Thresholds for Activation of Rabbit Retinal Ganglion Cells with Relatively Large, Extracellular Microelectrodes

Ralph J. Jensen,[1] *Ofer R. Ziv,*[1,2] *and Joseph F. Rizzo, III*[1,3]

Purpose. To investigate the responses of retinal ganglion cells (RGCs) to electrical stimulation, using electrodes comparable in size to those used in human studies investigating the feasibility of an electronic retinal prosthesis.

Methods. Rabbit retinas were stimulated in vitro with current pulses applied to the inner surface with 125- and 500-μm diameter electrodes while the responses of RGCs were recorded extracellularly.

Results. Both short-latency (SL; 3–5 ms) and long-latency (LL; $\geq$9 ms) responses were observed after electrical stimulation within the receptive field of an RGC. With short, 0.1-ms current pulses, the threshold current for the SL cell response was significantly lower than that for the LL cell response. With long (10- to 20-ms) pulses, the threshold currents for the SL and LL cell responses were very similar. The threshold current for the SL cell response increased more steeply than did the LL cell response when the electrode was displaced from the point of lowest electrical threshold, either above or along the surface of the retina. Stimulation of an RGC axon outside of the cell's receptive field produced only an SL response. For 0.1-ms duration pulses, the threshold current for the axonal response was significantly higher than the threshold current for the SL cell response. At pulse durations $>$ 1 ms, the thresholds were very similar.

Conclusions. RGC responses to electrical stimulation depend on the current pulse duration and location of the stimulating electrode. For an epiretinal prosthesis, short-duration current pulses may be preferable since they result in a more localized activation of the retina. (*Invest Ophthalmol Vis Sci.* 2005;46:1486–1496) DOI:10.1167/iovs.04-1018

Presently, there is no effective therapy or cure for vision loss due to retinitis pigmentosa or age-related macular degeneration. In both diseases, photoreceptors degenerate, resulting in blindness, whereas most other neurons of the retina survive.[1–5] To restore some vision in these blind patients, several research groups[6–11] are developing an intraocular electronic retinal prosthesis that has the potential to restore vision by electrically stimulating the remaining retinal neurons. An important aspect in the design of an electronic retinal prosthesis is the selection of electrode geometries and electrical stimulation parameters to enhance visual quality. It is our belief that rational selection of stimulus parameters requires an understanding of the response properties of retinal ganglion cells (RGCs) to electrical stimulation of the retina.

An electrical stimulus applied to the eye has long been known to evoke complex responses, consisting of multiple bursts of action potentials from RGCs.[12–14] These bursts are believed to be due to electrical activation of presynaptic retinal neurons, and recent pharmacological studies[15,16] have largely confirmed this. Direct activation of RGCs can also occur. Using an ultrafine, stimulating microelectrode, we examined in an earlier study[17] the spatial profiles of RGCs during direct activation with electrical stimuli applied to the inner retinal surface. The main finding of this study was that cathodal current pulses produce lower thresholds, more localized stimulation, and somewhat better selectivity for cell bodies over axons than do anodal current pulses. Although this study provided valuable information on the spatial sensitivity of ganglion cells to electrical stimuli, the threshold currents needed to activate RGCs are unacceptably high for the 5-μm-long conical electrode used in the study and would be likely to damage retinal tissue.[18]

In the present study, we examined the spatial profiles of individual RGCs in the rabbit retina in response to electrical stimuli applied with larger (125- and 500-μm diameter) electrodes. Specifically, we determined the amount of current needed to excite RGCs, both directly and indirectly, using various current pulse durations with the stimulating electrode placed at various positions, either above or along the surface of the retina. These larger electrodes are comparable in size to those currently being used for electrical stimulation of the inner surface of the retinas of blind patients.[7,8,19] Such large electrodes are currently being used so as not to exceed presumed safe charge limits. Some of the results of this study have been presented in abstract form (Jensen RJ, et al. *IOVS* 2003; 44:ARVO E-Abstract 5048; Jensen RJ, et al. *IOVS* 2004;45:ARVO E-Abstract 4191).

Methods

Subjects

All experimental methods and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the VA Boston Institutional Animal Care and Use Committee. Subjects were adult New Zealand White rabbits between 2 and 3 kg (Millbrook, Amherst, MA). The rabbits were maintained in a 12-hour light ($<$300 lux)/12-hour dark environment with high-fiber rabbit chow (Purina 5326; Ralston Purina, Richmond, IN) and water available ad libitum.

Retinal Preparation

Anesthetic induction was achieved with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg), followed by an intravenous injection of sodium pentobarbital (20–40 mg/kg).

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Under normal room lighting, an eye was enucleated and hemisected, and the vitreous humor removed with gentle suction applied with a Pasteur pipette. A strip (≈1 × 2 cm) of retina with attached sclera that included the optic nerve head was excised and laid flat, RGC side up, on a 10° inclined platform in a customized recording chamber. The retinal preparation was superfused continually (~2 mL/min) with bicarbonate-buffered Ames medium (Sigma-Aldrich, St. Louis, MO) bubbled with 95% O_2 and 5% CO_2 (pH 7.4) at 35°C to 36°C. Diffuse background light (~1 μW/cm^2 at the retina) was present throughout the experiments.

Electrophysiological Recordings

Extracellularly recorded action potentials from RGCs were obtained with glass-coated tungsten microelectrodes[20] with an exposed tip ~20 μm in length and ~3 μm in diameter at the base. The recorded potentials were amplified 10,000× and band-pass filtered between 0.3 and 3 kHz (DAM-80; World Precision Instruments, Sarasota, FL). The output from the amplifier was displayed on an oscilloscope (TDS 3012B; Tektronix, Gaithersburg, MD) and feed into an audio monitor (AM-2; Dagan Corp., Minneapolis, MN). In experiments in which electrical stimuli were applied within the receptive field of an RGC, recordings were made from the axon of the cell. In experiments in which electrical stimuli were applied near the axon of an RGC, recordings were made from the cell body of the RGC. Action potentials recorded from cell bodies could be differentiated from those recorded from axons by their slower time course and consequent lower pitch over the audio monitor. However, visual inspection of the tip of the recording electrode with respect to the location of the receptive field center of the RGC was always performed to substantiate the recording site.

Electrical Stimulation

The stimulating electrode was either a Teflon-insulated platinum-iridium wire of 125-μm diameter (A-M Systems, Carlsborg, WA) or a glass-insulated platinum wire of 500-μm diameter (Bioanalytic Systems, West Lafayette, IN). Only the disc-shaped ends of these wires were exposed. The return electrode was a 1.2-cm^2 Ag/AgCl foil that was located between the platform of the recording chamber and the sclera of the retinal preparation. Unless otherwise stated, electrical stimulation consisted of monophasic, cathodal pulses delivered at a frequency of 2 to 3 Hz through a constant-current stimulus isolation unit (PSIU-6; Grass-Telefactor, West Warwick, RI) attached to a stimulator (model S88; Grass-Telefactor). Stimulation thresholds were determined by increasing a subthreshold current until action potentials were elicited >50% of the time for five or more consecutive stimulations.

Experimental Protocol

Two sets of experiments were conducted with both sizes of stimulating electrodes. In one set of experiments, electrical stimuli were applied within the receptive field center of an RGC; in the other set, electrical stimuli were applied near the axon, far from the receptive field center of the RGC (Fig. 1).

In the former, a "blind" search was made for well-isolated activity from an axon of an OFF-center, brisk-transient RGC whose receptive field center was located ≥6 mm from the tip of the recording microelectrode. (The receptive field center of an RGC was found by flashing a ~300-μm diameter spot of light on the retina, and an OFF-center, brisk-transient RGC was distinguished from other types of ganglion cells by its responses to flashes of various size spots of light centered within the receptive field.[21]) Once such a cell was found, the tip of the stimulating electrode was positioned above the retina and over the receptive field center. While slowly lowering the stimulating electrode toward the retinal surface, we continually adjusted the current (typically, 1-ms cathodal pulses) to just stimulate the cell. As will be described in detail in the Results section, both short-latency (SL) and long-latency (LL) responses were observed after electrical stimulation of the cell within the receptive field. In preliminary experiments, it was our impression that the SL cell responses were more sensitive to electrode position along the retinal surface than were the LL cell responses. We therefore positioned the electrode on the surface of the retina that gave the lowest threshold for the SL cell response. This point of lowest threshold we called the "measured origin," and all other electrode positions were plotted in relation to it.

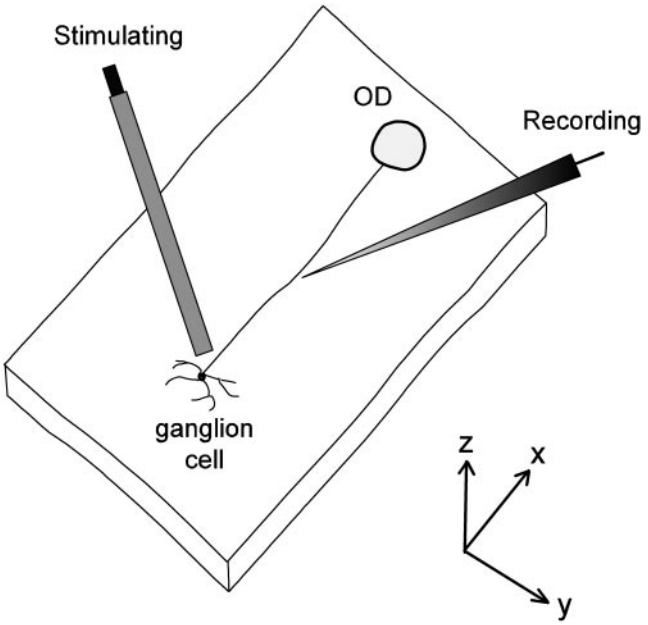

FIGURE 1. The experimental setup. In approximately one-half of the experiments, the stimulating electrode was placed within the receptive field near the cell body of an RGC, and the recording electrode was placed between the stimulating electrode and optic nerve head (OD). In the other experiments, the positions of the recording microelectrode and stimulating electrode were reversed. *Arrows*: orientation of the *x*, *y*, and *z* axes with respect to the surface of the retina. The *x*-axis was parallel to the presumed course of the axon, the *y*-axis was perpendicular to the *x*-axis and along the retinal surface, and the *z*-axis was perpendicular to the retinal surface.

In experiments in which electrical stimulation was made near an axon, we began by blindly searching for the cell body of an OFF-center, brisk-transient RGC in the peripheral retina. Once a cell was found, the tip of the stimulating electrode was positioned inferior to the visual streak and at least 6 mm from the center of an RGC's receptive field, along an imaginary line connecting that center and the optic nerve head. The point of lowest threshold was found by moving the stimulating electrode along the *y*-axis (i.e., parallel to the visual streak). The point of lowest threshold we again called the measured origin, and all other electrode positions were plotted in relation to it. When stimulating near axons, only SL responses were elicited.

In both sets of experiments (i.e., stimulation near the cell body and stimulation near the axon), stimulus strength–duration relations were obtained at the measured origin, using current pulse durations of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, and 50 ms. Threshold currents along the *z*- and *y*-axes were also determined as a function of distance from the measured origin at 12 points: 50, 100, 150, 200, 300, and 400 μm above the retina (i.e., along the *z*-axis) and 50, 100, 150, 200, 300, and 400 μm across the retinal surface (i.e., along the *y*-axis). With the 500-μm stimulating electrode, additional threshold current measurements were made 600 μm and 800 μm from the measured origin along the *y*-axis. When measuring thresholds along the *y*-axis, we raised the electrode 200 μm before attempting to reposition it for the next threshold measurement so as not to snag the retina. Electrode positions were determined from two digital micrometers (Digimatic Series 350; Mitutoyo, Aurora, IL) mounted on the micromanipulator.

Data Analysis

For comparison with results obtained previously with an ultrafine stimulating microelectrode,[17] only data obtained from OFF-center, brisk-transient RGCs were included for analysis. Furthermore, results were analyzed only from those cells in which complete data could be obtained for either the strength–duration relationship at the measured origin or the strength–distance relationship in which threshold currents were measured along *both* the *z*- and *y*-axes. Medians, rather than means, were used as a measure of central tendency, since the median is less susceptible to the small number of outlying values. Median absolute deviation (MAD), which is the median of the differences between each data point and the overall median for the data, was used as a measure of dispersion.

Results

Electrophysiological data were collected from 87 OFF-center, brisk-transient RGCs that had receptive fields located in the peripheral retina, approximately 1 cm inferior to the visual streak. Forty of these cells were electrically stimulated with a 125-μm electrode; the others were stimulated with a 500-μm electrode.

Basic Response Characteristics and Threshold Currents

Both SL (3–5 ms; measured from stimulus onset) and LL (≥9 ms) responses were observed on electrical stimulation of an RGC within its optical receptive field. The SL cell response was typically a *single* action potential, although a few cells elicited a second action potential that immediately followed the first. The LL cell response consisted of one or more bursts of typically two to five action potentials after stimulation at or slightly above threshold current. Unlike the SL cell responses, the LL cell responses were not tightly time locked to each stimulus pulse and were susceptible to bath application of agents that block glutamatergic input to RGCs[22] (Ziv OR, et al. *IOVS* 2002;43:ARVO E-Abstract 4474). Thus, the LL cell responses do not appear to be due to direct activation of RGCs but rather to presynaptic activation of retinal neurons.

Figure 2 shows an example of the responses that were obtained. Figure 2A shows the response of an RGC to the offset of a spot of light flashed in the center of the receptive field. A burst of 12 action potentials appeared with a latency of 54 ms. Figure 2B shows the responses of the same RGC to electrical stimuli applied within the receptive field center. In the top trace, a small amount of current (0.27 μA) evoked a single action potential with a latency of approximately 4 ms. This response is due to direct stimulation of the RGC. In the bottom trace, the current was increased further to 3.1 μA, and two bursts of action potentials appeared that were undoubtedly due to activation of presynaptic retinal neurons. The first burst had a latency of 10 ms and the second burst had a latency of 60 ms, nearly identical latency to that of the light-evoked response. For most cells, the individual bursts of action potentials had distinctly different threshold currents—the early burst of action potentials was usually evoked with less current than the later burst(s). We did not attempt in this study to characterize the detailed response properties of the individual bursts.

On electrical stimulation near the axon of an RGC, but far from its receptive field center, only an SL (3–5 ms) response was observed. Like the SL cell response, the axonal response was not susceptible to blocking glutamatergic transmission. We therefore attribute the SL response to direct stimulation of the RGC axon and not structures presynaptic to the RGC.

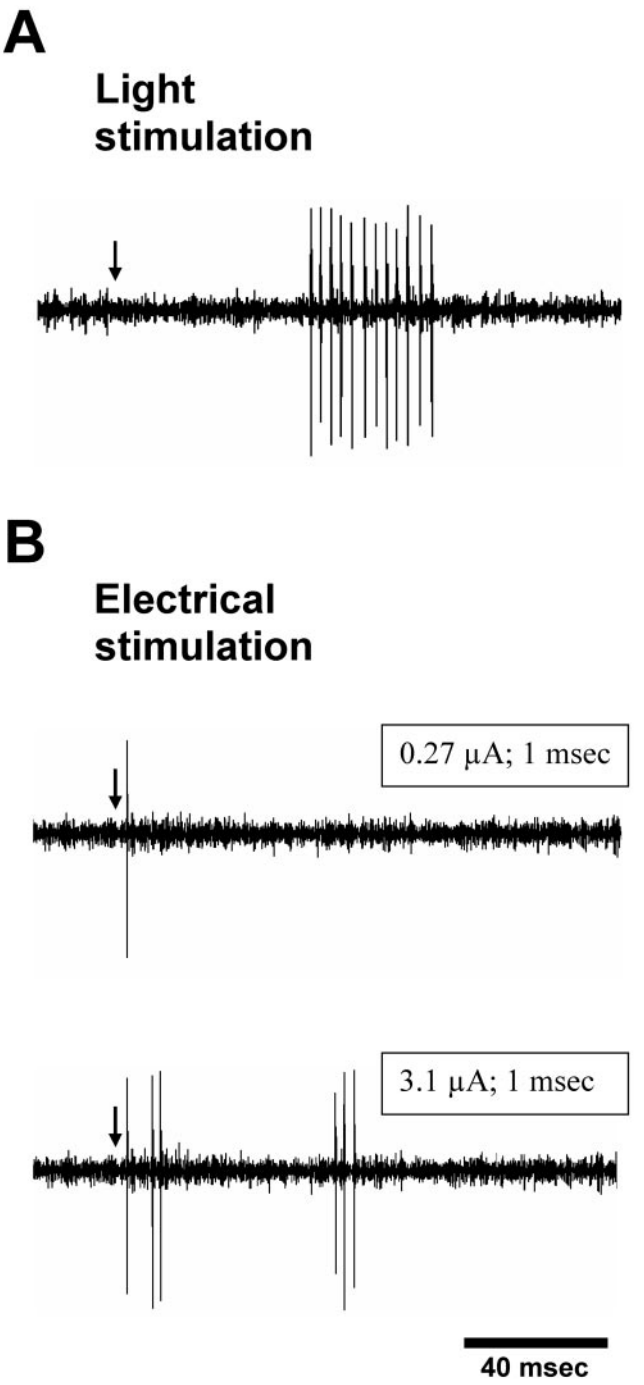

Figure 2. Example of recordings of an OFF-center brisk transient RGC to electrical and light stimulation. (**A**) Single-sweep response to the offset of a spot of light centered in the receptive field. *Arrow*: offset of the light stimulus (200-μm diameter spot; intensity 0.5 log unit above threshold). (**B**) Single-sweep responses to two current pulses delivered with a 125-μm diameter electrode within the optical receptive field. With sufficient electrical stimulation, at least two bursts of action potentials would have traveled down the optic nerve, in distinction to a single volley induced by light stimulation. The *arrow above* each trace marks the point of stimulus application. The shock artifact was eliminated from the traces for clarity. Time scale bar applies to both (**A**) and (**B**).

Strength–Duration Relationship

We examined the effect of stimulus pulse duration on the threshold current for activation of RGCs with the stimulating

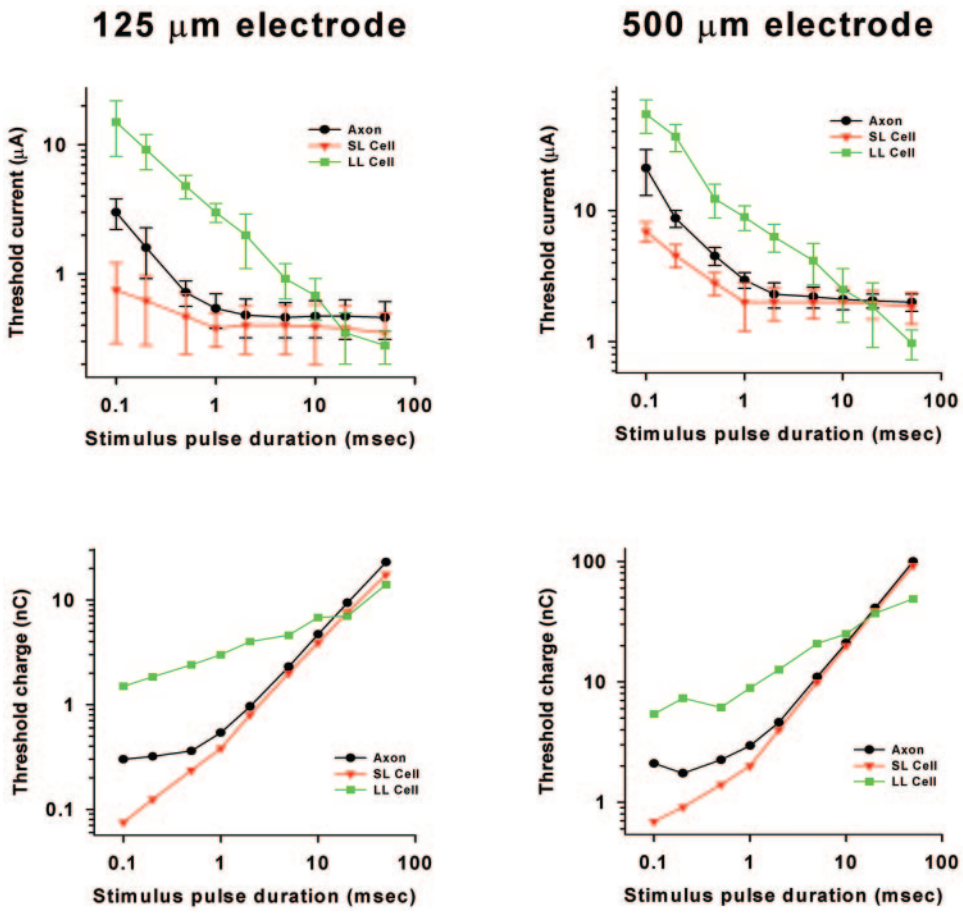

FIGURE 3. *Top*: threshold current for eliciting action potential(s) at the measured origin as a function of duration of the stimulating current with a 125-μm (*left*) or 500-μm (*right*) stimulating electrode. *Symbols*: medians from 13 axons and 11 cells with the 125-μm stimulating electrode and from 14 axons and 12 cells with the 500-μm stimulating electrode. The bars show $\pm$ MAD. *Bottom*: relationship between the threshold charge and stimulus pulse duration with a 125-μm (*left*) and a 500-μm (*right*) stimulating electrode. Total charge transfer is expressed in nanocoulombs (nC) and was calculated by multiplying median threshold stimulus current $\times$ stimulus pulse duration.

electrode positioned at the measured origin (see the Methods section). Figure 3 summarizes the results obtained with a 125-μm stimulating electrode (top left) and a 500-μm stimulating electrode (top right). With both size electrodes, the threshold currents for all three responses decreased as stimulus pulse duration increased. The threshold currents for the axonal and SL cell responses reached an asymptote at a stimulus pulse duration of approximately 2 ms. In contrast, an asymptote was not evident for the LL cell response with the pulse durations tested (up to 50 ms).

The difference in threshold currents between SL and LL cell responses was largest for short stimulus pulse durations (Table 1). With 0.1-ms current pulses, the median threshold current for the SL cell response was 20 times lower than the LL cell response with the 125-μm electrode and 7.8 times lower than the LL cell response with the 500-μm electrode. These differences between the median threshold currents at this pulse duration were statistically significant (Wilcoxon matched-pairs test; two-tailed $P \leq 0.001$). With increasing stimulus pulse duration, the difference in threshold current between SL and LL cell responses steadily decreased, and at the longest stimulus pulse duration (50 ms) tested, the median threshold current for the SL cell response was actually higher than the median threshold current for the LL cell response—1.25 times higher with the 125-μm electrode and approximately two times higher with the 500-μm electrode, although these differences were not statistically significant (Wilcoxon matched pairs test; two-tailed $P > 0.23$).

TABLE 1. Threshold Currents for Ganglion Cells at Measured Origin for Three Pulse Durations

	0.1 ms	2 ms	50 ms
125-μm electrode			
Axon	3	0.48	0.46
SL Cell	0.75	0.4	0.35
LL Cell	15	2	0.28
500 μm electrode			
Axon	21	2.3	2
SL Cell	6.9	2	1.9
LL Cell	54	6.3	0.98

Threshold currents are medians in microamps.

For both size electrodes, the greatest difference in threshold current between the SL cell response and axonal response was also obtained at the shortest stimulus pulse duration (0.1 ms) tested. The median threshold current for the SL cell response was three to four times lower than the median threshold current for the axonal response. These differences between the median threshold currents were statistically significant (Mann-Whitney test, two-tailed $P < 0.04$). For stimulus pulse durations > 1 ms, the threshold currents for the SL cell responses and the axonal responses were very similar.

Using the data from the top two plots of Figure 3, we calculated the threshold charge as a function of stimulus pulse duration. The results are displayed in the bottom two plots of the same figure. For all three responses, the amount of charge required to produce a response steadily decreased with decreasing pulse duration. With axonal stimulation, there appeared to be an asymptote for pulse durations < 1 ms, indicating that there is a minimum amount of charge needed to activate an axon. In contrast, for the SL cell response, an asymptote was clearly not present, and for the LL cell response,

Table 2. Chronaxies of Ganglion Cells at the Measured Origin

	125-μm Electrode	500-μm Electrode
Axon	0.43	0.66
SL Cell	0.14	0.35
LL Cell*	14.00	18.00

Data are expressed in milliseconds.
* Minimum chronaxies.

an asymptote appeared to be present only with 500-μm electrode stimulation.

Two measures can be extracted from strength–duration curves of the top two plots in Figure 3, the rheobase and the chronaxie. The rheobase corresponds to the horizontal asymptote, below which an action potential cannot be elicited regardless of stimulus pulse duration. The chronaxie is the stimulus pulse duration needed to excite the cell when the rheobase current is doubled. Using the threshold current at 50-ms pulse duration as the rheobase, the chronaxies for the axonal and cell responses were determined graphically and are given in Table 2.

With both size electrodes, the chronaxies of the SL cell responses were shorter than the axonal responses. With the 125-μm electrode, the chronaxie of the SL cell response was 3.1 times shorter than the axonal response. With the 500-μm electrode, the chronaxie of the SL cell response was 1.9 times shorter than the axonal response. In contrast, the chronaxies for LL cell responses were substantially (27–100 times) longer than the chronaxies for either the SL cell or axonal responses. The chronaxies for the LL cell responses may be even higher because the true rheobases may be lower, since a distinct asymptote was not obtained.

In our previous study,[17] in which we used a 5-μm tip electrode to stimulate rabbit RGCs electrically, we found that at the measured origin (which had been determined with cathodal current pulses) the threshold current for anodal stimulation was much higher. For axonal stimulation, the anodal threshold current was found to be approximately eight times higher than cathodal threshold current. In the present study, we examined whether a similar difference between anodal and cathodal threshold currents could be observed with our larger microelectrodes. As in our previous study, we found that the measured origin for axonal stimulation was the same point on the retina for both anodal and cathodal stimulation. Using the same current pulse duration (0.1 ms) as in our previous study, we found that with the 125-μm electrode the threshold current for anodal stimulation of axons was only 2.5 times higher than the threshold current for cathodal stimulation. With the 500-μm electrode, the threshold current for anodal stimulation was only 2.1 times higher.

The ratio of anodal to cathodal threshold current was dependent on the stimulus pulse duration. This was particularly evident with the 125-μm electrode. Figure 4 shows strength–duration curves obtained for RGC axons with the 125-μm electrode (top graph) and the 500-μm electrode (bottom graph). With the 125-μm electrode, the anodal-cathodal ratio ranged from 2.5 (obtained with 0.1-ms pulses) to 5.4 (obtained with 50-ms pulses). With the 500-μm electrode, the anodal-cathodal ratio ranged from 1.6 (obtained with 0.5-ms pulses) to 2.2 (obtained with 20-ms pulses).

Chronaxies, determined from the data in Figure 4, were shorter for anodal stimulation than cathodal stimulation. With the 125-μm electrode, the chronaxies for anodal and cathodal stimulation were 0.18 and 0.39 ms, respectively. With the 500-μm electrode, the chronaxies for anodal and cathodal stimulation were 0.42 and 0.61 ms, respectively.

Strength–Distance Relationship

The threshold currents were measured as the electrode tip was displaced from the measured origin, either above the retinal surface or along the retinal surface.

Threshold Currents along the *z*-Axis. The plots in Figure 5 show the threshold currents for axonal and cell responses as a function of electrode distance above the measured origin. For both stimulating electrodes, as the electrode moved farther above the measured origin, the threshold current for all three responses increased. In general, the rate at which the threshold current rose with distance was smallest for the LL cell response for all three stimulus pulse durations. Conse-

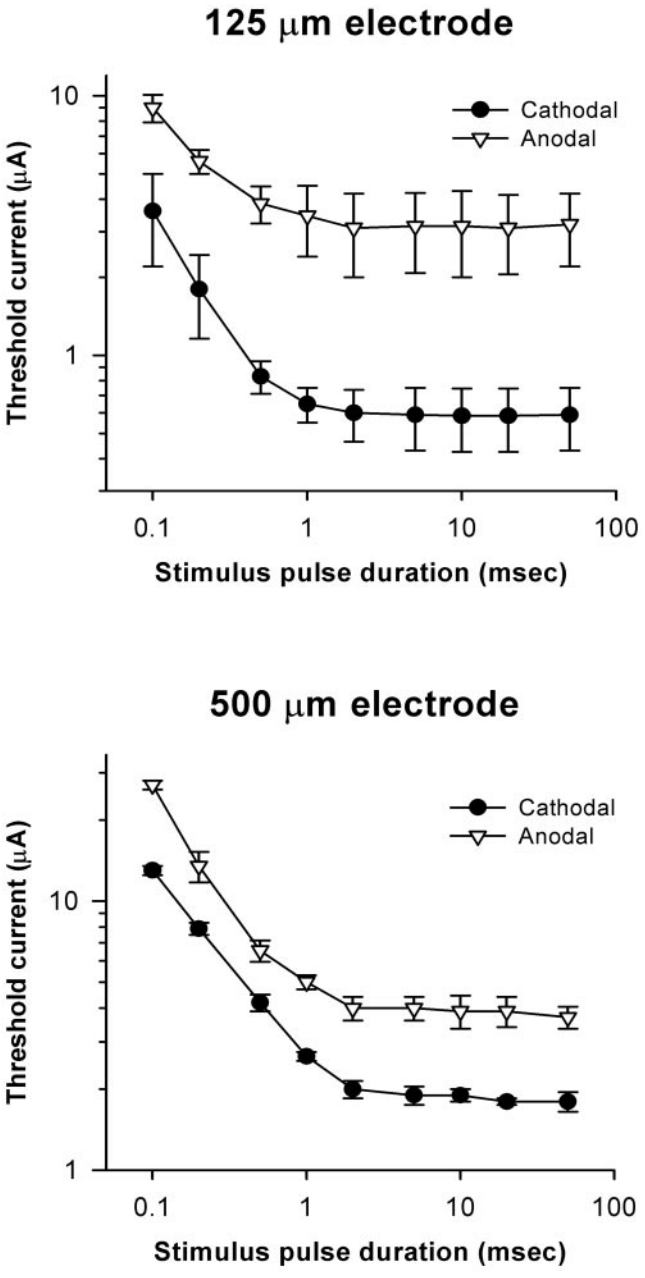

Figure 4. Comparisons of anodal and cathodal threshold currents for stimulation of RGC axons. *Top*: axons were stimulated with a 125-μm electrode; *bottom*: axons were stimulated with a 500-μm electrode. *Symbols*: medians from five axons with the 125-μm stimulating electrode and from eight axons with the 500-μm stimulating electrode. Bars, ±MAD.

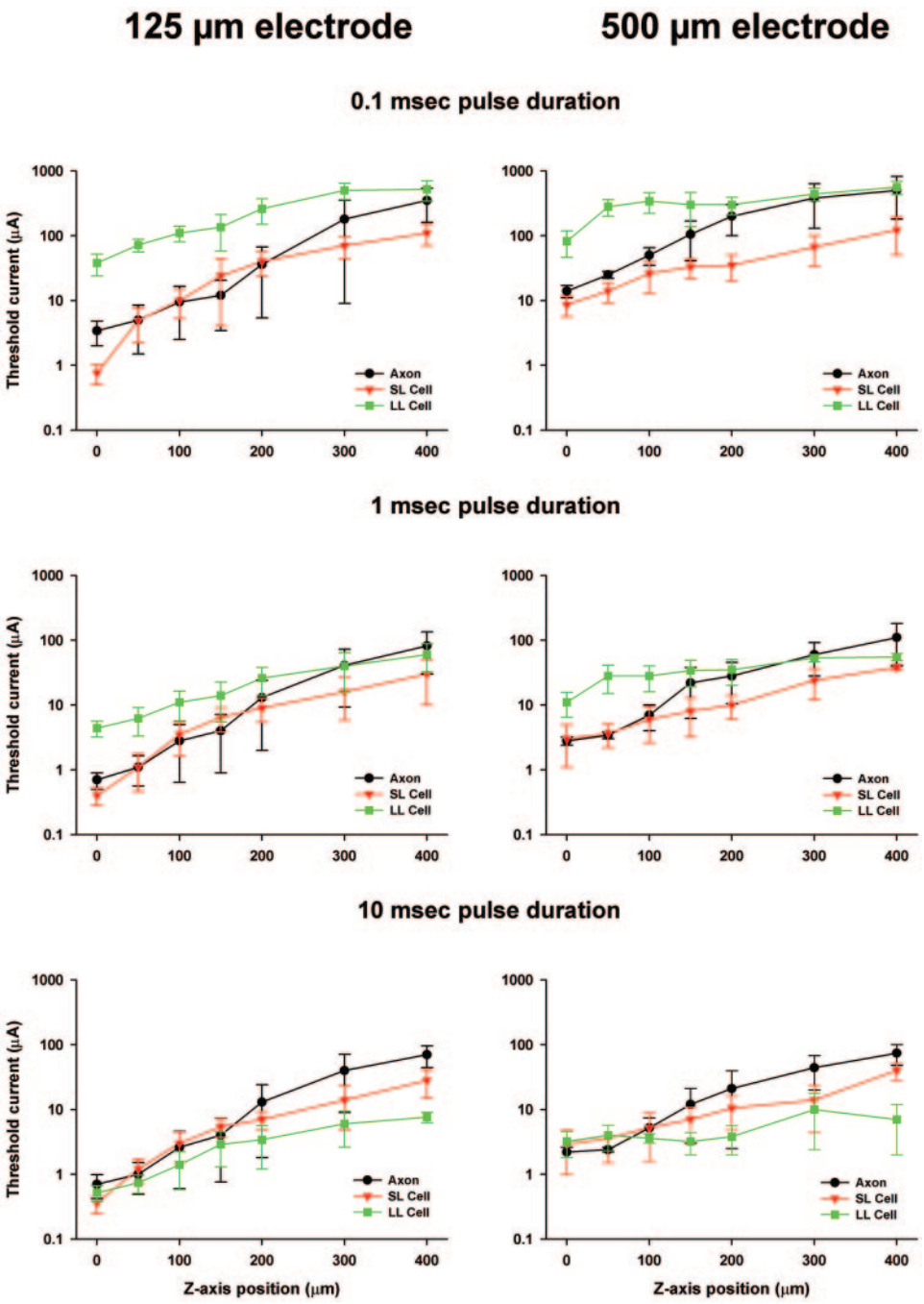

FIGURE 5. Threshold currents for the axonal, SL, and LL cell responses with respect to position above the measured origin with a 125-μm (*left*) and 500-μm (*right*) electrode at three stimulus pulse durations. Each data point is the median ± MAD of nine cells or nine axons.

quently, for 0.1- and 1-ms current pulses, the threshold current for the LL cell response approached that of the axonal and SL cell responses as the stimulating electrode moved away from measured origin.

For 10-ms current pulses, the strength–distance curves for axonal and SL cell responses were similar to those obtained with 1-ms pulse duration. This was expected based on data obtained from the strength–duration curves of Figure 3, which show that the threshold current for both axonal and SL cell responses began to asymptote at 1-ms pulse duration. In contrast, the strength–distance curves for the LL cell response shifted downward by approximately 0.9 log unit when changing from 1- to 10-ms current pulses. Consequently, as the stimulating electrode moved away from measured origin, the threshold current for the LL cell response became progressively lower than the threshold current for either the axonal or SL cell response. At 400 μm above the measured origin, the median threshold current for the LL cell response was significantly lower than the median threshold current for both the axonal (Mann-Whitney test, two-tailed $P < 0.01$) and SL cell responses (Wilcoxon matched-pairs test, two-tailed $P < 0.04$) for both stimulating electrodes.

Consistent with the data obtained from strength–duration curves (Fig. 3), the median threshold currents for the SL cell response were lower than the median threshold currents for the axonal response at the measured origin (i.e., $z = 0$ μm) when RGCs were stimulated with 0.1-ms current pulses (Fig. 5). However, this threshold difference disappeared when the 125-μm electrode was 50 to 200 μm above the measured origin. In contrast, with the 500-μm electrode, the median threshold current for SL cell response always remained lower than the median threshold current for the axonal response.

The graphs of Figure 5 collectively show that at a distance of 400 μm above the measured origin, the threshold currents for the three responses were essentially identical for both electrode sizes. In other words, the advantage of using a smaller electrode to obtain lower thresholds at the retinal surface is lost at a distance of 400 μm above the retina.

The data from the same cells in Figure 5 are replotted in Figure 6, as the relative threshold current (note the ordinate is a linear scale) as a function of distance above the retinal surface. In Figure 6, we see that the threshold currents changed substantially, even with small displacements from the measured origin. Furthermore, the threshold currents for the SL and LL cell responses were more sensitive to the position of the 125-μm electrode near the measured origin than with the 500-μm electrode. For axons, electrode size did not influence the rate of change in threshold above the retina.

Threshold Currents along the *y*-Axis. The plots in Figure 7 show the threshold currents for axonal and cell responses as a function of electrode distance from the measured origin on the retinal surface along the *y*-axis (i.e., perpendicular to the presumed course of axons to optic disc). Data were obtained from the same cells used to generate the plots in Figure 5.

For both stimulating electrodes, the threshold current for the LL cell response was relatively insensitive to electrode position along the retinal surface, at least up to the farthest distance tested from the measured origin (400 μm with the 125-μm electrode; 800 μm with the 500-μm electrode). With the 125-μm electrode, the median threshold current was only 1.7 to 2.1 times greater at 400 μm from the measured origin than that found at the measured origin. With the 500-μm electrode, the median threshold current was only 1.2 to 3.8 times greater at 800 μm from the measured origin than that found at the measured origin. By comparison, the threshold currents for both the axonal and SL cell responses increased more greatly with distance from the measured origin. With the 125-μm electrode, the median threshold current was 150 to 340 times greater at 400 μm from the measured origin than that found at the measured origin. With the 500-μm electrode, the median threshold current was 19 to 53 times greater at 800 μm from the measured origin than that found at the measured origin.

With 0.1- or 1-ms current pulses, as the stimulating electrode (125 or 500 μm) was moved away from the measured origin, the threshold currents for the three responses approached one another. With the 125-μm electrode, threshold currents for the three responses were about the same at 200 μm from the measured origin. With the 500-μm electrode, threshold currents for the three responses were about the same between 600 and 800 μm from the measured origin. With 10-ms current pulses, the threshold current for the three responses were about the same within 100 μm of the measured origin with the 125-μm electrode and within 400 μm of the measured origin with the 500-μm electrode. At greater distances from the measured origin, the threshold current for the LL cell response dropped below the threshold currents for both the axonal and SL cell responses.

Data from the same cells in Figure 7 are replotted in Figure 8 as the relative threshold current (note the ordinate is a linear scale) as a function of distance from the measured origin, along the *y*-axis on the retinal surface. For both the axonal and SL cell responses, the threshold current rose much more rapidly with electrode displacement for the 125-μm electrode than with the 500-μm electrode. With both electrodes, the threshold current for the SL cell response rose slightly faster with displacement from the measured origin than the threshold current for the axonal response. The threshold current for the LL cell response

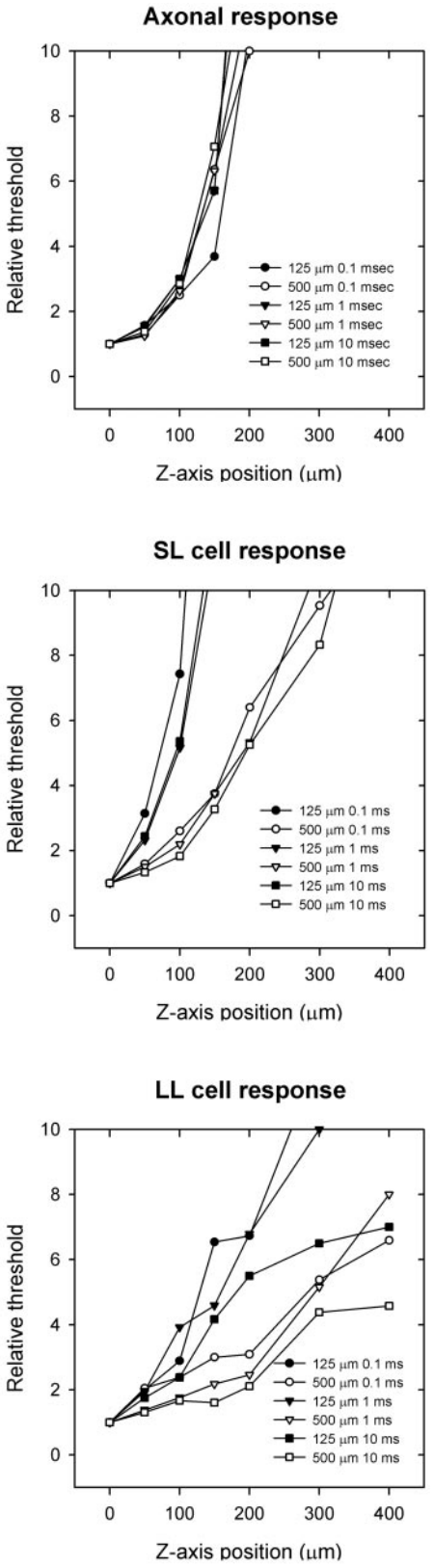

Figure 6. Relative threshold for the axonal, SL and LL latency cell responses with respect to position above the surface of the retina (i.e., along z-axis). The threshold current for each RGC was normalized to a value of 1 at $z = 0$ μm, and medians were then computed to produce the plots. For clarity, relative thresholds >10 are not illustrated.

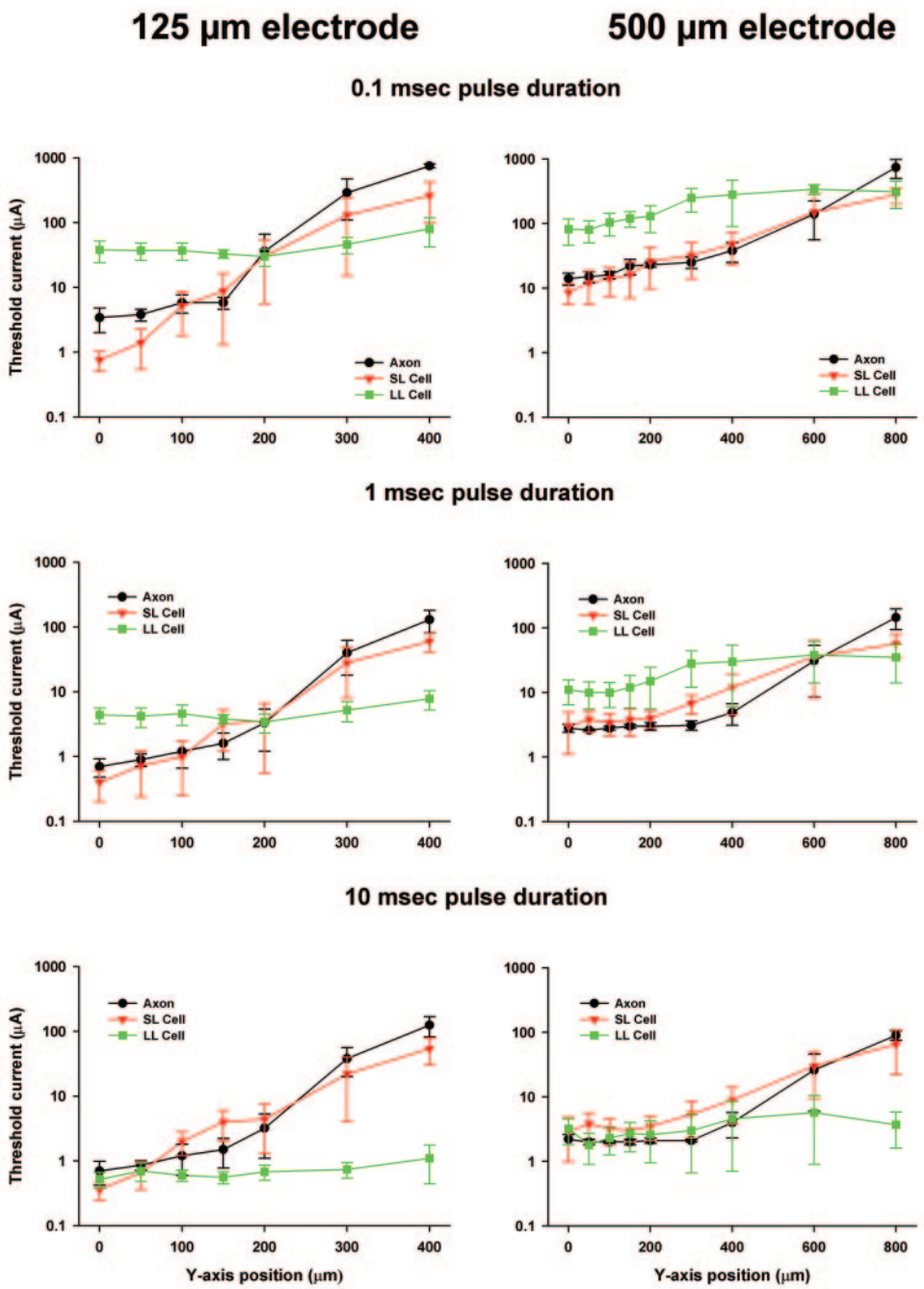

FIGURE 7. Threshold currents with respect to position along the surface of the retina. Each data point, except those taken at 800 μm from the measured origin (with the 500-μm electrode), is the median ± MAD of nine cells or nine axons. Each data point taken at 800 μm from the measured origin is the median ± MAD of six cells or six axons. The data were collected from the same cells as those in Figure 5.

was insensitive to electrode position along the *y*-axis for either size electrode.

DISCUSSION

In this study, we examined the responses of OFF-center, brisk-transient RGCs to electrical stimulation applied to the inner retinal surface with relatively large, extracellular disc electrodes. We found that the type of response elicited when the retina was electrically stimulated depended on both the current pulse duration and location of the stimulating electrode.

When the stimulating electrode was placed near the axon and far from the optical receptive field of an RGC, only an SL response (in most cases a single action potential) was observed. The latency of the recorded response was 3 to 5 ms. With the conduction velocity of intraretinal axons in the rabbit reported to be approximately 1 to 2 m/s,[23] much of this delay can be attributed to the time required for an action potential to travel from the point of stimulation to the recording site.

When the stimulating electrode was placed within the optical receptive field of an RGC, both SL and LL responses were observed. The SL cell response was very probably due to direct stimulation of the RGC. Unlike the LL cell response, the SL cell response was time locked to the electrical stimulus and was not abolished on blockade of synaptic transmission in the retina.[15,17] Although the site of action potential initiation that generates the axonal response is undoubtedly the axon itself, the site of action potential initiation that generates the SL cell response is uncertain. Others have suggested that even with a stimulating electrode positioned over the cell body or dendrites of a neuron, action potential initiation occurs in the proximal axon.[24–26] Unlike the SL cell response, the LL cell

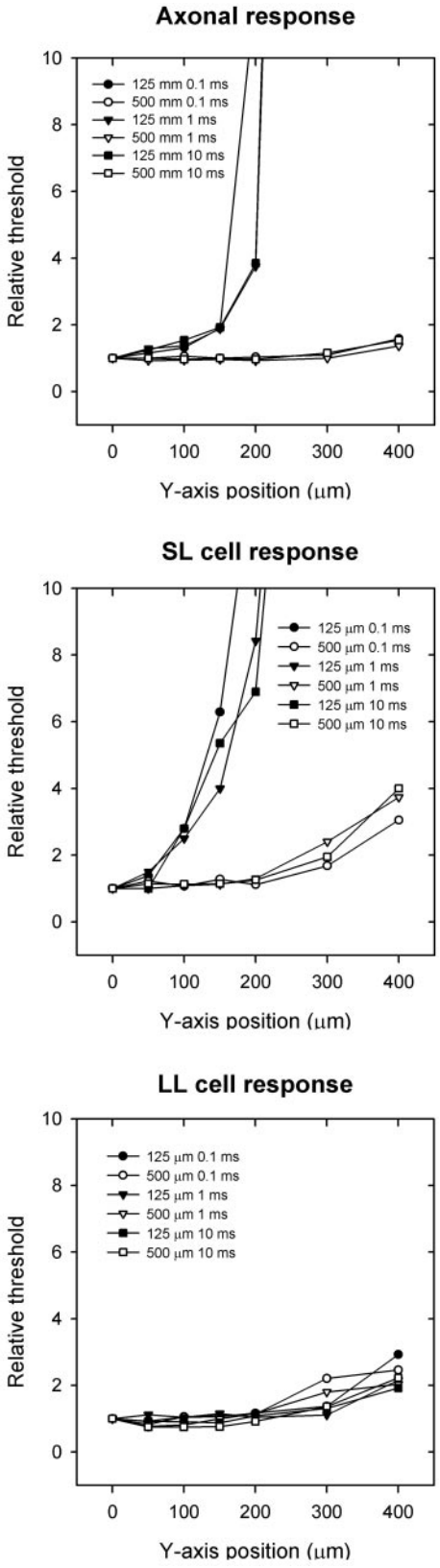

FIGURE 8. Relative threshold for the axonal response, SL and LL cell responses with respect to position along the *y*-axis on the surface of the retina. The threshold current for each RGC was normalized to a value of 1 at $z = 0$ μm, and medians were then computed to produce the plots. For clarity, relative thresholds >10 and positions >400 μm are not illustrated.

response consisted of one or more bursts of action potentials. LL cell responses are believed to be due to synaptic propagation of activity through neuronal circuits within the retina. Indeed, we[22] (Ziv OR, et al. *IOVS* 2002;43:ARVO E-Abstract 4474) and others[15,16] have found that the LL cell responses are susceptible to blockade of synaptic transmission in the retina. As will be discussed later, one can bias toward either an SL cell response or an LL cell response by manipulating the current pulse duration and the location of the stimulating electrode.

Effect of Current Pulse Duration

Strength–duration curves were obtained for axonal responses, SL cell responses, and LL cell responses with the simulating electrode located at the measured origin (site of lowest threshold for direct activation of an RGC). Data were obtained for both 125- and 500-μm diameter stimulating electrodes. The main difference in the findings between the two electrodes is the minimal amount of current needed to activate an RGC. At any given current pulse duration (0.1–50 ms), the median threshold currents for the axonal responses, SL cell responses, and LL cell responses were 2.6 to 9.2 times lower with the 125-μm electrode than with the 500-μm electrode. The reduction in current was not as great as one would predict based on the geometric area of the electrodes (the area of the 125-μm electrode is 16 times smaller than the area of the 500-μm electrode). A similar observation was recently reported by Johnson et al.[27], who used microwire glass electrodes. They found that reducing the area of retinal stimulation by a factor of 128 resulted in a current reduction by a factor of only 50. In our previous study,[17] in which the rabbit retina was stimulated with a 5-μm-long conical electrode, the median threshold currents for generation of an axonal response and an SL cell response are 0.95 and 0.50 μA, respectively, with 0.1-ms cathodal current pulses. In comparison, we found that with the 125-μm stimulating electrode, the median threshold currents for generation of an axonal response and SL cell response were 3 and 0.75 μA, respectively (Table 1). Although the area of the 125-μm electrode were markedly greater, the median threshold currents for the axonal and SL cell responses were only 1.5 to 3 times greater.

Greenburg[28] proposed that current pulses < 0.5 ms preferentially stimulate RGCs directly and that current pulses > 0.5 ms preferentially stimulate RGCs indirectly. Our findings show that when the stimulating electrode was positioned at the measured origin, short stimulus pulse durations preferentially stimulated the RGCs directly. However, only when current pulses were at least 50 ms in duration was there some indication of selective, indirect stimulation of RGCs. As will be discussed later, one may preferentially stimulate RGCs indirectly with pulse durations < 50 ms by positioning the stimulating electrode away from the measured origin.

Greenberg et al.[29] predicted, based on a computational model, that the threshold current for stimulation from a point source near the cell body of an RGC would be 58% to 73% lower than the threshold current for axonal stimulation and that this difference in threshold current would be even smaller for a disc electrode. In our previous study,[17] we used a 5-μm-long conical electrode to stimulate the rabbit retina. Although this stimulating electrode is not a true point source, we found that the median threshold current for "near cell body" stimulation is approximately two times lower than axonal stimulation with 0.1-ms monophasic cathodal current pulses, close to the prediction by Greenberg et al.[29] Using the same electrical stimulus in the present study, the median threshold current for activation of an RGC was three to four times lower when the stimulating electrode was placed over the receptive field than when it was placed over an axon, far away from the receptive

field (Table 1). This difference is much larger than the 20% difference predicted by Greenberg et al.[29] If anything, the large disc electrodes are more selective for "near cell body" stimulation than is the 5-μm-long conical stimulating electrode. The lower thresholds obtained with the stimulating electrode positioned over the receptive field of an RGC is an important finding, because it suggests that it is possible to avoid stimulating axons that are passing beneath the stimulating electrode with short-duration current pulses. Stimulation of axons passing underneath an active electrode can seriously degrade the percept a patient may experience with electrode stimulation of the retina.

Effect of Electrode Position

When the stimulating electrode was moved away from the measured origin *above* the retinal surface, the threshold currents for each response (axonal response, SL cell response, and LL cell response) did not change proportionally (Fig. 5). For example, the median threshold current for the SL cell response was significantly lower than the median threshold current for the axonal response when RGCs were electrically stimulated with the 125-μm electrode at the measured origin; however, when the same electrode was positioned 50 to 200 μm above the measure origin, the median threshold currents for the axonal and SL cell responses were similar. The median threshold current for the SL cell response was lower than the median threshold current for the axonal response when either size stimulating electrode was >100 to 200 μm above the measured origin. This finding suggests that it may be possible, even with long-duration current pulses, to stimulate RGCs directly and avoid axonal stimulation by positioning the stimulating electrode at some distance above the retinal surface. With regard to a retinal prosthesis, placing a stimulating microelectrode array several hundreds of micrometers above the retina has the disadvantage of potentially decreasing spatial resolution. At a distance of 400 μm above the retina, we found in fact that the size of the stimulating electrode became immaterial, in that the threshold currents for the three responses are essentially identical for both electrodes (Fig. 5).

As illustrated in Figure 5, we found that the median threshold current for the LL cell response did not in general increase as rapidly with distance above the measured origin as did the median threshold currents for the axonal and SL cell responses. Consequently, with 10-ms current pulses, the median threshold current for the LL cell response was significantly lower than the median threshold current for either the SL cell response or the axonal response when the stimulating electrode was positioned 400 μm above the measured origin. Thus, one may preferentially stimulate RGCs indirectly by using long pulse durations and positioning the stimulating electrode at some distance above the retina.

As the stimulating electrode was moved away from the measured origin *along* the retinal surface, the threshold currents for each response (axonal response, SL cell response, and LL cell response) again did not change proportionally (Fig. 7). This was especially evident when comparing the LL cell response to either the axonal response or SL cell response. The threshold current for the LL cell response was, remarkably, relatively flat. Although in this study we did not measure the size of the optical receptive fields of the RGCs, the receptive field centers may have been ~1 mm in diameter. Indeed, two groups[30–32] have shown that the dendritic field diameters of rabbit RGCs are nearly the same size as the receptive field centers, and in the peripheral retina, where our electrical stimuli were applied, the dendritic field diameters of rabbit α-RGCs (the morphologic correlate of brisk-transient RGCs) are quite large: 800 to 1000 μm (see Fig. 13 in Ref. 33). Given that the LL cell response results from activation of presynaptic retinal neurons that synapse on the broad dendritic tree of the RGCs, it is understandable that the threshold current for the LL cell response did not change appreciably with distance along the retinal surface. As a consequence, an LL cell response could be elicited from an RGC without eliciting an SL cell response when the stimulating electrode was at some distance from the measured origin. This was particularly evident with both size electrodes when 10-ms duration pulses were delivered (Fig. 7).

Applicability of Results to a Retinal Prosthesis

The findings from our study suggest that for an epiretinal prosthesis, it may not be possible to avoid direct stimulation of RGCs with stimulating electrodes positioned on the retinal surface. Direct RGC activation may yield a perceptual advantage, since the area of retina that is activated is more localized compared to indirect activation of an RGC. In human subjects, single electrode stimulation commonly induces a perception of a small spot of light.[7,34] For instance, Humayun et al.[7] reported that patients described a percept to be at times as small as a match head at arm's length when a 520-μm diameter electrode was used to stimulate the retina. It is tempting to speculate that this small percept is due to direct activation of a small cluster of RGCs beneath the stimulating electrode. Electrical stimuli that would indirectly activate RGCs would stimulate a larger area of the retina, and it would be reasonable to presume that a large percept would result. To avoid stimulation of passing (en passant) RGC axons, as well as neurons that are presynaptic to RGCs, our findings suggest that short-current pulses (0.1 ms or less) are preferable, in that the amount of current needed to generate an SL cell response is much lower than that needed to generate either an axon response or an LL cell response (Fig. 3). A short-current pulse has the additional benefit of reduced charge required for excitation. With reduced charge, the probability of electrode corrosion and tissue damage is lessened.

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