Response variability to high rates of electric stimulation in retinal ganglion cells

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Retinal prosthetics strive to restore vision to those blinded by outer retinal degenerative diseases such as macular degeneration and retinitis pigmentosa by electrically stimulating surviving retinal neurons (Humayun et al. 1996; Rizzo et al. 2003; Zrenner et al. 2009). Although the ability to create visual sensations is now well established, the quality and consistency of vision elicited by these devices is still highly variable. To improve the quality of elicited vision, much effort is being devoted toward understanding the neural activity arising from electric stimulation (Behrend et al. 2009; Margalit and Thoreson 2006; Sekirnjak et al. 2008; Ye and Goo 2007). It seems intuitive that a stimulation strategy that matched some (or all) of the signaling schemes that are used physiologically by the retina would lead to improvements in the quality of vision elicited by these devices.

Matching physiological signaling (spiking) patterns is not trivial, because such patterns are highly complex. There are at least 12 different types of ganglion cells (retinal output neurons) (DeVries and Baylor 1997; O’Brien et al. 2002; Rockhill et al. 2002; Roska and Werblin 2001); each is thought to extract different features of the visual world and uses distinct patterns of action potentials to transmit its signal to higher visual centers. The temporal properties of light-elicited patterns can vary considerably across types, suggesting that the prosthetic must be able to create a similarly wide range of temporal responses, including high-frequency bursts of spiking used by some cell types. Previous studies that have explored the temporal responsiveness of retinal ganglion cells have shown that a single, short-duration (~0.1 ms) stimulus pulse elicits a single action potential that is phase-locked to the onset of the cathodal pulse (Ahuja et al. 2008; Fried et al. 2006; Sekirnjak et al. 2006). In this manner, trains of short-duration pulses have been shown to elicit trains of action potentials. However, there are conflicting results as to the consistency with which elicited spikes can reliably follow pulse trains. Fried et al. (2006) found that each pulse in a 1-s train delivered at 250 pulses per second (PPS) reliably elicited a spike. Ahuja et al. (2008) showed that spikes could be reliably elicited by a 500-PPS train, although the duration for which this could be maintained was not specified. In contrast, Sekirnjak et al. (2006) found that not all pulses elicited spikes, even for rates as low as 100 PPS. This lack of consistency suggests a more systematic study of the temporal response is warranted.

Differences in the temporal response properties across ganglion cell types may arise from the heterogeneity of morphological and biophysical properties across types and raises the possibility that such differences may contribute to the differences in previous reports. One anatomic component that is likely to strongly influence the response to electric stimulation is a dense band of voltage-gated sodium channels located within the proximal axon (Boiko et al. 2003; Fried et al. 2009; Van Wart et al. 2007; Wollner and Catterall 1986); the band was shown to be the site with the highest sensitivity to electric stimulation (Fried et al. 2009), and it is therefore likely to be the site at which spikes are first initiated in response to electric stimulation. The anatomic properties of the band are different within each ganglion cell subpopulation (Fried et al. 2009), and it is therefore possible that these differences influence the ability of each ganglion cell type to follow pulse trains delivered at high rates. Additional variability may arise from the composition of ion channels within the band. Multiple sub-
types of voltage-gated sodium channels and at least one type of potassium channel have been identified in the band (Van Wart et al. 2007), and although heterogeneity of these distributions has not yet been demonstrated, the diversity of many other morphological and biophysical properties strongly suggests that ion channel distributions within the band are likely to be different as well.

Spikes initiated in the band propagate orthogradely along the axon, leading to synaptic communication with downstream neurons. Spikes also propagate retrogradely and “invade” the soma (generate a somatic spike) (Carras et al. 1992; Kole et al. 2007). The somatic spike has larger currents and broader spatial extent (compared with the spike generated in the band) and is therefore likely to be the response detected by extracellular recordings. The efficacy with which band-initiated spikes are converted into somatic spikes is thought to depend on properties of the band as well as the portion of the axon between the band and the soma (Hu et al. 2009; Mainen et al. 1995), and therefore differences in these properties across types are likely to underlie differences in the efficacy of “successful” invasion. This raises the possibility that certain forms of electric stimulation initiate action potentials that are more difficult for conventional recording methods to detect. This too has not been previously explored.

In the present study, we recorded the responses of five different types of retinal ganglion cells to 1-s trains of pulsatile electric stimulation with pulse rates ranging from 100 to 700 PPS. Only one cell type could reliably follow high-rate pulse trains, whereas several other types could not even follow rates of 200 PPS. In the types that could not follow high-rate trains, we observed a second type of response: a lower amplitude biphasic waveform that had latencies slightly faster than that of standard action potentials. Responses to a given train typically alternated between standard spikes and small waveforms, although the temporal patterns with which this occurred varied considerably across types. The implications of our findings are discussed in light of improving clinical outcomes with retinal prosthetics.

METHODS

Animal preparation and retina isolation. The care and use of animals followed all federal and institutional guidelines, and all protocols were approved by the Institutional Animal Care and Use Committees of the Boston Veterans Affairs Healthcare System and/or the Subcommittee of Research Animal Care of the Massachusetts General Hospital. New Zealand White rabbits (~2.5 kg) were anesthetized with injections of xylazine/ketamine and subsequently euthanized with an intracardial injection of pentobarbital sodium. Immediately after death, the eyes were removed. All procedures following eye removal were performed under dim red illumination. The front of the eye was removed, the vitreous was eliminated, and the eye cup was dissected so that the retina could be flattened. The retina was separated from the retinal pigment epithelium and mounted, photoreceptor side down, to a 10-mm-square piece of Millipore filter paper (0.45-μm HA membrane filter) that was mounted with vacuum grease to the recording chamber (~1.0-ml volume). A 2-mm circle in the center of the Millipore paper allowed light from below to be projected onto the photoreceptors.

Light responses and electrophysiology. Patch pipettes were used to make small holes in the inner limiting membrane, and ganglion cells were targeted under visual control. Spiking was recorded with a loose, cell-attached patch electrode (5–6 MΩ) filled with Ames medium. Two silver chloride-coated silver wires served as the return; each was positioned ~8 mm from the targeted cell and ~6 mm from each other.

The light stimulus and data acquisition software were controlled by custom software written in LabView (National Instruments) and MATLAB (The Mathworks) and written by G. Spor, T. Muench, and D. Balya. The electric stimulation software was written by D. Freeman. Light stimuli were projected onto the retina from below through a liquid crystal display projector (Dell) and focused onto the outer segments of the photoreceptors. A photopic background intensity was maintained throughout the experiment (~4 nW/m²) (Roska and Werblin 2001). Light stimuli consisted of stationary flashed squares (size range: 100–1,000 μm), 1-s duration, centered at the soma and moving bars (300 × 1,800 μm, moving at 600 μm/s in 4 orthogonal directions).

Cells were classified as directionally selective (DS) if their response to the flashed 200-μm square was ON-OFF and if their response to back-and-forth motion of the bars was directional, e.g., spiking levels were considerably higher in one direction vs. the other (Barlow and Levick 1965). These cells are more accurately classified as ON-OFF DS cells to distinguish them from a different type of DS cell that generates responses only at lights ON (ON DS cells). Cells were classified as local edge detectors (LEDs) based on physiological responses described in previous studies (Roska and Werblin 2001; van Wyk et al. 2006); that is, the cell gave ON and OFF responses to either light or dark stimuli and the responses were largest for small squares (100 μm). In addition, the light response was significantly reduced for squares of increasing size. Cells were classified as brisk transient/alpha cells (BT) if they responded with high frequency and transient bursts of spiking to stimuli centered in their receptive field (Cleland and Levick 1974; DeVries and Baylor 1997; Roska and Werblin 2001). Consistent with previous reports, responses were largest for larger squares and were typically small or nonexistent for small squares (≤100 μm). Similarly to previous studies, we found both ON and OFF varieties of these cells. OFF-delta cells exhibited robust spiking in response to light flashes; responses were maximum for mid-sized flashed squares (~300–500 μm) (Roska et al. 2006; Roska and Werblin 2001).

Electric stimulation. Electric stimulation was delivered via a 10-kΩ platinum-iridium electrode (MicroProbes); the exposed area was conical with an approximate height of 125 μm and base diameter of 15 μm, giving a surface area of ~5,900 μm², comparable to a 40-μm disk electrode. Two silver chloride-coated silver wires served as the return; each was positioned ~8 mm from the targeted cell and ~12 mm from each other. The height of the stimulating electrode remained fixed at 25 μm above the inner limiting membrane; the distance was calibrated by touching the surface of the inner limiting membrane with the tip of the electrode and then using the micromanipulator to raise the height by 25 μm. Pulse stimuli were controlled by MultiChannel Systems STG2004 hardware and software. The stimulating electrode was placed directly over the sodium channel band on the proximal axon (see below).

Location of the sodium channel band. In response to short-duration pulses, the location of the sodium channel band has been shown to correspond to the center of the region with the lowest threshold and is generally centered between 20 and 60 μm from the soma along the proximal axon (Fried et al. 2009). Using an iterative process, we were able to quickly find the center of the low-threshold region: movement of the stimulating electrode toward the center of the low-threshold region resulted in decreasing thresholds, whereas movement away from the center resulted in increasing thresholds. We used this location as the approximate center of the sodium channel band.

Rectangular pulses. Pulsatile stimuli were biphasic pulses (equal and opposite rectangular phases, cathodal first) delivered at rates of 10 and 100–700 PPS. Phase durations remained constant at 200 μs. For rates of 100–700 PPS, the interphase delay was equal to one-half of the period between consecutive pulses of the same phase, i.e., for 100 Hz, there was a 5-ms delay between the onset of consecutive cathodal
and anodal pulses. Although this approach introduced a variable delay between phases, it allowed the response to the cathodal phase to be studied in isolation, consistent with previous work (Fried et al. 2009; Sekirnjak et al. 2006). For the 10-PPS rate, the interval between cathodal and anodal was 10 ms, and the rate between anodal and cathodal was determined by the pulse rate.

**Stimulus threshold and statistical tests.** The threshold, T, was determined at 10 PPS before stimulation at the faster rates (100–300 PPS). The cells used in this study did not exhibit spontaneous firing, and therefore all recorded spikes were assumed to be stimulus-induced. Amplitudes at the faster rates were delivered in multiples of T. All statistical comparisons were performed using unpaired $t$-tests (GraphPad Software) unless otherwise specified.

**RESULTS**

We measured the response of retinal ganglion cells to pulsatile stimulation delivered at rates of 10–700 PPS. Stimulus waveforms were biphasic (cathodal first) and symmetric with fixed pulse durations (PD) of 0.2 ms (Fig. 1A, top). For pulse rates of 100–700 PPS, the interphase interval (IPI) was uniform between both phases (the cathodal-anodal and anodal-cathodal intervals were equal). For example, at 100 PPS, the interval between the onset of successive anodal and cathodal phases was 5 ms. The stimulating electrode was positioned over the proximal axon and centered approximately over the sodium channel band (METHODS). This location has previously been shown to have the highest sensitivity to cathodal pulses of electric stimulation (Behrend et al. 2009; Fried et al. 2009; Sekirnjak et al. 2008).

A portion of a typical response is shown in Fig. 1A, bottom. The large positive and negative waveforms (arrows) are the artifacts associated with the cathodal and anodal pulse, respectively. The biphasic waveform following each cathodal pulse is an elicited action potential and can be seen more clearly in an expanded view (Fig. 1B, left); these action potentials had similar amplitude and kinetics to spikes elicited by light stimuli (Fried et al. 2009; Sekirnjak et al. 2006) (not shown). Note that because currents are measured during voltage-clamp recordings, the depolarizing phase of the spike (inward current) is seen as a downward deflection (arrow), whereas the hyperpolarizing phase is upward. Pulses that elicited spikes could be clearly distinguished from pulses that did not (compare Fig. 1B, left and right).

Threshold was defined as the amplitude required to elicit spikes from 50% of the pulses when delivered at a pulse rate.

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**Fig. 1. Short-duration pulses each elicit 1 action potential.** A, top: the stimulus waveform consisted of 0.2-ms pulse duration (PD) cathodal and anodal pulses separated by an interpulse interval (IPI) that was determined by the pulse rate. Bottom: a portion of the response to a 100-pulse per second (PPS) pulse train. The artifact associated with each pulse (arrows) could easily be distinguished from elicited action potentials. B: expanded view of the response to 2 cathodal pulses; the one at left elicited a spike, whereas the one at right did not. C: each row contains the response of an OFF-brisk transient (BT) cell to a 1-s pulse train delivered at 200 PPS; each vertical line indicates an action potential. Stimulus levels were delivered in multiples of threshold (T): 1T, 2T, and 3T. D: the total number of action potentials elicited by a 1-s train of pulses at constant pulse amplitude is plotted as a function of amplitude for 3 different pulse rates. The total number of elicited action potentials was divided by the pulse rate to normalize responses.
of 10 PPS. We used this value (T) as a benchmark for stimulation at higher rates. For example, stimulus levels for all pulse rates >10 PPS were delivered in multiples of T; e.g., 2T, 3T, etc. The values of T (mean ± SD) for each of the five types tested in this study are shown in Table 1. Consistent with previous results (Fried et al. 2009; Jensen and Rizzo 2008), thresholds varied across types.

The amplitude required to elicit action potentials from 50% of pulses at higher pulse rates was different than that for 10 PPS. For example, when a 200-PPS train with amplitude T was delivered, only a small percentage of pulses elicited spikes (Fig. 1C, top row). Similarly, an amplitude of 2T elicited spikes for all pulses when delivered at 100 PPS (Fig. 1D) but elicited spikes in only ~50% of the pulses when applied at 300 PPS. The amplitude required to elicit one spike for each pulse increased as pulse rate increased (Fig. 1D) in all cells that we tested (n = 34). The relationship between pulse amplitude and the percentage of pulses that elicited spikes was highly consistent when measured within ganglion cells of the same type.

Across different types, however, the relationship between pulse amplitude and the percentage of pulses that elicited spikes varied considerably (Fig. 2). For example, whereas BT cells reliably followed all pulse trains, i.e., generated one spike for each pulse (Fig. 2A), most other types were not able to follow trains of 300 PPS, even at the highest stimulus amplitudes we tested (Fig. 2, B–D). In fact, a few types of ganglion cells could not even follow trains of 200 PPS (Fig. 2, B and C). Increasing the rate at which pulses were delivered (beyond 300 PPS) had little effect on the total

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>T Value</th>
<th>n</th>
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<tbody>
<tr>
<td>BT</td>
<td>13.2 ± 5.3</td>
<td>15</td>
</tr>
<tr>
<td>ON DS</td>
<td>20.8 ± 8.5</td>
<td>6</td>
</tr>
<tr>
<td>ON-OFF DS</td>
<td>15.0 ± 6.0</td>
<td>4</td>
</tr>
<tr>
<td>LED</td>
<td>21.3 ± 10.1</td>
<td>3</td>
</tr>
<tr>
<td>OFF-delta</td>
<td>29.7 ± 7.7</td>
<td>6</td>
</tr>
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</table>

Table 1. *T* values for each type of ganglion cell

Values are means ± SD for threshold (T); n is the number of brisk transient (BT), ON directionally selective (ON DS), ON-OFF DS, local edge detector (LED), and OFF-delta cells.

Fig. 2. The number of elicited standard spikes is variable across ganglion cell types. The total number of standard spikes elicited by trains of 100, 200, and 300 PPS is plotted as a function of pulse amplitude. Each plot contains the average of all responses from a given cell type: A, BT; B, local edge detector (LED); C, ON-OFF directionally selective (DS); and D, ON DS. Error bars indicate SD. Note that for pulse rates faster than 100 PPS, most types did not generate an action potential in response to each pulse.
number of spikes elicited in these types (Fig. 3, B–D). In contrast, BT cells could follow rates as high as 600 PPS (Fig. 3A), close to the theoretical limit for this cell type (the duration of a single action potential is ~1.5 ms).

To further explore the differences across types, we examined the temporal pattern of the response to each pulse in the train. Although the number of pulses that elicited spikes generally increased with amplitude in all types, the patterns with which spikes were elicited during the duration of the train varied across cell types. For example, in OFF Delta cells (Fig. 4a, left), there was an initial period during which each pulse elicited a spike followed by a period in which spikes were elicited only from approximately every other pulse. For ON-DS cells (right), after an initial period of all spikes, the pattern alternated between several consecutive pulses that did not elicit spikes and several consecutive pulses that did elicit spikes. While we did not perform a sufficient number of trials with which to statistically analyze these differences, several trends emerge from these patterns. First, when the average number of consecutive pulses that elicited spikes was plotted vs. the average number of consecutive pulses for which spikes were not elicited (Fig. 4b), the number of consecutive pulses that elicited similar responses was larger for ON-D cell types than for OFF-Delta cells, especially for the higher amplitude stimuli, consistent with the general appearance of the responses in Fig. 4A. The trend that emerges for higher amplitude stimulations can be better seen when responses for individual cells are plotted on the same axes (Fig. 4C). For the ON-D cells in which we made these measurements ($n = 3$), increases in amplitude resulted in a lengthening of the number of consecutive pulses that elicited spikes, consistent with the data of Fig. 4B in which the percentage of pulses that elicits spikes increased as amplitude increased, i.e., more points occurred to the right of the line of unity slope. The data of Fig. 4C also raise the possibility that there is an upper limit to the number of pulses that do not elicit spikes in these trains. The second trend is that the onset at which oscillations began was a function of both cell type and pulse amplitude (Fig. 4D). In general, both the pulse number at which onset began (compare OFF-delta vs. ON-DS cells) and the sensitivity of oscillation onset to increases in stimulus amplitude (compare OFF-delta vs. ON-OFF DS) were different across cell types.

Given that several previous studies reported that retinal ganglion cells follow high-rate pulse trains with one spike per pulse, we were somewhat surprised by the lack of this ability in certain ganglion cell types. Interestingly, in these types we found a second class of response (Fig. 5). This response was distinguished from the standard spike (Fig. 5A) by a much smaller amplitude and by different kinetics (Fig. 5A). Comparison of this small waveform with cases in which no response was elicited (Fig. 5A) as well as with cases in which the standard spike was elicited reveals the differences in amplitude and kinetics more clearly (Fig. 5A). This small waveform was elicited consistently in response to stimulation at 100–300 PPS in all cell types except for the BT. As a result, the analysis of the small

A

OFF BT

B

LED

C

ON OFF DS

D

ON DS

Fig. 3. Responses to higher pulse rates. The total number of standard spikes elicited by trains of 100–700 PPS is plotted as a function of pulse amplitude. Each plot contains the average of all responses from the same cell types as in Fig. 2: A, BT; B, local edge detector (LED); C, ON-OFF DS; and D, ON DS.
waveform response below is limited to only non-BT cell types ($n = 18$ cells).

We classified the responses to all pulses within a given train into a standard spike, a small waveform, or no response. Classifications were made by cross-correlating the raw response with a typical action potential (Fig. 5C, right), obtained by averaging action potentials elicited in response to light stimuli (no stimulus artifact). Before cross-correlation, the average stimulus artifact was subtracted at the appropriate location in the raw trace (obtained by thresholding); remaining artifacts were re-averaged and then subtracted again (Fig. 5C, left). The result of the cross-correlation between the modified raw trace and the average action potential contained a series of large and small peaks that were easily distinguished from each other (Fig. 5D). The time at which each peak occurred was used to extract a portion of the raw waveform. The extracted waveforms associated with the large peaks were highly consistent and closely resembled the light-elicited action potential (Fig. 5E1). Small waveforms were also highly consistent but different from the standard spikes (Fig. 5E2). For pulses that did not elicit spikes or small waveforms, the response was “flat” (Fig. 5E3); in conjunction with the uniform responses of Fig. 5E2, this suggests that the small waveform response is homogeneous and probably the result of an all-or-nothing (spike) mechanism.

Fig. 4. Temporal patterns of standard spiking are heterogeneous. A: the response to each pulse in a 200-PPS train is plotted as a function of pulse number for 2 different cell types: OFF delta (left) and ON DS (right). B: the average number of standard spikes between oscillations is plotted vs. the average number of nonspikes for amplitude levels of 3T (left), 4T (middle), and 5T (right). C: the average number of spikes between oscillations is plotted vs. the average number of nonspikes for 3 ON DS cells and 3 OFF delta cells. For most cells, the 3 points correspond to amplitudes of 3T, 4T, and 5T. For ON DS cell 1 and OFF Delta cell 3, the 4 points correspond to amplitudes of 3T, 4T, 5T, and 6T. For OFF delta cell 2, the 2 points correspond to amplitudes of 3T and 4T. D: the onset of oscillation is plotted as a function of amplitude for 3 different cell types; each line is the response from a different cell.
We compared the amplitude of the small waveforms with that of the standard spike (Fig. 5F). The peak-to-peak amplitudes could not be directly compared because the peak minimum value, especially for the small waveform, was often obscured by the stimulus artifact. Therefore, we compared the peak magnitude of the positive current in the small waveform (Fig. 5E2) with the peak magnitude of the afterhyperpolarization in the standard spike (Fig. 5E1). Figure 5F shows a typical histogram of the amplitude of the response to all pulses in a train. The mean amplitude of the standard spike was 0.379 ± 0.014, whereas the mean amplitude of the small waveform was 0.051 ± 0.006. Similar results were found in 18 of 18 cells; P values for all comparisons were <0.001, suggesting that the two response types are distinct.

We added the total number of standard spikes elicited by every pulse in a given train to the total number of small waveforms elicited from the same train and plotted the sum as a function of pulse amplitude (Fig. 6). Interestingly, the total of the number of standard spikes plus the number of small waveforms was consistently equal to the pulse rate for all cell types studied. However, because standard spikes could obscure the presence of a small waveform, we could not rule out the possibility that both were sometimes (or frequently) elicited. Nevertheless, the result suggests that either a standard spike or a small waveform (or both) is generated by every pulse in the train.

**DISCUSSION**

Ability to follow pulse trains is variable across ganglion cell types. Somewhat surprisingly, the responses to pulse trains were different for each of the cell types tested in this study. BT ganglion cells were the only cell type that reliably followed pulse trains regardless of rate, generating one spike for each pulse, even at rates as high as 600 PPS (Figs. 2 and 3). In contrast, neither LED nor ON-OFF DS cells could follow rates of 200 PPS (or higher) for 1-s durations. For cells that could not follow the faster rates for the full 1-s duration, there were further differences in the patterns with which spikes were generated. For example, in some types the pattern alternated...
nearly every pulse between spike and no-spike, whereas in other types spikes were elicited for several consecutive pulses followed by several consecutive pulses that did not elicit spikes. Most types could follow trains of 100–300 PPS for a portion of the 1-s train, i.e., the first ~200 ms (Fig. 4, A and C).

Clinical studies have explored the effect of pulse rate on the threshold required to elicit psychophysical percepts. For example, Horsager et al. (2009) found that the thresholds for eliciting percepts decreased as the rate of stimulation was increased. Presumably, changes in the rate of stimulation alter the rate at which spikes are elicited in targeted ganglion cells. Our present findings provide some support for this notion. Because the duration of the pulse train used in their study was limited to 200 ms, our results suggest that most ganglion cells generated one spike for each pulse in their trains. If so, the higher stimulation frequencies that they used would have been correlated with faster rates of spiking, and therefore faster spike rates in ganglion cells may be correlated with lower thresholds. Interestingly, our findings also suggest that if Horsager et al. had used a longer pulse train, most ganglion cells might have stopped generating one spike for each pulse part way through the train. This likely would have altered the properties of the percept midway through the stimulus and might also have contributed to the relatively fast fading of percept brightness that occurred in other clinical trials (Perez Fornos et al. 2010).

*Toward effective stimulation of the retina.* The optimum stimulation and coding schemes for retinal prosthetics have not yet been determined. Based on our understanding of the signaling methods used by the retina under physiological conditions, it is likely that the prosthetic will need to create high-frequency bursts of spike trains in ganglion cells, e.g., many cell types in the retina generate action potentials with interspike intervals of ~10 ms, and some can generate spikes at frequencies considerably ~100 Hz (DeVries and Baylor 1997; O’Brien et al. 2002; Roska and Werblin 2001). However, the number of spikes elicited in a given type was generally correlated to the spike rate elicited in response to other types of stimulation, e.g., visual (DeVries and Baylor 1997; Roska and Werblin 2001) or current injection (O’Brien et al. 2002). For example, in this study LEDs generated the fewest number of spikes in response to pulse trains, and they also had the lowest spike frequency in response to light stimuli (of the types tested presently). Therefore, further testing is needed to determine whether the inability of all ganglion cell types to follow high-rate trains will be an impediment to the creation of high-quality vision with a prosthetic. Importantly, all of our experiments were performed in the normal rabbit retina; further

Fig. 6. The total of standard spikes plus small waveforms matches the pulse rate. A–D: the total number of standard spikes plus the total number of small waveforms elicited by trains of 100, 200, and 300 PPS is plotted as a function of pulse amplitude for different cell types: A, BT; B, local edge detector (LED); C, ON-OFF DS; and D, ON DS. Lines represent the average of all cells in each types; error bars indicate SD.
testing is also needed to determine whether the response properties of ganglion cells in the human degenerate retina are similar.

Although the results of Horsager et al. (2009) in conjunction with our findings suggest that higher rates of spiking are associated with lower thresholds, it is important to point out that uniform delivery of high-rate pulse trains may not be the optimum stimulation strategy. Different types of ganglion cells each typically respond to visual stimuli with different spike frequencies (DeVries and Baylor 1997; Roska and Werblin 2001), and therefore it is probably not desirable to generate similar rates of high-frequency spiking in all types. Also, in response to natural scenes, retinal ganglion cells generate bursts of spikes that are temporally sparse, further suggesting that uniform and prolonged high-rate trains may not be optimum for creating high-quality percepts. The fact that the ganglion cell types that we tested could generally follow high-rate pulse trains for short durations suggests that the creation of “natural” signaling patterns may be feasible, although there are two important limitations to the implementation of this stimulation strategy. First, the short-duration pulses that were used to activate ganglion cells in this study are also effective for activating axons (Behrend et al. 2009; Grunet et al. 2000; Jensen et al. 2003). As a result, it is likely that the spatial extent of elicited activity will extend beyond the local vicinity surrounding the stimulating electrode. Second, each type of ganglion cell uses different patterns of spiking to represent the visual scene. However, since activation thresholds are thought to vary by less than an order of magnitude across types (Fried et al. 2009), it is likely that similar patterns of neural activity will be created in all ganglion cells (regardless of type) that are close to the stimulating electrode. This may be modulated somewhat by the variability in thresholds for different types of ganglion cells (Fried et al. 2009) as well as by their proximity to the stimulating electrode. Nevertheless, the creation of similar patterns of activity in all ganglion cell types is nonphysiological and may reduce the quality of elicited percepts. Therefore, improved stimulation methods that can preferentially target specific types of ganglion cells would allow more physiological patterns of activity to be created and possibly improve the quality of elicited vision.

What factors influence variability across types? The variability in responsiveness to pulse trains most likely arises from differences in the spike-generating machinery of each type. Almost certainly, the responses in the present study arose through direct activation of the ganglion cell: previous studies consistently indicate that pulse durations comparable to those used in our study activate ganglion cells directly without simultaneously activating presynaptic neurons (Fried et al. 2006; Sekirnjak et al. 2006), and also, the bursts of spiking that are characteristic of presynaptic activation (Fried et al. 2006; Jensen et al. 2005) were not observed in these experiments. Within the ganglion cell it is likely that spikes were initiated within the dense band of sodium channels within the axon initial segment (AIS): this region is known to have the highest sensitivity to extracellular electric stimulation (Behrend et al. 2009; Fried et al. 2009; Sekirnjak et al. 2008), and the stimulating electrode was consistently positioned directly over its center. The anatomic properties of the band are known to vary across types (Fried et al. 2009), raising the possibility that band differences underlie the response differences observed presently. Support for this notion comes from a recent modeling study (Jeng et al. in press) that found that band differences contribute to the variability in thresholds found in response to single pulses.

The efficacy with which ganglion cells followed pulse trains was dependent on stimulus amplitude. For example, in ON DS cells, the amplitude required to elicit one spike per pulse at 200 PPS was larger than that required to elicit one spike per pulse at 100 PPS (Fig. 2D). The same was true for BT cells: higher amplitude levels were required to elicit one spike per pulse as the pulse rate was increased (Fig. 2A). It is not clear why this is the case, but the temporal patterns of spiking elicited by high-rate trains (Fig. 4A) suggests that the inability to follow pulse trains at a given amplitude occurred because some aspect of the spike initiation process “fatigues.” It seems unlikely that this could arise from the properties of the voltage-gated ion channels that underlie spiking because the kinetics of activation, inactivation, and/or de-inactivation are all considerably faster than the intervals between pulses (Hille 1992), especially at the lower pulse rates. For example, increasing pulse rate from 100 to 200 PPS reduced the interval between consecutive anodal and cathodal phases from 5 to 2.5 ms. However, the duration of the action potential in BT cells was ~1.5 ms, indicating that the kinetics of the ion channels that underlie spiking are faster and thus should not require a stronger level of activation at faster rates. Furthermore, the hyperpolarizing anodal phase should facilitate the inactivation and de-inactivation mechanisms, thereby making more channels available to be activated by the upcoming cathodal phase. Recent findings in Purkinje neurons suggest that the spike initiation process occurs in two phases. Action potentials are first initiated at the distal portion of the AIS; this region consists primarily of the Na,1.6 subtype of voltage-gated sodium channels. Na,1.6 channels are activated at relatively hyperpolarized levels of membrane potential (low threshold). The strong depolarization of the distal AIS activates the higher threshold Na,1.2 sodium channels located within the proximal portion of the AIS. Although the proximal portion of the AIS in retinal ganglion cells is thought to comprise the Na,1.1 subtype, presumably a similar two-phase activation process is utilized. If the conversion from Na,1.6 spikes to Na,1.1 spikes was not 100% efficient at high rates of stimulation, higher amplitude pulses might target Na,1.1 sodium channels in the proximal axon directly, thereby overcoming any inefficiency. Other factors, including a contribution from the variability in duration between stimulus phases at the different pulse rates we used, could also explain our findings, and further exploration is warranted.

Relevance of small waveforms. The responses elicited by individual pulses from pulse trains delivered at 100–300 PPS fell into two categories (Fig. 5). The amplitude and kinetics of the first type of response were identical to that of light-elicited action potentials, consistent with much previous work that found that short-duration pulses elicit single action potentials (Fried et al. 2006; Jensen et al. 2005; Sekirnjak et al. 2006). The amplitude of the second type of response was considerably smaller than the standard spike (Fig. 5F). Although our measurements did not directly reveal the origin of this smaller waveform, it is important to consider the possibility that the small waveform reflects that an action potential was elicited. This could occur, for example, if AIS-elicited action potentials
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REFERENCES


