Electrical stimulation of retinal neurons in epiretinal and subretinal configuration using a multicapacitor array

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Eickenscheidt M, Jenkner M, Thewes R, Fromherz P, Zeck G. Electrical stimulation of retinal neurons in epiretinal and subretinal configuration using a multicapacitor array. J Neurophysiol 107: 2742–2755, 2012. First published February 22, 2012; doi:10.1152/jn.00909.2011.—Electrical stimulation of retinal neurons offers the possibility of partial restoration of visual function. Challenges in neuroprosthetic applications are the long-term stability of the metal-based devices and the physiological activation of retinal circuitry. In this study, we demonstrate electrical stimulation of different classes of retinal neurons with a multicapacitor array. The array—insulated by an inert oxide—allows for safe stimulation with monophasic anodal or cathodal current pulses of low amplitude. Ex vivo rabbit retinas were interfaced in either epiretinal or subretinal configuration to the multicapacitor array. The evoked activity was recorded from ganglion cells that respond to light increments by an extracellular tungsten electrode. First, a monophasic epiretinal cathodal or a subretinal anodal current pulse evokes a complex burst of action potentials in ganglion cells. The first action potential occurs within 1 ms and is attributed to direct stimulation. Within the next milliseconds additional spikes are evoked through bipolar cell or photoreceptor depolarization, as confirmed by pharmacological blockers. Second, monophasic epiretinal anodal or subretinal cathodal currents elicit spikes in ganglion cells by hyperpolarization of photoreceptor terminals. These stimuli mimic the photoreceptor response to light increments. Third, the stimulation symmetry between current polarities (anodal/cathodal) and retina-array configuration (epi/sub) is confirmed in an experiment in which stimuli presented at different positions reveal the center-surround organization of the ganglion cell. A simple biophysical model that relies on voltage changes of cell terminals in the transretinal electric field above the stimulation capacitor explains our results. This study provides a comprehensive guide for efficient stimulation of different retinal neuronal classes with low-amplitude capacitive currents.

neural prosthetics; retina

THE ACTIVATION OF NEURONS by extracellular current is the major principle underlying implantable neuroprosthetic devices. Clinical applications include cochlear implants (Wilson and Dorman 2008), deep brain stimulation for the treatment of Parkinson’s disease (Krack et al. 2003; Montgomery and Gale 2008), and, most recently, retinal implants (Ahuja et al. 2011; Zrenner et al. 2011). Although all approaches show remarkable success, improvements in electrode properties are required to enhance prosthetic performance and stimulus protocols are needed that target specific neuronal classes within a circuit.

To study the mechanism of neuronal stimulation in brain tissue we used a multicapacitor array that is part of a complementary metal oxide semiconductor (CMOS)-based silicon chip (Lambacher et al. 2011). For technological reasons the entire CMOS chip is insulated by a thin and chemically inert oxide layer (TiO2/ZrO2). The insulation prevents faradaic processes that may damage the interfaced neural tissue (reviewed in Merrill et al. 2005) or may lead to electrode deterioration (Cogan 2008; Merrill et al. 2005). On the other hand, the insulation lowers the specific electrode capacitance compared with metal electrodes; as a consequence the maximum capacitive stimulation current is lowered. However, even small capacitive currents can elicit neuronal activity if a tight contact with neurons and brain tissue is provided (Hutzler and Fromherz 2004; Schoen and Fromherz 2007, 2008). Specifically for the rabbit retina, it has been demonstrated recently that such a tight contact exists with the same all-oxide CMOS silicon chip used here (Zeitler et al. 2011). We therefore applied a purely capacitive stimulation to selectively stimulate neurons in the well-defined layered nervous tissue of the retina.

The electrical stimulation of the ex vivo mammalian retina with metal-based electrodes has been extensively studied in both epiretinal (Fried et al. 2006; Jensen et al. 2003; Sekirnjak et al. 2008) and subretinal (Jensen and Rizzo 2006, 2009; Stett et al. 2000, 2007; Tsai et al. 2009) configurations. In the epiretinal configuration the stimulation electrode is close to the retinal projection neurons, the ganglion cells, whereas in the subretinal configuration the stimulation electrode is close to the outer retina. The activation of preselected retinal circuitry is currently investigated in both configurations: Examples include the selective activation of retinal ganglion cells with small electrodes (Sekirnjak et al. 2006, 2008) and the preferential activation of bipolar cells or photoreceptors with sinusoidal stimuli with appropriate frequencies (Freeman et al. 2010). Restricted stimulation geometry, however, hampers a thorough investigation of the biophysical mechanisms underlying neuronal stimulation stimulation in the retina (but see Fried et al. 2009). Using a multicapacitor array with 400 stimulation sites (50 × 50 μm2 within 1 mm2), we applied stimuli of different sizes and of different polarities at different positions to the same retinal circuit converging onto one ganglion cell. In this study we focused mainly on the ON retinal pathway, which is activated by light increments. We demonstrate the activation of different neuronal classes within this pathway by combining the different stimulation protocols and using pharmacological receptor antagonists. A protocol is identified that hyperpolarizes photoreceptor terminals, similar to the physiological response to light increments and analogous to a promising recent optogenetic approach that targets remnant photoreceptors in blind retinas (Busskamp et al. 2010). Finally, we present a simple model for capacitive stimulation of bipolar cells and photoreceptors that
can be generalized to other cylinder-like neuronal structures in defined electric fields.

METHODS

Semiconductor chips with multicapacitor arrays. Capacitive stimulation of neuronal activity was performed with multicapacitor arrays that are integrated in a CMOS-based silicon chip used in previous studies for extracellular recording (Lambacher et al. 2011; Menzler and Zeck 2011; Zeck et al. 2011). To evoke action potentials in retinal neurons, a capacitor array comprising 400 equally spaced stimulation sites with a total area of 1 mm² was used. Each individual capacitive stimulation site extends over an area of 50 × 50 µm² and is separated from the adjacent capacitors by a gap of ~0.5 µm (Fig. 1, B and C). The capacitor consists of a platinum electrode separated from the conductive electrolyte by a thin (~30 nm) TiO₂/ZrO₂ layer. Arbitrary subsets of individual capacitors can be combined by a shift register implemented in the CMOS chip that contains the information concerning the sites to be selected. Once the shift register is loaded, a single external waveform generator applies the stimulus to the specified capacitors. The CMOS-based chips are wire-bonded to standard ceramic packages (CPGA, Spectrum, San Jose, CA). A custom-made Perspex chamber with an inner area of 12 mm² is attached to shield the bond contacts and to expose the stimulus array to culture medium.

Measurement of specific oxide capacitance. For capacitive stimulation, voltage ramps were applied between the chip and an Ag/AgCl electrode immersed in the electrolyte. The voltage ramps are generated at 250 kHz by a high-speed I/O DAQ device (NI6259, National Instruments).

The stimulation current I (Fig. 1D) is calculated from the voltage drop measured across a serial 50 Ω resistor (Schoen and Fromherz 2007). The resistor is in series with the Ag/AgCl electrode and represents the only ohmic contact to the bath. The voltage drop is amplified in a customized amplifier. The stimulation current density i_s is calculated according to i_s = I/A, with A being the known stimulation area (Fig. 1E). The current density, stimulation voltage amplitude ΔV_s, and stimulus time Δt are used to calculate the specific oxide capacitance c_s = i_s Δt/ΔV_s. In a second experiment (Fig. 1F), AC and DC currents at different holding potentials were measured with a potentiostat (PARSTAT 2263; AMETEK Princeton Applied Research, Oak Ridge, TN) as described by Wallrapp and Fromherz (2006) and used to calculate the specific capacitance at different frequencies.

Preparation of retina and interfacing to multicapacitor array. All experimental procedures were carried out in compliance with the institutional guidelines of the Max Planck Society and were approved by the local government (Regierung von Oberbayern; Statement of Compliance no. A5132-01). All animals were killed prior to the

Fig. 1. Characterization of the capacitive stimulation array. A: micrograph of the complementary metal oxide semiconductor (CMOS)-based silicon chip that embodies an array of capacitive stimulation sites. B: top view of the array of stimulation sites comprising 400 equally spaced stimulation sites (marked by the white square). The size of each capacitive site is 50 × 50 µm². Capacitors are separated from each other by only 0.5 µm. C: schematic cross section through the capacitive stimulation array. A thin oxide layer (~30 nm) separates the silicon chip from the ionic electrolyte that is held at ground potential with a Ag/AgCl electrode. Each capacitor consists of a 50 × 50-µm² platinum electrode covered by a TiO₂/ZrO₂ dielectric. A shift register with switch driver circuitry contains the data that control a switch related to each site. This connects the respective Pt electrode to an external voltage generator. Dimensions are not to scale. Vs, stimulation voltage D, top: falling voltage ramps applied to the array of capacitive sites (areas: 50 × 50 to 900 × 900 µm²). Bottom: capacitive currents (I) measured in response to the voltage ramp between the Ag/AgCl electrode immersed in the recording solution and the system ground. Individual traces are recorded for different capacitive areas on the same array. The constant current reflects a purely capacitive stimulation current. E: current amplitude vs. capacitive area. Each symbol represents the minimal current measured for 1 capacitive area (stimulus shown in D). The linear relationship indicates constant current density without edge effects. F: specific capacitance (c_s) measured with a potentiostat. The specific capacitance does not depend on the bias voltage and shows little frequency dependence in the range of 0–1 V.
removal of organs in accordance with the European Commission Recommendations for the euthanasia of experimental animals (Part 1 and Part 2). Housing and euthanasia of the rabbits were fully compliant with applicable German and European laws and regulations concerning care and use of laboratory animals.

The preparation of the retina follows previous reports (Zeck et al. 2011; Zeck and Masland 2007). Rabbits (strain New Zealand White, 8–12 wk of age, Charles River) were dark adapted for 1 h, anesthetized, and euthanized as reported. The enucleated eye was hemisected under dim red light and the retina peeled from the sclera. Retinal portions (~4 × 4 mm²) from the midperiphery inferior to the visual streak were cut under a dissecting microscope, transferred to the chip chamber, and placed on the multi-electrode array. Prior to the experiment, CMOS chips were gently cleaned with detergent (Ticoptop R36, 5%, Stamm/Berlin, 80°C) and rinsed with ultrapure water (resistivity 18 MΩcm). After the chip surface was dried in a nitrogen stream, an ~500-μl (1 mg/ml) volume of poly-L-lysine (P1399, mol mass 150–300 kDa, Sigma) was applied for at least 2 h. The chips were rinsed prior to positioning of the retina with Ames medium (A1420, Sigma). The chip comprising the attached retina was mounted on a custom-made circuit board attached to an upright microscope (BXW 51W, Olympus) and continuously perfused with oxygenated Ames medium (flow rate 2–4 ml/min, temperature 32–35°C).

Electrical stimulation and signal detection. Stimulation pulses were monophasic, with an interpulse interval of 100 ms. Negative voltage ramps of amplitude ΔV, and duration Δt elicit a cathodal (negative) capacitive current, while positive voltage ramps elicit anodal currents. The stimulation voltage amplitude was limited to 1.3 V to avoid faradaic currents. Three different stimulation protocols were used. 1) At a fixed stimulation area A of 500 × 500 μm² negative and positive voltage ramps of different amplitudes (maximum amplitude 1.3 V, increment 0.1 V) and different durations (0.05–100 ms) were applied to the retina. 2) Rectangular stimulation sites (100 × 1,000 μm²) were used to map the spatial stimulus selectivity of the retinal ganglion cell. The current amplitude and stimulus duration were fixed (voltage ramp: 1 V/1 ms). 3) Square-shaped stimulation sites with variable areas (50 × 50 μm² to 900 × 900 μm²) were used to estimate the threshold current density. In these protocols the pulse duration was 1 ms while the pulse amplitude varied between 0.1 and 1.0 V. Within each protocol each stimulus was applied 50 times.

Action potentials were detected with an extracellular tungsten electrode (TM31C20KT 2-MΩ impedance, World Precision Instruments) connected to a bridge-amplifier (BA-03X, NPI Electronics). The band-pass (1 Hz to 30 kHz) filtered signal was sampled at 60 kHz by a Multi-I/O board (NI 6259, National Instruments) using galvanic separation with a cutoff frequency of 30 kHz. Capacitive stimulation and signal detection were performed with custom-written software (LabVIEW 8.5, National Instruments).

Light stimulation. Light-induced extracellular activity was used for physiological identification of the retinal ganglion cells. Light stimuli were projected onto the retina by a microdisplay (OLED, SVGA+ XL Rev2; eMagin, Bellevue, WA) and focused onto the photoreceptor layer through a ×10 objective (LMPlan; Olympus Optical, Tokyo, Japan). A photopic background intensity was maintained throughout the experiment (9 mW/m²). Transient ON and OFF ganglion cells were identified on the basis of their response properties to flashed stimuli (500-μm spot diameter) centered on the soma. The firing rate of transient cells (of both ON and OFF type) to flashed spots decays below 20% of their maximal value within 100 ms. Sustained cells (of both ON and OFF type) maintain a constant firing rate above the 20% level throughout the stimulus presentation time (300 ms). Sustained cells and direction-selective cells were excluded in this study, as their complex spike pattern cannot be assigned to specific cell types with pharmacological agents. Light stimulus protocols consisted of five stationary flashed spots (diameter range 100–1,000 μm, presentation duration 0.3 s).

Data analysis. Extracellular electrode data were analyzed off-line with LabVIEW (National Instruments) and Origin (Microcal). Spike detection was performed off-line. Spikes were identified if the extracellular voltage of five consecutive data points exceeded 5 times the RMS voltage computed over 1 s. The spikes are assigned to one ganglion cell if consecutive waveforms do not differ in shape and if no violation of a refractory period of 1 ms occurs. Repeated electrical stimuli give rise to stimulus locked responses. This confirmed that ganglion cell responses and no artifacts were recorded.

All spikes that occur within 1 ms after stimulus onset are hidden in the stronger response of the resistive retinal tissue. The average tissue response was computed for 50 stimulus repetitions. Subtraction of this average response from each individual stimulation trace revealed the ganglion cell response (Fig. 2). This method applied for stimulation near threshold. Above stimulation threshold an average tissue response was calculated as a linearly scaled response of a subthreshold stimulus. This average voltage does not contain ganglion cell signals and was subtracted from individual recordings.

The occurrence of ON ganglion cell activity was evaluated for each stimulus condition within the two time intervals (2–30 ms and 30–60 ms; see Fig. 4). The response probability was approximated by a cumulative Gaussian distribution. The stimulus threshold was extracted for the value of 80% response probability.

Measurement of specific retinal resistivity. For a simple model of neuronal stimulation above a capacitive area, the tissue resistivity ρretina is measured with a silver-chloride micropipette. The sharp pipette is lowered to the capacitor surface and stepped through the retina. At calibrated equidistant positions Δz above the capacitor surface the steady-state voltage ΔV following a long current pulse is measured. The mean tissue resistivity ρretina is calculated according to

\[ ρ_{\text{retina}} = \frac{1}{i_S} \frac{ΔV}{Δz} \]

Voltage change at the terminal of a cylinder-like neuron in a homogeneous electric field. The stimulation of bipolar cells or photoreceptors is modeled with a simple biophysical model of a cylinder-like nonleaky passive cell in a homogeneous electric field. Classic theory predicts that a cable of finite length placed in a longitudinal static electric field is hyperpolarized along its anode-facing half and depolarized along its cathode-facing half. The maximum voltage drop occurs at the end of the cable and amounts to \( ΔV = E \cdot L/2 \) for a nonleaky cell with electrolytic distances much larger than the cell length L (Plonsky and Barr 2007). The electric field is given by \( E = ρ_{\text{retina}} i/ \), assuming a homogeneous tissue resistivity ρretina and a spatially constant current density i in the tissue. During the application of the capacitive stimulation current there are no other current sources in the retina or in the electrolyte above the retina. Thus the voltage drop across the terminal of a cable-like cell is given by \( ΔV = ρ_{\text{retina}} i S L/2 \).

To account for spatially restricted stimulation areas and for the finite retinal dimension we numerically calculate the extracellular voltage change in a homogeneous volume conductor above a planar square-shaped surface. The voltages within the retina (Vretina) and in the electrolyte (Velectrolyte) obey a Laplace equation: \( \nabla^2 V = 0 \). The boundary condition at the capacitor-retina interface is

\[ \frac{\partial V_{\text{retina}}}{\partial z} \bigg|_{z=0} = -ρ_{\text{retina}} i_S \]

There is a homogeneous electrolyte volume between the retina and the ground electrode (Ag/AgCl). The height of the retina in our model is \( h = 100 \mu m \); the electrolyte extends 2 cm above the retina. At the...
transition between retina and electrolyte the voltages obey the following equations:

\[ V_{\text{retina}}(x, y, h) = V_{\text{electrolyte}}(x, y, h) \]

and

\[ \frac{1}{\rho_{\text{ret}}} \frac{\partial V_{\text{retina}}}{\partial z} \bigg|_{z=h} = \frac{1}{\rho_{\text{el}}} \frac{\partial V_{\text{electrolyte}}}{\partial z} \bigg|_{z=h} \]

The general solution of the voltage \( V_{\text{retina}}(x, y, z) \) is proportional to the stimulation current density \( i_s \). The voltage \( V_{\text{retina}}(x, y, z) \) further depends on the specific resistivities of the retina and of the electrolyte and on the geometry of the square-shaped capacitor (Muzychka et al. 2003). The voltage in the retina is numerically calculated with Mathematica 5 (Wolfram Research) and is displayed for selected square-shaped capacitors in Fig. 7A.

**RESULTS**

**Characterization of capacitive stimulation array.** The results presented in this study were obtained with 400 square-shaped stimulation sites that form a mult-capacitor array (Fig. 1, A and B). The entire surface of the array is insulated by a chemically inert oxide layer (METHODS). Underneath the oxide, each stimulation site (50 \( \times \) 50 \( \mu \)m\(^2\)) is defined by a platinum contact (Fig. 1C). The distance between adjacent oxide-covered electrodes is only 0.5 \( \mu \)m. To probe the nature of the stimulation...
current we applied voltage ramps to the chip. An Ag/AgCl electrode immersed in the electrolyte served as counterelectrode. At a chip holding potential up to 1.3 V there was no measurable (upper bound 0.3 μA/cm²) current flow across the oxide (Fig. 1D). During the voltage ramp a constant current was detected at the Ag/AgCl electrode (Fig. 1D; METHODS). After the termination of the voltage pulse the current trace returned to zero. We thus infer that the current induced by the voltage ramp is purely capacitive. The stimulus parameters (voltage amplitude 1 V, stimulation time 10 ms, and measured current density 0.3 mA/cm²) were used to estimate the value of the specific oxide capacitance to 3 μF/cm². Increasing the stimulation area while keeping all other parameters constant led to a linear increase of the capacitive current (Fig. 1E). The small gaps between capacitive stimulation sites did not distort the capacitive current. The falling voltage ramps shown in Fig. 1D lead to monophasic cathodal currents. Rising voltage ramps lead to constant anodal currents without faradic components.

In a second experiment we probed the frequency-dependent specific capacitance with a potentiostatic method as described by Wallrapp and Fromherz (2006) and in METHODS. At each of the three frequencies tested (100 Hz, 1 kHz, 10 kHz) the specific capacitance remained constant within the voltage range of 0–1.2 V (Fig. 1F). The upper bound of putative leakage current is given by the accuracy of the potentiostat (0.5 μA/cm²). In the remainder of the study all values are calculated with the specific oxide capacitance of 3 μF/cm². The presented multicapacitor array shows on one hand perfect insulation to the electrolyte and on the other hand capacitance values attractive for neuronal stimulation. In the following we applied purely capacitive monophasic currents (amplitude range: 3 × 10⁻³ to 60 mA/cm²) of different durations (0.05–100 ms) to retinal tissue.

Identification of electrically evoked action potentials in epiretinal and subretinal configuration. Retinal portions were isolated from the rabbit retina and interfaced to the capacitive array (METHODS). The retina is a layered neural tissue comprising three major excitatory neuronal cell classes: photoreceptors, bipolar cells, and ganglion cells (Fig. 2A). The inhibitory cell classes (horizontal cells and amacrine cells) acting on bipolar and ganglion cells, respectively, are omitted in Fig. 2. In the epiretinal configuration (Fig. 2A) a tungsten electrode penetrated the retina and approached a single retinal ganglion cell until spontaneously elicited extracellular action potentials were recorded. We recorded a ganglion cell approximately in the center of the array as confirmed by visual inspection of the tungsten electrode tip. In the subretinal configuration (Fig. 2B) we recorded a ganglion cell ~100 μm above the center of the stimulating capacitor surface.

We first consider the electrical tissue response to anodal and cathodal currents in either epiretinal or subretinal configuration. Cathodal currents elicit negative voltage changes in the retina (Fig. 2C), while anodal currents elicit positive voltage depolarizations (Fig. 2D). In the epiretinal configuration (Fig. 2C) a cathodal capacitive current (i_c = 2.1 mA/cm² across the 500 × 500-μm² stimulation site) induced a voltage change of ~15 mV. The electric characteristics of the tungsten electrode and of the resistive tissue properties low-pass filter the detected voltage. We detected extracellular voltage changes caused by capacitive stimulation (“passive” response) regardless of neuronal activity. Repetition of the same stimulus led to identical voltage changes, regardless of the type of extracellular electrode. Qualitatively similar passive voltage traces were recorded for cathodal-epiretinal and anodal-subretinal currents.

We identified single-cell activity in the recorded voltage traces (Fig. 2, C and D) by increasing the stimulation current above threshold. If the recording electrode was in close proximity to a ganglion cell, the voltage trace contained an “activity” signal in addition to the “passive” tissue response. The stimulation-induced electrical activity was identified as follows. We applied each stimulus 50 times. The average response was subtracted from each individual recording. Above a certain current threshold in the difference signals voltage deflections became visible that are similar in shape to extracellular ganglion cell signals (Fig. 2Ciii). The first of such signals appeared within 1 ms. To confirm that the difference signals indeed reflect neuronal activity, we added the sodium channel blocker TTX (0.2 μM) to the perfusion solution. Under these conditions all stimulated ganglion cell activity was inhibited (Fig. 3). We thus conclude that ganglion cell action potentials were detected without delay after stimulus onset. A similar methodology has been applied recently with metal-based electrodes (Sekirnjak et al. 2008). The procedure for identification of action potentials applies to epiretinal and subretinal configurations (Fig. 2, C and D). In epiretinal or subretinal configuration a burst of action potentials with latencies in the range of 0.5–80 ms was recorded. For display purposes, only the first 12 ms are shown in Fig. 2, C and D. In the following we investigate the cellular origin of the activity burst.

Identification of stimulated action potential’s cellular origin. The cellular origin of electrically evoked spikes was first identified with pharmacological experiments. In the epiretinal configuration two monophasic stimuli of different polarity (duration 1 ms, current density 1.8 mA/cm², stimulus area 500 × 500 μm²) separated by 100 ms were repeatedly applied (Fig. 3A). We recorded spiking activity from an ON ganglion cell (Fig. 3A) as confirmed by light stimuli prior to electrical stimulation. The spiking activity in the subretinal configuration is discussed in a subsequent paragraph.

The anodal epiretinal stimulus evoked a burst of action potentials. The first spike appeared ~18 ms after the stimulus. Application of the metabotropic glutamate receptor blocker L-AP4 (20 μM) to the electrolyte inhibited these signals. L-AP4 is known to selectively block the transmission at the photoreceptor-to-ON bipolar cell synapses (Slaughter and Miller 1981). On the basis of this pharmacological experiment we inferred that anodal epiretinal stimulation excites photoreceptors. Under unperturbed conditions the photoreceptor excitation is transmitted by intraretinal circuitry to the recorded ON ganglion cell.

The cathodal epiretinal stimulus evoked a complex burst pattern of action potentials, with the first spike appearing within 1 ms after stimulus onset. The application of L-AP4, as described above, inhibited the last burst of ganglion cell spikes, which appeared within 30–60 ms after stimulus onset (Fig. 3B, 2nd trace). Thus, as for anodal stimulation, the cathodal epiretinal stimulus activates photoreceptors. In a second experiment, in addition to L-AP4 we added the ionotropic glutamate receptor blocker CNQX (20 μM) to the perfusion. CNQX blocks ionotropic glutamate receptors that are expressed on OFF bipolar cells, amacrine cells, and, most important for this
conclude that a single cathodal epiretinal stimulus (500 grov et al. 2010; Fried et al. 2009; Sekirnjak et al. 2008). We been analyzed recently for the epiretinal configuration (Boina-

cated that intermediate spikes recorded from the ON retinal
cell spike (4th row). The stimulus current parameters were amplitude 2 mA/cm² and duration 1 ms.

Fig. 3. Anodal and cathodal epiretinal stimulation elicit a complex burst of ganglion cell spikes. A: schematic of the excitatory signal flow in the retina from photoreceptors (top) to bipolar cells to ganglion cell (bottom). The synapse between photoreceptors and ON bipolar cell can be blocked by L-AP4. The synapse between ON bipolar cell and ON ganglion cell can be blocked by the ionotropic glutamate receptor blocker CNQX. The sodium channel blocker TTX inhibits action potentials in all spiking retinal neurons. B: appearance of a burst of extracellular voltage deflections near an ON ganglion cell following an anodal current pulse (amplitude 1.8 mA/cm²; duration 1 ms). The burst is inhibited by L-AP4 and thus attributed to photoreceptor activation. C: appearance of a complex burst of action potentials of the cell shown in B following a cathodal current pulse. After the application of L-AP4 (2nd row) the late RGC spikes (~30 – 60 ms after stimulus onset) are inhibited. The addition of CNQX inhibits all but 1 spike (3rd row). Addition of the sodium channel blocker TTX inhibits the first ganglion cell spike (4th row). D: appearance of a burst of extracellular voltage deflections near an OFF ganglion cell following anodal current pulse (left) and cathodal current pulse (right). The stimulus current parameters were amplitude 2 mA/cm² and duration 1 ms.

experiment, ON ganglion cell dendrites. CNQX thus blocked the transmission between ON bipolar and ON ganglion cells. The stimulated activity in the presence of CNQX and L-AP4 was limited to one single spike, which appeared within 1 ms after cathodal stimulus onset (Fig. 3C). This experiment indicated that intermediate spikes recorded from the ON retinal ganglion cell range 2–30 ms) are caused by the activation of excitatory input to ganglion cells (Fried et al. 2006). The first stimulated spike (delay with respect to stimulus onset <1 ms) represents a direct activation of the ganglion cell and could be inhibited when the sodium channel blocker TTX was added to the electrolyte (Fig. 3, B and C). This type of stimulation has been analyzed recently for the epiretinal configuration (Boina-
grov et al. 2010; Fried et al. 2009; Sekirnjak et al. 2008). We conclude that a single cathodal epiretinal stimulus (500 × 500 μm²) excites all three excitatory retinal cell classes.

A similar complex burst pattern was measured for OFF ganglion cells as well (Fig. 3D). However, for the OFF ganglion cell pathway L-AP4 does not block the photoreceptor-OFF bipolar transmission, and thus a thorough pharmacologi-
cates symmetry in the ON ganglion cell response pattern

Mirror-symmetry between epiretinal and subretinal stimulation. To investigate biophysical principles underlying the stimulation of bipolar cells and photoreceptors we compared the experiment performed in the epiretinal configuration (Fig. 3) with an experiment in the subretinal configuration with otherwise identical stimulus parameters.

The spike trains recorded in 50 stimulus repetitions of the protocol shown in Fig. 3 are summarized in Fig. 4, A and B. The activity of the ON ganglion cell following monophasic anodal currents in the epiretinal configuration has a single maximum ~18 ms after stimulus onset (Fig. 4A). The post-stimulus histogram following cathodal currents has three peaks: one peak within 1 ms, a second peak ~5 ms after stimulus, and a third peak ~44 ms after stimulus (Fig. 4B). The first peak is not presented and will not be considered in the remainder of this report. The second peak of the ganglion cell activity (Fig. 4B) was inhibited by application of CNQX and L-AP4 and thus attributed to bipolar cell activation (Fig. 3C). The late ganglion cell response was abolished by L-AP4 alone and was thus attributed to the activation of photoreceptors.

Activity between the two peaks (5 and 44 ms) most likely arises from time-shifted bipolar cell response or from sponta-

In a second experiment we applied the same stimulus protocol (duration 1 ms, current density 1.8 mA/cm², stimulus area 500 × 500 μm²) to another retina interfaced to the multicapacitor array in the subretinal configuration. The induced activity was recorded again from an ON ganglion cell (Fig. 4C) for 50 stimulus repetitions. The anodal subretinal pulse elicits a complex burst pattern. The poststimulus time histogram of the ganglion cell response has three peaks: a very fast peak (1-ms latency, not shown in Fig. 4C), an intermediate peak (latency ~6 ms), and a late peak (~40 ms) (Fig. 4C). This behavior is similar to the ganglion cell response to a cathodal epiretinal pulse. In contrast, the cathodal subretinal pulse elicited ganglion cell spikes with an average latency at ~20 ms (Fig. 4D). These spikes did not appear when L-AP4 was added to the electrolyte (data not shown). This response pattern is similar to the anodal epiretinal pulse.

This simple experiment using one stimulus protocol indicates symmetry in the ON ganglion cell response pattern between epiretinal cathodal and subretinal anodal stimulation on the one hand and for epiretinal anodal and subretinal cathodal stimulation on the other hand. We confirmed this mirror-symmetry for two OFF ganglion cells (Fig. 4, E–H). The poststimulus time histograms were qualitatively similar to the ON ganglion cells (Fig. 4, A–D). However, we measured considerably longer spike latencies for all response phases of
For each epiretinal stimulus the ganglion cell response was evaluated. Ganglion cells in the subretinal configuration were compared to ganglion cells in the epiretinal configuration and three ON spontaneous or jittered activity (Fig. 4). Measurements for five stimulus presentation times the amplitude of the voltage is considered the threshold current. We selected this relatively high threshold to avoid errors in threshold evaluation due to spontaneous or jittered activity (Fig. 4). Measurements for five ON ganglion cells in the epiretinal configuration and three ON ganglion cells in the subretinal configuration were evaluated. For each epiretinal stimulus the ganglion cell response was separated in three parts (see Fig. 4, A and B): anodal spikes, intermediate-latency (2–30 ms) cathodal spikes, and long-latency cathodal spikes (30–60 ms). On the basis of the result presented in Fig. 4, C and D, for each subretinal stimulation protocol the ON ganglion cell response is separated in three parts as well: cathodal subretinal spikes, intermediate-latency (2–30 ms) anodal spikes, and long-latency anodal spikes. For each of the three response parts—as suggested by the symmetry presented in Fig. 4—we evaluated the threshold current densities for each stimulus duration (Fig. 5, A–C). Two important features were common to each stimulus-response protocol: 1) the current threshold densities increased when stimulus duration was reduced (Fig. 5, A–C), and 2) the current threshold densities were similar (within the standard error) in both configurations (epiretinal and subretinal) for each of the three response parts at a given stimulus duration. An exponential approximation of the strength-duration curves (Lapicque 1907) allows us to fit a stimulus strength for infinite stimulation (rheobase) and the pulse duration at twice rheobase (chronaxie). These parameters are assumed to be characteristic for electrical stimulation (Plonsey and Barr 2007). In our experiments the approximated rheobase and chronaxie values for epiretinal anodal (subretinal cathodal) currents (Fig. 5A) were...
0.018 mA/cm² (0.06 mA/cm²) and 106 ms (80 ms), respectively. These chronaxie values are, however, longer than the ganglion cell response latency (Fig. 4, A and D) and are therefore not an appropriate stimulation parameter here. For intermediate-latency spikes following cathodal epiretinal and anodal subretinal currents (Fig. 5 B) the rheobase and chronaxie were similar in the epiretinal and subretinal configurations (rheobase: 0.31 mA/cm² epiretinal, 0.65 mA/cm² subretinal; chronaxie: 6.5 ms epiretinal, 3 ms subretinal). We could not elicit intermediate spikes with current pulses longer than 10 ms (Fig. 5 B). Late spikes (Fig. 5 C) following subretinal anodal stimuli displayed a rheobase of 0.12 mA/cm² and a chronaxie of 15 ms. However, late spikes following epiretinal cathodal stimuli display a rheobase of 0.03 mA/cm² and a chronaxie values of 58 ms. This mean chronaxie value is longer than the response latency (~40 ms; Fig. 4, B and C) and therefore not an appropriate stimulation parameter. In summary, individual current thresholds were mirror-symmetrical for epiretinal and subretinal configuration. However, as threshold current densities do not change for stimuli longer than 20 ms (Fig. 5, A and C) the approximation using Lapique’s theory failed on one hand but indicated on the other hand that photoreceptors have a lower rheobase than bipolar cells.

Aiming for a biophysical description of the stimulation mechanism, we conducted an additional set of experiments. We investigated the area dependence of the threshold current density for different capacitor areas (range 100 × 100 μm² to 900 × 900 μm²) (Fig. 5, D–F). We evaluated the experiments for a subset of the ON ganglion cells presented in Fig. 5, A–C (n = 3 in subretinal and n = 2 in epiretinal configuration). The stimulus presentation time was 1 ms in all experiments. In Fig. 5, D–F, we present the current threshold densities for each cell and for each response latency individually (Fig. 4). Except for one ON ganglion cell in the epiretinal configuration and for small stimulation areas (100 × 100 μm²) the stimulation thresholds were similar. We note a general increase of stimulation threshold for smaller capacitor areas.

The presented symmetry between retina-array configuration and current polarity (Fig. 4 and Fig. 5) can be explained by the polarization of cylinder-like cells in a homogeneous electric field (METHODS). In the electric field above the relatively large capacitive electrodes the terminals of the cylinder-like bipolar cells that are close to the ganglion cell (Fig. 2, Fig. 4, Fig. 5, B and E) may be depolarized by cathodal epiretinal or anodal subretinal current. The bipolar cell axon terminals contain most of the cell’s voltage-gated channels (Euler and Masland 2000) that open upon depolarization and lead to ganglion cell activation. A related mechanism may govern the photoreceptor activation by currents of opposite polarity (Fig. 2, Fig. 5, A and D). It is well known that hyperpolarization of photoreceptor terminals leads to action potentials in ON ganglion cells. This hyperpolarization is achieved by anodal currents in the epireti-
nal configuration and by cathodal current in the subretinal configuration. However, using the capacitor areas centered on the ganglion cell we cannot infer why photoreceptor-induced ganglion cell spiking is measured in both configurations with both current polarities. Therefore we performed an additional set of experiments in which we stimulated photoreceptors at different positions with respect to the ganglion cell soma.

Receptive field mapping of ganglion cell with stripelike capacitors. We used rectangular stimulation areas (100 x 1000 μm²) that were presented at equally spaced (distance 50 μm) positions across the array (Fig. 6A). A similar protocol using rectangular electrodes has been shown to stimulate roughly the ganglion cell’s receptive field center (Jensen and Rizzo 2009; Stett et al. 2000). The receptive field center of a ganglion cell corresponds to the retinal area covered by photoreceptors that excite the cell via excitatory bipolar cells. Photoreceptors in the so-called receptive field surround inhibit a retinal ganglion cell by signal transmission through inhibitory interneurons (horizontal cells and amacrine cells).

We first studied the receptive field center and surround of ON ganglion cells in the subretinal configuration. Cathodal pulses (current density 3 mA/cm², stimulus presentation time 1 ms) evoked action potentials in an ON ganglion cell (Fig. 6B) over a distance of 467 μm [full width at half maximum (FWHM)]. This activity is caused by the activation of photoreceptors (Fig. 4). We determined the response probability by counting the percentage of evoked single spikes in a repetition series of 50. Assuming circular symmetry, barlike stimuli activate a ganglion cell within a radius of ~240 μm. The response profile roughly corresponds to the receptive field center of the ganglion cell, as confirmed by mapping the receptive field with circular stimuli (see METHODS). A similar response profile (FWHM 419 μm) of the same ON ganglion cell was recorded by evaluating the intermediate-latency spikes upon anodal stimulation (Fig. 6B). Such spikes are caused by the activation of bipolar cells (Fig. 4) in the ganglion cell’s receptive field center. The slight decay of the response probability in the midst of the receptive field center (Fig. 6B) may be caused by differential excitability of the receptive field microstructure (Brown et al. 2000) and is not found for all cells.

The anodal subretinal stimulus evokes late ganglion cell spikes (30–60 ms after stimulus onset) that are attributed to photoreceptor stimulation (Fig. 4). The spatial extension of the response profile (FWHM 920 μm) is more than twice as large as that measured for the photoreceptor or bipolar cell-induced intermediate-latency spikes (Fig. 6B). This wide stimulation range most likely corresponds to the receptive field surround.

Fig. 6. Stimulation of ON ganglion cell receptive field in epiretinal and subretinal configuration. A: schematic of the retinal stimulation using stripelike stimulation areas. The capacitive stimulus of 100 x 1000 μm² (black rectangle) is presented at 19 equidistant locations (separation 50 μm). The applied current density is 3 mA/cm² for a stimulus time of 1 ms. B: response profile recorded from an ON ganglion cell in subretinal configuration. At each position the capacitive stimulus is presented 50 times. The percentages of presentations that elicit at least 1 spike after a cathodal stimulus are marked by squares. This photoreceptor-induced activity (Fig. 3) is obtained between stimulus positions 300 and 700 μm. The anodal bipolar cell stimulation [intermediate latency (IS) spikes, circles] is obtained over a similar spatial extent. The anodal stimulation of photoreceptors [long-latency (LS) spikes, triangles] is feasible over a range of ±500 μm from the ganglion cell soma. C: response profile recorded from a second ON ganglion cell in epiretinal configuration. At each position the capacitive stimulus is presented 50 times. The percentages of presentations that elicit at least 1 spike after a cathodal stimulus are marked by squares. This photoreceptor-induced activity (Fig. 3) is obtained between stimulus positions 300 and 700 μm. The anodal bipolar cell stimulation [intermediate latency (IS) spikes, circles] is obtained over a similar spatial extent. The anodal stimulation of photoreceptors [long-latency (LS) spikes, triangles] is feasible over a range of ±500 μm from the ganglion cell soma. D: left: summary of ratio of the full width at half-maximum (FWHM) for bipolar cell stimulation (center IS spikes) and photoreceptor stimulation (center) in epiretinal and subretinal configuration. Right: ratio of FWHM for surround activation and center activation (photoreceptor). The 4 symbols represent different experiments in subretinal configuration. Each dotted line connects the results from 1 cell. The continuous line connects the mean ratios.
We further studied the receptive field center and surround of ON ganglion cells in the epiretinal configuration, using the same set of striplike stimulus presentations (Fig. 6A). Anodal epiretinal stimulation (Fig. 6C) evoked a response within a distance of 540 μm (FWHM). The spatial scale roughly corresponds to the ganglion cell’s receptive field center. The response probability for cathodal epiretinal stimulation was evaluated separately for intermediate-latency spikes and long-latency spikes. Intermediate-latency ganglion cell spikes were evoked in this ON ganglion cell by stimulus presentations over a distance of 460 μm (FWHM). As these spikes are elicited by bipolar cell activation, we conclude that the spatial scale of electrical bipolar cell stimulation roughly corresponds to the light-induced response profile. The long-latency spikes following epiretinal cathodal stimuli were elicited with a lower probability compared with anodal subretinal long-latency spikes. The long-latency ganglion cell spikes were evoked outside the receptive field center, suggesting the same “surround” mechanism as for subretinal stimulation. In the epiretinal configuration photoreceptors are the most distal cells from the electrode. Membrane changes in these neurons might not be transmitted through the multisynaptic circuitry (photoreceptor–horizontal cell–bipolar cell).

The experiments using striplike capacitors in epiretinal and subretinal configurations are summarized in Fig. 6D. The mean ratio of the FWHM of the two center responses (photoreceptor vs. bipolar) evaluated for 10 experiments is 0.9. The ratio of the center response FWHM to the surround FWHM evaluated in subretinal configuration is 1.5. The dashed lines in Fig. 6D connect experiments from individual ganglion cells. For some ganglion cells in the subretinal configuration the surround extended beyond the stimulation area, while for epiretinal stimuli we could not quantify the extension of the surround.

We conclude that bipolar cells are excited within the ganglion cell’s receptive field center, while currents of different polarities excite photoreceptors within and outside the receptive field center in both configurations. In the following we present a simple model that explains the experimental results (Figs. 3–6) based on selective de- or hyperpolarization of cell terminals in an electric field.

Model of bipolar cell and photoreceptor stimulation. The results presented in Figs. 3–6 can be explained assuming de- and hyperpolarization of cell terminals of nonleaky elongated retinal interneurons in a homogeneous tissue (METHODS). To estimate the voltage drop above capacitive areas of different size we first calculated a mean tissue resistivity $r_{\text{tissue}}$ (1,300 Ωcm) from subretinal ($n = 3$) and epiretinal ($n = 5$) recordings (see METHODS for experimental details). The linear decay of the voltage (Fig. 7A) above different stimulation areas indicates a homogeneous electric field perpendicular to the capacitor surface. The voltage at the retinal surface ($z = 100 \mu m$) does not decay to zero because of the finite electrolyte resistivity. Theoretic estimates of intraretinal voltage above the center of capacitors of different size (Fig. 7A) were calculated as described in METHODS. The assumption of maximum voltage drop at the cell terminals relies on the large electrotonic length of the cablelike interneurons. Considering reported values for intracellular resistivity ($R_m = 130 \Omega \mu m$), the specific cell membrane resistance ($R_m = 24 \kappa \Omega \mu m^2$), and a fiber diameter of $\sim 0.7 \mu m$ (Olmedal and Hartveit 2010), we obtain an electrotonic length in the centimeter range. The stimulation model of cylinder-shaped neurons in a homogeneous retina is shown in Fig. 7B and is quantified in the following for a specific cell length in epiretinal or subretinal configurations.

We disregard the variety of photoreceptor and bipolar cell subtypes (Euler and Masland 2000) and assume for either photoreceptor or bipolar cell a cable length of 40 μm (Reichenbach et al. 1991). The adult rabbit retina is $100 \mu m$ thin (Reichenbach et al. 1991) with these two cell types superposed as shown schematically in Fig. 2, A and B. We further assume

![Fig. 7. Voltage changes in the retina perpendicular to the capacitive stimulation sites. All presented voltage changes are normalized to the steady-voltage amplitude $V_{\text{stim}}(z = 0)$ at the capacitor surface (900 × 900-μm² area). A: extracellular voltage in the retina above square-shaped stimulation areas. The voltage $V_{\text{stim}}(z)$ is measured at different positions $\Delta z$ above a large (900 × 900 μm²) capacitor (open symbols). Average values are presented ($n = 5$ epiretinal and 3 subretinal configurations); error bars denote SD. Theoretical estimates of the retinal voltage above smaller square-shaped capacitors are shown as continuous lines. The change above the rectangular capacitive electrode (Fig. 6A) is shown as a dashed line. The voltage above the retina does not drop to 0 because of the finite resistivity of the retina and of the electrolyte. B: voltage change across a cylinder-shaped nonleaky neuron (40 μm long) embedded in a homogeneous electric field of a volume conductor. Half of the voltage drop occurs at each cell terminal, as there is no voltage change within the cell. The voltage drop decreases for smaller stimulation areas (900 × 900, 200 × 200, and 100 × 100 μm, respectively). C: voltage drop (see B) across theoretic photoreceptor and bipolar cell terminals in epiretinal (epi) or subretinal (sub) configuration above square-shaped capacitors of different sizes. The position of the bipolar cell terminals (20 or 80 μm above surface) and of the photoreceptor terminals (60 and 40 μm above surface) in the 2 configurations (epiretinal or subretinal) determines the expected voltage drop.](http://jn.physiology.org/doi/10.1152/jn.00909.2011)
for the ganglion cell layer a vertical extension of 20 μm. Following Fig. 7, A and B, the voltage drop above large capacitors across bipolar cells and photoreceptors represents ~40% of the total voltage change within the retina (Fig. 7C). The nonlinear electric field above small capacitors (Fig. 7A) implies a reduced polarization of distant cells (Fig. 7C). The area-dependent voltage drop across bipolar cells and photoreceptors relates to the increase in current threshold measured for different capacitors (Fig. 5, D–F). Note that barlike stimuli (Fig. 6) evoke a voltage change similar to a 200 × 200-μm² capacitor area.

We finally translate this simple stimulation model into a simplified retinal scheme (Fig. 8). The anode-facing membranes of bipolar cells and photoreceptors are hyperpolarized, while the opposite cell membranes are depolarized by a monophasic current. Thus anodal epiretinal or cathodal subretinal currents hyperpolarize the photoreceptor endings that are presynaptic to the bipolar cell (Fig. 8A). Hyperpolarization leads to a decrease of the dark current, i.e., decrease of the glutamate release from photoreceptors. The metabotropic glutamate receptors of the ON-type bipolar cell transform this signal into a depolarization of the bipolar cell and—above stimulation threshold—into spiking activity of ON ganglion cells. Anodal epiretinal or cathodal subretinal currents hyperpolarize the bipolar cell axon terminals and thus do not lead to bipolar cell-induced spiking in the ganglion cell. This stimulation strategy (Fig. 8A) leads to an activation of photoreceptors similar to the physiological response upon light increments. Cathodal epiretinal and anodal subretinal currents (Fig. 8Bii) depolarize bipolar cell terminals, which in turn depolarize the postsynaptic ganglion cell. Bipolar cell terminals release glutamate and evoke intermediate-latency ganglion cell spikes (Figs. 3–6). Photoreceptors in the surround of the ganglion cell’s receptive field may depolarize horizontal cells (Fig. 8Bii), which excite ON bipolar cells (Duebel et al. 2006) and finally the ON ganglion cell. This multisynaptic retinal circuitry leads to long-latency spikes (Figs. 3–6). The presented stimulation scheme for ON ganglion cells is supported by pharmacological evidence (Fig. 3) and localized stimulation (Fig. 6). However, we cannot exclude amacrine cell pathways that may contribute to the ganglion cell surround (Fried et al. 2006). Neurons in the OFF pathway cannot be isolated pharmacologically and also receive cross-inhibition from the ON pathway (Liang and Freed 2010), making it harder to disentangle their relative contributions to OFF ganglion cell spikes. The presented model (Figs. 7 and 8) summarizes the experimental results for capacitive retinal stimulation of photoreceptors and bipolar cells with monophasic current pulses.

**DISCUSSION**

In this study the electrical stimulation of different neuronal classes with a multicapacitor array has been demonstrated. Specifically, ex vivo rabbit retinas were interfaced and activated in either epiretinal or subretinal configuration with low current densities. Two sets of experiments (pharmacology and position-dependent stimulation) suggest a simple biophysical model of bipolar and photoreceptor cell stimulation. In the following we discuss the advantages of capacitive stimulation arrays and discuss our results with respect to neuroprosthetic applications.

**All-oxide layer on multicapacitor array.** The integration of hundreds of electrodes is necessary to achieve high-resolution stimulation in the retina (Palanker et al. 2005; Weiland and Humayun 2008) and other neural tissues (Kipke et al. 2008). Integration of electrical circuitry in small, implantable devices requires CMOS technology (Chen et al. 2010; Eversmann et al. 2003). The inert and biocompatible oxide used in this study has a twofold advantage: 1) It prevents tissue-induced damage of the CMOS chip, and 2) it prevents damage of the interfaced tissue. The layer of TiO₂/ZrO₂ was chosen for its high dielectric constant and its compatibility with the CMOS chip. It has been shown to be chemically stable and biocompatible with organotypic brain slices (Hutzler et al. 2006). In addition, it had been shown previously that TiO₂ does not deteriorate during electrical stimulation and that it is chemically far more stable than an insulating layer of SiO₂ under similar culture conditions (Hutzler and Fromherz 2004). The disadvantage of an oxide-covered stimulation electrode is the reduced specific electrode capacitance compared with the double-layer capacitance of metallic surfaces in electrolyte. The specific capacitance reported here (ε, ~3 μF/cm²) is similar to values obtained for high-kappa metal insulator-metal capacitors in electronic devices (Cheng et al. 2008; Lin et al. 2009). It exceeds the value reported for TiO₂ on silicon substrate (Wallrapp and Fromherz 2006) but is smaller than the Helmholtz capacitance (10–20 μF/cm²) surrounding metallic stimulation electrodes immersed in electrolyte (Merrill et al. 2005). Safe stimulation without faradaic current was limited here to 1.3 V. This limits the amount of stimulation current (Fig. 5) to ~30–60 mA/cm² and therefore stimulation with small capacitive electrodes, which will enhance spatial resolution of retinal prosthesis (Palanker et al. 2005). However, for 100 × 100-μm² capacitive stimulation sites epi- and subretinal stimulation was demonstrated with low
response is feasible (Fig. 2). Since the resistance of the cell membrane is much higher than the cellular interior that quickly becomes equipotential because their morphology changes from a cylinder-like to a spheroid shape. Selective electrical stimulation of the OFF pathway remains a challenge, as the ON bipolar cells need to be omitted. Long monophasic stimuli (>10 ms; Fig. 5) with low current amplitudes are currently being tested. Future research must prove whether the presented strategy can be transferred to degenerated retinas, where circuit remodeling may occur (Marc et al. 2003). In summary, we have demonstrated the feasibility of capacitive retinal stimulation and suggested—on the basis of ex vivo experiments—stimulation strategies that may lead to more efficient neural prostheses. The presented concepts of selective membrane depolarizations and hyperpolarizations may extend beyond the application to retinal prostheses. The intricate neuronal geometry interfaced to the stimulation electrodes must be carefully considered (Plonsey and Barr 2007; Rattay 1986). ACKNOWLEDGMENTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.E., P.F., and G.Z. conception and design of research; M.E. performed experiments; M.E. analyzed data; M.E. and G.Z. interpreted results of experiments; M.E. and G.Z. prepared figures; M.E., M.J., R.T., P.F., and G.Z. edited and revised manuscript; M.E., M.J., R.T., P.F., and G.Z. approved final version of manuscript; P.F. and G.Z. drafted manuscript.
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