of no better than 20/946. Threshold voltage showed only a modest increase across these distances. **Conclusion:** The existence of significant hysteresis requires that future investigations of electrical retinal stimulation control for such long-memory adaptation. The spread of electrical activation beyond 200 μm suggests that neighbouring electrodes in epiretinal implants based on indirect stimulation of RGCs may be indiscriminable at interelectrode spacings as large as 400 μm .

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Introduction

There are several visual prostheses that stimulate different parts of the visual pathway, such as the optic nerve [1] and the visual cortex [2-4]. Retinal stimulation, however, appears to possess more advantages than stimulation of other parts of the visual system [5]. Nevertheless, retinal implants remain far from a perfect solution. The *in vitro* study of

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neural activities can offer valuable insights for successful clinical applications of prosthetic vision. It is essential to inspect the spatial extent of retinal ganglion cell (RGC) responses to understand the limitations in spatial resolution that are experienced by implanted patients. Retinal stimulation can be roughly divided into two subgroups – epiretinal and subretinal – based on which side of the retina the electrodes are placed [6]. As is common, using a monopolar electrode configuration in which the return electrode is implanted in a distant part of the eye or body, the flow of current through the retina near the electrode is approximately perpendicular to the surface of the retina [7]. Accordingly, for examinations of the spatial spread of electrical stimulation, the two configurations can be considered equivalent to a first approximation [8].

The spatial extent (characterized by the electrical point spread function, EPSF) is important because it dictates the limits of electrical cross-talk for multiple electrodes and ultimately the electrode spacing of an implant. In recent decades, many research groups have investigated the spatial extent of RGCs evoked by single electrode or spatially patterned stimulation. Stett et al. recorded spike activity from the chick retina with a dense hexagonal microelectrode array (Hex-MEA), with varying electrode diameters (10, 20, 30 μm) and interelectrode distances [9]. They were able to evoke responses up to 200 μm away from the subretinal needle electrode and reported a half-width at half-maximal response (HWHM) of 100 μm using an anodal voltage pulse. In an earlier study Stett et al used a bar-like spatial stimulation pattern (0.8, 1.2 and 1.6 V amplitude) by selecting a row or a column on a 8×8 MEA (diameter 10 μm , spacing 100 μm) which also yielded an increase in spike rate out to at least 100 μm in the chick retina [10]. Eickenscheidt et al showed that rectangular cathodal epiretinal stripe-like stimulation (areas 100 $\times$ 1000 μm^2 , step spacing 50 μm) across a multicapacitor array can evoke responses in rabbit ON RGCs which extends over HWHM distance of 230 μm [8]. Lorach et al. reported both the smaller ($\sim$ 100 μm) and larger ($\sim$ 250 μm) HWHM values in healthy and degenerated rat retina using a subretinal photovoltaic electrode array [11]. Similar distances for direct RGC stimulation were reported by Sekirnjak et al. who confirmed that RGCs as far as 160 μm away can be stimulated in the rat retina [12]. Recently, our group used a MEA with 30 μm electrode diameter and 200 μm interelectrode distance to show that RGC responsiveness in wild type mice declines swiftly from 200 μm to 283 μm [13]. Importantly, however, we found reliable RGC responses in a small subset of RGCs even for distances up to 1000 μm . This result has been confirmed by Stutzki et al. who found electrical responsiveness to extend out to 500-1000 μm [14]. Due to the large 200 μm interelectrode spacing of this array, we were unable to sample responses more densely or at shorter distances from the stimulating electrode. Therefore, we set out to further study the spatial extent of electrical stimulation in the retina with a denser MEA using the same stimuli as in our previous study.

Materials and Methods

Animals

Adult wild-type C57Bl/6J (Jackson Laboratory, Bar Harbor, ME, USA), postnatal day 30-45 mice were used. The mice were kept under a standard white cyclic lighting. They had free access to food and water. All possible efforts to minimize the number of animals used and their suffering were made. All animal experimental procedures were approved by state authorities (Regierungspräsidium, Tübingen) and conducted under the supervision of the Tübingen University facility for animal welfare (Einrichtung für Tierschutz, Tierärztlichen Dienst und Labortierkunde) in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Retinal Preparation

Inhalation of CO_2 was used to anesthetize each mouse. After checking for withdrawal reflex by a pinch of the tissue between toes, the mouse was euthanized by cervical dislocation. The eyes were then

removed under normal room light and placed in carbogenated (95% O₂ and 5% CO₂) artificial cerebrospinal fluid (ACSF) solution containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃ and 20 Glucose; pH 7.4. During the dissection process (under red light to reduce photoreceptor bleaching) the cornea, ora serrata, lens and vitreous body were removed. Next the optic nerve was cut at the base of the retina and finally the retina was detached from the pigment epithelium. Next all traces of vitreous material on the inner surface of the retina were removed. Retinas were maintained in carbogenated ACSF until needed. All recordings were performed with a planar microelectrode array (MEA). Retinas were cut in half and a retinal half was oriented and flattened onto the MEA, ganglion cell side down, with two miniature forceps and was allowed to adapt for >30 min. Care was taken not to damage the retina or MEA during this process. In order to create better contact between the retina and the electrodes, a dialysis membrane (CelluSep, Membrane Filtration Products Inc., Seguin, Texas, USA) mounted on a custom Teflon ring was lowered onto the retina [15]. The MEA was then placed under the preamplifier and continuously superfused with carbogenated ACSF (~4 ml/min, Peristaltic Perfusion System PPS2 perfusion pump; Multi Channel Systems, Reutlingen, Germany) maintained at 33°C using both a heating plate and a heated perfusion cannula (HE-Inv-8 & PH01; Multi Channel Systems).

MEA and Data Acquisition System

A planar MEA with hexagonal layout (Hex-MEA) containing 59 circular TiN electrodes (60HexaMEA40/10, diameter: 10 µm, interelectrode and row spacing: 40µm; Multi Channel Systems, Reutlingen, Germany) as shown in Fig. 1b with electrode tracks insulated by Si₃N₄ on a glass substrate was used for recording from RGCs. The electrodes had impedances of approximately 250 kΩ at 1 kHz. The data acquisition system consisted of the MEA60 system (MCS, Reutlingen, Germany) including a 60 channel preamplifier with integrated filters and a blanking circuit (MEA 1060-Inv-BC). The blanking circuit was used to reduce recording noise by disconnecting amplifiers during stimulation pulses. The MEA_Select software was used to ground any defective electrodes and to choose the stimulating electrode. All data collection was done using the MC_Rack program. The computer was also fitted with MC_Card data acquisition hardware, an RS-232 serial port via which MEA_Select communicated with the amplifier, and an analog input card to record stimulus trigger signals. The raw data was sampled at a rate of 50 kHz/channel. Using a filter with a bandwidth of 1 Hz-3 kHz and a gain of 1100. To confirm the contact of the retina on MEA, the retina was imaged by a microscope-mounted camera (DCC1645C, Thorlabs Inc, Newton, New Jersey, USA).

Stimulation

A stimulus generator (STG 2008, Multi Channel Systems, Reutlingen, Germany) was used to generate electrical stimulus pulses that were delivered from the ganglion cell side of the retina (epiretinally) via only one of the 59 electrodes. Stimulating electrodes were chosen based on proximity to electrodes with robust neural signals to ensure a maximum number of recorded neurons. In these epiretinal experiments, electrodes of the Hex-MEA were used to simultaneously stimulate and record from the retina. The stimulus consisted of monophasic cathodic rectangular voltage pulses of 1000 µs duration with the following amplitudes 100, 300, 500, 1000, 1500, 2000, and 2500 mV (Fig. 1c). Stimulus pulses were safely below the safe charge injection limit specified by the manufacturer for these electrodes (16 mA for 1 ms; corresponding to 4000 mV at an assumed impedance of 250 kΩ) curves. Above these limits, faradic reactions occur that will lead to electrolysis of the electrode. The 100 mV stimuli were subsequently found to not be reliably delivered by the stimulator and, consequently, are not included in the analysis presented here.

At the beginning and end of each experiment, spontaneous activity was recorded for ~30 s. We presented 5 voltage blocks, proceeding in an up/down sequence containing 14 pulses, repeated 5 times with an interval of 20 s after each block of 70 pulses to allow the recovery of RGC responsiveness before visual stimulation. The post-time setting in MC_Stim was set to 100 µs to facilitate active discharge of the stimulating electrode. Additionally, a wait time of 1 ms was set in the MEA_Select software to extend the blanking period before recording was resumed – thus minimizing stimulation artifacts.

To better sample various distances, upon completion of the full experiment, the electrical stimulation was often moved to a new electrode location and another full experiment was conducted with the same tissue. Accordingly, some cells yielded data from activation of multiple separate electrodes – each delivering a stimulus during a different, but sequential experiment. The reported distances are the distance from the

stimulating electrode and the electrode on which the cell was recorded. Of the total 58 cells recorded, 5 yielded samples from 5 separate stimulating electrodes and 28 yielded samples from 4 separate electrodes, the remaining 25 cells were only sampled once. Each of the 162 sample was independently tested for responsiveness, yielding 76 separate electrically responsive samples, and only included in Fig. 4 if the cell was found to be responsive for that particular stimulating electrode.

To monitor tissue health, six visual stimulus blocks were interleaved before, after, and between electrical stimulation blocks. Each visual stimulus block consisted of a full-field 'flash' stimulus of 2 s maximum brightness (ON, ~40 klx) followed by 2 s minimum brightness (OFF, ~20 lx), cycled 25 times without pause. Visual stimuli were presented with a commercial projector (Acer k10, US and Canada) through a custom optical pathway.

Data Processing and Analysis

The stored raw data were processed using commercial spike sorting software (Offline Sorter, Plexon Inc, TX, USA). To process the data, voltage traces were first high-pass filtered with a 12-pole Bessel filter with a cut off frequency of 51 Hz. Following this, putative action potential events (spikes) whose filtered amplitude was greater than four standard deviations below the mean were detected. These events were sorted into clusters with an automated routine (T-distribution expectation maximization) to assign noise events as well as spikes from up to 3 cells recorded on each electrode to their own separate 'units'. Finally, as a quality control step, the multiple automated sorting solutions were manually inspected and the best one was modified as necessary in order to minimize Type I and Type II errors in attribution of events to different sources. Only units with a distinct waveform, stability during the whole measurement, an interspike interval lockout corresponding to a neuronal refractory period, and cross-correlograms indicative of good sorting were considered to contain the spike train from a single RGC and included in the analysis presented here. Time stamps assigned to the detection threshold crossing of these sorted spikes were collected with NeuroExplorer (PlexonInc, TX, USA) and exported to MATLAB (The Mathworks, Natick, MA, USA) for further analysis.

In all experiments, we processed and analysed all RGC responses recorded on electrodes other than the stimulating and reference electrodes. For each cell, the spiking response was integrated over the interval spanning 10 to 100 milliseconds after pulse onset, and averaged across the 25 repetitions of each unique stimulus. Likewise, a spontaneous rate, chosen to best characterize the baseline firing rate within the context of ongoing electrical stimulation, was calculated from the 1 s of recording time before each pulse and averaged across all samples. The response integration window of 10 to 100 was chosen to exclude spikes generated by either direct RGC stimulation which have a latency <10 ms [12, 16, 17] or spontaneous spikes which make a significant contribution at latencies >100 ms [18, 19].

Our data was collected from 58 well-isolated RGCs from 3 retina halves – each from a different mouse. Of these, 36 cells were responsive to at least one of the 7 voltages. The vast majority were only responsive to -2000 and -2500 mV. In light of previous experiments [13], we interpret this to reflect less current for a given voltage due to smaller electrodes and higher impedance relative to previous experiments. A cell was considered 'responsive' if the average response to one of the voltages was above threshold (3 standard deviations above the spontaneous rate) and with a minimum average response rate of 60% - at least 3 spikes for every 5 stimulus repetitions. While matching voltage stimuli are separated into upward and downward sequences for Figure 3 to examine hysteresis, the upward and downward sequences were combined into a single voltage response function for Figure 4 and the associated analysis. All analyses and statistical tests were performed in MATLAB.

Results

The present observations demonstrate the spatial extent (from 0 to 300 μm) of network-mediated electrical activation of retinal ganglion cells (RGCs) with a dense Hex-MEA in the epiretinal electrode configuration (Fig. 1).

Raw voltage traces were filtered so that spike times relative to electrical stimulus pulses could be extracted (Fig. 2). The spiking response was integrated from 10 to 100 ms following

Fig. 1. Experimental design. a) Stimulation was delivered epiretinally by placing the ganglion cell side of the retina on the microelectrode array. b) The hexagonal array (Hex-MEA) used enabled dense sampling of the spatial spread of electrical stimulation. c) Prior to and after each experimental block, spontaneous activity (SP) was recorded for ~30 s. We presented 5 electrical stimulation blocks (ES), proceeding in the up/down sequence of 14 pulses shown repeated 5 times per block, and 6 visual stimulation blocks (VS) with a stimulus of 2 s ON (~40 klx) and 2 s OFF (~20 lx) that was cycled 25 times per block. An interval of about 20 s after each block was recorded in order to allow the recovery of RGC responsiveness. The electrical stimuli consisted of monophasic cathodic rectangular voltage pulses of 1000 μ s duration with the following amplitudes, -100, -300, -500, -1000, -1500, -2000, and -2500 mV. The 100 mV stimulus was not included in analysis because it could not be reliably delivered by the stimulator.

Fig. 2. Representative ganglion cell recording. a) Digitized voltage trace of responses to three stimuli. Stimulus artefacts indicated with arrows. Large positive deflections are perfusion drip artefacts. b) Zoom of the 300 ms surrounding the selected -2500 mV pulse from the upward half of the stimulus series. Spikes identified with arrows. c) Rastergram of all responses for this cell. The same spike train shown in b) are identified with arrows. d) Peristimulus time histogram binned at 2 ms intervals for all responses. Smoothed histogram (Gaussian smoothing filter, sigma = 4 ms) is overlaid. Light grey bar covering b-d indicates the 10 ms excluded from response. Darker grey bar is the 90 ms of response integration. e) Voltage-response tuning curve during rising and falling phases of the stimulus sequence. Direction of sequence indicated by arrows. Error bars are standard deviation of the 25 repetitions.

each pulse. This allowed us to examine the effects of indirect RGC stimulation via the activation of presynaptic neurons while excluding directly stimulated RGC spikes. Spikes with a latency

Fig. 3. Voltage-response tuning curves showing response hysteresis. a) Normalized tuning curve of 76 individual RGC samples. After subtracting spontaneous firing rate for each cell, responses were normalized to the maximal response for that cell and averaged across all 25 repetitions. b). Mean $\pm$ standard deviation across all normalized tuning curves. Supplemental time axis indicates elapsed time during the up/down sequence. Inset highlights hysteresis of -2000 mV responses from the upward and downward segments of the stimulus sequence. Lines are shaded according to whether the response increased or decreased. Thick line is the population mean.

longer than 100 ms were excluded because spontaneous spikes make a progressively larger contribution to the recorded response at such latencies. From Figure 2b it is clear that while -2000 and -2500 mV stimuli elicited robust responses, weaker voltages did not. This result was consistent across our population, in contrast to previous reports [13]. After careful consideration, we judge the difference to result from the higher impedance of the electrodes used here as well as higher thresholds for such small electrodes. While the MEAs used in our previous experiments delivered 0.57 mC/cm^2 for a 1000 mV pulse, the Hex-MEAs used here delivered 1.27 mC/cm^2 for the same pulse. However, it has previously been shown that despite this 2-fold increase in charge density, the expected threshold increase for smaller electrodes would be 2-fold higher, if not more [12]. Electrical stimuli consisted of monophasic rectangular cathodic voltage pulses (-300, -500, -1000, -1500, -2000, and -2500 mV) in an up/down sequence.

Hysteresis

The interpulse interval of 5 seconds was chosen to allow the retina to return to baseline activity between stimulus pulse intervals so that each response could be considered in isolation. Nevertheless, in the course of a previous study, we observed evidence that even such long intervals might not be sufficient to return the retina to baseline for electrical stimulation [13]. Therefore, in addition to investigating the spatial spread of electrical stimulation, we designed the present experiments in an up/down sequence to test whether electrical stimulus history at such long intervals influences responses. Hysteresis of the voltage response function demonstrating history dependence of the response can be seen for the example cell in Figure 2e. The 76 response samples of 36 cells (sometimes at multiple separate stimulating electrodes, see Methods) to the full up/down voltage sequence, averaged across 25 repetitions, are shown in Figure 3a. In the population average (Fig. 3b), it can more clearly be seen that responses to -2500 and -2000 mV were weaker when following stronger stimuli. The inset compares responses to -2000 mV during the rising and falling sequences. The average response decrease of 40% shown here was found to be statistically significant ($p < 0.05$; paired, one-tailed t-test) (Fig. 3b-Inset of the figure). Such differences in response to identical stimuli presented 15 s apart amounts to significant stimulus history-dependent hysteresis in the voltage response tuning curve at a longer time interval than is

Fig. 4. Spatial spread of electrical stimulation. a) Mean normalized firing rate of RGCs according to pulse amplitude and stimulation distance. b) Fraction of RGCs responsive to each pulse according to distance between stimulating and recording electrodes (see Methods for definition of 'responsive'). c) Mean population threshold vs. stimulation distance. Error bars in a) and b) are SD. Error bars in c) are 95% binomial confidence intervals. Data were fit with linear (a and b) and exponential (c) functions, shown along with dashed confidence bounds. Sample number is shown above the error bar for each distance.

currently considered to be relevant in retinal prostheses. Thus, RGCs have a 'memory' on the time scale of seconds for electrical stimulation. The implications of this long memory are examined in the Discussion.

Spatial Extent

We next examined how electrical responsiveness across the RGC population varies with distance from the stimulating electrode. Stronger voltages (-2500 vs. -2000 mV) yielded stronger responses at a given distance as expected, with firing rates generally decreasing with increasing distance from stimulating electrode (Fig. 4a). We estimated the electrical point spread function (EPSF) by fitting a line to the data. From the fitted lines, we can estimate the half-width at half maximal response (HWHM) to be 303 μm and 1034 μm for -2000 and -2500 mV, respectively. Using the Rayleigh criterion to calculate the limit of spatial resolution

based on the HWHM of these EPSFs and assuming 288 $\mu\text{m}/\text{degree}$ in the human retina, we find corresponding acuities of 20/1263 or 1.80 logMAR for the -2000 mV curve and 20/4308 or 2.33 logMAR for the -2500 mV curve [9].

To complement the analysis based on how firing rate varies with distance, we also examined how the fraction of electrically responsive RGCs varies with distance (Fig. 4b). As in Fig. 3, more RGCs were recruited with stronger voltages. Applying the same analysis methods we find HWHM to be 227 and 364 μm for -2000 and -2500 mV, respectively. Corresponding visual acuities are 20/946 or 1.67 logMAR for -2000 mV and 20/1517 or 1.88 logMAR for -2500 mV. We can see from these and the previous results that spatial spread increases with stimulus amplitude, reducing the visual acuity that can be realized under otherwise ideal conditions. This supports the common practice of using the minimal level of stimulation necessary to yield a reliable visual percept.

Finally, we examined whether stimulation threshold varied with distance (Fig. 4c). We found a modest increase in threshold voltage magnitude with distance (about -0.58 mV/ μm from 0 to 300 μm , $r^2=0.097$). We attribute this low correlation the fact that only 2000 and 2500 mV pulses yielded responses from any RGCs with the present electrodes – resulting

in the large error bars shown. To more precisely characterize how threshold changes with distance, we conclude that threshold amplitudes will need to be sampled much more finely in the future.

Discussion

The present observations describe the spatial spread of stimulation for RGCs of the whole-mount retina in response to voltage pulses using high density electrode array. We show that voltage-response curves express hysteresis, reflecting adaptation to electrical stimulation. Evoked firing rate decreased irregularly from 0 to 300 μm , dependent on the strength of stimulation. The percentage of RGCs responsive to electrical stimulation also diminished from 0 to 300 μm in a voltage dependent manner. Finally, we found little difference in threshold voltage across these distances.

Our results confirm earlier studies which all find a decrease in electrical response with increasing distance from the stimulating electrode [8, 9, 20]. According to our previous report, the fraction of responsive RGCs in wild type mice on a 'standard' MEA (30 μm electrode diameter and 200 μm interelectrode distance) diminished speedily from about 50% at 200 μm to 20% at 283 μm with little additional drop up to 1000 μm [13]. Nevertheless, we find inconsistencies in the literature regarding the choice of how to define a threshold response or the reporting of RGC recruitment versus the average RGC firing rate. Stimulation threshold, average response firing rate and the fraction of the RGC population recruited are all distinct but interconnected population measures of the extent of electrical stimulation. These are complimented by measurements of the electrical receptive fields of individual RGCs [8-10]. Because of the great diversity in RGCs and their responses to electrical stimulation, the choice of which measure to examine strongly impacts conclusions on the spatial limits of electrical stimulation. In order to better understand such matters we believe that better methods must be developed to differentiate the multitude of RGCs according to their 30 or more functional types [21] during *in vitro* electrical stimulation experiments. Only then can variability due to different cell types be controlled to uncover the true (presumably much lower) variability within distinct RGC types.

Hysteresis

The existence of hysteresis in electrical stimulation experiments has important implications for prosthetic stimulation research. The hysteresis we have reported implies a memory of at least 5 seconds (the interval between pulses). Ironically, this interval was chosen based on an awareness of such adaptational effects in an effort to yield fully independent responses. It has been shown that RGCs have a primary time constant of 150 ms [22]. Although their secondary time constant is 15 s, it is so weak that we suspect adaptation within the retinal network to be the primary factor in the hysteresis observed here. In an absence of independence (hysteresis), researchers must take careful measures to account for adaptation to electrical stimuli. One method is to deliver 'probe' stimuli within the context of ongoing electrical stimulation [23]. An alternate method is to randomize the presentation of pulses, and average repetitions with different preceding stimulation histories [13]. Further research is needed to fully characterize the time course and strength of such adaptation effects for network-mediated RGC stimulation.

Impedance Effects

In striving to connect the present data with our previous experiments, we were reminded of the significant role that impedance plays in electrical stimulation – especially when the voltage rather than the current of each pulse is controlled. When voltage-controlled pulses are used, the actual current delivered is dependent on the impedance of the electrode/tissue interface in accordance with Ohm's Law. This means that identical voltage pulses can

deliver vastly different currents in arrays with high impedance such as old MEAs or MEAs with smaller electrodes (as in the present study). Likewise different retinal preparations or locations on the retina can have significantly different interface impedances. This variability amplifies the response variability already present in the diverse population of RGCs. In contrast, when current-controlled pulses are used, only the necessary voltage changes with impedance. This is significant because electrode current has a much more significant influence in driving neurons to response than electrode voltage. While the voltage gradient across neurons induces ion channels to open and produce an action potential, the voltage gradient across the retina is determined by the charge injected into the retina, not the voltage at the electrode surface which is strongly attenuated by a high-impedance electrode/tissue interface.

Nevertheless, even controlling for current density on the electrode, the threshold current and charge densities decrease significantly as impedance decreases (with increasing electrode diameter from 5 to 25 μm) [12]. This, combined with the higher impedance of smaller electrodes, explains why our previous experiments with 30 μm electrodes (impedance $\sim 50\text{ k}\Omega$) yielded much lower and more varied thresholds than the present experiments with 10 μm electrodes (impedance $\sim 250\text{ k}\Omega$). To control for such differences when stimulating with voltage-controlled pulses, we recommend matching the actual current delivered through different electrodes.

Implications for Visual Acuity in Retinal Prostheses

Stimulation of an implant's electrode activates a region of neurons. If this region is larger than the interelectrode spacing of the implant, crosstalk between electrodes occurs [24]. Even with significant crosstalk, phosphenes generated by neighbouring electrodes can be discriminated so long as their collective activation profile has sufficient contrast between electrodes to be detectable. The Rayleigh criterion which yields the resolution limit based on a point spread function is a useful boundary for two-point discrimination estimations.

Taken as a whole, the studies summarized in the introduction and the present results seem to indicate that the half-width at half-maximal response (HWHM) that characterizes the electrical point-spread function (ePSF) for stimulation of the population of retinal ganglion cells (RGCs) is approximately 250 μm . Within this population, a majority of RGCs seem to have smaller ePSFs with HWHM around 100 μm . Recently, Stutzki et al. have mapped the spatial extent of the electrical receptive field (eRF) for various RGCs [14]. The many single-cell receptive fields they report exhibit a broad range of ePSFs corresponding to those previously reported. Importantly, these eRFs also exhibit a wide range of spatial profile shapes as well as both onset and offset responses to electrical stimulation. Future work should continue to elaborate on such methods for probing the spatio-temporal response profile of individual RGCs. One such elaboration is the use of white-noise mapping which has the advantage that it is easily scalable to recording from tens to hundreds of simultaneously recorded RGCs. Although well-known in the world of visual neurophysiology, such linear systems methods have only recently been applied to visual prosthesis research [23, 25]. As a better understanding of the dozens of different RGC types develops [21], it may become possible to target only RGCs with the smallest eRFs in order to provide the highest-possible prosthetic visual acuity.

Based on the current lower HWHM limit of 100 μm , it appears that the best visual acuity achievable stimulating RGCs with such ePSFs may be around 20/400 Snellen, or 1.3 logMAR. Indeed, the best visual acuity reported for a visual prosthesis is 20/546 or 1.43 logMAR [26]. However, it may be possible to achieve even better acuity. One possibility reflected in the present study is that the ePSF decreases modestly as stimulation amplitude decreases. By selectively activating a more homogeneous subset of the RGC population, it may be possible to fine-tune the stimulus such that it remains barely above threshold to produce the smallest possible ePSF. How small this may be first requires a full characterization of the electrical responses of the dozens of different RGC types. Just as importantly, given the

many specializations of the human fovea where high-acuity vision is localized, it will soon be necessary to extend present research into primate retina.

At present, the highest resolution implants have electrodes spaced about 70 μm apart [11, 26]. If stimulation with such implants were to produce a field of mutually discriminable phosphenes, the best possible acuity would be 20/292 or 1.16 logMAR. Such spacing is mostly limited by the need to separate relatively large electrodes on the order of 50 μm in diameter. As electrodes using modern materials become smaller than this, stimulation threshold quickly approaches the limits of tissue and electrode damage. Therefore, although much work remains to minimize the ePSF, innovations in electrode materials and design will also be needed before perfect vision can be realized.

Conclusion

We have built upon previous studies by measuring the spatial extent of stimulation across the RGC population and applying an alternate method to characterize electrical responsiveness. In designing our experiments to also examine hysteresis of the voltage-response function, we have demonstrated that network stimulation of RGCs is influenced by stimuli at least 5 seconds prior. Accordingly, future research should carefully control for adaptation to electrical stimulation. We have affirmed that the pulse amplitude range used in retinal prostheses should be determined carefully by considering the potential reduction of spatial resolution which can be caused by undesirable stimulation of distant RGCs due to unnecessarily high stimulation amplitudes. In keeping with previous reports, we conclude that the spatial extent of electrical stimulation likely varies depending on the species, RGC type, and experimental methods used. Significantly more work will be needed to fully understand which spatial effects limit visual acuity in retinal prostheses and how they might be overcome.

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Disclosure Statement

None.

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