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A Method for Generating Precise Temporal Patterns of Retinal Spiking Using Prosthetic Stimulation

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Fried, S. I., H. A. Hsueh, and F. S. Werblin. A method for generating precise temporal patterns of retinal spiking using prosthetic stimulation. J Neurophysiol 95: 970–978, 2006. First published October 19, 2005; doi:10.1152/jn.00849.2005. The goal of retinal prosthetic devices is to generate meaningful visual information in patients that have lost outer retinal function. To accomplish this, these devices should generate patterns of ganglion cell activity that closely resemble the spatial and temporal components of those patterns that are normally elicited by light. Here, we developed a stimulus paradigm that generates precise temporal patterns of activity in retina ganglion cells, including those patterns normally generated by light. Electrical stimulus pulses (≥1-ms duration) elicited activity in neurons distal to the ganglion cell; this resulted in ganglion cell spiking that could last as long as 100 ms. However, short pulses, <0.15 ms, elicited only a single spike within 0.7 ms of the leading edge of the pulse. Trains of these short pulses elicited one spike per pulse at frequencies ≤250 Hz. Patterns of short electrical pulses (derived from normal light elicited spike patterns) were delivered to ganglion cells and generated spike patterns that replicated the normal light patterns. Finally, we found that one spike per pulse was elicited over almost a 2.5:1 range of stimulus amplitudes. Thus a common stimulus amplitude could accommodate a 2.5:1 range of activation thresholds, e.g., caused by differences arising from cell biophysical properties or from variations in electrode-to-cell distance arising when a multielectrode array is placed on the retina. This stimulus paradigm can generate the temporal resolution required for a prosthetic device.

INTRODUCTION

Retinal degenerative diseases such as retinitis pigmentosa and age-related macular degeneration are the two leading causes of blindness in the United States (Bunker et al. 1984; Curcio et al. 1996; Friedman et al. 2004). Currently there is no cure for either disease. Loss of visual function arises from a degeneration of the outer retina, primarily the photoreceptors, but these diseases do not target inner retinal neurons; large populations of inner retinal neurons remain morphologically intact, even in patients blind for many years (Humayun et al. 1999; Santos et al. 1997; Stone et al. 1992; but also see also Jones and Marc 2005; Marc and Jones 2003; Marc et al. 2003). During clinical trials in patients blind from retinal degenerative diseases, electrical stimulation from electrodes placed close to the ganglion cell layer elicited light percepts (Humayun et al. 1994, 1996; Rizzo et al. 2003a,b). These findings suggest that ganglion cells (the output cells of the retina) not only remain morphologically intact, but remain functionally viable as well.

Several research groups are developing retinal prosthetic devices with the hope of providing meaningful visual information to blind patients (Chow et al. 2004; Humayun et al. 2003; Rizzo et al. 2003a,b; Zrenner et al. 1997). To be effective, these prosthetic devices should generate patterns of activity that resemble patterns normally evoked by light. This means that the device should be capable of replicating the temporal properties of light-elicited spike trains from normal retina, while spatially confining the response to a focal region—ideally a single ganglion cell. In addition, there are ~12–15 different types of ganglion cells (Rockhill et al. 2002; Roska and Werblin 2001); populations of each type generate unique spiking patterns that are carried to distinct downstream visual sites (Roska and Werblin 2001) (both cortical and noncortical areas). This means that the prosthetic device must also be capable of targeting the appropriate spiking pattern to the correct ganglion cell subtype.

There are several challenges associated with replicating the temporal properties of normal light-elicited spike trains. For example, maximum spike rates in normal retina can exceed 250 Hz (O’Brien et al. 2002), but not all cell types respond with an equal number and/or pattern of spikes. Therefore the prosthetic device must be capable of generating a wide range of spike frequencies and patterns. Even within a single cell type, there are considerable variations in the light-elicited spike trains, such as changes in spike rates used to code information about stimulus contrast (Smirnakis et al. 1997). In addition, several studies now indicate that spike timing between multiple retinal neurons is often very precise (Mastronarde 1989; Meister et al. 1995), suggesting that prosthetic devices may need to generate spikes with high temporal precision.

Electrical stimulation of the retina activates multiple classes of retinal neurons complicating the generation of precise temporal patterns of spiking. Jensen et al. (2005a) reported that the spiking response to electrical stimulation consists of short- and long-latency components and that the latency of the late component ranged from 8 to 60 ms. They further showed that the long component arose from activation of bipolar cells, excitatory presynaptic neurons in the inner nuclear layer. In response to light, bipolar cells deliver excitatory input to both ganglion cells and amacrine cells (see Wassle 2004 for a review). Activated amacrine cells deliver inhibitory input in a feed-forward manner to ganglion cells and through feedback to bipolar cells. The precise interplay between these excitatory and inhibitory signals shapes the retinal response. Because electrical stimulation targets bipolar cells, it is possible that amacrine cells are targeted as well. Activation of amacrine cells is likely to lead to long-lasting inhibitory signals feeding...
and yielded results that were consistent with bath-applied methods. Spiking was blocked with 1 mM TTX. The cocktail of synaptic blockers consisted of 5 mM curare, 1 mM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 10 mM DL-2-amino-7-phosphono-heptanoic acid (AP-7).

**Light stimulus and data acquisition**

The stimulus presentation and data acquisition software was written by E. Eizenman and G. Spor. Light stimuli were projected onto the retina from below through an LCD panel (CRL OPTO) and focused on to the photoreceptor outer segments. Retinas were light adapted before the start of electrophysiological recordings. Light stimuli consisted of stationary flashed squares (100, 200, 300, 500, or 1,000 µm), 1-s duration, and centered at the soma. Contrast levels were set to 200% unless specified. Data were analyzed in Matlab (MathWorks).

**Electrical stimulation**

Electrical stimulation consisted of charge balanced biphasic pulses: cathodic and anodic pulses were typically equal and opposite square wave pulses (MultiChannel Systems hardware and software). Cathodic pulses were delivered first, and intervals between phases were large enough so that the neural response to the cathodic pulse was completed before the onset of the anodic phase (typically 5 ms; range 2–100 ms). In some of the higher stimulation frequency experiments, the duration of the anodic pulse was increased 2–10 times. The amplitude was adjusted to keep the total charge constant. Electrical pulses were delivered using Platinum-Iridium electrodes (Micro Probes, impedances 10–100 kΩ). Stimulation was applied epi-retinally; the stimulating electrodes were placed (under visual control) ~15 µm from the somata of targeted ganglion cells.

**Charge density calculations**

Electrodes (10 kΩ) were conical, with a length of ~125 µm and a base radius of ~15 µm. The corresponding geometric surface area was ~5,890 µm². The actual surface area was higher because of the rough nature of the platinum surface. We estimated a 50% increase in surface area leading to an effective surface area of 8,836 µm². With this electrode, typical early phase spike thresholds were 137 µA with a 0.06-ms pulse width, resulting in a calculated charge density of 0.093 mC/cm². The threshold levels for late phase spike activation represents a maximum stimulation level and were ~322 pA, resulting in charge densities of 0.219 mC/cm². These calculated levels of charge density assume uniformity across the surface of the electrode (but also see McIntyre and Grill 2001).

**RESULTS**

**Electrical stimulation elicits two distinct phases of spiking**

**DISTINGUISHING NEURAL ACTIVITY FROM STIMULUS ARTIFACT.** We measured responses to light and electrical stimulation in 65 ganglion cells from 30 different retinas using a combination of cell-attached and whole cell patch-clamp recordings (see METHODS). The initial response to pulses of electrical current consisted of large transient currents (Fig. 1A, horizontal arrows) that were temporally correlated with the onset and offset of both phases of the stimulus pulse (cathodic and anodic). The second portion of the response consisted of a series of biphasic waveforms (asterisks). These waveforms had similar magnitude and kinetics to light-elicited action potentials (Fig. 1B, inset). TTX, a blocker of neuronal spiking, eliminated the
FIG. 1. Spiking response to electrical stimulation consists of 2 phases. A: response to a 15-μA biphasic stimulus pulse (timing at top) consists of large transient currents (horizontal arrows) followed by a series of biphasic waveforms (asterisks). B: in TTX, all the biphasic waveforms are eliminated. In addition, response is also modified in the region immediately after onset of the cathodic pulse (compare responses in A and B in shaded region). Inset: subtracting the TTX trace from control reveals a single spike; onset immediately follows onset of cathodic pulse (vertical dotted line). Average light-elicited spike is shown for comparison (dotted line).

biphasic waveforms (n = 5/5), confirming that they were conventional voltage-gated Na+ spikes (Fig. 1B).

TTX also modified a portion of the response associated with the first large transient current (compare the shaded region in Fig. 1, A and B). This suggested that one or more spikes were buried within this transient current. To reveal the spike(s), we reasoned that the response in TTX did not contain neural activity and was therefore mainly electrical artifact generated by the stimulus pulse. Subtraction of the electrical artifact (TTX response) from the control response revealed an additional pulse-elicited spike (inset, solid trace, n = 5/5). The waveform of this spike was nearly identical to the average light-elicited spike for this cell (inset, dotted trace). We will refer to this single spike as the early-phase spike and refer to the subsequent series of multiple spikes (biphasic waveforms) as late-phase spikes. We found similar results in all ganglion cell types of the rabbit retina (data not shown). These results are consistent with the short- and long-latency spiking responses recently reported by Jensen et al. (2003), although our threshold values and latency measurements differ (see Discussion). The onset of the TTX-extracted pulse-elicited spike closely followed the onset of the cathodic pulse (mean = 580 μs, range = 400–680 μs, n = 5). Without TTX, it was difficult to precisely determine the onset of the elicited spike, but comparison of control records in 5 TTX and 15 non-TTX experiments were similar, providing further support that the elicited spike closely and consistently follows the stimulus pulse onset.

ALL LATE PHASE SPIKES WERE BLOCKED BY SYNAPTIC BLOCKERS. To determine whether pulse-elicited spiking arises from direct activation of ganglion cells or whether it arises as a result of activation of presynaptic excitatory neurons, we measured responses in the presence of a pharmacological cocktail (CNQX, AP-7, and curare) that blocked all excitatory synaptic input to ganglion cells (Fig. 2). All late-phase spiking was eliminated in the presence of this cocktail (compare Fig. 2, A and B; n = 3/3), suggesting that excitatory synaptic input underlies the late-phase response (Jensen et al. 2005). In contrast, the early-phase spike was not affected by the cocktail, suggesting that this spike is not synaptically driven. Taken together, these results suggest that the spiking response to electrical pulses arises from two different mechanisms: 1) direct activation of the ganglion cell generates a single spike immediately after the onset of the cathodic pulse (early phase) and 2) activation of presynaptic excitatory neurons (likely bipolar cells) generates increased release of excitatory neurotransmitter on to ganglion cells, causing depolarization and spiking (late phase).

Stimulus pulses activate bipolar and amacrine cells

STIMULUS PULSES ELICIT EXCITATORY AND INHIBITORY CURRENTS IN GANGLION CELLS. To better understand the activation of presynaptic cells, we directly measured the input currents to ganglion cells using whole cell patch-clamp recordings. The isolated excitatory input current to ganglion cells provides a measure of the activation level of presynaptic excitatory neurons (most likely bipolar cells); increased release from these cells appears as an outward current in ganglion cells. Similarly, the isolated inhibitory current provides a measure of the activation level of presynaptic inhibitory neurons (most likely amacrine cells); increased release from amacrine cells appears as an inward current in ganglion cells. We used the magnitude and duration of the input currents to provide a more precise means of determining the activation level of presynaptic neurons in response to different stimuli configurations.

We measured robust excitatory input currents in response to electrical stimulus pulses (Fig. 3A). By convention, increases in excitatory currents are shown as downward deflections and downward peaks are depicted by vertical arrows. The presence of excitatory input currents indicates that bipolar cells were activated by electrical stimulation (n = 4/4). Stronger stimulation levels generally elicited more excitatory activity (n = 4/4), suggesting that bipolar activation increases with increasing pulse duration.

We also measured inhibitory currents in response to these pulses (n = 3). Increases in inhibitory currents are drawn as upward deflections and upward peaks of inhibitory activity are again indicated by vertical arrows; the second peak is consid-

FIG. 2. Early and late phase spiking arise from different mechanisms. A: response to a 20-μA biphasic stimulus pulse (in a different cell from Fig. 1) again consists of early phase (shaded region) and late phase (asterisks) spiking. B: cocktail of synaptic blockers [6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), ti.-2-amino-7-phosphono-heptanoic acid (AP-7), and curare] eliminates late phase spikes but leaves early phase spike intact.
FIG. 3. Robust excitatory and inhibitory currents underlie late phase spiking response. A: spiking response (middle) and underlying excitatory (bottom) and inhibitory (top) input currents in response to a 15-μA biphasic stimulus pulse. Two late phase spikes (asterisks) are present while the early phase spike, buried within the stimulus artifact (large arrow), is seen in the expanded time scale (asterisk, inset). Excitatory and inhibitory currents each contain several peaks (vertical arrows, top; upward deflections indicate increasing inhibitory input, bottom; downward deflections indicate increasing excitatory input). B: expanded time scale from A reveals temporal alignment between spikes (dashed line) and input currents.

generally longer and stronger than the first. The presence of inhibitory currents in the ganglion cell indicates that amacrine cells are activated by electrical stimulation. There are two possible mechanisms by which this might arise: amacrine cells are either activated directly by electrical stimulation or they are synaptically driven by electrically activated bipolar cells (bipolar cells provide excitatory input to amacrine cells).

Our results do not allow us to distinguish between these possibilities.

STIMULUS PULSES ELICIT REVERBERATING ACTIVITY AT THE INNER PLEXIFORM LAYER. We examined the temporal correlation between spiking and the peak levels of excitatory and inhibitory input. Pulse-elicited spikes occurred during the onset (leading downward slope) of excitatory input currents (Fig. 3B); a similar temporal relationship exists between spiking and excitatory currents in response to light. The inhibitory current peaks were out of phase with the excitatory peaks so that inhibition was maximal in-between and after the late-phase spikes. In general, the peaks of excitation and inhibition alternated, suggesting that electrical stimulation elicits a reverberation of the underlying circuitry. For larger amplitude stimuli, we observed additional alternating peaks of excitation and inhibition lasting as long as 80 ms, although we did not quantify these responses or study the underlying mechanism (n = 3).

Amacrine cells activation disrupts the response to subsequent pulses

In healthy retina, amacrine cells deliver both feedforward inhibition to ganglion cells and feedback inhibition to bipolar cells. This raises the question of whether electrically stimulated amacrine cells will inhibit either ganglion or bipolar cells or both. Inhibitory currents can last for ~100 ms (Fig. 3A), so the spiking response to pulses spaced at <100-ms intervals might be affected.

LONG-LASTING INHIBITION REDUCES EXCITATION. To evaluate the effect of prolonged inhibition, we measured the excitatory input to ganglion cells as stimulation frequency increased (Fig. 4). Because the inhibitory input to an individual stimulus pulse persisted for ~100 ms, we reasoned that inhibition would not affect responses for low stimulation frequencies (<10 Hz), but would affect responses at higher stimulation frequencies. We measured excitatory currents in response to stimulus pulses delivered at 1 Hz (Fig. 4, top; n = 4) and found them to be consistent from trial to trial. When the stimulation frequency was raised to 2 Hz, however, the amplitude of the excitatory input current was reduced immediately: the magnitude of excitation was largest for the first pulse and reduced consistently for the second and subsequent pulses (Fig. 4, middle). This suggests that an inhibitory signal was acting at the release sites (bipolar cell terminals) to reduce excitation. The reduction was largest at the second pulse; excitatory currents increased over the next few pulses but did not return to original levels. This affect is most evident in the 2-Hz traces (Fig. 4, middle). At higher stimulation frequencies, the amplitude of the excitatory input current continued to decrease (Fig. 4, bottom) and by 10 Hz, the excitatory input current was no longer detectable (data not shown). The reduction of excitatory input with increasing stimulation frequency was summarized by averaging the elicited excitatory input per pulse at each stimulation frequency (Fig. 4B). These results suggest that the release of excitatory transmitter from bipolar cells is suppressed by long duration stimulus pulses and that the suppression persists for ≥500 ms. It is likely that this suppression is

FIG. 4. Higher frequencies of stimulation suppress excitatory input. A: excitatory input is reduced in response to increasing frequency of stimulation. 1 Hz: initial stimulus pulse (left vertical line) generates a robust excitatory input to ganglion cells (downward deflection) that remains constant for subsequent stimulus pulses (vertical lines). At higher stimulation frequencies (2-4 Hz), excitatory input is robust for the 1st pulse but reduced in response to subsequent pulses. B: average excitatory input for 1-, 2-, and 4-Hz stimulation frequencies.
.mediated by activation of amacrine cells that supply inhibition to the release sites on the bipolar cell terminals, although we did not perform experiments to support this.

Separating the early and late phase responses

Is it possible to independently activate either the early- or late-phase spiking responses? Our results thus far suggest that the spiking response to electrical stimulation is the result of a direct spike in ganglion cells (early phase) as well as a complex interaction between presynaptic neurons that results in a variable amount of spiking (late phase). We observed that the excitatory input elicited by electrical stimulation was a function of pulse duration: longer duration pulses generated larger excitatory inputs (Fig. 5A; \( n = 2/2 \)). This suggested that decreasing the pulse duration might reduce or eliminate excitatory input to ganglion cells and therefore the late phase spikes. Short-duration pulses were not likely to eliminate the direct spike because the onset of the spike closely followed the leading edge of the cathodic pulse (Fig. 1B, inset) suggesting that the leading edge of the pulse was generating the early-phase spike.

The response to short-duration cathodic pulses (Fig. 5B) consisted of large transient currents temporally correlated with the stimulus pulse but short pulses did not generate late-phase spiking. The transient currents were TTX-sensitive, indicating the presence of one or more spikes (\( n = 5/5 \)). Subtraction of the TTX response from control revealed the presence of a single spike (Fig. 5C). We measured similar control responses in 16 additional cells. Several additional cells (\( n = 3 \)) responded to short-duration pulses with two spikes: These cells had on-off responses to light flashes but were not studied further.

ONE SPIKE PER PULSE IS CONSISTENT AT HIGHER STIMULATION FREQUENCIES. The ability to generate a single spike from a single electrical pulse is important and suggests that we can use short pulses to generate specific predetermined patterns of spiking. Ganglion cells generate light elicited spiking at rates \( \leq 250 \text{ Hz} \); thus we evaluated whether short pulses could generate one pulse per spike at higher stimulation frequencies (Fig. 6, A and B). High-frequency trains of short-duration stimulus pulses (Fig. 6A, top) elicited one spike (asterisks) per pulse. We extracted the time interval from 1 ms before to 2 ms after each cathodic pulse for all 250 pulses: the individual traces are overlaid in Fig. 6B. The individual responses were extremely consistent and comparison with the response in TTX (dotted trace) indicates that each pulse elicits a single spike. We observed similar responses in 12 cells at all frequencies tested (\( \leq 250 \text{ Hz} \)).

ONE SPIKE PER PULSE IS GENERATED OVER A WIDE RANGE OF STIMULUS AMPLITUDES. We were able to consistently generate one spike per pulse over a wide range of stimulus amplitudes (Fig. 6C). Above threshold levels, stimulus pulses elicited spikes (vertical arrow, left gray box). The threshold at which spiking occurred was determined by observing the change in shape of the early phase response (cf. control and TTX traces in Fig. 5B). The early-phase response persisted for all stimulus levels above threshold. At higher amplitude levels, however, stimulus pulses elicited late-phase spiking (Fig. 6C, right). Further increases of stimulus amplitude generated increased amounts of late-phase spikes. For this cell, the range of amplitude that elicited a single spike was 120 – 340 \( \mu \text{A} \). For the population (\( n = 13 \)), the range of effective amplitude was 193 \( \pm 64 \mu \text{A} \). The spike was elicited by the cathodic phase of the pulse; in almost all cases, anodic pulses did not elicit spiking, even up to the late-phase spike threshold (\( n = 12/13 \)).

REPLICATING LIGHT-ELICITED SPIKE PATTERNS. We used programmed sequences of short pulses to replicate light-elicited spike patterns (Fig. 7). We measured the spiking response to the flash of a small square of light (Fig. 7, A and B) and calculated the latency of each spike. This was used to program a sequence of short-duration pulses (Fig. 7B, top) such that the latency of individual cathodic pulses matched the latency of individual spikes; charge-balancing anodic pulses followed each cathodic pulse. The programmed sequence of pulses was delivered to the ganglion cell, and the net result was a pattern of elicited spikes whose temporal pattern precisely matched the pattern of light-elicited spikes (Fig. 7C). Jitter between individual light- and pulse-elicited spikes was < 0.5 ms. Changes in the light stimulus elicited different spike patterns that were replicated with different programmed arrays of short pulses (data not shown).

DISCUSSION

Light-elicited spike patterns can be simulated with electrical stimulus pulses

We developed a method to replicate light-elicited spiking patterns in individual ganglion cells using electrical stimulation. One short-duration cathodic pulse (~ 0.1 ms) generated one spike, time-locked to within 0.7 ms of the pulse onset (Fig. 5C). One spike per pulse was elicited at stimulation frequen-
cies ≤250 Hz (Fig. 6, A and B), the upper limit of light-elicited spiking responses. This suggests that precise temporal patterns of spiking, including those patterns elicited by light, can be generated using programmed arrays of short pulses (Fig. 7). Matching the normal light spike patterns provides a method to send a more natural (physiological) signal to the brain that may lead to the generation of more meaningful percepts.

There will be variability in the distance between individual electrodes and targeted ganglion cells when an epi-retinal array of stimulating electrodes is placed on the retinal surface. The variations arise because ganglion cell somata are situated at different depths within the ganglion cell layer and also because the stimulating array may not lie perfectly flat on the retinal surface (Humayun et al. 2003). The threshold for activation increases with increasing distance between the stimulating electrode and targeted ganglion cell (BeMent and Ranck 1969), suggesting that stimulation methods will need to be effective over a wide range of amplitude levels. The large window of effective stimulation for short pulses (~2.5:1) suggests that variations in distance can be accommodated by this method (Fig. 6C).

LATENCY OF THE EARLY-PHASE RESPONSE IS <0.7 MS. We found the latency between the onset of the stimulus pulse and the

FIG. 6. Short electrical pulses reliably elicit 1 spike per pulse. A: response to 10 consecutive 0.1-ms pulses at 200 Hz (bottom). Top: stimulus timing (downward deflections are cathodic pulses). Each cathodic pulse elicits a single spike (asterisk). B: response to 200 consecutive 0.1-ms pulses delivered at 200 Hz (overlaid black lines). Comparison with the response in TTX (dotted trace) reveals that each record contains a single spike. C: response to increasing amplitude 100-μs pulses at 2 different time scales. Left: early phase spiking. Right: late phase. Threshold for early phase spiking is ~100–120 μA (shaded region, vertical arrows) and for late phase spiking is 360 μA (shaded region, vertical arrow).

FIG. 7. Programmed sequences of short electrical pulse replicate light responses. A: spiking response to a 1-s light stimulus (horizontal bar). B: bottom: expanded time scale from A reveals individual spike latencies. Top: programmed sequence of short pulses derived from individual spike latencies: each cathodic pulse is arranged 0.5 ms before corresponding spike. C: spikes elicited by programmed sequence of short pulses (red) precisely match the light elicited spike pattern (black) from B.
onset of the elicited spike to be <0.7 ms (Fig. 5C). This value differs from a recent study (Jensen et al. 2005) that reported latencies of 3–5 ms. The most significant difference between the studies is the position of the recording electrode. We placed patch-clamp electrodes directly onto the surface of the ganglion cell soma (cell-attached patch). This measurement provided a robust spiking signal that could be visualized within the stimulus artifact; comparison with the TTX response allowed us to isolate the spike and precisely determine its latency. Previous studies (Jensen et al. 2005a) using extracellular recordings placed the recording electrode several millimeters from the stimulating electrode. Under these conditions, the measured latency included a delay that was a result of spike propagation along the axon to the recording site. The long delay is effective for separating spikes from the stimulus artifact but misrepresents the actual timing between the stimulus pulse and the spike onset.

**Long-lasting activation of amacrine cells suppresses responses to subsequent stimulation**

LONG PULSES ACTIVATE NEURONS PRESYNAPTIC TO GANGLION CELLS. Long pulses of electrical stimulation (≥1 ms) elicited excitatory and inhibitory currents in the ganglion cell, indicating that both bipolar and amacrine cells are activated by these pulses (Fig. 3). Bipolar cell activation is supported by previous studies (Greenberg 1998; Jensen et al. 2005), in which a portion of the pulse-elicited spiking response disappeared in the presence of excitatory synaptic blockers. We were able to determine the level of bipolar cell activation by measuring the magnitude of excitatory input currents in ganglion cells and found that shorter pulses resulted in progressively weaker bipolar cell activation (Fig. 5A). This led us to test shorter pulses as a means of silencing bipolar cell-mediated spiking.

ACTIVATION OF AMACRINE CELLS SUPPRESSES PRESYNAPTIC ACTIVITY. In the normal retina, amacrine cells synapse onto and inhibit bipolar, ganglion, and other amacrine cells. This suggests that activated amacrine cells might not only inhibit the direct early-phase response (by raising activation thresholds), but might also reduce the amount of late phase spiking by suppressing activation of bipolar cells to subsequent pulses. The long duration of the pulse-elicited inhibitory current (Fig. 3A) suggests that the response to subsequent pulses could be inhibited for ≤100 ms, which would interfere with stimulation at frequencies >10 Hz. We observed a reduction of excitatory input at stimulation frequencies as low as 2 Hz (Fig. 4). Our results do not allow us to unravel the mechanism by which this occurs, but it seems likely that the reduced excitatory input to subsequent pulses arises from an increased level of amacrine cell-mediated inhibition that arrives at the bipolar cell terminal. The duration of this effect was ≥500 ms, even longer than the ~100-ms duration of the direct inhibitory signal. The difference in duration between the two inhibitory effects (direct vs. reduction of excitatory input) suggests that more than one population of amacrine cells is activated: one population delivers direct inhibitory input to ganglion cells, and the other population inhibits the release of excitatory transmitter at the bipolar cell terminal. It is possible that additional populations of amacrine cells are also activated, although we did not encounter their effects in our measurements.

The diverse effects of activated amacrine cells suggest that long stimulus pulses may compromise our ability to generate closely spaced patterns of spiking activity in retinal ganglion cells. Whereas short pulses can reliably elicit spikes every few milliseconds, long pulses elicit prolonged inhibitory activity that can adversely affect the spiking response to subsequent pulses. On the other hand, inhibitory activity from long stimulus pulses may provide a mechanism to temporarily reduce the spiking output of the retina, e.g., if spontaneous activity is increased in degenerated retina (Stasheff 2004).

**Are small diameter electrodes more suitable for retinal stimulation?**

SMALL DIAMETER ELECTRODES CAN RELIABLY ELICIT SPiking at SAFE STIMULATION LEVELS. In this study, we used small-tipped electrodes to elicit spiking in ganglion cells—the surface area of our largest electrodes was comparable with a 40-μm-diam disk electrode, considerably smaller than currently used implanted electrodes (Humayun et al. 2003). Smaller electrodes are advantageous for stimulating the retina because they generate more focal stimulation; however, they require higher charge densities that can cause breakdown of the electrode as well as adverse tissue reactions. The recommended upper limit of charge density for platinum electrodes is 0.3–0.35 mC/cm² (Brummer and Turner 1977). In this study, charge densities at threshold were ~0.093 mC/cm². Most of our experiments were performed at ~150% of threshold, corresponding to a charge density of ~0.139 mC/cm². These charge density levels assume a uniform distribution across the surface of the electrode that may not hold true for small-tipped conical electrodes (McIntyre and Grill 2001). However, even if the nonuniformities in our electrodes increase the charge density slightly beyond safe limits, further testing of small electrodes is still warranted. New electrode materials under development have higher charge density limits (Cogan et al. 2005), and electrodes with symmetrical designs (circular, hemispherical) will reduce the elevated charge densities arising from irregular shapes. Reducing electrode size will allow focal activation of small groups of neurons which will lead to higher resolution patterns of prosthetic-elicited activity that are closer to light-elicited patterns.

SHORT PULSES LOWER CHARGE DENSITY LEVELS. Typical thresholds for 1-ms pulses were ~15 μA, corresponding to a charge density of 0.17 mC/cm², nearly twice the current density associated with short pulses. These results are consistent with recent results from Jensen et al. (2005), who reported similar percentage increases between short and long pulses. Short pulses, by reducing the total charge delivered, will allow us to use smaller electrodes and still remain within the acceptable limits of charge density.

VARIATIONS IN THRESHOLD ACROSS STUDIES. The thresholds reported by Jensen et al. (2003) were ~1 μA, lower than our average of ~15 μA. It is likely that the differences arise because of differences in electrode impedance between the two studies. Although, Jensen et al. did not report impedance levels, they describe their electrode as conical with 5 μm height and 2 μm base diameter. Our smallest electrode was 15 μm height and 7 μm base diameter. It is likely that the smaller surface area of the electrode of Jensen et al. results in higher
impedance levels of their electrodes. Higher impedances result in higher current densities with corresponding lower threshold levels.

WHY ARE PERCEPTUAL THRESHOLDS HIGHER THAN SPIKE THRESHOLDS? Current levels required to generate spikes in in vitro preparations are lower than current levels for perception in implanted patients. Why is this so? It is likely that properly coordinated physiological activity of populations of ganglion cells normally generated in response to light (Roska and Werblin 2001) are not achieved during electrical stimulation; therefore, although cells are spiking, their activity does not lead to perception. In this study, we found that temporal properties of (long) pulse-elicited spike trains do not match the temporal properties of light-elicited spike trains. In human psychophysical experiments, the large diameter stimulation electrodes (250–500 μm; Humayun et al. 2003) cannot match the precise spatial patterns generated by light stimuli. A third discrepancy between light-elicited versus pulse-elicited patterns of activation arises from the presence of multiple types of retinal ganglion cells (e.g., ON and OFF). Under normal conditions, light stimuli do not simultaneously activate ON and OFF ganglion cells, whereas large diameter electrodes likely do. It is likely that the specific features (temporal, spatial, and cell-type) of the normal patterns are necessary for optimal detection of the retinal signal. Without the precise signaling patterns, it is likely that the brain’s ability to detect the retinal signal is diminished. The method we have developed here matches the temporal component of these patterns but not the spatial and cell-type components. Stimulation methods that match these two other components may reduce the thresholds for percept detection down to the levels for spiking in individual ganglion cells (in vitro).

Future work

TARGETING INDIVIDUAL CELLS. Our results indicate that light-elicited spike patterns in ganglion cells can be replicated using trains of short pulses that each elicit a single spike. Considerable challenges remain, however, along the path toward focal stimulation of local populations of individual ganglion cells. For example, methods that limit activation to small focal regions and methods that separately target individual ganglion cell types must both be developed. In addition, the threshold for activation of ganglion cells is only slightly lower than the threshold for activation of axonal fibers (Greenberg 1998; Jensen et al. 2003). Therefore methods that avoid stimulating axons must be improved as well.

TECHNICAL CHALLENGES FOR IMPLEMENTING SHORT PULSES. Short pulses have lower total charge requirements but require higher stimulation current levels and will likely require higher power supply voltage levels. The higher current levels associated with short pulses may lead to increased heating of the retina. Finally, stimulation methods that employ short pulses to elicit individual spikes may require more sophisticated signal processing regimens. The considerable benefits associated with replicating light-elicited spike patterns with short pulses will have to be evaluated in light of these additional engineering challenges.

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REFERENCES


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