A method for generating precise temporal patterns of retinal spiking using prosthetic devices

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Abstract

To restore meaningful vision to blind patients, retinal prosthetic devices should elicit spiking patterns in ganglion (output) cells that match the patterns normally generated by light. Here we have developed a method to generate high temporal precision spike trains that can mimic light-elicited patterns. Cell-attached patch clamp recordings were used to measure spiking responses from individual retinal ganglion cells in the flat mount rabbit retina. Biphasic electrical stimulus pulses were delivered epi-retinally by small-tipped platinum-iridium electrodes. Long duration electrical pulses (≥1 msec) elicited a single short-latency spike followed by a train of much longer latency spiking which was variable and depended on pulse amplitude levels. Short duration pulses (~0.1 msec) also elicited the single short-latency spike but no longer latency spikes. High frequency trains of short duration pulses also reliably elicited a single spike per pulse, suggesting that short duration pulse trains can be used to generate precise temporal patterns of spiking in ganglion cells. The short pulse stimulus paradigm allows us to mimic physiologically relevant light evoked responses, e.g., transient or sustained cells, as well as the spiking patterns that code for changes in intensity and contrast.

1. INTRODUCTION

Retinitis pigmentosa and age-related macular degeneration are two of the leading causes of blindness, resulting in more than 100,000 cases per year in the US alone1,2. Each disease has many etiologies2 but they both lead to a degeneration of photoreceptors, resulting in a loss of visual function. Several research groups are developing devices that target remaining downstream neurons in the retina with the hope of restoring activity in these cells. To elicit meaningful percepts in patients, these prosthetic devices should generate patterns of activity that resemble patterns normally evoked by light. Here, we have developed a paradigm to generate precise temporal patterns of spiking activity in retinal neurons.

2. METHODS

We measured spiking responses in ganglion cells, the output cells of the retina, using cell-attached patch clamp recordings in the flat mount rabbit retina. The extraction of the retina, development of the preparation and maintenance of the tissue are described elsewhere3. Electrical stimulation was delivered by small tipped platinum-iridium (Pt-Ir) electrodes (Impedance: 10 – 100 kΩ) and consisted of biphasic (cathodic first), charge balanced square wave current pulses. Amplitudes ranged from 1 to 400 µA and pulse durations ranged from 60 µsec to 6 msec. Light responses were measured at the start of each stimulus trial to ensure stability of the recording setup.

3. RESULTS

A typical spiking response to electrical stimulation is shown in Figure 1a (solid line). Large transient currents (horizontal arrows) were recorded which temporally correlate with the onset and terminations of the individual phases of the stimulus pulse. This electrical ‘artifact’ that occurred during the stimulus pulse tended to obscure a neural action potential (spike) buried in the trace. In TTX, a blocker of neuronal spiking, the response was different in
the region immediately following the onset of the pulse. Subtracting the TTX response from the control response unmasked a single spike (Fig. 1b, solid line) which was similar in magnitude and kinetics to light evoked spikes (Fig. 1b, dotted line). In response to the anodic phase of the stimulus pulse, there was no difference between the control and TTX responses (data not shown).

The spike was elicited immediately after the onset of the pulse (Fig. 1b), suggesting that it was elicited at the leading edge of the pulse. To test this, we shortened the duration of the stimulus pulse. The responses to a 0.2 ms pulse under control conditions and in TTX (Fig. 1c, solid and dotted lines respectively) were subtracted again, revealing the presence of a spike (Fig. 1d). The response to a short pulse in control conditions was always triphasic, consisting of an upward, downward and then second upward deflection. In TTX, the response was always biphasic (no second upward deflection). In later experiments with short duration pulses the second upward deflection was a reliable marker to indicate that a spike had been elicited.

Longer duration pulses elicited longer latency spiking that occurred after completion of the stimulus pulse spikes (Fig. 2a, left, asterisks). These spikes were also eliminated in the presence of TTX (Fig. 2a, right). The number and timing of these spikes was variable and generally increased with increasing pulse amplitude or duration. Short duration pulses (~0.1 ms) did not elicit any of these spikes at all (Fig. 2c, right). We refer to spiking responses that occur immediately following the onset of the cathodic pulse as early phase (Figure 1) and spikes that are elicited following completion of the entire pulse as late phase (Figures 2a-c, left).

Figure 1: Single spikes are elicited by the leading edge of electrical pulses. (a) Voltage clamp response to a 3 ms 50 µA cathodic pulse (solid trace). Large current transients are seen (horizontal arrows) at pulse onset and offset (timing given by square wave above). The response was sensitive to TTX (dotted trace) but only in the time period immediately following the pulse onset (grey box). (b) Subtracting the TTX record from control extracts the spike which is similar to light-elicited spikes (dotted trace). (c) Voltage clamp response to a 200 µsec 50 µA electrical pulse (solid line). Large transient currents are again seen at pulse onset and offset (horizontal arrows). The response here was also TTX sensitive (dotted trace). (d) Subtracting the TTX record from control extracts the spike.

Figure 2: Early and late phases of spiking are activated by different mechanisms. (a) Left trace: A second phase of spiking (individual late-phase spikes indicated by asterisks) is elicited after completion of long duration pulses (vertical arrow indicates completion of pulse in a-c). Spikes are eliminated in TTX (right trace). Stimulus pulse waveform and timing is represented at top. (b) Late phase spiking in a different cell (left trace) is completely eliminated by 1 mM CNQX, 10 mM AP7 and 5 mM Curare (right trace). Inset: expanded time scale of the interval between the dotted lines from control (dotted) and excitatory synaptic blocker (solid) traces. The normalized TTX response from a different cell is shown for comparison. The early phase spike remains intact. (c) Late phase spiking initiated by long pulses (left, asterisks) is eliminated by reducing the duration of the pulse to 0.1 ms (right trace). The scale bar in (c) applies to all traces. Cathodic and anodic pulses were 3 ms for long pulses (a-c) and 0.1 ms for short pulse (c). Interval was 5 ms for all pulses.
All of the late phase spiking was eliminated in the presence of a cocktail that blocked excitatory synaptic input to ganglion cells (Fig. 2b, compare left and right panels). This indicates that excitatory synaptic input to ganglion cells is required for the generation of these spikes, and suggests that long-duration pulses activate presynaptic neurons that release excitatory neurotransmitter. The early phase spike was not eliminated in the presence of these blockers (Fig. 2b, inset), indicating that it arises from direct activation of the ganglion cell.

At higher stimulation frequencies, short duration pulses continued to elicit one spike per pulse (Fig. 3a). The responses to 200 individual pulses, applied at 200 Hz (Fig. 3a, solid lines) are all tri-phasic, indicating the presence of a neuronal spike in each. Comparison with the TTX response (dotted line) confirms that every stimulus pulse elicited a spike.

A single spike per pulse could be reliably elicited over a wide range of pulse amplitudes as shown in Figure 3b. For this cell, pulse amplitudes above 120 µA consistently elicited a single spike. Above 360 µA, late phase spikes were elicited, likely due to activation of presynaptic neurons.

### 4. DISCUSSION AND CONCLUSIONS

We have developed a stimulus paradigm that elicits one neuronal spike per stimulus pulse and is effective over a wide range of stimulation frequencies and amplitudes. This is significant because it will allow electrical prosthetic devices to mimic the spike patterns sent to the brain during normal vision.

Short pulses are likely to simplify the generation of spatially complex patterns of activity because they activate only ganglion cells. Longer pulses appear to activate bipolar cells. Amacrine cells are also activated, either directly or via synaptic input from bipolar cells. Activation of these presynaptic elements elicits long latency excitation as well as long latency inhibition that can spread over broad spatial regions. The use of short pulses avoids the long latency and broad spatial activity.

Short pulses elicit spiking in ganglion cells using less total charge than long pulses. This may allow for reduced electrode size thereby generating a more focal response.

### References


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