The impact of calcium current reversal on neurotransmitter release in the electrically stimulated retina

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Received 24 November 2015, revised 23 February 2016
Accepted for publication 23 May 2016
Published 14 June 2016

Abstract

Objective. In spite of intense theoretical and experimental investigations on electrical nerve stimulation, the influence of reversed ion currents on network activity during extracellular stimulation has not been investigated so far. Approach. Here, the impact of calcium current reversal on neurotransmitter release during subretinal stimulation was analyzed with a computational multi-compartment model of a retinal bipolar cell (BC) that was coupled with a four-pool model for the exocytosis from its ribbon synapses. Emphasis was laid on calcium channel dynamics and how these channels influence synaptic release. Main results. Stronger stimulation with anodic pulses caused transmembrane voltages above the Nernst potential of calcium in the terminals and, by this means, forced calcium ions to flow in the reversed direction from inside to the outside of the cell. Consequently, intracellular calcium concentration decreased resulting in a reduced vesicle release or preventing release at all. This mechanism is expected to lead to a pronounced ring-shaped pattern of exocytosis within a group of neighbored BCs when the stronger stimulated cells close to the electrode fail in releasing vesicles. Significance. Stronger subretinal stimulation causes failure of synaptic exocytosis due to reversal of calcium flow into the extracellular space in cells close to the electrode.

Keywords: retinal prosthesis, ribbon synapse, electric stimulation, computer simulation

(Some figures may appear in colour only in the online journal)

Abbreviations

| Abbreviation | Definition                  |
|--------------|-----------------------------|
| BC           | Bipolar cell                |
| FEM          | Finite element method       |
| EPSC         | Excitatory postsynaptic current |
| AC           | Amacrine cell               |
| GC           | Ganglion cell               |
| RP           | Releasable pool             |
| RRP          | Rapidly releasable pool     |

1. Introduction

One major goal in neural engineering is the restoration of an adequate level of vision to the blind. Electrically driven visual neuroprostheses can be implanted along the visual pathway at several locations such as the retina, the optic nerve, the lateral nucleus geniculate and the visual cortex. The retina as implantation site, however, seems to be the most promising location [1–9]. Aside from electrical stimulation several new approaches such as optogenetics [10], small-molecule photoswitches [11] and gene therapy [12] may become important alternatives to restore visual sensations; see reviews for the state of the art and future investigations [13–15].
Retinal implants can be placed at either the inner face (epiretinally) or at the outer portion (subretinal or suprachoroidal) of the retinal tissue. Epiereitical implants stimulate primarily elements close to the active electrodes which are the axons of ganglion cells (GCs). In general, the axon is the most excitable part of a neuron [16–18] and especially their ‘sodium channel band’ is most sensitive for extracellular stimulation [18–21]. Contrary to the epiereitical approach, subretinal electrodes, placed in the photoreceptor region, are supposed to stimulate the retinal network in a more natural way generating graded potentials in bipolar cells (BC). The network of stimulated BCs, horizontal and amacrine cells (AC) communicates with the GCs via synaptic contacts. Although electric synapses are also included within this network, the artificial generation of synaptic release by activation of voltage sensitive calcium channels seems to be the key element for subretinal stimulation. No matter which cell types (photoreceptors or ACs) feed their inputs to BCs, physiological GC excitation is always initiated by synaptic (glutamateric) release from BCs. This synaptic transmission is mainly depending on the level of intracellular calcium concentration ([Ca$^{2+}$]) in the BC terminals which is controlled by voltage sensitive calcium channels [22].

An interesting phenomenon observed during extracellular stimulation is the so-called upper threshold phenomenon [23–25]. A spherical model neuron stimulated extracellularly by a microelectrode may respond to a train of increasing stimuli with subthreshold membrane voltage, followed by spiking and finally by spike suppression [23]. Within the excitation window the spike is generated by sodium current influx across voltage sensitive ion channels. However, sodium ion flux changes its direction from inside to outside of the cell in regions where transmembrane voltage exceeds the Nernst potential of sodium as consequence of strong stimulation. The observed block of action potentials in retinal GCs at high stimuli was explained by reversed sodium currents at strongly depolarized regions of the neuron [24]. Whereas this explanation holds for spike block in neurons without neurites, it was shown that the block observed in the soma does not exclude one-sided firing of spikes conducted along the axon [26]. It should be noted that most phenomena observed during extracellular stimulation can be explained with a multi-compartmental approach but not with a single compartment model [17, 27, 28].

One advantage of stimulation from the subretinal space is to substitute, at least for some extend, the physiological role of lost photoreceptors by generating neurotransmitter release in BCs, the second order retinal neurons. Especially for this cell type reversal of calcium currents seems to be critical for stronger stimuli.

Therefore, this modeling study aims to (i) elucidate the importance of calcium channel dynamics on intracellular calcium concentration, (ii) describe the underlying mechanism of the current reversal during extracellular stimulation, (iii) to present the resulting implications on synaptic activity and (iv) to discuss their consequences for retinal implants.

2. Methods

2.1. Model neuron

The modeled neuron resembles the morphology of a rat type 9 ON BC which was extracted from a two-dimensional depiction from the literature and used in previous studies [29–33]. Beside the spherical soma all 106 compartments were modeled as cylinders with a maximum length of 5 µm. The BC had 10 synapses modeled as axon-terminal compartments each between 1.5 and 3 µm long. Somatic diameter was set to 10.96 µm, axonal and dendritic diameters varied between 1.1 and 2.2 µm. Synaptic compartments had a diameter of 2.2 µm.

2.2. Compartment model

The response of a multi-compartment model to extracellular stimulation can be computed by solving equation (1) [17]. In this equation, $V_n$ and $V_e$ denote the transmembrane voltage (extracellular potential–intracellular potential) and the extracellular applied voltage at the nth compartment, respectively, $R_n$ stands for the axial resistance of the nth compartment and $C_n$ for the membrane capacitance. The term $I_{\text{ion}}$ is computed by a Hodgkin–Huxley type model. Specific axial resistivity was fixed at 130 Ω cm

\[
\frac{dV_n}{dt} = \left[ -I_{\text{ion},n} + \frac{V_{n-1} - V_n}{R_{n-1}/2 + R_n/2} + \frac{V_{n+1} - V_n}{R_{n+1}/2 + R_n/2} + \ldots \right] \\
+ \frac{V_{e,n-1} - V_{e,n}}{R_{n-1}/2 + R_n/2} + \frac{V_{e,n+1} - V_{e,n}}{R_{n+1}/2 + R_n/2} + \ldots \\
\frac{1}{C_n}. \]

The presented BC membrane dynamics are simplified to a minimum. A thorough previous study by Benav [29] revealed a large number of different voltage sensitive ion channels located in BCs. As these channels were shown to have no significant influence on the membrane potential in terminal compartments only a leak current is incorporated into the model and a L-type calcium channel (Ca,1,4) as mainly expressed in BC terminals [34, 35] in the terminal compartments in order to compute the intracellular calcium concentration close to synaptic ribbons.

The calcium channel kinetics taken from Sikora and coworkers [36] were originally fitted to tiger salamander data from experimental studies [37, 38]. The presented dynamics, however, also resemble the current–voltage relationship determined in rat quite closely (see results). Thus, ion currents of a BC were computed by

\[
I_{\text{ion},n} = (i_{\text{Ca,n}} + i_L) A_n, \\
i_{\text{Ca,n}} = g_{\text{Ca}} C_n (V_n - E_{\text{Ca}}) \quad \text{and} \\
i_L = g_L (V_n - E_L). \quad (2)
\]
$g_{Ca}$ and $g_L$ denote cell membrane conductivities. $g_{Ca}$ was 0 mS cm$^{-2}$ in all non-terminal compartments and 1 mS cm$^{-2}$ in terminals. $g_L$ was set to 1 and 2 mS cm$^{-2}$ in non-terminal compartments and terminal compartments, respectively. $A_n$ denotes the surface area of the $n$th compartment. Furthermore, the gate variable $c$ was defined by [36]

$$\frac{dc}{dt} = \alpha_c (1 - c) - \beta_c c \text{ and } \alpha_c = \frac{-0.3 (V + 70)}{\exp(-0.1(V + 70)) - 1},$$

$$\beta_c = 10 \exp(-(V + 38)/9).$$

(3)

The resting potential of the BC was set equal to $E_L = -50$ mV which is close to values used in the past (−60 mV [39] and −53 mV [29]). Note that the reversal potential $E_{Ca}$ in equation (2) is a Nernst potential and depends on the ratio of intracellular and extracellular calcium concentrations where the intracellular variations dominate. By using the original parameters given by Fohlmeister [40] a Nernst potential varying around 130 mV is plausible. Calcium channels, however, are also selective for potassium ions and therefore $E_{Ca}$ is also influenced by $E_K$ (−60 to −90 mV, [29, 41]). An equal weighting of both Nernst potentials results in an $E_{Ca}$ of approximately 10–40 mV which was fixed to 20 mV in the simulations according to $I$–$V$ curves of L-type calcium channels in rat [42, 43].

Intracellular calcium concentration $[Ca^{++}]_i$, in synaptic terminals was assumed to depend on the compartment’s surface ($A$) to volume ($V$) ratio as well as on the calcium current across the cell membrane [40]. Furthermore, a passive sequestering process extrudes calcium above the residual level from the intracellular space

$$\frac{d[Ca^{++}]_i}{dt} = \frac{-i_{Ca} A}{2FV} \frac{[Ca^{++}]_i - [Ca^{++}]_{res}}{\tau},$$

(4)

with the Faraday constant $F = 96485.33$ C mol$^{-1}$, the standing calcium level $[Ca^{++}]_{res}$ and the time constant $\tau$ (10 ms) of the passive extrusion process. The calcium residual level was set to 0.34 μM. Calcium ions were assumed to be evenly distributed within the terminal compartments.

The model was solved using a fixed-step size backward Euler integrator in MATLAB R2013b (The Mathworks Inc., Natick, Massachusetts, United States). Since no spiking activity was included into the model a rather large time step of 0.01 ms was used. A comparison of time steps between 0.01 and 0.001 ms resulted in differences of membrane voltage smaller than 0.5% (data not shown).

2.3 Synapse model

The concept of modeled synaptic release is shown in figure 1. Generally, the filling of the four vesicle pools is determined by their current pool states and four rate constants for refill and release. The intracellular calcium concentration in BC synaptic terminals is a key parameter for exocytosis.

The model is able to generate release with a fast transient component and a slow sustained component as determined in experiments (see results). The model consists of four vesicle pools: (i) the cytoplasmatic pool (C) acts as a source of free vesicles which can bind to the release sites; (ii) the vesicles of the releasable pool (RP) are tethered to the synaptic ribbon; (iii) the vesicles of the rapidly releasable pool (RRP) are located close to the fusion site and are already primed for release; (iv) all vesicles which have undergone exocytosis enter the exocytosis pool (E).

![Figure 1. Schematic diagram of the synapse model—Four pools are modeled to mimic experimentally determined results from rat bipolar cells [44]. The cytoplasmatic pool C refills vesicles to the two release pools RP and RRP rate constants $\rho_{RP}$ and $\rho_{RRP}$. The RP characterizes vesicles located in the upper rows of the ribbon. Thus, the RP releases vesicles slowly but in a sustained manner. The RRP, on the other hand, is responsible for a fast and transient release of vesicles into the synaptic cleft. The vesicles in the RRP are tethered at the ribbon close to the actual fusion site and are already primed for release. Release from the RP and RRP is simulated as Poisson process and is mainly determined by the intracellular calcium concentration $[Ca^{++}]_{i}$, and the release constants $\rho_{RP}$ and $\rho_{RRP}$. RP and RRP release their vesicles into the exocytosis pool (E).](image)

The refill rates for the RP and the RRP are modeled by

$$\rho_{RP} = \frac{\rho_{RP} R_{RP} - R_P}{C_0}$$

and

$$\rho_{RRP} = \frac{\rho_{RRP} R_{RRP} - R_{RRP}}{C_0}$$

(5)

with $C_0$, $R_P$, and $R_{RRP}$ being the initial fillings of the three pools C, RP and RRP, respectively. $R_P$ and $R_{RRP}$ denote the current pool state at time $t$. $\rho_{RP}$ and $\rho_{RRP}$ are the refill rate constants of the two release pools and both are set to 0.25 s$^{-1}$ in agreement with a previous experimental study [44].

Release of vesicles from the RP and RRP to the exocytosis pool is modeled by a Poisson process. The exocytosis rate constants are further dependent on the current intracellular calcium concentration near the ribbon and the current pool state of the release pools. If the conditional expressions

$$\text{rand} \leq \text{rate}_{RP} \text{ and rand} \leq \text{rate}_{RRP}$$

with rand being Gaussian (0, 1) and

$$\text{rate}_{RP} = 1 - \exp(-\alpha_{RP} [Ca^{++}]_i R_P \Delta t)$$

and

$$\text{rate}_{RRP} = 1 - \exp(-\alpha_{RRP} [Ca^{++}]_i R_{RRP} \Delta t)$$

(6)
are true a vesicle is released either from the RP or the RRP. Thereby, \( \alpha_{\text{RP}} \) \((60 \text{vesicle M ms}^{-1})\) and \( \alpha_{\text{RRP}} \) \((5000 \text{vesicle M ms}^{-1})\) are the release rate constants of the RP and RRP, respectively, and \([\text{Ca}^{2+}]\) denotes the intracellular calcium concentration in the synaptic terminal. Again, RP, and RRP, are the current pool states. \( \Delta t \) stands for the simulation time step.

Initial pool sizes were fixed in all simulations and all pools were completely filled at the beginning of all simulations except otherwise stated. The fast pool of one ribbon had a capacity of 6 vesicles [44], the slow pool was 5 times larger (30 vesicles as determined in mouse, rat and goldfish; [39, 44–47] and the cytosplasmatic pool had a size of 360 vesicles. This factor \((10 \times (\text{RP + RRP}))\) was derived from the fact that in goldfish this pool has a size of 1000 vesicles per tethered vesicle but is suggested to be more than 100 fold smaller in mouse BCs [48–50], reviewed in [51]).

### 2.4. Electrode models

Two different approaches were used in order to compute extracellular potentials. An analytical solution of the potential distribution generated by a point source in a homogeneous surrounding is given by

\[
V_e(r) = \frac{I_{\text{sim}} \rho_{\text{ext}}}{4 \pi r}
\]

with \( I_{\text{sim}} \) being the applied current, \( \rho_{\text{ext}} \) being the electric resistivity of the surrounding medium and \( r \) being the Euclidean distance to the electrode. \( \rho_{\text{ext}} \) was set to \( 10^3 \Omega \cdot \text{cm} \) (see FEM model) in all simulations.

Additionally, finite element (FEM) computations were performed in COMSOL Multiphysics 4.4 (electric currents (ec) module, [https://comsol.com/]) to model stimulation with disc electrodes embedded in an isolated carrier. Computed potential distributions \( (V_e) \) were stored in .mat files and, if necessary, loaded by the framework. In the next step, the FEM solution was linearly interpolated at the compartment centers. For each electrode size only one run has to be performed since the resulting external potentials are linearly dependent on the applied electrode current and can therefore easily be scaled.

The FEM model had a size of \( 2000 \, \mu \text{m} \times 2000 \, \mu \text{m} \times 400 \, \mu \text{m} \) and consisted of three layers: the electrode carrier located subretinally in the region of degenerated photoreceptors (thickness \( = 100 \, \mu \text{m} \)), the retina (thickness \( = 200 \, \mu \text{m} \)) and the (replaced) vitreous at the epiretinal portion of the retina (thickness \( = 100 \, \mu \text{m} \)). The electrode was embedded into the electrode carrier and had a height of 5 \( \mu \text{m} \). Electrode diameter was varied from 50 to 200 \( \mu \text{m} \). The electrode carrier was electrically shielded to the retina. During monopolar stimulation (single disc electrode) the outer boundaries of the model were grounded. The electric resistivity of the four modeled volumes was set to the following values: carrier \( = 10^3 \Omega \cdot \text{cm} \), retina \( = 10^6 \Omega \cdot \text{cm} \), epiretinal volume \( = 10^8 \Omega \cdot \text{cm} \) and the electrode \( = 10^{-4} \Omega \cdot \text{cm} \). Since the carrier was electrically shielded to the retina its resistivity is not of importance. Retinal resistivity was chosen to be in the mid-range of previously reported values [52–54]. We assumed the vitreous at the epiretinal side to be replaced by silicone oil as reported in a clinical study [55] and therefore specified a high resistivity as suggested previously [29]. Electrode resistivity was in the range of resistivity of platinum. Aside from the four volumes describing the retina and its surrounding, an additional volume close to the electrode was incorporated which provided a fine mesh and therefore high quality of the computed results (not shown in figure 2).

### 3. Results

The purpose of this study was to analyze the response of BCs to electrical stimulation from the subretinal space. Time course of the membrane potentials as well as intracellular calcium concentration in terminal compartments were examined. Furthermore, synaptic release from ribbon synapses at BC terminals was computed.

#### 3.1. Cell-polarization during subretinal stimulation

BCs stimulated from the subretinal space are aligned perpendicular to the electrode and therefore are depolarized at their terminals and hyperpolarized at their dendrites when an anodic pulse is applied (figure 3(B)). Cathodic pulses, on the other hand, increase membrane potential at the dendritic end of the neuron and hyperpolarize the axonal and terminal compartments (figure 3(C)). The stimulus was applied via a point source electrode located 30 \( \mu \text{m} \) distant to the end of the dendritic tree in the subretinal space (figure 3(A)).

#### 3.2. Intracellular calcium concentration in synaptic terminals

In a next step it was investigated how the intracellular calcium concentration in BC terminals changes during subretinal stimulation. Intracellular calcium is known to be the crucial parameter for exocytosis at ribbon synapses and therefore for the functional outcome during electrical stimulation.

Using the presented dynamics (equations (2)–(4)) \([\text{Ca}^{2+}]\) in the terminals is determined by several parameters and two major physiologic mechanisms: (a) influx and outflow of calcium ions through calcium channels embedded into the cell membrane and (b) removal of calcium ions from the terminals via a passive sequestering process.

#### 3.2.1. Influx of calcium into terminals

The opening of calcium channels followed by an influx or outflow of calcium ions is mainly determined by the cell’s membrane potential and the time constant \( (\tau_c) \) of the channel state variable \( c \). Displaying steady state current densities at different clamp voltages results in an \( I-V \) curve which is depicted for the presented L-type calcium channel in figure 4. At membrane voltages close to the resting potential of \(-50 \text{mV} \) almost all calcium channels are closed. When the membrane is depolarized from its resting state calcium
channels open and lead to an influx of calcium ions into the intracellular space. The minimum (i.e. the negative peak of the $I-V$ curve) is located at approximately $-15$ to $-20$ mV and denotes the membrane voltages at which most calcium channels are open in steady state conditions (i.e. the channel time constant can be neglected). If this peak potential is exceeded the inward-directed (negative) calcium current is first weakened and further even changed into an outward current. This implies that stronger depolarization does not necessarily lead to a higher calcium concentration. As soon as the calcium reversal potential is exceeded (vertical dashed line in figure 4) the unwanted effect of outflowing calcium occurs. The inset in figure 4 confirms the close agreement between an experimentally determined [42] and modeled $I-V$ relationship.

3.2.2. Effect of calcium reversal potential. The following example of subretinal BC stimulation demonstrates the effect of reversed calcium current for stronger stimulation in one of its synaptic terminals. A BC was stimulated as in figure 3(A) with 1 ms pulses of 6 and 15 $\mu$A, respectively. As expected, the 15 $\mu$A pulse leads to stronger depolarization (thick) compared to the weak stimulus (thin) (figure 5(A), upper curves). Whereas the weaker pulse causes only inward calcium current shown as negative current (figure 5(A), lower curves, thin), the current resulting from the strong pulse
changes polarity as soon as the reversal potential is reached (thick, shaded regions). During repolarization the membrane potential traverses from the right (depolarized) to the left (resting potential) of the \(I-V\) curve and therefore also crosses through the minimum of the \(I-V\) curve. This opens a maximum number of calcium channels and generates the minima of the two current traces. Larger channel time constants thereby shift these minima further to the right because of the slower deactivation during repolarization (not shown).

Consequently, the resulting intracellular calcium concentration is higher for the weak pulse (figure 5(B)). The 6 \(\mu\)A pulse leads to an almost linear increase of calcium concentration during the pulse (thin). The strong pulse (thick) results in a dip in the calcium trace that arises from the weaker and further reversed calcium current (figure 5(B)). Therefore, stronger pulses do not necessarily lead to higher intracellular calcium concentrations. A summary of this characteristic is shown in figure 5(C). A linear increase of depolarization (thick) is coupled with a nonlinear relationship of intracellular calcium ion concentration (thin). After a peak at \(~6\mu\)A higher amplitudes weaken the efficacy of stimulation. Pulses with amplitudes higher than 15 \(\mu\)A do not elevate intracellular calcium significantly and are therefore not suitable to activate synaptic exocytosis.

3.3. Synaptic release

The impact of intracellular calcium concentration on discrete synaptic vesicle release was simulated with a four pool model (figure 1). A cytoplasmatic pool C fills the rapidly releasable

or fast pool RRP and the releasable or slow pool RP. These two pools release their tethered vesicles into the synaptic cleft (exocytosis pool E). The presented model does not incorporate any mechanism for endocytosis and calcium binding and unbinding. Only short-term simulations were performed and therefore no need for refill of the cytoplasmatic pool was needed.

Singer and Diamond [44] conducted an experimental study on vesicle depletion and synaptic depression in synapses between rod BCs and postsynaptic AII ACs in in vitro rat preparations. Presynaptic voltage clamps led to sustained vesicle release with a large transient and a small sustained component of the postsynaptic EPSC (see also [56–58]. Furthermore, voltage clamps for 100 ms resulted in prolonged calcium currents as expected from the non-inactivating L-type calcium current located in synaptic boutons. With seven vesicles they estimated the size of the fast pool.

To confirm the physiologic behavior of the presented synapse model the voltage clamp experiments by Singer and Diamond were mimicked (figure 6, figure 1(C) in [44]). The ON BC model neuron was clamped from its resting potential of \(-50\) mV to \(-20\) mV for a period of 100 ms (figure 6(A)). In this configuration no axial currents between neighboring compartments are evoked and therefore all compartments are in a virtual mono-compartment mode. During the clamp a constant calcium current in the range of measured currents (tens of pA in [44]) was evoked (not shown). Intracellular calcium concentration was elevated to a maximum of 30 \(\mu\)M and dropped back to its resting state after the clamp was removed (figure 6(B)). Also synaptic release fits experimental results properly (figure 6(C)). Total exocytosis is in the range of 100 vesicles during the 100 ms clamp (thick). The fast pool empties all vesicles within the first 20 ms (middle). The slow pool (thin) continuously releases vesicles during higher levels of \(Ca^{++}\) with a rate of approximately 0.5 vesicles ms\(^{-1}\) (dashed lines in figure 6(C)) as determined by Singer and Diamond [44]. Thus, the presented simulation exhibits the same major kinetic characteristics as measured results in in vitro preparations.

3.4. Implications on subretinal stimulation

3.4.1. Release and stimulus amplitude. The impact of stimulus amplitude on synaptic release was simulated for extracellular point source stimulation according to figure 3(A) for 2 ms pulses. Similar to intracellular calcium concentration (see figure 5) also synaptic release does not increase monotonically at higher amplitudes (figure 7(A)). At each stimulus amplitude (0.75 \(\mu\)A steps) 10 simulation runs were performed and the total release of 10 ribbons (=360 vesicles tethered in total) was monitored (asterisks). Simulation time was set to 30 ms to also monitor vesicle release after pulse offset due to sustained higher levels of \([Ca^{++}]\). The mean release of all 10 runs is depicted by the solid line. Below a stimulus strength of 1.5 \(\mu\)A no release is triggered. Maximum release is evoked at an electrode current of approximately
μA and release is switched off when amplitudes exceed 12 μA.

In order to determine robustness of the computed results pulse width and configuration was varied. First, shorter pulse durations in the range as applied in clinical applications (0.5–1 ms, e.g. [60]) and in vitro experiments (0.1 ms, e.g. [61]) were used. As shown in figure 7(A) a pulse duration of 0.5 ms (dashed) leads to the same release characteristics (lower and upper limit for exocytosis), however, the total number of release is decreased strongly. Single pulses <0.5 ms were not able to elicit synaptic release.

Charge balanced biphasic pulses (cathodic- and anodic-first) as used in clinical applications were also tested (figure 7(B)). No significant difference to monophasic stimulation was observed. The cathodic pulse in biphasic configuration did not influence calcium currents essentially because it only accelerated re- (and hyper)-polarization of the membrane without influencing intracellular \([Ca^{++}]\), distinctly. Consequently, synaptic release was not substantially affected.

### 3.4.2. Center-surround effect

An important implication of the relationship between synaptic release and stimulus amplitude is the artificial generation of an unexpected center-surround effect for stronger stimuli. Whereas during natural excitation a depolarization level close and above the reversal potential of the calcium channel will not be reached this might happen during electrical stimulation with subretinal implants.

In order to examine how release is influenced by cell location relative to a stimulating electrode 1681 ON BC positions were aligned on a \(41 \times 41 \times 5 \, \mu m\) grid in \(x-y\)
Two stimulus amplitudes were applied 10 times and the mean response (i.e. overall released vesicles) of all 1681 BCs was monitored. The first chosen amplitude was 6 μA which caused the strongest possible release for the cell closest to the electrode. Increased BC distance from the electrode resulted in weaker synaptic responses and BCs far away (>60 μm) from the electrode were not depolarized strong enough to initiate synaptic release (figure 8(A)).

Stronger pulses were shown to decrease synaptic activity because of a hyper-depolarization of the calcium channel. The outcome of this fact during stimulation of multiple BCs is a pronounced ring-shaped exocytosis pattern (center-off, surround-on), e.g. for 15 μA (figure 8(B)). Thereby, BCs closest to the stimulation electrode are not responding at all (no synaptic release) but BCs in a mid-range between approximately 20 and 90 μm released vesicles. Far distantly located BCs (>90 μm) again do not respond because of the decreased influence of the applied electric field.

Figure 9 summarizes the center-surround mechanism for a wide range of amplitudes. The mean number of released vesicles from all 1681 cells is plotted against stimulus amplitude.
amplitude (9(A)). The total number of initially tethered vesicles is 1681 \times 10 \times (30 + 6) = 605 160. Higher amplitudes lead to more neurotransmitter release and therefore are likely to generate stronger network activity, however, at locations which are not close to the electrode center. Release maps for the range of applied amplitudes show the transition from an increasing spot of exocytosis to a ring-shaped release pattern (9(B)).

3.4.3. Stimulation with disc electrodes. In order to get closer insights on synaptic release caused by retinal implants point source simulations were repeated with subretinal electrodes according to figure 2. In the first example stimulus amplitude was set to be most effective for BCs just below the electrode, i.e. to evoke maximum release. Three electrode diameters (50, 100 and 200 μm) were investigated. Synaptic release for BCs located along one axis through the middle of the electrode...
was computed (figure 10(A)). Stimulation with disc electrodes leads to release which peaks above the surface of the electrode and gradually drops towards zero outside of the edge of the electrode.

In a second series of simulations, the impact of stimulus strength on the center-surround effect was examined for the 50 μm diameter electrode. Qualitatively, the current reversal mechanism led to the same release patterns as demonstrated before by a point source. The almost constant release for BCs located inside the edge of the electrode, however, led to more abrupt (i.e. evoked by only small changes in stimulus amplitude) change between vesicle release in BCs close to the electrode and the ring-shaped exocytosis pattern (figure 10(B)). The center-off region typically had approximately the size of the electrode at amplitudes little above the reversal potential.

4. Discussion

The key finding of this work is that a computational model supports the hypothesis that subretinal stimulation strongly influences calcium channel dynamics and synaptic release in BC terminals. In consequence, surprising and unwanted effects might occur during stimulation with subretinal implants. Whereas too low stimulus amplitudes will not sufficiently activate calcium channels in BC terminals also high amplitudes prevent synaptic signal transmission due to reversed calcium currents.

4.1. Upper threshold for spikes and synaptic release

Current–voltage relationship, as well as evoked current traces during voltage clamp experiments, allows deriving mathematical descriptions of many types of ion channels. Some of the resulting implications, which can be made from these kinetics, however, were not elucidated thoroughly in the past. The consequences of current reversal is investigated only in a few studies and resulted in divergent conclusions [23–26]. Therefore, it is not clear yet if current reversal phenomena can be evoked during extracellular stimulation (i) at all, (ii) in exceptional cases, or (iii) regularly and estimated by simple rules.

Whereas the previously mentioned studies used neurons, which transduce information via all-or-nothing spikes to our knowledge, no former work was conducted about implications of reversed currents on synaptic transmission. Stimulating strategies used in current neural prostheses, which aim to trigger synaptic release in a controlled way, do not consider the current reversal phenomenon. As suggested by the presented results, however, synaptic release at BC terminals is strongly influenced by reversed calcium currents during stimulation with high amplitudes. Thus, the upper threshold for calcium current inflow and the presented center-surround

Figure 10. Stimulation with disc electrodes and center-surround effect—(A) Release from BCs aligned along the main axis of the electrodes (50, 100 and 200 μm in diameter) was monitored. Regions below (according to figure 2) the electrode surface show constant release which gradually decreases outside of the electrode edges as long as calcium current reversal is avoided. (B) Stimulation at sub- and supra-reversal amplitudes for a 50 μm electrode (black circle). Like for point source electrodes a center-surround pattern arises at strong stimuli. Because of the release pattern depicted in (A), however, the size of the center-off region has approximately the size of the electrode.
mechanism is expected to have implications on the generated phosphenes in patients when stimulated subretinally.

4.2. Cathodic versus anodic stimulation

The presented biophysical model (figure 3) explains why anodic polarity is capable of eliciting synaptic release in BCs when stimulated from the subretinal space: synaptic release is triggered by an increase of intracellular calcium which can only be initiated if terminal compartments are depolarized (figures 4 and 5). Cathodic stimulation, however, hyperpolarizes terminal compartments and, thus, does not lead to an influx of calcium ions. In two in vitro and in vivo studies a higher efficacy of anodic stimulation to activate the retinal network was also shown [62, 63]. The presented model only consists of BCs without getting inputs from ACs which modulate synaptic BC output [22]. From a modelers point of view, indirect GC excitation without photoreceptor input during cathodic stimulation is (as shown in experimental studies, e.g. [64]) possible through at least two mechanisms: (i) activation of all ACs connecting to ON BCs via gap junctions (excitation via rod pathway, ON GCs activated only) and (ii) activation of BCs due to more parallel oriented BC terminal regions.

4.3. Pulse parameters

This study uses a default monophasic pulse with a duration of 2 ms to present first insights into current reversal during electrical stimulation. Shorter pulses were tested too and resulted in decreased synaptic release. This resulted from a shorter period of inflowing calcium ions into the intracellular space and, therefore, to a lower level of [Ca\(^{2+}\)]. Current subretinal implants use pulse lengths in the range of 1 ms [60] which is on the long side of pulse durations in electrical neurostimulators. Another recent clinical study with an epiretinal implant also reports that longer pulses elicit the network stronger [65]. In sum, the presented model supports these findings that longer pulses are generally more suitable to generate synaptic (network) activity.

4.4. Calcium channel dynamics and vesicle release

Aside from the current reversal phenomenon also other dynamics of the L-type calcium channel determine the synaptic release during electrical stimulation. Whereas the current reversal is fully determined by the calcium reversal potential the temporal behavior (i.e. how fast a gating variable responds to changes in membrane voltage) of activation and deactivation of the channel are specified by the channel’s time constant \(\tau_c\).

Preliminary results (not presented) suggest that the time constant can be responsible for synaptic release despite stimulation in the current reversal regime (supra-reversal stimulation). If the current reversal occurs calcium flows out of the intracellular space and drops quickly to the residual level during the pulse. This happens for a wide range of time constants and does not lead to vesicular release. When the pulse is switched off, however, the time constant starts to play an important role. For small time constants the state variable \(c\) almost immediately drops back to its state at the holding potential (fast deactivation). This does not activate a calcium current sufficient to elevate \([Ca^{2+}]\) necessary for synaptic release. Larger time constants, on the other hand, lead to a slow progression of the state variable towards its resting state (slow deactivation) and therefore activate a sustained inward calcium current and consequently synaptic release. However, more simulations and comparisons to experimental data have to be made in order to state more concrete assertions.

4.5. Artificially evoked synaptic release and fading percepts

A clinical study reported that 8 out of 9 tested subjects experienced fading percepts during a stimulation of 10 s with an epiretinal prosthesis [66]. At stimulus onset, all patients reported well-localized and bright sensations, which faded away in a very short period of time. The authors concluded an activation of the retinal network to be the reason for this fading phenomenon. The presented calcium channel dynamics might be another explanation for fading sensations during prolonged electrical stimulation. If long and or repetitive strong pulses keep terminal membrane voltage at a constant high level close to the inward current peak of the calcium channel (−20 to −10 mV) the intracellular calcium concentration reaches a constant high level. This constant high level of \([Ca^{2+}]\) further leads to a fast but also sustained release of vesicles which can almost deplete both the RP and RRP within a short period of time, i.e. in the range of seconds. Vesicle depletion caused by too strong stimuli therefore can be one reason for fading percepts during electrical stimulation. This assumption is further supported by the fact that rectangular pulses lead to an unnatural depolarization process. By applying strong stimuli the membrane voltage almost immediately (<5 ms, figure 3) follows the time course of the pulse and therefore gets clamped from its resting potential to a certain voltage until the pulse is switched off. This artificial clamp condition has two important implications: (i) the intracellular calcium concentration will rise rapidly and (ii) therefore release (and consequently the GC EPSC) will have a large transient component. In natural vision, on the other hand, the response of the membrane voltage to light inputs can take up to 100 ms [67] and therefore also shows quite different release kinetics [57]. In sum, artificially evoked responses of the retinal network by electrically driven implants do not reflect natural excitation and therefore limitations in clinical outcome such as temporal fading of percepts might occur. Thus, the ion channel time constant, pulse shape, stimulus amplitude and frequency seem to be crucial parameters which should be systematically investigated in the future.

4.6. BC morphology and current reversal

This study investigated the response of a rat type 9 ON BC [31] to electrical stimulation from the subretinal space. BCs, however, can be classified by their morphology into multiple (∼10) subtypes in rat [31] and mouse [68]. Whereas ON BCs
stratify in layers closer to the inner retinal border OFF BCs have shorter axonal processes and therefore their stratification layers are closer to the outer retinal border. It was shown that the stratification level (i.e. the length of BCs), aside from other factors such as soma size and location and geometry of synaptic terminals, is the most dominant factor for cell depolarization [33]. ON BCs are stronger depolarized during anodal stimulation from the subretinal space than OFF BCs. Since depolarization of the membrane potential determines the calcium current reversal, stimulation for ON and OFF BCs significantly different patterns of synaptic release are expected. Because of their different depolarization characteristics the calcium current reversal will occur at lower stimulating amplitudes in ON than in OFF BCs.

Moreover, since the exact distance between the implant and its target neurons (BCs) is not the same for each electrode small differences in this distance can lead to large differences in depolarization of BCs. Furthermore, electric properties of the surrounding tissue are highly anisotropic and therefore a certain amplitude pulse might have different impact on different BCs.

4.7. AC activation

ACs are a diverse group (>25 subtypes) of laterally working second-order neurons in the retina [69]. ACs connect to GCs but also make inhibitory synapses on BC axons. Therefore, the responses of ACs during electrical stimulation of the retina also seem to be of high importance on clinical outcome. In a simplified picture, ACs are aligned in parallel to the stimulating element and therefore their activation can be estimated by the activating function (i.e. the curvature of the electric potentials along the neuron [27] and the electric field at the terminals [17]). Their synaptic activity during electrical stimulation is therefore also strongly influenced by the geometric alignment of synaptic terminals. Current depictions of ACs in the literature, however, mostly only show the general layout of ACs without examining terminal regions closely. Therefore, without more detailed knowledge of AC morphology, especially the alignment of the synaptic terminals, it is not possible to make general assertions on the activation of ACs during subretinal stimulation. Due to their larger distance (~50–100 μm, depending on stratification level in the inner plexiform layer and electrode placement) from the stimulating electrode, however, it is likely that ACs will not be activated strongly at amplitudes sufficient to activate synaptic release in BCs. Thus, the influence of ACs on artificially generated visual percepts may be rather small during subretinal stimulation.

4.8. Direct GC activation and current reversal

An experimental study investigated the suitability of direct activation of retinal GCs from the subretinal space [70]. In their results Tsai and coworkers, however, stated that direct stimulation of GCs was accompanied with unpredictable long-latency activity of the network. In order to compare our finding of the center-surround mechanism with these results spiking threshold for subretinal anodic stimulation of a model GC was determined (50 μm disc electrode, 2 ms pulse, data not shown). Activation thresholds varied between 3 and up to 11 μA (depending on electrode location) which is in a similar range as amplitudes that can evoke the current reversal phenomenon (see figure 10). Therefore, it is likely that direct stimulation of GCs from the subretinal space will correlate with supra-reversal stimulation of BCs and thus, because of the surround-ON behavior at high amplitudes, might generate visual sensations of low spatial and temporal accuracy.

4.9. Procedures to experimentally determine current reversal

Future experimental studies should evaluate the predicted calcium current reversal phenomena during subretinal stimulation. Oltedal and Hartveit previously showed the effect of current reversal in one of their figures, however, did not further investigate the underlying mechanisms [39]. Therefore, systematic investigations should clarify unknowns and further explore effects on synaptic release. By placing either a single stimulating electrode or a microelectrode-array in the space between retinal pigment epithelium and the outer plexiform layer and consequent stimulation of BCs the theoretically described mechanism can be elucidated. In order to determine the occurrence and effect of calcium reversal several experimental methods can be used: (i) measuring of calcium currents in synaptic terminals of BCs close to the stimulating element (e.g. [39]); (ii) observation of change in terminal intracellular calcium concentration with fluorescence calcium imaging (e.g. [43]); (iii) quantification of terminal [Ca++], with two-photon Ca++ imaging (e.g. [71]); and (iv) determination of synaptic release by measuring changes in cell membrane capacity [39].

We currently design a project to experimentally identify the proposed mechanisms.

4.10. Implications on other chemical synapses and neuroprosthetic devices

The calcium current reversal phenomenon is not limited to ribbon synapses in the retina but according to this study it is expected to occur in general at synapses in the close vicinity of microelectrodes when they are used to stimulate neural tissue extracellularly. Recently it was reported that intracortical microstimulation activates layer 5 pyramidal neurons mainly transsynaptically [72]. This observation seems to be in conflict with the generally accepted assumption that the axon and especially the axon initial segment and nodes of Ranvier are the most excitable parts of a neuron [16, 18, 19, 21, 73]. However, finding the most excitable region of a neuron is based on experiments where a microelectrode scans threshold current in constant distance, e.g. 50 μm, along the surface of a neuron. The threshold current for a 100 μs pulse from a microelectrode 50 μm above the axon initial segment of a pyramidal cell is about 17 μA [73]. During subthreshold stimulation of this target cell with a 15 μA pulse the same microelectrode can excite many tiny axons and dendrites which are densely packed around the tip of the electrode.
Whereas a small number of single pulses may result in a lack of temporal summation of excitatory and inhibitory postsynaptic potentials, repetitive stimulation trains have been shown to promote transsynaptic neural activation [74, 75]. The reduced excitation by calcium current reversal is therefore of high interest for such pulse trains where the synaptic excitation is expected in animal experiments or via neuroprostheses in medical applications.

4.11. Additional aspects/limitations

4.11.1. Synapse model. In order to keep the presented synapse model simple and because only short time periods were investigated no mechanism for endocytosis—re-uptake of released vesicles into the cytoplasmatic pool—was incorporated. Furthermore, binding and unbinding of calcium ions as suggested by other models [76–78] was neglected. The refill process of the fast pool is generally faster than of the slow pool, however, works in the range of several seconds for both pools (time constants between 4 s (RPP) and 8 s (RP) in rat [44] and goldfish [79–81]) and therefore has only small influence on the presented results. In different species an accelerated refill process was examined during prolonged calcium influx which was not included into the presented model (e.g. [44, 46, 82]).

4.11.2. Synaptic calcium concentration. The amount of free calcium in the intracellular space of synaptic terminals governs exocytosis in chemical synapses [83, 84]. Therefore, in the presented model \([\text{Ca}^{++}]\) is the most crucial parameter when simulating synaptic release. Several complex models were proposed in the past in order to estimate intracellular calcium concentrations in different cell types [85–87]. Such models also incorporate detailed descriptions of multiple subsections within a compartment, low- and high-affinity buffers, calcium-induced calcium release (CICR), Na–Ca exchangers and the Ca++ ATPase in order to describe calcium homeostasis. However, in order to reduce complexity and parameter space the simple approach proposed by Fohlmeister and coworkers [40] was used in this work. Additionally, a former study [29] stated that the complex model by Usui and the simple model by Fohlmeister result in similar calcium concentrations and time-courses.

Aside from the influx of calcium into the cell the other important process determining \([\text{Ca}^{++}]\) is the simple passive extrusion mechanism which is governed by only one time constant \(\tau_{\text{Ca}}\) and the calcium residual level. For a prolonged release of neurotransmitter from the ribbon synapse a sustained calcium level in the close proximity of the vesicles has to be maintained. In the case of pulses that short as applied in retinal implants (e.g. 1 ms) \([\text{Ca}^{++}]\) will be elevated during the pulse and will fall back to its resting level after pulse offset. Therefore, the \(\tau_{\text{Ca}}\) for removing calcium above the residual level from the intracellular space has strong implications on synaptic transmitter release during repetitive stimulation.

4.11.3. Action potentials in BCs. Recent studies changed the view on BCs and their signal transmission via graded potentials. In multiple animal models also transient membrane fluctuations resembling action potentials were found (e.g. [88–90]). These spikes are generated by either calcium or sodium channels and differ strongly in amplitude and shape. The arrival of a spike at the synaptic terminal is supposed to trigger an instantaneous release of all docked vesicles. This induced transient release is further followed by a short time of depression [91]. These new findings are of high importance not only from an electrophysiological point of view but also will strongly influence the functional outcome during electrical stimulation using retinal implants.

4.11.4. T-type calcium channels in mammalian BC terminals. Also T-type calcium channels were found in the mammalian retina [42, 92, 93]. These channels are activated at lower membrane voltages and show transient and smaller sustained components [56, 92, 93]. The role of T-type Ca++ channels in controlling and regulating synaptic release is still unclear and needs further investigation. Due to their stronger inactivation (in contrast to L-type channels) and subsequent more transient kinetics they are thought act as an initial booster for synaptic transmission [56]. Furthermore, as discussed previously (‘artificially evoked synaptic release and fading percepts’), the temporal kinetics of calcium influx also play an important role in synaptic release and therefore T-type channels might be responsible for the transmission of fine temporal details.

5. Conclusion
An analysis of synaptic release at BC terminals during subretinal stimulation revealed the crucial mechanism of current reversal. By utilizing multi-compartment models and a simple mechanism for synaptic exocytosis we were able to show that the activation of the retinal network is strongly influenced by the kinetics of calcium channels in BC terminals. Synaptic release does not monotonically increase with stimulus amplitude but the exocytosis patterns depend on pool states, time constants and the current reversal phenomenon. More quantitative data on calcium channel types and their presence in the >10 groups of BCs is needed to predict the impact of current reversal phenomena such as the center-surround effect on visual sensations elicited by electrical stimulation of the retina. Aside from affecting restoration of vision with (sub) retinal implants the calcium current reversal mechanism is also expected to strongly influence synaptic activity during the usage of other neuroprosthetic devices such as stimulators of the cortex.

Acknowledgments
This work was supported by the Austrian Science Fund (FWF), Grant No. P 27335-B23.
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