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Influence of the sodium channel band on retinal ganglion cell excitation during electric stimulation - A modeling study

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Abstract

Electric stimulation using retinal implants allows blind people to re-experience a rudimentary kind of vision. The elicited percepts or so called 'phosphenes' are highly inconstant and therefore do not restore vision properly. The better knowledge of how retinal neurons, especially retinal ganglion cells, respond to electric stimulation will help to develop more sophisticated stimulation strategies. Special anatomic and physiologic properties like a band of highly dense sodium channels in retinal ganglion cells may help to achieve a focal activation of target cells and as a result better restoration of vision. A portion of retinal ganglion cell axons, about 40 μm from the soma and between 25 and 40 μm in length, shows a specific biophysical property. Electrode locations close to a band of highly dense sodium channels which was identified immunochemically show lowest thresholds during electric stimulation. The (modeled) thresholds for this kind of structure result in lowest thresholds as well. The influence on the location where action potentials are generated within the axon is far reaching. When a stimulating electrode is positioned far outside the actual band region the site of spike initiation still remains within the sodium channel band. These findings suggest to further examine the key mechanisms of activation for retinal ganglion cells because focal activation without influencing passing axons of neurons located far away can improve the outcome of electric stimulation and therefore the development of retinal implants.

Keywords

retinal implant; sodium channel band; external electric stimulation; spike initiation; activating function; compartment model

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1. Introduction

Several research groups are trying to restore vision to people blinded by diseases like retinitis pigmentosa and age-related macular degeneration by the means of electric stimulation (Humayun et al. (1996); Rizzo et al. (2003); Zrenner et al. (2011)). Large progress has been made during the last two decades in retinal implants, however, there are still many obstacles to overcome before a higher quality of vision can be achieved.

One of the main goals in the development of retinal implants is to gain better knowledge of which retinal structures are activated during external electric stimulation. Many experimental investigations (e.g. Jensen et al. (2003); Sekirnjak et al. (2006, 2008); Cai et al. (2011); Eickenscheidt et al. (2012)) but only few modeling studies (e.g. Jeng et al. (2011); Tsai et al. (2012)) have been performed to enlighten the recruitment order of the electrically stimulated retinal circuitry.

A conclusion from the first computational study about epiretinal stimulation was that the lowest electrode threshold occurred when the stimulating electrode was positioned over the soma (Greenberg et al. (1999)). 100 μ s cathodic stimuli with disc electrodes were reported to be 20% more effective over the soma than over the axon using Hodgkin-Huxley (1952) membrane dynamics. The ratio increased further to 1.73/1 for excitation from a point-source and evaluation with a membrane model derived from salamander cell (Fohlmeister et al. (1990)). However, the axon was preferentially stimulated versus the soma when the model was evaluated with a passive membrane, i.e. voltage sensitive ion-channel gating mechanisms are neglected and membrane conductance is kept constant.

Rattay presented a theoretic framework for which neuronal structures will be preferentially activated during electric stimulation (Rattay (1999)). The activation is strongly influenced by geometric conditions, i.e. for the case that the given ion channel density is uniform throughout neuronal structures, a long structure (axon) is activated easier than a spherical (soma) one. If the sodium channel density is increased in certain axonal parts, like the sodium channel band in retinal ganglion cells (Fried et al. (2009)), the threshold for activation decreases. Therefore axons have a lower activation threshold than dendrites (lower sodium channel density) having the same diameter (Nowak and Bullier (1998); Rattay and Wenger (2010)). These findings refute the results presented by Greenberg et al. (1999) who did not presume a high sodium channel density in the axon initial segment (AIS).

This study uses previously obtained anatomical data of a high-density sodium channel band (Van Wart et al. (2007); Fried et al. (2009)) to establish a computational model of retinal ganglion cells (RGCs) in order to explore the key components for the focal activation during direct external electric stimulation. We analyzed the stimulation with electrodes in the vicinity of the band of highly dense sodium channels of RGCs as the band is supposed to be the most excitable part of these cells.

As starting point for our computational investigations we used the threshold maps provided previously (Fried et al. (2009)). In this experimental study, the thresholds for spike initiation in retinal ganglion cells were identified to be lowest around the soma and proximal axon

parts (Fig. B.1, Table A.1). Fried and coworkers experimentally determined two dimensional threshold maps for retinal ganglion cells (in rabbit) during external electric stimulation. The threshold map in Fig. B.1 plots the sensitivity to stimulation from a small electrode as it is moved across the soma and proximal axon regions of a retinal ganglion cell. Each pixel indicates the threshold when the stimulating electrode was positioned at that location. Spacing between adjacent pixels was $10\mu\text{m}$ and the electrode was held fixed at a constant height above the retina ($\sim 30\mu\text{m}$). The stimulus was a 0.2ms cathodal pulse (pseudomonophasic). Under these conditions, thresholds varied from $12\mu\text{A}$ to $45\mu\text{A}$. Thresholds were lowest in a central region (darkest red) and increased monotonically with distance from the approximate center of this region. This region of lowest threshold was found to be spatially aligned with a dense band of voltage-gated sodium channels, identified immunochemically, suggesting that the band is the site of spike initiation in response to extra-cellular electric stimulation.

If the sodium channel band is in fact the site of spike initiation in response to extracellular electric stimulation, it is likely that one or more properties of the stimulus (i.e. the induced electric field) are consistent across the band region each time a spike is generated. Thus, the hypothesis was that some property of the induced field would be consistent across the band region for each combination of stimulating electrode location and stimulus level that successfully generated a spike. The goal of these computer simulations was to determine whether this was the case and if so, what properties of the stimulus were essential for activation. Therefore, we compared two-dimensional threshold maps obtained by Fried et al. (2009) with computational results calculated with a multi compartment nerve fiber model. Sodium channel densities, having an important influence on activation threshold, were estimated by comparing experimental thresholds (see Fig. B.1) with computed thresholds for different sodium channel densities in the band region. The immunochemical experiments revealed a higher sodium channel density in the band region, however, no quantitative assertion (i.e. sodium channel density gNa) could be made with this method. Therefore, the computed 'best fit' was further used to investigate the most crucial properties of the sodium channel band. Furthermore, our model resembles the characteristics of the measured thresholds, lowest thresholds are located in the close proximity of the sodium channel band and increase concentrically with greater distances.

This study can be divided into two major parts: First, the key components of neural activation are examined to investigate the validity of the activating function within a structure with a region of highly dense sodium channels. Furthermore, various electric properties of the stimulating pulse are investigated to find a reliable predictor of the site of spike initiation in an inhomogeneous fiber. Second, the influence of the sodium channel band during extracellular stimulation on threshold and site of spike initiation is explored.

2. Methods

Two major modeling steps had to be performed: First, we had to calculate the electric field for a given electrode geometry in a heterogeneous media. Second, we used the Fohlmeister-Coleman-Miller (Fohlmeister et al. (1990)) model as multi-compartment model to simulate the response of a single nerve fiber.

Electric field calculations

To calculate an electric voltage distribution for an electrode within a heterogeneous medium the finite element approach can be used. To optimally imitate the physiological experiments we modeled a three/seven layer model with finite element software (COMSOL Multiphysics). The retina was either modeled as a single layer with an isotropic electric resistance or with five layers with different resistances (see Fig. B.2A and methods section 'Three-layer model vs. Seven-layer model'). The electrode was embedded in an ames solution which had an isotropic electric resistivity of $1.1\Omega\cdot\text{m}$ (Jeng et al. (2011)). As electrode material we chose Platinum (electric resistivity: $1.05\cdot 10^{-7}\Omega\cdot\text{m}$ (Comsol Multiphysics Material Library)), the modeled electrode geometry was the same as used by Fried et al: a cone shaped electrode, $30\mu\text{m}$ in base diameter and $35\mu\text{m}$ in height. The sclera was modeled as a single layer with an isotropic electric resistivity of $505\Omega\cdot\text{m}$ (10 times retina resistivity). The whole system had a length varying from 1-4mm (depending on the fiber length) and a width and height of 1mm, respectively, system boundaries were grounded ($V = 0\text{V}$). To find the same angle between electrode and stimulated tissue that was used in the experiments we performed various calculations for different angles and compared the results (voltage profiles) to the voltage profiles measured experimentally. Calculated results for 10° (see Fig. B.3 for geometry) fitted the data best so all our further simulations were performed with this configuration. The computed voltage profile was exported to MATLAB and the first (electric field) and second derivative of the extracellular potential V_e were calculated.

Compartment model & activating function

To analyze the response of a target nerve fiber we used the approach of compartment modeling. The current flow along a stimulated fiber is simulated by a network of compartments with given electrical (membrane) properties (Rattay (1986, 1999)). Every compartment is electrically described by a single virtual point in the center in which all electric currents are calculated. Applying Kirchoff's law for compartment n gives following equations:

$$I_{C,n} + I_{ion,n} + I_{ohm,n} = 0 \quad (1)$$

which further results in:

$$\frac{d(V_{i,n} - V_{e,n})}{dt} C_n + I_{ion,n} + \frac{V_{i,n} - V_{i,n-1}}{R_n/2 + R_{n-1}/2} + \frac{V_{i,n} - V_{i,n+1}}{R_n/2 + R_{n+1}/2} + \dots = 0 \quad (2)$$

V_i , R_n and C_n denote the intracellular potential, axial resistance and membrane capacity, respectively (Rattay (1999)). With introducing the reduced membrane voltage $V = V_i - V_e - V_{rest}$ one is able to deduce the equation to compute the time courses of V_n for every compartment:

$$\frac{dV_n}{dt} = \left[-I_{ion,n} + \frac{V_{n-1} - V_n}{R_{n-1}/2 + R_n/2} + \frac{V_{n+1} - V_n}{R_{n+1}/2 + R_n/2} + \dots + \frac{V_{e,n-1} - V_e}{R_{n-1}/2 + R_n/2} + \frac{V_{e,n+1} - V_e}{R_{n+1}/2 + R_n/2} + \dots \right] \frac{1}{C_n} \quad (3)$$

The direct stimulating influence of the applied external potential on compartment n is described by the activating function which has the physical unit of V/s.

$$f_n = \left[\frac{V_{e,n-1} - V_{e,n}}{R_{n-1}/2 + R_n/2} + \frac{V_{e,n+1} - V_{e,n}}{R_{n+1}/2 + R_n/2} \right] \frac{1}{C_n} \quad (4)$$

The activating function gives the very first influence of the electric stimulation on the nerve fiber which corresponds to the slope of membrane voltage V_n at stimulus onset (Rattay (1989)). The expression within the brackets of the last equation is a virtual injected current that is the driving term in compartment n resulting from the applied electrical field. Note that this driving term, and consequently the value of the activating function, is independent from the electrical properties of the membrane. For a homogeneous fiber with constant diameter the activating function is proportional to the second difference quotient of the extracellular membrane which becomes the second derivative for compartment length $\rightarrow 0$ (Rattay (1986)).

Fohlmeister-Coleman-Miller, channel dynamics

To simulate the electric properties of the nerve fiber membrane we incorporated the ion channel dynamics of the Fohlmeister-Coleman-Miller (Fohlmeister et al. (1990); Fohlmeister and Miller (1997); Sheasby and Fohlmeister (1999)) model. The ion currents are: a sodium current (I_{Na}), a calcium current (I_{Ca}), a leakage current (I_L) and three types of potassium currents: a delayed rectifier (I_K), a potassium type A (I_{KA}) and the calcium activated potassium current ($I_{K,Ca}$).

To analyze the impact of the band we increased the sodium channel conductance in the band region by the 2-30 fold respective to the neighboring axonal segments.

Nerve fiber parameters

Our model-neuron was a straight fiber with a length of up to 3000 μ m and a diameter of 1 μ m. The length of the sodium channel band was set to 40 μ m. Compartment size varied from 5 μ m to 20 μ m which was small enough to minimize the computational error to the order of 1% (Rattay (1999)). The internal resistance varied from 0.55 Ω ·m to 5.5 Ω ·m, the specific membrane capacity was held at 1 μ F/cm². The resting potential was set to -65mV. In the actual experiments, the examined retinas had a temperature of 36° (=were superfused by Ames with 36°). Rattay and Aberham (1993) showed that temperature changes the waveform of action potentials (higher temperature leads to shorter/faster action potentials) but has a small impact on actual threshold. Since the used biophysical model does not include temperature correcting factors (Q10), all simulations were computed at a temperature of 22°C.

For stimulation we used either a 0.2ms monophasic cathodic pulse like in the physiologic experiments or a biphasic pulse form with various pulse lengths (0.2ms, 0.5ms, 1ms). Sodium channel density was held at 70mS/cm² for the axonal parts of the fiber neighbored to the sodium band.

Self-spiking of the model neuron

During the first simulations an unexpected result could be observed: The activation-strength of the modeled sodium channel band was responsible for so called self spiking after some time. This means that although no stimulating pulse was delivered the fiber started to spike anyway. Depending on the sodium channel density this self spike occurred approximately 100-150ms after the start of the simulation. We fixed the instability of the model neuron by also increasing the leak channel conductance in the band compartments. For example, a five-fold higher sodium channel conductance requires a 11 times higher leak conductance to bring the neuron into a stable state. In spite of this modification the resulting leakage/sodium channel conductance ratio is with approximately 1/3800 very low and consequently it had an insignificant impact on the threshold.

Determination of thresholds

As criteria for detecting an action potential (AP) we looked for a zero-crossing of membrane voltage of at least one compartment within 10ms after stimulus onset. When we recorded an action potential the applied stimulus amplitude was taken as threshold level. With this method we were able to draw one- and two-dimensional threshold maps, respectively, for various electrode positions relative to the model-neuron. The compartment which first crossed the 0mV border was taken as site of spike initiation. This allowed us to determine whether the band region or the axonal part of the neuron was the section where the spike occurred.

Three-layer vs. Seven-layer model

A simple three-layer model was compared to a more complex seven-layer model. Both models had a total size of $4000 \times 1000 \times 1000 \mu\text{m}$ as seen in Fig. B.2A.

The computational model based on experimental results obtained by Karwoski et al. (1985) who measured the internal resistivity within frog retina for various layers. The experimentally determined average resistivity between inner and outer limiting membrane was $50.5 \Omega \cdot \text{m}$ (total thickness of frog retina was $180 \mu\text{m}$ which is comparable to human retina in the foveal pit (Landau et al. (1997))). In the seven layer model the retina consisted of the five following layers (also see Fig. B.2A, right and Table A.2):

- Ganglion cell bodies and optic nerve fibers (GC/ONF)
- Inner plexiform layer (IPL)
- Outer- and inner nuclear layer (ONL-INL)
- Subretinal space (SUBS)
- Pigment epithelium (RPE)

A simple model containing three-layers (see Fig. B.2A, left and Table A.2) was implemented to compare if the more realistic seven-layer model has a crucial influence on computation of external potentials. In this case the retina was modeled as a single homogeneous layer with a resistivity of $50.5 \Omega \cdot \text{m}$. For these investigations a model neuron with the same parameters as described before was used.

3. Results

Three-layer vs. Seven-layer model

In the first computer simulations the fiber center was positioned straight below the electrode and the applied current amplitude was increased in $0.1\mu\text{A}$ steps until the first AP occurred (time window of observation = 10ms). The observed pulse amplitude was considered as 'threshold level'. Shifting the electrode and a consequent measuring of thresholds along the stimulated fiber results in threshold profiles as shown in Fig. B.2B. The complex model shows slightly lower thresholds than the simple model but we felt that the average difference of 10% was small enough that it did not warrant the considerable increase in computational effort required with the complex model. In all consequent calculations the three layer (simple) model was used.

Model simplifications and cell geometry

Fig. B.3A describes the geometrical simplifications which were made for the performed simulations. A retinal ganglion cell is virtually embedded into the three-dimensional Finite Element model, the left panel shows a simplified geometry of a retinal ganglion cell. In this case the electrode is positioned epiretinally. Therefore the axon (thin bar) and the sodium channel band (thick bar) are located closer to the electrode. The vertical distance between axon and soma (black dot) is approximately $15\mu\text{m}$.

The right panel of Fig. B.3A shows a even more simplified model of a retinal ganglion cell. This model neuron consists of a long straight uniform axon (thin bar) with a centered band of highly dense sodium channels (thick bar). Again there are no dendrites included, the soma was neglected.

To determine the accuracy for both model neurons one-dimensional threshold profiles were calculated to examine the influence of the soma (Fig. B.3B). The computed results show considerable threshold differences, especially for electrode positions close to the soma. However, since in both cases the region of lowest thresholds was located close to the sodium channel band the simple geometry was used in the following simulations. This was made to spare computation time and to understand the influence of the sodium channel band without influence from geometric side effects.

Key components of activation

The activating function is a good predictor for the initial depolarizing and hyperpolarizing effects in a uniform nerve fiber when an electrical field is applied (Rattay (1986, 1990)). Complex geometries and special biophysical properties of the cell membrane make it more difficult to predict the site of spike initiation within a fiber. Depolarized compartments are likely to initiate an action potential. However, the axial current flow during the stimulus pulse is also important for spike initiation. Regions that contain high numbers of voltage gated sodium channels may be the site of spike initiation even in cases with negative activating functions. Lower thresholds in these regions lead to unexpected activation phenomena. The further simulations try to reveal the key components of action potential generation in fibers containing a band of highly dense sodium channels.

Electric properties

To investigate whether stimulation at threshold level generated a consistent effect in and around the band region for different locations of the stimulating electrode, the extracellular voltage generated from six locations of the stimulating electrode within the threshold map of Fig. B.1 was explored. The x-y coordinates and thresholds for each location are given in Table A.1. The points were chosen as follows: points P1 and P2 were the points at which the observed thresholds were lowest, P3 corresponded to the approximate location of the soma, P4, P5 and P6 were chosen randomly across other locations. The extracellular voltage elicited by the stimulus pulse along the length of the axon at threshold for two points, P1 and P6, is shown in Fig. B.4A (dashed blue and green lines, respectively). The peak value of the applied external voltage occurs directly under the stimulating electrode (0 μ m offset for P1; 70 μ m offset for P6). The amplitude of the stimulus delivered at P6 was reduced to 75% of threshold and the resulting voltage profile from this (reduced amplitude) pulse was plotted (thin black line in middle and bottom panels). This (reduced) voltage profile had a similar shape and amplitude (but mirrored) in the band region to the voltage profile arising from threshold stimulation at location P1. However, the profile from location P1 resulted in an action potential while the (reduced) profile arising from location P6 did not. This suggests that the applied external voltage profile across the band by itself does not underlie spike initiation. The one-dimensional electric field of each voltage profile was calculated by taking the derivative of the external voltage along the x-coordinate of the fiber (dV/dx). Similar to the voltage profiles, the electric fields across the band arising from threshold stimulation at locations P1 and P6 (red and green dotted lines, respectively) showed little similarity suggesting that the field is also not the key component underlying action potential initiation.

Because much previous work with axonal stimulation indicates that the activating function is a good predictor for the site of spike initiation the activating function arising from threshold stimulation at each location by calculating the second derivative of the external voltage (d^2V/dx^2) was approximated (blue and green solid lines). The activating function provides an indication of how each compartment will respond initially to external stimulation: positive regions of d^2V/dx^2 correspond to compartments with positive activating function values and indicate the compartment will initially depolarize in response to stimulation: axonal regions in which the activating function is highest are generally the regions in which the action potential is initiated. Surprisingly, the second derivatives of the voltage profile arising from stimulation at the six locations described in Fig. B.1 and Table A.1 revealed no apparent similarities in and around the band region (Fig. B.4B). For example, the second derivative profile across the band region was completely positive for stimulation at some locations (e.g. P1) but almost completely negative for stimulation arising from other locations (e.g. P6). Because the negative second derivative suggests that the band's initial response to stimulation will be hyperpolarization, these results bring into question whether the band is actually the site of spike initiation for all stimulus locations.

Nerve fiber activation / time course

The lack of consistency across the band regions, especially for the second derivative profiles, led to further simulations. Until then, all simulations were restricted to Finite

Element computations (voltage/electric field/second derivative profiles) but no (computational) nerve fiber stimulation was performed. Therefore, the next round of simulations was restricted to an axon fiber model as shown in Fig. B.3A (right, trace 'b').

Again, a 40 μ m long band of voltage-gated sodium channels was modeled in the center of the axon by increasing the sodium channel conductance in the band compartments; conductance in these compartments was increased to five-fold of the conductance in the rest of the axon. In this configuration thresholds for P1-P6 for the model fiber ranged from 15-23 μ A. This range is comparable to but slightly smaller than the actual thresholds measured *in vitro* (Table A.1).

Because the activating function is a static measurement, i.e. it only applies to the time period immediately following pulse onset, the dynamics of individual compartments during the 0.2ms duration of the pulse were examined. Panel A in Fig. B.5 shows the time course of the stimulus: A monophasic, cathodic 0.2ms rectangular pulse was used for all calculations like in the physiological experiments of Fried et al. (2009). In panel B of Fig. B.5 the axial current flow within a fiber during the pulse is shown schematically. Before pulse onset no current flows in the axial direction (top) because there is no current unbalance. At pulse onset only a small region directly below the stimulating electrode is depolarized whereas all other fiber parts are hyperpolarized (middle). This corresponds to the principle of the activating function. The width of the depolarized region broadened throughout the duration of the pulse (see Fig. B.5B bottom). This widening can be seen as a spread of positive charge from the central regions to the negatively charged regions immediately adjacent.

Thus there were three different categories of response that arose in individual compartments (see Fig. B.5C): First, some compartments had a positive activating function and remained depolarized throughout the duration of the pulse. These were typically the compartments closest to the site of stimulation (labeled as '++' in Fig. B.5C). Second, some compartments had negative activating functions and remained hyperpolarized throughout the pulse (labeled as '—'). Finally, some compartments hyperpolarized at pulse onset (negative activating function) but depolarized during the course of the pulse (labeled as '—+').

The extent of these three regions for stimulation from location P1 was calculated and plotted in Fig. B.5D. Compartments with a positive activating function were constrained within the two innermost vertical lines during the course of the 2ms pulse. The extent of the compartments that were depolarized expands to incorporate the region between the two outermost vertical lines (Fig. B.5D). The time course of the compartments shown in Fig. B. 5C correspond to 'X' locations of 0, 50 and 200 μ m in Fig. B.5D. The inset at the top left of Fig. B.5C shows the response to stimulation immediately following pulse onset (activating function) for each type of response.

Finally, membrane voltage at the end of the pulse for all six stimulating electrode locations was plotted. Now all compartments within the band region were depolarized (Fig. B.5E). This suggests the possibility that band regions that initially hyperpolarize might still initiate spikes.

Site of spike initiation: Band vs. no Band

To gain further insight into how the sodium channel band influences action potential generation over the duration of the stimulus the response of a long, uniform, unmyelinated axon was compared to the same fiber with a band of high-density sodium channels located at the center (Fig. B.6A).

As previously mentioned the sodium channel band was modeled by increasing the sodium channel conductance in the regarding fiber region (Fig. B.6A, right panel, below the fiber) and the band length was always 40 μ m. The sodium channel density in Fig. B.6 and 7 was increased to five-fold of the axon conductance. The density of sodium channels in all non-band regions was fixed at 70mS/cm² - identical to the sodium channel density of the uniform fiber. All other properties of the two fibers were identical. Simulations compared the response to stimulation from a point source located 30 μ m above the fiber and 60 μ m (Fig. B.6) and 200 μ m (Fig. B.7), respectively, from its center. A compartment centered under an electrode which is 60 μ m away corresponds to the '—+' region in Fig. B.5 whereas the 200 μ m distant compartment corresponds to the previously mentioned '—' region.

The compartments corresponding to a region subtended by a 70° angle from the point source had a positive activating function and depolarized initially in response to the stimulus pulse (as predicted by theory, Rattay (1989)). The extent of the subtended region is indicated by the two inner dotted vertical lines below the fiber in Fig. B.6B; the left inner line is labeled t=0ms. As described in Fig. B.5 the portion of the axon that was depolarized expanded during the course of the pulse - the range of this expansion is indicated by the two outer dotted vertical lines, the rightmost of which is labeled t=0.2ms.

In Fig. B.6 the response to stimulation over time in the same two compartments from each fiber was observed: the first was located directly below the point source and was 60 μ m from the fiber center (corresponding to the '—+' region in Fig. B.5C) while the second was located at the center of the fiber (6C, blue and red traces respectively). As expected spike initiation in the uniform fiber occurred directly below the point source (left panel). The triggered spike propagated along the fiber and was observed at the fiber center a short while later.

For the fiber with an incorporated band it was found that the spike was initiated prior to the spike in the compartment directly below the point source although in this region the activating function was negative. Because of the broadening effect during the pulse the band compartments were depolarized after the end of the pulse and therefore capable to be the site of spike initiation.

In Fig. B.7 the response to stimulation of an electrode far outside the actual band region (180 μ m away) was examined which means the compartment directly under the electrode corresponded to the '—' region in Fig. B.5C. In case of the no-band situation of course again the compartment directly under the stimulating electrode was the site of spike initiation (see Fig. B.7B, left; as activating function predicts) but surprisingly in the fiber with the band, the spike in the band region was initiated prior the spike in the compartment directly below the point source (see Fig. B.7B, right). Further, this occurred even though the activating function was negative in the center compartment and the compartment remained

hyperpolarized throughout the duration of the stimulus pulse ('—' region). The threshold for the fiber with the band was a bit less than that for the uniform fiber, most likely because of the higher sodium channel conductance.

One-dimensional threshold maps for both fibers were computed to explore how the presence of the band influences threshold. When moving a stimulating electrode along the axis of the uniform fiber the thresholds remained constant regardless of the position of the stimulating electrode (see Fig. B.6D left). For the fiber with the sodium channel band, threshold was lowest in the band area and surrounding regions (Fig. B.6D right).

Epi- vs. subretinal stimulation

To investigate the neural response during subretinal stimulation on RGCs we increased the electrode-fiber distance from 30 μ m to 200 μ m. The activation of other neural structures (bipolar cells, horizontal cells, amacrine cells) was neglected because we were only interested in the direct activation of RGCs.

The region of lowest thresholds was once more the band of highly dense sodium channels with a threshold of 137 μ A and increased when moving the electrode along the fiber axis. A stimulus amplitude of 180 μ A was necessary to initiate an axonal spike. Again, it was possible to elicit action potentials in the band compartments although the stimulating electrode was located far outside the actual band region (results not shown).

Parameter variation

To find the crucial parameters in the presented model several input variables were varied to see their influence on action potential generation. First, the internal resistivity ρ_i was either decreased by a factor of 2 from 1.1 Ω ·m to 0.55 Ω ·m or increased by the factor of 2 (2.2 Ω ·m) and 5 (5.5 Ω ·m), respectively. Furthermore, the sodium channel density within the band was varied in the range from 140mS/cm² to 1400mS/cm². Axon sodium channel conductance was held fixed at 70mS/cm² which means that the ratio between axon and sodium channel band conductance varied from 1:2 to 1:20.

Fig. B.8A shows computed threshold profiles for two fibers having the same band to axon sodium channel ratio (5:1) but having different internal resistivity (1.1 Ω ·m for trace ('b') and 5.5 Ω ·m for ('a')). Axonal threshold for electrode positions far outside the actual band region was higher for the fiber with a higher internal resistance. 'I' and 'II' indicate the beginning of the region where threshold varied by less than 0.1 μ A. The influence of the sodium channel band on the site of spike initiation was bigger for 'b' (constant threshold at 290 μ m) than for the fiber that had a higher internal resistance (threshold started to be constant for regions over 110 μ m away from band center).

In panel B of Fig. B.8 two fibers having the same internal resistance (1.1 Ω ·m) were compared. Trace 'a' resulted from a five-fold increase of the sodium channel conductance in the band whereas 'b' resulted from a twenty-fold increase in the band region. Both threshold profiles showed the same thresholds for axonal spiking but the higher sodium channel conductance in trace 'b' led to a bigger influence (=lower thresholds) in the periphery of the band.

Fig. B.8C and D summarize the results. A higher internal resistance led to smaller axial currents which reduced the effect of the passive current propagation along the fiber and consequently axon thresholds increased. A decrease of the sodium channel density within the band lowered the influence because the threshold for spike initiation increased.

Anisotropic nerve fiber layer

Since the optic nerve fiber layer contains many parallel oriented RGC axons, this structure is likely to be electrically anisotropic. Therefore, an additional anisotropic layer (thickness 20 μ m) was incorporated in the retina of the three-layer model with a 5 times higher axial (i.e. in axonal direction) conductivity than in all other directions. A comparison of the evoked external potentials in fiber direction with a stimulus current of 10 μ A is shown in Fig. B.9. The voltage profile for a higher axial conductivity along the fiber is decreased in maximum amplitude and slightly broader than for the standard parameters.

Biphasic pulses

Fig. B.10 shows two-dimensional threshold maps for the model neuron when different biphasic pulses are applied. If the standard stimulus pulse (0.2ms, monophasic, cathodic, dashed line) is changed to a biphasic pulse with doubled pulse length (0.4ms, cathodic first, solid line) this will lead to a significant increase in threshold in the band and axonal regions. This effect gets smaller for 0.5ms pulses and disappears for pulses with a length of 1ms.

4. Discussion

Predictor for neural activation

The activating function is a good predictor for de- and hyperpolarization in a homogeneous fiber with constant diameter. Therefore, it is also a predictor for the probable location where an action potential will arise. However, when a fiber with constant diameter shows inhomogeneity of its ion channel composition, the activating function is not able to predict the site of spike initiation anymore. The distribution of the applied external potentials as well as the resulting electric field and second derivative are not capable to make reliable assertions about where a spike will occur. As the results show, the sodium channel band can also be the site of spike initiation although the stimulating electrode is far away from the actual band region. Furthermore, regions (compartments) with negative activating function and with a hyperpolarized membrane voltage throughout the stimulating pulse are capable to be the location where an action potential is fired. This study started to systematically investigate various properties of the stimulating pulse (e.g. external voltage, electric field, derivative of field, activating function) to find a predictor for the site of spike initiation in a nerve fiber showing inhomogeneity in its ion channel density.

Activation of passing axons

One of the major problems in retinal implants is the activation of passing axons from neurons located far away. Previous clinical studies (e.g. Rizzo et al. (2003)) reported of unwanted perceptions when stimulating RGCs which can be explained by this activation phenomenon. When these axons are activated unintentionally, the expected round

perceptions will become blurred and therefore are not useful to restore a higher quality of vision.

The band of sodium channels and the corresponding low-threshold region may be helpful to focally activate RGCs. If the applied stimulus amplitude is sufficient to activate the band region without activating any passing neural structures a focal stimulation can be achieved.

Epi- vs. subretinal stimulation

When the stimulating electrode is positioned subretinally, i.e. the distance between electrode and fiber is increased, the former described effects of the sodium channel band are qualitatively the same as in the epiretinal configuration. The low threshold region also corresponds to the band of highly dense sodium channels and it is possible to initiate spikes in the band compartments when the stimulating electrode is located far outside the actual band region.

In previous studies it was assumed that during high frequency electric stimulation synaptic (indirect) RGC responses start to fade because of inhibitory inputs from amacrine cells (Fried et al. (2006); Margalit and Thoreson (2006)). Freeman and Fried (2011) found evidence for more complicated synaptic activities contributing to firing rate reduction in trains of stimuli. Perez Fornos and coworkers (Pérez Fornos et al. (2012)) supposed this phenomenon to be the reason for unwanted fading of elicited perceptions in clinical trials. Therefore, the 'direct' activation of the underlying retinal network will likely be the dominant way when stimulating subretinally. Although subretinal, and thus more natural, stimulation might be the preferred strategy to activate the remaining retinal neurons it might also be a strategy to stimulate RGCs directly with a subretinal implant. This study shows that the most important difference in direct activation of RGCs during epiand subretinal stimulation, respectively, is that during epiretinal stimulation the ratio of axonal threshold to the threshold in the band region is approximately 1.5:1 whereas during subretinal stimulation this ratio is only 1.3:1. This means that for subretinal positioned electrodes the operating window (axonal threshold minus band threshold) to solely activate the band region is smaller than in the epiretinal case. Furthermore, in the subretinal configuration the lowest threshold was 137 μ A which was more than 10 times more than the lowest threshold (14 μ A) when stimulating epiretinally. Since charge limit is an important factor in implant design the higher stimulus amplitude during subretinal stimulation leads to larger electrodes to avoid exceeding charge limit.

Resistivity of the retina

Previous studies (e.g. Karwoski et al. (1985); Greenberg et al. (1999); Kasi et al. (2011)) provided numbers for retinal resistivity which differed by almost two orders of magnitude. In the presented simulations we used the results provided by Karwoski et al. which led to similar thresholds compared to the actual experiments. In contrast, simulations that used the numbers provided by Greenberg resulted in three times higher thresholds than the thresholds measured by Fried et al. (2009, results not shown).

Furthermore, the retina as a heterogeneous medium containing many cell types and neural structures is likely to show an anisotropic resistivity which will lead to different results.

Since the optic nerve fiber layer has a strongly oriented structure we also examined voltage distribution in our FEM model with an anisotropic RGC layer to check the influence of this parameter. Therefore, we increased the electric conductivity in axial direction of the RGC-axons by factors up to 5. The results show that the distribution of the external potential becomes broader and the amplitude decreases slightly.

Further investigations will be needed to have preciser input parameters for future modeling studies. For many investigations a simple model of the retina is sufficient, however, computational power increases steadily and therefore also more complex models should be established to refine results in further studies.

Biphasic pulses

In the presented results mostly monophasic, cathodic pulses with a length of 0.2ms were examined. However, in clinical application the pulse form has to be adjusted in a certain way because of technical reasons. For example, charge limit is a crucial parameter in functional electric stimulation. Therefore, the applied pulse has to be charge balanced, i.e. a (second) compensation pulse has to be adjusted which has the same magnitude of charge as the first pulse (i.e. biphasic stimulation). We also investigated the influence of biphasic stimulation (cathodic first) to see if the major findings are modified by this type of stimulation. Our results suggest that short (0.2ms each pulse) biphasic pulses lead to higher thresholds. One reason for this seems to be the hyperpolarizing effect of the second (anodic) pulse that forces the transmembrane voltage back to resting potential. A longer pulse length (up to 1ms) leads to a smaller difference in threshold (compared to monophasic stimulation), most likely because the action potential already gets initiated by the cathodic pulse and is not stopped anymore by the compensation pulse. Further studies are necessary to find out the most efficient and applicable pulse form and length for implants of the next generation.

Site of spike initiation

The activating function (Rattay (1986)) predicts a region of 70° which will be depolarized when a cathodal stimulating pulse is delivered. When the distance between the stimulating electrode and the neural tissue is small, this region is substantially shorter than the space (or length) constant λ which describes how far current will travel along an axon and consequently will influence the membrane potential. Thus, the axial current within the axon is capable of having an effect on the sodium channel band located far away in comparison to the distance between electrode and neuron.

This study shows a threshold decrease far outside the actual band region, however, the threshold reduction at this location was smaller than 1/1000 which can be calculated but is not important in real experimental stimulations because naturally occurring variations, e.g. ion channel fluctuations in fibers like retinal ganglion cells are expected to be in the lower percent area (Verween and Derksen (1968); Rattay et al. (2001)).

Previous studies showed that action potentials in RGCs arise at the proximal portion of the axon which also gives this region its name (Carras et al. (1992)): AIS, axon initial segment. However, it is also possible for dendrites to generate spikes which are forwarded to the axon through the soma. Currents which are below threshold level in the dendritic parts of a

neuron can be transferred to the AIS as well where an action potential is initiated. This phenomenon can be observed during natural excitation as well as during intra- and extracellular stimulation, respectively (Stuart et al. (1997); Gasparini et al. (2004); Rattay and Wenger (2010)). This study supports these findings, the AIS or region of highly dense sodium channels is likely to be the site of spike initiation even if the stimulating element is located hundreds of microns from the actual band region. For the standard parameters, a five fold higher sodium channel conductance in the band compartments (350mS/cm^2) and an internal resistance of $1.1\Omega\cdot\text{m}$, the border between a 'band' spike and an 'axonal' spike is located approximately $300\mu\text{m}$ outside the center of the sodium channel band (stimulus amplitude step = $0.01\mu\text{A}$, see Fig. B.8A, trace 'b'). These results suggest to re-interpret the principle of the activating function. However, as previously mentioned, these theoretical numbers are not important for actual applications because this precise breakpoint between 'band' and 'axonal' spike only exists theoretically and becomes a region where a spike might be triggered or not. Considering diameter changes along the fiber and ion channel fluctuations it might be recommendable to remain about 25% below these values during actual focal stimulation. This means, as can be seen in Fig. B.8A (trace 'b'), that with a stimulating amplitude of about $16\mu\text{A}$ (75% of axonal threshold) it might be possible to stimulate the band region focally without stimulating passing axons although the electrode is about $30\mu\text{m}$ away from the edge of the band. These findings are also consistent with a modeling study (Tsai et al. (2012)) which reported that the actual electrode position is a poor predictor for the site of spike initiation.

Different types of sodium channels

There are various types of sodium channel which show different excitation kinetics and sensitivities (Trimmer and Rhodes (2004)). In RGCs two different types of voltage gated sodium channels have been shown (Van Wart et al. (2007)). Studies in the central nervous system (CNS) reported that sodium channels of the type Nav1.6 which are located in the band region have lower thresholds than Nav1.2 type channels that are located in somatic and dendritic parts, respectively (Hu et al. (2009)). For stimulation in the CNS using micro electrodes close to the soma or even further away from the actual band region this means that the AIS is likely to be the site of spike initiation (Rattay and Wenger (2010); Rattay et al. (2012)). We do not know yet if this special sodium channel distribution also can be of relevance in the stimulation of retinal ganglion cells.

Limitations of the model

The modeling of neural structures and their activation during extracellular stimulation has certain limitations. First, several parameters (conductivities, resistivities, ion channel distributions) have to be estimated and therefore the results have to be regarded carefully. Second, in the presented Finite Element Model the sclera was modeled but was actually removed during dissection of the eye in the actual experiments. However, this fact is not expected to have a major qualitative or quantitative influence on the obtained results.

Appendix A

Table A.1

Coordinates and thresholds for six points on threshold map

Name	x [μm]	y [μm]	Threshold [μA]
P1	00	00	12
P2	-50	10	12
P3	50	00	25
P4	30	-40	22
P5	-30	60	27
P6	-70	10	22

z-distance between fiber and electrode is always 30μm. P1 and P2 are the two points showing lowest threshold on threshold map, P3 represents the soma, P4, P5 and P6 are randomly chosen. All stimuli are cathodic 200μs pulses.

Table A.2

Thickness and corresponding specific resistivity ρ for different layers of the two finite element models

Three layer	Ames		Retina				Sclera
thickness [μm]	500		200				300
ρ [Ω·m]	1.1		50.5				505
Seven layer	Ames	GC/ONF	IPL	ONL-INL	SUBS	RPE	Sclera
thickness [μm]	500	20	40	60	60	20	300
ρ [Ω·m]	1.1	70.6	18.2	60	12.7	1230	505

Appendix B

Figure B.1. Threshold map for electric stimulation of a retinal ganglion cell; see Table A.1 for exact locations and thresholds of indicated points

A two dimensional colormap shows thresholds for each electrode position. Thresholds to a monophasic cathodic pulse of $200\mu\text{s}$ varied from $12\mu\text{A}$ to $45\mu\text{A}$ and are given by the colorbar (right). The approximate location of the high density sodium channel band is indicated by the thick bar, the soma is at P3. The thin horizontal line indicates the approximate position for the axon. The conical stimulating electrode was positioned approximately $30\mu\text{m}$ above the fiber.

Figure B.2. FEM model for calculation of external potentials

(A) The left panel shows the simple model geometry consisting of three layers: sclera, retina and a layer of Ames solution. The stimulating electrode (not shown) was embedded in the Ames layer. The right panel depicts the 5 layer model for the retina suggested by Karwowski et al. (B) The comparison of thresholds for the single layer retina (a) and the 5 layer retina (b) shows slightly (about 10%) lower thresholds for the 5-layer model.

Figure B.3. Geometry of two different model neurons

(A) The left panel shows a retinal ganglion cell that includes soma (large black circle), initial segment (angled line), sodium channel band (thick line) and axon (thin horizontal line). To avoid geometric side-effects a model consisting only of an axon (thin line) and sodium channel band (thick line) was used (right). The angle between the stimulating electrode and the retina layer in the model was 10° as in the actual experiments. (B) Thresholds for a complex cell geometry (a) and the straight, uniform axon (b). The threshold increase on the right side results from the incorporated large soma. The gray shaded region indicates the extent of the band section which shows lowest thresholds for both cell geometries. Sodium channel conductance in the band region was five times higher than in the axon ($=350\text{mS}/\text{cm}^2$).

Figure B.4. Electric properties along a straight, uniform axon

(A) Geometry (top), voltage, electric field and derivative of electric field distribution along the axon for points P1 (middle) and P6 (bottom). In both panels the 75% voltage profile of P6 is shown (thin black line, see text). Grey shaded region indicates the extent of the sodium channel band. (B) Second derivatives for all six points of Fig. B.1. Note that for some locations of the stimulating electrode, e.g. P6, the value of the second derivative is almost totally negative within the band region.

Figure B.5. Some compartments with negative activating functions are depolarized at end of pulse

(A) Time course of the applied pulse. (B) The extent of the region activated during cathodic pulse application gets broader over time due to the flow of balancing currents. (C) Membrane voltage versus time for the three different compartments (vertical lines in panel

D) define three types of regions: '++', '-+' and '--'. In the '++' area all compartments have positive activating functions and membrane voltages are positive after the pulse end. Compartments in the '-+' region have a negative activating function but are depolarized by the end of the pulse. Both, activating function and ending membrane voltage, are negative in the '--' region. (D) Activating function (dashed) and membrane voltage (scaled) at end of pulse. The zero crossings of the activating function and the membrane voltage at pulse offset define the borderlines of the three types of activated functions marked by vertical dotted lines. Three 'X's indicate compartments centered at 0 μ m, 50 μ m and 200 μ m offset (see panel C). (E) Membrane voltage at the end of the pulse for the six locations of the threshold-map depicted in Fig. B.1. Note that in contrast to Fig. B.4B all compartments in the band region are now depolarized.

Figure B.6. A band of high density sodium channels influences the site of spike initiation
 (A) Comparison of two different biophysical structures: A straight uniform axon without (left) and with (right) a centered high density band of voltage gated sodium channels (g_{Na} is five times higher than in the axonal compartments). (B) The circle depicts a point source stimulating both structures at the same relative position. Dotted lines and arrows at the bottom indicate the border between depolarization and hyperpolarization regions for activating function ($t = 0\text{ms}$); solid vertical lines indicate membrane voltage at pulse offset ($t = 0.2\text{ms}$) (see Fig. B.5A and B). (C) Membrane voltage vs. time for two compartments: The red curve ('b') indicates response of the center-compartment whereas the compartment corresponding to the blue trace ('a') is located directly under the electrode. Stimulus

amplitude is set to threshold level on both sides. (D) Threshold level for different electrode positions along fiber shows a threshold decrease that extends far beyond the actual band.

Figure B.7. A band of high density sodium channels influences the site of spike initiation
 (A) Like in Fig. B.6, the sodium channel density is five times higher in the band compartments than in the axon region. The circle depicts a point source stimulating both structures at the same relative position. Dotted lines and arrows at the bottom indicate the border between depolarization and hyperpolarization regions for activating function ($t = 0\text{ms}$); solid vertical lines indicate membrane voltage at pulse offset ($t = 0.2\text{ms}$) (see Figure 5A and B). (B) Membrane voltage vs. time for two compartments: The red curve ('b') indicates response of the center-compartment whereas the compartment corresponding to the blue trace ('a') is located directly under the electrode. Stimulus amplitude is set to threshold level on both sides.

Figure B.8. Band parameters strongly influence the site of spike initiation

(A) One-dimensional threshold map for internal resistances of 5.5 $\Omega \cdot m$ (a) and 1.1 $\Omega \cdot m$ (b). Red and blue crosses indicate begin of region where threshold varies by less than 0.1 μA . (B) Threshold map for sodium channel density (gNa) ratios between axon and band of 1:5 (a) and 1:20 (b). Again, red and blue crosses indicate begin of region where threshold varies by less than 0.1 μA . (C) Plots of internal resistance versus the SSI (=site of spike initiation) where threshold varies by less than 0.1 μA for four different gNa ratios between band and axon (2:1, 5:1, 10:1, 20:1). The points labeled 'I' and 'II' correspond to 'I' and 'II' in panel (A). (D) Plots of gNa ratio between band and axon versus points where threshold varies by less than 0.1 μA for four different internal resistances (0.55, 1.1, 2.2, 5.5 $\Omega \cdot m$). The points labeled 'I' and 'II' correspond to 'I' and 'II' in panel (B).

Figure B.9. Anisotropic fiber layers change the distribution of external potentials

The three-layer model (see methods) was extended by an additional anisotropic layer (20 μm in height) to examine the influence of the strongly parallel oriented RGC axons. External potential distribution in fiber direction was compared for the standard case (solid line) with the evoked potentials when the axial conductivity is increased by a factor of 5 (dashed line). For the anisotropic case the maximum amplitude of the generated potentials is decreased by approximately 15%. Another effect of the anisotropy is a slightly broader distribution of the evoked external potentials.

Figure B.10. Biphasic stimuli change neural response during extracellular stimulation

Longer pulses reduce the influence of biphasic pulses. A biphasic pulse form (0.2ms each pulse, cathodic first, solid line) leads to significant higher thresholds compared to monophasic stimulation (dashed line). This effect disappears when the pulse length is increased: results are shown for pulse lengths of 0.5ms and 1ms, respectively.

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- The axonal sodium channel band lowers electrode threshold currents
- Electrodes distant from the band can initiate spikes within the band via intra-axonal current
- The density of sodium channels strongly influences the site of spike initiation