Two mathematical frameworks for vesicle release from a ribbon synapse of a retinal bipolar cell

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Abstract

Background: Bipolar cells communicate with amacrine and ganglion cells of the retina via both transient and sustained neurotransmitter release in ribbon synapses. Reconstructing the published quantitative release data from electrical soma stimulation (voltage clamp experiments) of rat rod bipolar cells were used to develop two simple models to predict the number of released vesicles as time series. In the experiment, the currents coming to the All amacrine cell originating from releasing vesicles from the rod bipolar cell were recorded using paired-recordings in whole-cell voltage-clamp method. One of the models is based directly on terminal transmembrane voltage, so-called 'modelV', whereas the temporally exacter modelCa includes changes of intracellular calcium concentrations at terminals. Results: The intracellular calcium concentration method replicates a 0.43-ms signal delay for the transient release to pulsatile stimulation as a consequence of calcium channel dynamics in the presynaptic membrane, while the modelV has no signal delay. Both models produce the quite similar results in low stimuli amplitudes. However, for large stimulation intensities that may be done during extracellular stimulations in retinal implants, the modelCa predicts that the reversal potential of calcium limits the number of transiently released vesicles. Adding sodium and potassium ion channels to the axon of the cell enable to study the impact of spikes on the transient release in BC ribbons. A spike elicited by somatic stimulation causes the rapid release of all vesicles that are available for transient release, while a non-spiking BC with a similar morphometry needs stronger stimuli for any transient vesicle release. During extracellular stimulation, there was almost no difference in transient release between the active and passive cells because in both cases the terminal membrane of the cell senses the same potentials originating from the microelectrode. An exception was found for long pulses when the spike has the possibility to generate a higher terminal voltage than the passive cell. Simulated periodic 5 Hz stimulation showed a reduced transient release of 3 vesicles per stimulus, which is a recovery effect. Conclusions: We presented two mathematical concepts for vesicle release in ribbon synapses and explained decreasing efficiency in retinal implants for suprathreshold stimulation.

Background

A synaptic ribbon is a protein structure that is perpendicular to the active zones (Sterling and Matthews 2005; Matthews and Fuchs, 2010; Schmitz, 2009) and is characterized by rows of connected vesicles on each of its two faces (Maxeiner et al. 2016; Singer and Diamond 2006). Ribbons have been found in hair cells of the cochlea and vestibular organ (Wersall et al. 1965; Smith and Sjostrand, 1961), retinal photoreceptors and bipolar cells (BCs) (Kidd 1962; Dowling and Boycott 1966; Sjostrand 1953, 1958), electroreceptors and lateral line receptors of fish and pinealocytes (Wachtel and Szamier 1966; Hama 1965; Vollrath and Huss 1973), as well as some neuromuscular junctions (Kosaka and Ikeda 1983; Katz et al. 1993). The shape of ribbons varies from planar in photoreceptors (Sterling and Matthews 2005), platelet in BCs (Graydon et al. 2014), spheroid in hair cells (tom Dieck and Brandstaetter 2006), to T-shape in drosophila photoreceptors (Prokop and Meinertzhagen 2006).

Three types of vesicles are present in axon terminals of the cells containing ribbons. There are (i) a few vesicles primed for fast release (< 10 ms, Singer and Diamond, 2003), making the rapidly (or readily) releasable pool (RRP). (ii) Vesicles are also tethered to the ribbon making the releasable pool (RP), and their population is larger than the vesicles in the RRP. The RP pool can be depleted over several hundreds of milliseconds. (iii) The freely diffusible vesicles in the terminal make up the cytoplasmic pool (CP), and their number reaches the thousands (Sterling and Matthews, 2005). Tethered vesicles cannot leave the ribbon to be added to CP, but they can fill the empty places in the RRP, if there are any. When a vesicle leaves the ribbon, a vesicle from the CP is replaced (Graydon et al. 2014).

Vesicles in the RRP are called 'docked'. Docked vesicles are connected via a protein linker to the cell membrane (Szule et al. 2012). The release of docked vesicles depends on the calcium concentration in the terminal. This intracellular calcium concentration increases when the terminal membrane depolarizes, which results in opening voltage-gated calcium channels, mainly L-types (Hartveit 1999; Pan 2000, 2001), thereby enabling calcium ions to enter the terminal. The calcium ions bind to the calcium-sensitive protein linker that is connected to the docked vesicles to cause neurotransmitter release into the synaptic cleft. Two types of vesicle release are defined according to their release rate. Vesicle release by a quick large depolarization of the terminal membrane is called 'transient vesicle release' and is responsible e.g. for contrast adaption (Oesch and Diamond 2011). In prolonged stimulation, transient release is replaced by sustained releases with a slower rate; leading e.g. to luminance adaption in the retina (Oesch and Diamond 2011). Continuous vesicle release from the ribbon in response to prolonged stimuli can be explained by RP pool dynamics (Heidelberger et al. 2005; Matthews and Fuchs 2010). Released neurotransmitters bind to the receptors located on the surface of the postsynaptic cells, which depolarizes or hyperpolarizes the postsynaptic cell by opening the ion channels close to the receptors (Pan 2001).

Rod BCs (RBC) receive inputs from rod photoreceptors (Dowling and Boycott 1966) and put their outputs on AII and A17 amacrine cells at their axon terminals (Famiglietti and Kolb 1975; Nelson and Kolb 1985). Paired-pulse voltage-clamp recording from a synaptically connected RBC and an amacrine cell allowed the quantification of vesicle release of the RBC from the excitatory postsynaptic current (EPSC) of the AII (Oesch and Diamond 2011). Several key parameters of the proposed models are based on this technique. In the experiment, the transmembrane voltage of a rat RBC increases from -70 mV to other potentials with a step voltage of 5 mV for one second followed by a second voltage step fixed at -20 mV (Fig 1A). The recorded EPSCs of the AII (Fig 1B) are closely related to the number of released vesicles from the RBC. When the transmembrane voltage of RBC changes from -70 mV to any value > -45 mV, a transient release of vesicles was observed in form of a current that entered the AII cell (Fig 1, dashed red window). The lack of such EPSCs in the AII during the changes in the voltage step from -70 mV to -55 or -50 mV means that no transient vesicle is released from the RBC. Transient vesicle releases are also seen when the transmembrane voltage of the RBC changes to -20 mV from other potentials (Fig. 1, dashed blue window) and the number of transiently released vesicles increased with the first voltage step. The RBC voltage change from -25 to -20 mV shows no transient release as the RRP becomes empty when the voltage of the RBC changes from -70 mV to -25 mV or the voltage changes from -25 mV to -20 mV is so small. No

transient release during the voltage change from -25 mV to -20 mV is also observable from Fig 3A-bottom in which the All-ESPC does not change during the process (the grayest line). Despite the transient release, sustained release is seen for each arbitrary voltage with a specific rate, and EPSCs between the dashed red and blue windows represent the sustained releases. The rates of sustained release depend on the terminal transmembrane voltage of the cell.

Here, the standard value for the pool size RRP is 10 (Graydon et al. 2014). Assuming a maximum of 10 vesicles in every RRP of an RBC ribbon and considering that the total size of the RRP equals 55 (Oesch and Diamond 2011), 5.5 ribbons in an RBC can be obtained as an average value. The maximum All EPSC amplitude of 300 pA according to the RBC voltage change from -70 to -25 mV (Fig 1B) corresponds to the release of all 55 RRP vesicles. According to the dashed red window (Fig 1B), the transient EPSCs are 0, 0, 58, 147, 209, 274, and 300 pA, respectively, when the RBC voltage changes from -70 mV to -55, -50, -45, -40, -35, -30, and -25 mV, which leads to a total transient vesicle release of 0, 0, 11, 27.5, 38.5, 49.5, and 55 for the relevant voltage shift. The transient release of several vesicles at a voltage step can be considered as a single event. By dividing the total transient vesicle release numbers by 5.5, the number of transiently released vesicles for a single ribbon becomes 0, 0, 2, 5, 7, 9, and 10, respectively, for the related transmembrane voltage change.

In the presented release and refill models, in addition to transient and sustained releases, there are two types of vesicle feeding: refill and recovery.

Because sodium channels in the membrane of amacrine and ganglion cells had been detected, these two types of neurons were for many years the only candidates to generate spikes in the retina; all BCs were considered to have passive membranes (no sodium channel on the membrane). The detection of sodium spikes in BCs (Cui and Pan 2008; Puthussery et al. 2013) as well as recent progress in retinal implants has led to questions such as 'what is the role of the spike on vesicle release in a BC?', 'How do spiking and non-spiking bipolar cells behave in an external electric field?', and 'Why does contrast detection gradually decrease in patients using retinal implants?'. To address such questions, in the following, we use the 3 dimensional morphology of a rat RBC and simulate this (passive) cell but with L-type calcium channels on the terminals plus a leak current on all compartments according to the experiment shown in Fig. 1. We present two vesicle release models for ribbon synapses. ModelV is based on terminal transmembrane voltage, and modelCa is based on the intracellular calcium concentration of the terminal, each of which is separately explained by two time-dependent equations. Additionally, a spiking RBC was simulated by using the same cell morphology in which some parts of the axon contain sodium and potassium channels according to the observed position of DB4 BC in a macaque's retina (Puthussery et al. 2013, see also Rattay et al. 2017). By comparing the responses of passive and active cells stimulated intracellularly, this type of stimulation is close to reality when BCs are stimulated through photoreceptors. Spikes were shown to empty the RRP and thus cause the high-amplitude transient release, whereas a cell with a passive membrane does not release the vesicle transiently in the same situation. The effect of extracellular stimulation generated by a single microelectrode on transient release, which is more similar to situations in retinal implants, was also tested. The test suggested no difference between active and

passive cells for small pulse durations because the BC terminal membrane senses the same potential in both cases (Rattay et al. 2018). However, for long pulses, spiking BCs exhibit more transient release because the generated spike would reach the terminal quickly, leading to a magnification of the membrane depolarization. The effect of periodic signals on RRP recovery when the cell is stimulated both intra- or extracellularly also suggested that the RRP recovers only for three vesicles at time intervals of 200 ms between two pulses.

Results

ModelV: A vesicle release and refill model based on terminal transmembrane voltage

According to the experiment in Fig 1, a simple release and refill model will be developed in the following based on terminal transmembrane voltage $V_T(t)$ as an input parameter. In addition to transient and sustained release, two temporal components are included: refill and recovery. Each of the terms is explained below in detail.

Transient release

Transient vesicle release refers to the number of vesicles from the pool RRP that are released rapidly upon depolarization. As noted in the introduction, an RBC voltage change from -70 mV to -55, -50, -45, -40, -35, -30, and -25 mV, respectively, leads to the release of 0, 0, 2, 5, 7, 9, and 10 transiently vesicles from a single ribbon in a time unit. Figure 2 shows a fitted step function to this data where the steps are defined as (see Equation 1 in the Supplemental Files)

where $\lfloor \cdot \rfloor$ is the floor function, which is a function that takes a real number X as an input and produces the greatest integer less than or equal to X as an output. This is necessary to bring an integer concept to the number of released vesicles.

The relationship showing the number of transiently released vesicles during a single time step when the transmembrane voltage of the terminal changes from V_1 to V_2 is simulated as (see Equation 2 in the Supplemental Files)

Sustained release

In comparison to transient release, sustained vesicles are released in essentially larger time intervals as stochastic events with specific rates (number/second). To find the rates for one ribbon, we redrew an item (Fig 3A) based on a previous work (Oesch and Diamond 2011, Fig 3c). Integration with the EPSC-time diagrams exhibits the amount of charges coming to the All cell that is proportional to the amount of released neurotransmitters. Rescaling the diagrams by $1/5.5$ in order to find the amount of charge originating from a single ribbon and once again by $1/0.36$ to make the size of the RRP equal 10 shows the cumulative vesicle release versus time from a single ribbon (Fig 3B). Figure 3B shows both the transient and sustained releases; transient releases occur rapidly after depolarization and vesicle release

continues afterwards with linear rates showing sustained releases. Thus, the slope of each diagram shows the average number of sustained released vesicles per second at each transmembrane voltage. Then, by fitting a function to the steady state of the $I_{ca}(V)$ diagram, the sustained vesicle release rate becomes a function of the terminal voltage. We preferred to explain the sustained release rate based on $I_{ca}(V)$ because vesicle release is stopped as soon as the terminal transmembrane voltage reaches the calcium Nernst potential or passes through that. In these states, the inward calcium current is replaced by outward calcium current and no calcium ions would be available in the terminal to bind to the vesicles and make them ready to be released (Werginz and Rattay 2016). According to (Fig 3C) and Eq. 24, the sustained release rate is (see Equations 3 and 4 in the Supplemental Files)

where c_{∞} is the steady state of the gate at voltage V , and 1.3 is a number with the dimension of the vesicle/(s.mV), which is used to obtain the sustained rates at voltage V for a standard ribbon with 10 vesicles in RRP at the initial point. α_c and β_c are explained in the Materials and Methods section.

To consider the stochastic release, an equally distributed random number between $(0,1000]/\Delta t$ is generated at each time step Δt , $\Delta t = 1$ ms, by $F_{Sustained}(t, V_T)$ compared to the related value in $Sus()$. If this number is less than the related number in $Sus(V_T)$, a vesicle is released from the pool; otherwise, it is not. The number 1000 is used to change the second dimension to milliseconds. The maximum release rate of 45 vesicles/s (Fig 3C) means that the average time between two releases is 22.22 ms. Thus, a time step appears to be appropriate. The sustained release is simulated as (see Equation 5 in the Supplemental Files)

Refill

The RRP should be fed via the same rates as the sustained releases because the rate of sustained vesicle release remains constant at each transmembrane voltage even when the pool is empty. The pool is empty when the transmembrane voltage reaches -25 mV from -70 mV and stays at -25 mV for one second; see Fig 1 and Fig 5. Thus, the same strategy as sustained release is applied for the refilling process. In the refilling process, a random number between $(0,1000]/\Delta t$ is generated in each time step and is then compared with the related number in $Sus(V_T)$. If the random number is less than the related number in $Sus(V)$, one vesicle is injected to the pool; otherwise, it is not. The refill process is explained by $F_{Refill}(t, V_T)$ as follows (see Equation 6 in the Supplemental Files)

Two series of random numbers, random1 and random2, are generated by $F_{Sustained}(t, V_T)$ and $F_{Refill}(t, V_T)$ for the same time step to consider the independence between the refill and release terms. Although any bell-shaped curve; like Poisson or Gaussian distribution, can also be used instead of the term $Sus(V_T)$, we preferred to use the term $Sus(V_T)$ as it depends on calcium current in each voltage as well as not existing experimental data for transmembrane voltages more than -20 mV. Note that because of L-type calcium ion channels included in vesicle release that remain open as far as the membrane depolarizes, sustained vesicle release should depend on kinetics of the channels or equivalently the calcium current.

Recovery

The time required for the RRP to become full again is called the 'recovery' time. The recovery time was also investigated in two paired-pulse experiments (Singer and Diamond 2006, Fig 5) where the RBC was stepped from -60 mV to +90 mV to make the RRP pool empty, then stepped again to -60 mV, causing recovery without any sustained release. Then for different time intervals, the cell was stepped again to +90 mV while the All EPSC was simultaneously measured. To find the recovery term, a function to the data from this double pulse experiment was fitted (Fig 4). The circles in Fig 4 show the replotted experimental data, and the solid line represents the modelled recovery time explained by the following equation: (see Equation 7 in the Supplementary Files)

where $\lfloor \cdot \rfloor$ is the floor function as previously explained.

A mathematical framework for the occupancy of the RRP

The equation explaining the occupancy of the RRP versus time is composed of four terms $F_{\text{Sustained}}(t, V_T)$, $F_{\text{Refill}}(t, V_T)$, $F_{\text{Transient}}(t, V_T)$, $F_{\text{Recovery}}(t, V_T)$, which respectively stand for sustained vesicle release from the RRP, vesicles coming to the pool, rapid (transient) vesicle release, and recovery. The equation is (see Equation 8 in the Supplementary Files)

The terms showing vesicle release from the pool are marked with negative signs, and the terms showing the entrance of vesicles to the pool are marked with positive signs. Pool occupancy versus time is shown in Fig 5A for the voltage protocol used in Fig 1A; here, the average of $N=10000$ trials was used to reduce the stochastic influence. When the RBC transmembrane voltage jumps from -70 mV to -55 mV and -50 mV, the pool remains full, jumping to -45 mV to make 20% of the pool empty; this becomes 50%, 80%, 90%, and 100% for voltage jumps to -40, -35, -30, and -25 mV, respectively. Afterwards, when the voltage reaches -20 mV, the pool again becomes empty. Fig 5B shows the recovery time of the pool. The pool becomes full in different time intervals depending on the previous occupancy state of the pool. In other words, the emptier the pool, the more time it needs to become full. For example, it takes only 48 ms to become full when the cell is stepped from -45 to -60 mV as the pool was 80% full. The other time intervals for the states of -40, -35, -30, and -25 mV to become full are 0.96, 2.9, 6.6, and 15 s, respectively.

As the refilling and sustained release terms obstruct each other's effect for trials (which is usually more than $N=1000$ trials), the number of transient vesicles released from the RRP is actually the result of the RRP occupancy change for when the change is negative, while a positive RRP occupancy change shows RRP recovery. Thus, the number of released vesicles (NRV) from the RRP at time t when the terminal membrane voltage V_T is governed by RRP occupancy changes and is added to the term showing sustained release: (See Equations 9 and 10 in the Supplementary Files)

where $\Delta\text{RRP}(t, V_T)$ stands for RRP occupancy changes at time t that is the average of $N=10000$ trials, and T_0 and t_0 stand for the time at which the RRP occupancy starts and stops decreasing, respectively. Note that when $\Delta\text{RRP}(t, V_T)$ is less than zero, some vesicles have left the pool; when it is positive, some vesicles

have been injected into the pool, which is the recovery process. Thus, the condition $\Delta RRP(t, V_T) < 0$ in Eq. 10 actually shows the number of transient released vesicles from the RRP by considering RRP occupancy, but it is not necessarily equal to $F_{Transient}(\Delta V_T)$. On the other hand, because the RRP occupancy has to be also considered for number of transient release vesicles, the term $\Delta RRP(t, V_T)$ shows number of transient released vesicles when the pool contains less vesicles than what $F_{Transient}(\Delta V_T)$ suggests. This phenomena is vital during periodic signals, see Fig. 13. It is obvious that $\Delta RRP(t, V_T)$ is equal to $F_{Transient}(\Delta V_T)$ if there are enough vesicles in the RRP, and $\Delta RRP(t, V_T) < F_{Transient}(\Delta V_T)$ if not. T_0 would be too small for voltage clamp experiments because the membrane voltage changes quickly in these experiments; however, T_0 would be larger for the cases in which the terminal membrane voltage depolarizes via spike or non-spike stimulations. $F_{Sustained}(t, V_T)$ is the sustained vesicle release term from the RRP explained by Eq. 5. Figure 6 shows the results of Eq. 9 for the protocol of Fig 1A using a time step and $T_0=1\text{ms}$. $T_0=5\text{ms}$ for the spike and terminal membrane depolarization presented in Fig 11 and Fig 13 because it takes 5 ms for the membrane to reach to its maximum amplitude, $T_0=7\text{ms}$ (Fig 12).

ModelCa: Vesicle release model based on intracellular calcium concentration

A possible step to improve the release model is to consider the calcium concentration in the terminal as the key parameter. Vesicle release depends on the degree of depolarization of the membrane of the axon terminals that control the voltage-gated calcium channels. In BCs, these are mainly L-type channels, located linearly parallel to the bottom surface of the ribbon. The intracellular calcium concentration $[Ca^{2+}]$ depends on the calcium current across the terminal membrane, the surface area and volume of the terminal (A, V), and the decay time constant τ (Fohlmeister et al. 1990). (see Equation 11 in the Supplementary Files)

where $F=96485.33$ is the Faraday constant, $[Ca]_0$ is the initial calcium concentration set to $0.34 \mu\text{M}$ and $\tau=10$ ms (Werginz and Rattay 2016); time step $dt=10 \mu\text{s}$ was used in solving this equation.

For terminal voltage < -60 mV, there is no calcium current flow, but the onset of depolarization causes an inward calcium current flow (Fig 7A and B). The inward calcium current eventually reaches a maximum if the terminal transmembrane voltage remains constant. An increment in the calcium current results in an increased intracellular calcium concentration to a steady state value (maximum value) (Fig 7C and Eq. 11). Thus, a specific intracellular calcium concentration value corresponds to each transmembrane voltage. By finding the maximum value of the calcium concentration for each transmembrane voltage, the corresponding sustained release rate is easily obtained by using Fig 3C and can be fitted (Fig 8) as (see Equation 12 in the Supplementary Files)

where 20.14 has a dimension of μM , 1 and 0.84 are dimensionless numbers, and 57.11 has the dimension of the vesicle/s.

The stochastic release process (sustained release vesicle) can be simulated as (See Equation 13 in the Supplemental Files)

where $F_{\text{sustained}}(t, [\text{Ca}^{2+}])$ works like $F_{\text{sustained}}(t, V_T)$. In other words, a random integer number between $(0, 1000]/dt$, $dt=0.01$ ms is generated at each time step by $F_{\text{sustained}}(t, [\text{Ca}^{2+}])$. All of the numbers in the interval have the same probability to be chosen compared to the related value in $\text{Sus}([\text{Ca}^{2+}])$; if this number is less than the related number in $\text{Sus}([\text{Ca}^{2+}])$, one vesicle is released from the pool, otherwise, it is not.

The refilling process can be calculated with the same algorithm as sustained release: (see Equation 14 in the Supplemental Files)

Again, to show the independence of the randomly generated numbers in $F_{\text{sustained}}(t, [\text{Ca}^{2+}])$ and $F_{\text{Refill}}(t, [\text{Ca}^{2+}])$, we show them as random1 and random2.

The recovery process is simulated with the same formalism as previously: (See Equation 15 in the Supplemental Files)

By calculating the time derivation of the intracellular calcium concentration (Fig 7D), a criterion for transient vesicle release appears. The local maximum points of intracellular calcium concentration changes would correspond to the number of transient vesicles released for any change in terminal voltage (Fig 7E and using Fig 2). For example, when the terminal voltage changes from -70 mV to -45 mV (see Fig 2), two transient vesicles are released. Thus, two-vesicle release is equivalent to $0.004 \mu\text{M}\cdot\text{s}^{-1}$; see the red parts in Fig 7. The transient release is explained by (see Equation 16 in the Supplemental Files)

where 16.2 and 16.39 have the dimension of $\text{Vesicle}\cdot\text{s}^{-1}$, and 1 and 0.75 have no dimension. 27.02 is a normalizing number with a dimension of $\text{s}\cdot\mu\text{M}^{-1}$. $\lfloor \cdot \rfloor$ is the floor function as previously discussed. To find the transient release versus time, the times in which the local maximum points take place ($T_1, T_2, T_3, \dots$) are extracted at first; these points are the roots of the second time derivation of the intracellular calcium concentration and show the times in which the transient releases take place. Using the indicator function and other terms already explained, the RRP occupancy reads as (see Equation 17 in the Supplemental Files)

where T is a set containing the times at which the local maximum points of the time derivation of the intracellular calcium concentration take place. Time step $dt=10 \mu\text{s}$ was used to solve this equation.

The number of released vesicles at time t will be governed by solving (see Equations 18 and 19 in the Supplemental Files)

Although we used special kinetics for calcium ion channels, any type of kinetics can be used without affecting the generality of the problem. Fig 9 shows the experimental data versus the data calculated by these equations.

One of the differences between modelCa, Eq. 18, and modelV, Eq. 9, is the signal delay, i.e., the time interval between stimulation of the bipolar cell and the release of the first vesicle. The modelV has no

signal delay, while the modelCa has a maximum signal delay of 0.43 ms, similar to other works that suggest millisecond delay (Baden et al. 2011, Singer and Diamond 2003; see discussion) (Fig 10). Figure 10 shows the first transiently released vesicles versus time when the terminal membrane changes from -70 to other potentials with a step voltage of 5 mV in a voltage clamped experiment. Transiently released vesicles have a time delay of 0.27, 0.34, 0.40, 0.43, and 0.43 ms when the transmembrane voltage changes from -70 mV to -45, -40, -35, -30, and -25 mV, respectively.

To compare vesicle release in spiking and non-spiking BCs, two cells with the same geometry are considered in extracellular stimulation because the geometry of the cells defines the shape and amplitude of the potential exerted to the compartments (Rattay et al. 2018). Thus, an imaginary spiking BC with the same shape was made by adding sodium and potassium channels to the axon. When the two spiking and non-spiking BCs are stimulated intracellularly with a 100 ms rectangular pulse of 500 pA, the terminal transmembrane voltage of the non-spiking BCs reaches -48 mV, leading to no transient vesicle release; however, the voltage membrane of the terminal in spiking BCs passes through -10 mV, which means that the terminal depolarizes for 50 mV, leading to the release of all 10 vesicles existing in the pool (Fig 11). Both models in Eq. 9 and 18 produced the same results. Thus, spikes make the RRP empty. Here, sustained release is not of interest because the pulse duration is not long. In average three sustained vesicles are released during a 100 ms pulse stimulation in spiking BCs, while this number is less than one in the cell with a passive membrane.

When both spiking and non-spiking bipolar cells are stimulated extracellularly with small pulse amplitudes, between 0.9-3 μ A and a long pulse duration of 10 ms (Fig 12), the number of transiently released vesicles increases linearly versus the pulse amplitude for the non-spiking cell while it increases exponentially in the spiking cell because the spike has sufficient time to reach the terminal and make the terminal more depolarized. Both cells with active and passive membranes have the same behavior for short pulse durations, e.g. 3 ms, since the terminal in both cells senses the same voltage originating from the microelectrode and the spike does not have sufficient time to reach to the terminal. The major difference between the two models appears when the pulse amplitudes pass through 4 μ A; the number of transiently released vesicles starts decreasing in modelCa but remains constant in modelV (Fig 12). The reduction in the number of transiently released vesicles for large pulse amplitudes is because of the outward calcium currents that take place when the terminal transmembrane voltage depolarizes more than $E_{Ca} = -20$ mV.

Figure 13 shows the responses of a spiking BC to pulse trains. The spiking BC is stimulated with a pulse amplitude of 1500 pA and 3 μ A in intra- and extracellular stimulations, respectively, with a pulse duration of 1 ms followed by a 200 ms interval (5 Hz stimulation). Spike amplitudes are 52, 45 mV in intra- and extracellular stimulations, respectively. In both cases all of the 10 vesicles existing in the pool are released by the first pulse and 3 vesicles by the following pulses in the recovery time of 200 ms (Fig 13).

Discussion

The available BC experimental data show that the largest variations are seen between fish and mammals. In goldfish, the MB1 BC contains 50 synaptic ribbons (von Gersdorff and Matthews 1996; Neves and Lagnado 1999) in which around 110 vesicles are connected to each ribbon (Lagnado et al. 1996; von Gersdorff and Matthews 1996) and 22 of the 110 vesicles are readily releasable (von Gersdorff and Matthews 1996; Neves and Lagnado 1999). The average diameter of the vesicles in Mb1 is 36 nm (Lagnado et al. 1996). Different recovery time constants have been reported for these cells ranging from 4 s (Mennerick and Matthews 1996) to 11.8 s (Palmer et al. 2003). Rat RBC, which was the main object of this study, contains 36 synaptic ribbons (Singer et al. 2004), while each ribbon contains 22-48 vesicles according to a recent experiment (Graydon et al. 2014), which is in correspondence to the previous experiment showing 35 vesicles per ribbon (Singer and Diamond 2006). The vesicle diameter in the rat RBC is 38 nm (Graydon et al. 2014) and is close to the vesicle diameter in Mb1. The size of the RRP in rat RBC has been reported as 7 (vesicles) using computational methods (Singer and Diamond 2006) and from 5.7 to 11.9 in experiments (Graydon et al. 2014). The reported recovery time constant in the rat RBC is 3.9 s (Singer and Diamond 2006). Different numbers have been reported for the number of ribbons in the mouse RBC; the minimum number shows 23 ribbons (LoGiudice et al. 2008), while the maximum is 46 (Tsukamoto et al. 2001). Another reported value, 34, can be considered as an average (Wan et al. 2008). The average vesicle diameter in mouse RBC is 33 nm (LoGiudice et al. 2009), although other numbers have been reported, such as 29 to 35 nm (Spiwoks-Becker et al. 2001). The number of vesicles tethered to each ribbon in mouse RBC is 12-35 (Wan et al. 2008), while 8 (Wan et al. 2008) or 10 (Wan et al. 2010) vesicles make the RRP. The recovery time constant in mouse RBC is 417 ms (Wan et al. 2008). Cb2 BC in ground squirrels has a time constant of 70-140 ms (Light and DeVries 2007; Grabner et al. 2016), while this number for the Cb3 BC is 524-888 ms (Light and DeVries 2007; Grabner et al. 2016) and for the Cb1 is 482-734 ms (Grabner et al. 2016). To our knowledge, the recovery time constant of BCs in rabbit and monkey have not yet been investigated. Diameter of vesicles are of interest in different animals because it may affect size of RRP and time constant of releasing.

The key data of the presented two models are based on experiments by Diamond and coworkers who estimated the transient and sustained release from the postsynaptic currents of rat RBC (Oesch and Diamond 2011, Singer and Diamond 2003). In modelV, the release and refill processes are directly related with the transmembrane voltage of the terminal, while in modelCa the intracellular calcium concentration of the terminal is the key variable. Furthermore, both models were combined with a multi-compartment model to demonstrate characteristic differences in vesicle release when spikes or graded potentials are the driving forces. Each model is made of four terms explaining the refill, recovery, transient release, and sustained release. The calcium ion channel gating in the presynaptic membrane causes modelCa to respond with a time delay of 0.43 ms for the transient release when the largest voltage steps are applied (Fig 10), while in modelV, this signal delay is missed. The other priority of modelCa in proportion to modelV is the calcium reversal potential that is not included in modelV and so modelV predicts wrong behavior of the ribbon during large extracellular stimulation amplitudes (Fig 12, see also Werginz and Rattay 2016). Although it is impossible to measure the signal delay with a less precision of 10 ms, the

number 0.43 ms implies on fast opening of the L-type calcium channels. According to experiments by Singer and Diamond (2003, Fig 2), it needs around 2.3 ms after stimulation of BCs to see the EPSC in synaptically connected amacrine cells. In addition to the ion channel gating times of the pre- and postsynaptic membrane, the recorded 2.3 ms delay includes several other time-consuming processes. Calcium influx also originates from T-type calcium channels in RBCs (Pan et al. 2001) when the cells depolarize from -100 mV (Singer and Diamond 2003); therefore these type of channels are called low voltage-activated channels. As the transient release is definitely dominated by the kinetics of L-type calcium channels (Singer and Diamond 2003) as well as we did not investigate vesicle release in hyperpolarized conditions, we did not take T-type channels into consideration in the modelCa.

Although other modeling works on the synaptic refill and release on BCs are available, (e.g., Jarsky et al. 2011, Sikora et al. 2005, Werginz and Rattay 2016), to our knowledge this is the first mathematical framework explaining transient and sustained releases together with a refill and recovery term. Oesch and Diamond (2011) also presented a pure computational model for vesicle release, but their model does not include the recovery process, F_{Recovery} , so can not be used for periodic stimulations. There is also an unpublished work based on Bayesian inference scheme for a stochastic model of glutamate release at the ribbon synapse using totally different scheme (Schroeder et al. 2019). Presenting ModelV was crucial since the related experiments were based on the membrane voltage. In addition, as most recent simulation works related to the topic were mainly based on membrane voltage (Oesch and Diamond 2011, Jarsky et al. 2011, Graydon et al. 2014) as well as to see the differences between the models generated by membrane voltage and intracellular calcium concentration, we needed to take the models into consideration.

Although both $F_{\text{Refill}}(t, V_T)$ and $F_{\text{Recovery}}(t, V_T)$ terms imply on the same phenomena in which the RRP is fed from the ribbon and both terms can be written in a single term, we preferred to use the two different terms because of the recovery process during periodic stimulations. In the periodic signals with big enough pulse amplitude; 1500 pA and 3 μ A respectively in intra- and extracellular stimulations; Fig. 13, the membrane voltage reaches -25 mV or more leading to make the RRP empty and then the membrane voltage comes back to its resting value (-60 mV) for 200 ms. During the process, if only the recovery process from the term $F_{\text{Refill}}(t, V_T)$ is considered according to Fig. 3C, the RRP recovery will be recovered for < 1 vesicle in 200 ms in average, and so there should be no other transient release for the next signals. But, 4 vesicles are actually injected to the pool in less than 120 ms according to Fig. 4. Note, both terms can be written as a single term since the term $F_{\text{Refill}}(t, V_T)$ implies on RRP recovery when the membrane potential is > -60 mV and the other term $F_{\text{Recovery}}(t, V_T)$ refers to the states when the membrane potential is < -60 mV. The other issue of writing the recovery terms separately refers to the number -60 mV because of lacking of experimental data; like what has been done in Fig. 4, for potentials more than -60 mV. For example, when the membrane potential changes from -70 to -25 mV (to make the RRP empty), then jumps to -40 mV in different time intervals and then comes back to -25 mV again. Thus, as we were sure for potentials < -60mV, we added the term $F_{\text{Recovery}}(t, V_T)$ from one experiment and calculated the average values as a refilling term, $F_{\text{Refill}}(t, V_T)$, from the other experiment. It is obvious that whenever the

experiment of Fig. 4 was done for other membrane potentials, the term $F_{\text{Refill}}(t, V_T)$ can be eliminated and the term $F_{\text{Recovery}}(t, V_T)$ would be expanded in more precious details.

To simulate vesicle release from a spiking BC, sodium and potassium ion channels were added to the axon, similar to those found in a Db4 BC in the macaque retina (Puthussery et al. 2013). Unfortunately, position of sodium channels is rarely determined in different cells since it is hard to be detected. But the cited paper has determined the exact position of the channels, thus we prefer to put the channels to the same position. Tough, both kinetics and position of the channels can be replaced by any arbitrary choice without changing the generality of the issue.

The intracellular stimulation of both spiking and non-spiking BCs suggests that for small pulse amplitudes, only spiking BCs are releasing transient vesicles (Fig 11, Puthussery et al. 2013). Intracellular stimulation is similar to the natural situation in which BCs receive input from photoreceptors. Here, BCs were also stimulated extracellularly, which is similar to the stimulation via electrodes of a subretinal implant (Chuang et al. 2014; Resatz and Rattay 2003; Werginz et al. 2015). The extracellular stimulation has some different features in proportion to the intracellular one in both passive and active membranes. The effect of extracellular stimulation on transient release almost suggests no difference between the responses of the spiking and non-spiking BCs in short pulses because the terminal membrane of the cells in both cases senses the same potentials originating from the microelectrode. However, spiking BCs release more transient vesicles compared to non-spiking BCs when stimulated with pulses long enough to profit from the amplification of the spike. In addition to the signal delay, a difference between modelCa and modelV is their number of transiently released vesicles; this decreases in modelCa for large pulse amplitudes but remains constant in the other model. This effect originates from exceeding the calcium reversal potential (Fig 12). To our knowledge, there is no experiment explaining behavior of ribbon synapse in spiking bipolar cells except for the work qualitatively suggesting release of total number of vesicles of the RRP (Baden et al. 2013). This phenomenon was shown by both modelV and modelCa quantitatively.

One of the deficiencies of retinal implants is the eventual reduction in contrast detection. We tested this issue by investigating the effect of extracellular periodic stimulation for inter-pulse intervals of 200 ms. For such pulse trains, only three transient vesicles are released from a single ribbon (Fig 13), which could explain the efficiency reduction of retinal implant types that stimulate primarily BCs and not the axons of retinal ganglion cells. Thus, for subretinal implants, longer pulses with low amplitudes may have several advantages. First, according to intracellular simulations (which is closer to the natural situations), a passive BC is not able to release transient vesicles totally (Fig 11), and only some spiking BCs are the candidates to release transient vesicles in the retina. Second, we showed that extracellular stimulation has a direct effect on transient vesicle release independent of whether the cell is spiking or non-spiking, which originates from its direct effect on terminal voltage change. Finally, when the amplitude of a pulse decreases but its duration increases, a spike is generated in the cells.

Conclusion

The synaptic output of retinal bipolar neurons has two components, i.e., a transient and a sustained release, which is uncommon for chemical synapses. This uniqueness originates from the existence of an extra protein structure called 'ribbon'. Transient outputs take place immediately after a large enough stimulus, e.g. after an action potential, while sustained outputs occur at any state with a different rate. We presented two mathematical frameworks for the cells' output to study the general behavior of ribbon synapses in different states. In addition, the models are applicable on retinal prostheses where arrays of microelectrodes enable the blind to restore vision. We show that increasing the pulse duration might reduce the efficiency of the prosthesis although it is supposed that pulse increments may lead to more intense percepts.

Materials And Methods

Morphology

RBCs are the simplest BC types concerning their poorly branched dendritic tree of usually not more than three terminal branches. These features simplified the simulation of the 3D morphology of a rat RBC following its previous 2D depiction (Oltedal et al. 2009) with good accuracy using Encke's method. In this method, the positions and local diameters of the neuron parts are determined from the 2D image, and the missing coordinates of the third dimension are estimated through a normally distributed random function (Encke et al. 2013) (Fig 14). After reconstruction, the cell has a total surface area of $525 \mu\text{m}^2$, of which the dendrite, soma, and axon + terminal make up 100, 254, and $170 \mu\text{m}^2$, respectively, the same as the amounts previously presented (Oltedal et al. 2009).

Compartment model

Response of the cell to intra- or extracellular stimulation is computed using a multi-compartment model concept (Rattay 1999), Eq. 20. The compartments are simulated as cylinders, and their interactions with neighbors and the extracellular space are governed by equivalent electrical circuits representing the electrophysiological properties of the cell. *The compartments* connect to their neighbors by their axial resistances. (see Equation 20 in the Supplemental Files)

where V_n , R_n , and C_n stand for transmembrane voltage, axial resistance and membrane capacitance of the n th compartment, respectively. The axial resistance R of a compartment with length L , cross section area A , and specific axial resistivity ρ_i is $R = \rho_i L / A$. Specific axial resistivity and membrane capacitance were fixed at $132 \Omega\text{cm}$ and $1.18 \mu\text{F}/\text{cm}^2$, respectively, according to previous work (Oltedal et al. 2009). The term i_{ion} represents the transmembrane currents according to the Hodgkin–Huxley formalism (Hodgkin Huxley 1952). $i_{intra-stim,n}$ is the current injected to the n -th compartment in case of intracellular stimulation, whereas this term is replaced by Eq. 21 when the cell is stimulated extracellularly (see Equation 21 in the Supplemental Files)

where V_n is the extracellularly applied voltage at the n-th compartment calculated via (see Equation 22 in the Supplemental Files)

where I is the applied current to the electrode, ρ is the electric resistivity of the surrounding medium, and r is the distance from the center of each compartment to the electrode. A backward Euler method with time steps of 10 μ s was used to solve Eq. 20.

RBC, passive membrane

Although the RBC contains a variety of ion channels such as potassium (Hu and Pan 2002), hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels (Ma et al. 2003), and L-type calcium channels (Oltedal et al. 2007), the presented BC membrane dynamics are simplified to a minimum that only contains calcium channels (in the terminals) and a leak current. Calcium channels were taken into account because they are used to compute the intracellular calcium concentration of the terminals that is mandatory to explain vesicle release. The potassium and HCN channels were also shown to have no significant influence on the membrane potential of the terminals (Benav 2012).

The leakage current existing on all compartments of the cell is (see Equation 23 in the Supplemental Files)

where $G_m = 1.0 \text{ mS/cm}^2$ is the maximum membrane conductivity and $V_m = -60.05 \text{ mV}$ is the reversal potential.

L-type Ca channels

The kinetics of the calcium ion channel, which are located at the terminals, are determined with the kinetics previously presented (Werginz and Rattay 2016). The maximum conductivity of the calcium channels, G_{Ca} , was considered to be 2 mS/cm^2 for the terminal and 0 for the non-terminal compartments (Werginz and Rattay 2016). As calcium channels are also conductive for potassium (Lee and Tsien 1982) with $E_K = -60 - -90 \text{ mV}$ as well as barium ions (Catterall et al. 2005) with $E_{Ba} = 40 - 60 \text{ mV}$, should be in the range of 10-40 mV. According to the I-V diagram in Werginz and Rattay (2016), $E_{Ca} = 20 \text{ mV}$ was used in (see Equation 24 in the Supplemental Files)

where μ A, mV and ms are respectively units for current, voltage, and time. c is the gating variable (open probability) that changes over time calculated via (See Equations 25 through 27 in the Supplemental Files)

where α and β are rate coefficients for opening and closing the ion channel.

Active BC

In addition to L-type calcium channels and the leaky membrane, the active BC also contains sodium and potassium channels located on the axon with maximum conductivities G_{Na} and G_{K} . Sodium and potassium currents are (Sterratt et al. 2011) (see Equations 28-38 in the Supplemental Files)

where α and β are the rate coefficients for opening and closing the ion channel, $E_{Na}=50\text{mV}$, $E_K=-70\text{mV}$. Both passive and active cells have the same morphologies.

The resting potential of the BC in both the passive and active cases was -60 mV (Veruki et al. 2006), which is similar to those of previous studies (-60 mV (Thoreson 2000), -53 mV (Benav 2012)).

Abbreviations

BC: bipolar cell, RBC: rod bipolar cell, ms: millisecond, s: second, V: voltage, EPSC: excitatory postsynaptic current, V_t : transmembrane voltage of the terminal, RRP: rapidly (or readily) releasable pool, RP: releasable pool, CP: cytoplasmic pool

Declarations

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Author Contributions

HB and FR conceived the studies and analyzed data, HB prepared figures and wrote the main manuscript, FR review and edited the final manuscript.

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Data availability

All relevant data are within the manuscript and at <http://www.doi.org/10.6084/m9.figshare.7961087>

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Vesicle release from a rat RBC visualized in a synaptically coupled RBC-All pairs experiment. RBC is maintained at -70 mV and is then stepped to specific voltages. This ranges from -55 mV to -25 mV for 1 s with a voltage step of 5 mV, which then jumps to -20 mV lasting also for 1 s (A), while the EPSCs of the All cell are recorded simultaneously (B). Thus, the amplitudes of All EPSCs are proportional to the number of vesicles released from the RBC. Any sufficiently large change in the transmembrane voltage of RBC causes a rapid response in the All cells, showing a transient vesicle release, as highlighted in the dashed red and blue windows. Sustained vesicle releases also are low amplitude EPSCs taking place at any transmembrane voltage with a specific rate. The strongest amplitude of 300 pA corresponds to the total depletion of all previously full RRP's containing 55 vesicles in total. This case (lowest line in B) corresponds to a first step to -25 mV and is without a second transient response for the step to -20 mV. The diagram was replotted from (Oesch and Diamond 2011, Fig 3a).

Figure 2

Number of transiently released vesicles from a single ribbon in a voltage change from -70 mV. Circles represent experimental data of Fig 1B, and the solid line is the fitted function, Eq. 1, showing each voltage step that causes one additional vesicle to be released.

Figure 3

The rate of charge coming to the All cell represents a criterion for sustained vesicle release. A) amount of charge coming to the All cell versus time. The (bottom) diagram was replotted from a previous work (Oesch and Diamond 2011, Fig 3c) and was obtained by integrating All EPSC traces from Fig 1B. B) cumulative vesicle release versus time from a single ribbon. The slope of each diagram, θ , defines the average number of sustained released vesicles per second for a standard ribbon with 10 vesicles in RRP initially. The voltage protocols shown at the top of A and B represent the voltages at which the RBC is clamped according to Fig 1A. C) Circles correspond to the slopes of the lines in B using the same gray intensity, and the solid line is the fitted function based on Eq. 3.

Figure 4

Recovery time of the RRP based on a double pulse experiment. The transient response of the second pulse (not shown in the stimulus) depletes the pool again and thus the corresponding number of released vesicles defines the number of vesicles in RRP during recovery. Black circles show the number of incoming vesicles until the second pulse is applied (only some normalized points were extracted from Singer and Diamond 2006, Fig 5). The solid line is the fitted function plotted by $F_{\text{Recovery}}(t, VT)$. Although ~40% of the RRP is recovered during only a 120-ms time interval, it takes ~15 s for complete refilling.

Figure 5

Pool occupancy continuously varies with the changing transmembrane voltage of RBC. A) Schematic of the voltage protocol used in the paired-pulse recordings in Fig 1A (top) and the related RRP occupancy versus time (bottom). B) Schematic of the voltage protocol used in A except for being stepped to -60 mV instead of -20 mV (top) and the related RRP occupancy versus time (bottom). The RRP starts being recovered as soon as the terminal transmembrane voltage reaches its resting membrane potential, -60 mV, in different time intervals according to the previous state of pool occupancy.

Figure 6

Comparison of coupled RBC-All paired-pulse experiment of Fig 1 and number of released vesicles simulated for a single ribbon. Transient and sustained releases show the same characteristics, e.g., the weakest pulse (darkest, black) causes a single released vesicle (sustained) and no transient release, but all 10 vesicles of the pool are transiently released at the beginning of the second strong stimulus, -20 mV.

Figure 7

The intracellular calcium concentration changes as a criterion for transient release. A) voltage clamped stimulus is increased 2-fold for 25 mV. B) The L-type calcium current, shown with a positive sign, is much larger for the second step, which is a consequence of the gating variable c in Eq. 24. C) The intracellular calcium concentration with an initial intracellular calcium concentration of $0.34 \mu M$ calculated by Eq. 11. D) The intracellular calcium concentration changes versus time, i.e., the time derivation of C. E) The

number of transiently released vesicles versus local maximum points of D (circles) and a fitted function obtained by Eq. 16. According to Fig 1B, a transmembrane voltage change from -70 mV to -45 m causes the release of 2 transient vesicles; the maximum calcium concentration change during the same voltage change is 0.004 $\mu\text{M}/\text{ms}$, which is shown with red circles in D and C. The dashed red line in E explains that 0.004 $\mu\text{M}/\text{ms}$ would release two vesicles.

Figure 8

Rate of sustained released vesicles as a function of the intracellular calcium concentration. The steady state intracellular calcium concentration as in Fig 7C is fitted to the experimental data of Fig 3C. In other words, any steady state intracellular calcium concentration refers to a specific membrane voltage, Fig. 7C, and as magnitude of average number of sustained released vesicles in any membrane voltage is also obtainable from Fig. 3C, the relation between the steady state intracellular calcium concentrations and average number of sustained released vesicles would be calculated by equalling the two terms. The circles show the relationship. The solid line is the fitted function according to Eq. 12.

Figure 9

The model based on the intracellular calcium concentration. Left: experimental data from Fig 1. Right: The data modeled by Eq. 18 showing the number of released vesicles versus time from the pool.

Figure 10

ModelCa based on the intracellular calcium concentration has a maximal time delay of 0.43 ms. A) Voltage of the terminal membrane is clamped at -70 mV and then changed to other voltages with a voltage step of 5 mV. B) Calcium current coming to the terminal with a positive sign and C) intracellular calcium concentration for the same protocol as A. D) Calcium concentration changes versus time (solid lines) as well as local maximum points (circles). E) Released vesicles versus time calculated by $\text{NRV}(t, [\text{Ca}^{2+}])$.

Figure 11

Vesicles released in a passive vs. active membrane in intracellular stimulation. Passive compartments are highlighted in blue, and compartments containing L-type calcium channels are highlighted in red. The yellow part represents sodium and potassium channels that amplify the stimulus (top). The released vesicles from the terminal for three trials are both calculated by $NRV(t, [Ca^{2+}])$ and $NRV(t, V_T)$. For the passive membrane, the terminal transmembrane voltage does not reach -45 mV and therefore no transient release occurs. This is in contrast to the active case in which the spike causes a transient release of all 10 vesicles calculated by both mathematical models of $NRV(t, [Ca^{2+}])$ and $NRV(t, V_T)$.

Figure 12

Extracellular stimulation makes the RRP empty for small pulse amplitudes. A microelectrode located 30 μm above the soma generates isopotential surfaces that scale inversely with distance r from the tip of the microelectrode, and the potentials are for anodic 1 μA pulse (left). The number of transiently released vesicles versus amplitude for 10 ms pulses (right). Bottom: the active cell membrane has a smaller stimulation window for the graded vesicle releases. The red (green) lines show the number of transiently released vesicles calculated by $\text{NRV}(t, [\text{Ca}^{2+}])$ ($\text{NRV}(t, V_T)$). Number of vesicles decrease when increasing the pulse amplitude in ModelCa because big enough pulse amplitudes depolarize the membrane more than calcium reversal potential. This process is not seen in ModelV since the model does not consider calcium reversal potential.

Figure 13

Vesicle release under pulse train stimulation. The number of transiently released vesicles reaches a constant value in spiking BCs both for intra- (left) and extracellular stimulation (right) while using a periodic pulse with pulse amplitudes of 1500 pA and 3 μ A, respectively. The terminal transmembrane voltage reaches -8 mV for intracellular stimulation, while in extracellular stimulation, the terminal depolarizes up to -15 mV, as shown in the green diagrams. The simulation of vesicle release both by $NRV(t, [Ca^{2+}])$ and $NRV(t, V_T)$ suggests that spike generation makes the RRP empty in both the intracellular and extracellular stimulations after the first pulse. When the RRP becomes empty, transient

releases are also observed in the next stimulations because of recovery. The red lines represent transient releases and black lines show sustained releases.

Figure 14

D model of a rat RBC. The cell consists of 151 cylindrical compartments, 9 of which belong to the soma highlighted in gray. 85 compartments make the axon (cyan), and 14 of these 85 are the axon terminals (yellow). 57 are dendritic compartments (blue). The diameters of the axonal, terminal and dendritic compartments vary between 0.11-1.3 μm , while in the soma they vary between 0.3-5.2 μm and the total length of the cell is 98.7 μm in the z direction.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Equations.pdf](#)