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A computational model of the ribbon synapse of bipolar retinal cells

im Rahmen des Studiums

Biomedical Engineering

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Maria Ioana Bordeus

Matrikelnummer 1176634

ausgeführt am Institut für Analysis und Scientific Computing
der Fakultät für Mathematik und Geoinformationen der Technischen Universität Wien

Betreuung Ao.Univ.Prof. Dipl.-Ing. Dr.rer.nat. Dr.sc.med. Dr.techn. Frank Rattay
Projektassistent Hassan Bassereh

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Abstract

Synaptic transmission is a main issue for the understanding of higher functions in the central nervous system (CNS). Processing of visual information within the retina depends in large measure upon a complement of chemical neurotransmitters which are released at synaptic contacts between adjacent neurons.
Retinal implants are developed to help people with degenerative retinal diseases such as retinitis pigmentosa and age-related macular degeneration (AMD) where the optic nerve and the visual centers in the brain are still functioning but the patient has lost light or sight perception due to degeneration of the outer layer of the retinal photoreceptor cells. On the other hand, in retinal diseases such as retinitis pigmentosa and AMD where the photoreceptors are damaged, the inner bipolar and ganglion layers are relatively intact and still functioning.
In order to restore some form of light perception and vision perception, a retinal implant would need to focus on replicating the sensation of light and darkness by artificially hyperpolarizing and depolarizing remaining photoreceptor and subsequent bipolar cells in a damaged retina.
Retinal implants can be epiretinal where the implant is inserted on the surface of the retina with electrodes extending into the internal layers of the retina to stimulate either the bipolar or ganglion cells. In subretinal implants, the implant is inserted inside the retina in the photoreceptor region. These subretinal implants generate graded potentials in bipolar cells. Artificial generation of synaptic release by depolarizing voltage sensitive calcium channels has the main role in subretinal stimulation.
Retinal bipolar cells form a part of the direct visual information from a light source to the brain. They are located in the outer and inner plexiform layer of the retina, between the photoreceptor and ganglion cell layers. Photoreceptors can receive light and ganglion cells can fire action potentials to the optic nerve.
The bipolar cells, horizontal and amacrine cells communicate with the ganglion cells via synaptic contacts. Bipolar cells, along with amacrine and horizontal cells, have the role to control the detection of light by graded impulse responses of glutamate which are released by the photoreceptors. The ganglion cells are depolarized by synaptic glutamatergic release from bipolar cells. The synaptic transmission depends on the level of intracellular calcium concentration in the bipolar cells.
In this thesis, the influence of the membrane potential over the calcium channel dynamics and calcium current was simulated and analyzed. Calcium ion flux changes its direction from inside to outside of the cell in regions where the voltage membrane exceeds the equilibrium potential of calcium. A computational study was performed in order to get a better insight of the roll of the bipolar cell in the transduced signal. Furthermore, it was aimed to investigate the intracellular calcium concentration in the bipolar cell terminals using voltage clamp or space clamp technique. The intracellular calcium concentration has the main role in triggering neurotransmitter release and in this purpose it is analyzed how it changes during a stimulus of different wave-forms, with different amplitudes and time duration. In the last section, the impact of different intracellular calcium concentrations on the vesicle release was investigated. The discrete synaptic vesicle release is simulated with a four pool model and it doesn't include any endocytosis mechanism because the simulations were performed on short terms. In this study, L-type voltage-gated calcium channels was used to explain calcium current in type 9 ON bipolar cell in the rat retina.
The results of this thesis may give an insight on how various computer simulations could resemble physiologic process in retinal bipolar cells. In this scope, the influence of sinusoidal stimuli of different frequencies (from 20 Hz to 5 kHz) and amplitudes (30 μA to 50 μA) or by using repetitive rectangular pulses was investigated.

A constant high level of intracellular calcium concentration leads to a fast and sustained release of vesicles.
In case of a square wave, the depolarization membrane follows immediately the time course of the pulse and it gets clamped until the pulse is switched off. In this situation, the intracellular calcium concentration will rise rapidly and the vesicle release will have a large transient component. This doesn't describe right the process that happens by light inputs. The voltage membrane responds to light stimuli in a much larger time interval (up to 100ms).
The pulse shape and stimulus amplitude and frequency are very important parameters in further investigating of the impact of the subretinal stimulation in restoration of vision.

Kurzfassung

Die synaptische Übertragung ist ein Hauptproblem für das Verständnis höherer Funktionen im zentralen Nervensystem (ZNS). Die Verarbeitung von visueller Information innerhalb der Retina hängt in hohem Maße von einem Komplement von chemischen Neurotransmittern ab, die bei synaptischen Kontakten zwischen benachbarten Neuronen freigesetzt werden.

Netzhautimplantate werden entwickelt, um Menschen mit degenerativen Netzhauterkrankungen wie Retinitis pigmentosa und altersbedingter Makuladegeneration (AMD) zu helfen, bei denen der Sehnerv und die Sehzentren im Gehirn noch funktionieren, der Patient jedoch aufgrund von Degeneration der äußeren Schicht der retinalen Photorezeptorzellen, Licht- oder Sichtwahrnehmung verloren hat. Andererseits sind bei Netzhauterkrankungen wie Retinitis pigmentosa und AMD, bei denen die Photorezeptoren geschädigt sind, die inneren Bipolar- und Ganglionschichten relativ intakt und funktionieren immer noch.

Um eine Form der Lichtwahrnehmung und der Sehwahrnehmung wiederherzustellen, müsste sich ein retinales Implantat darauf konzentrieren, die Empfindung von Licht und Dunkelheit durch künstliche Hyperpolarisierung und Depolarisierung des verbleibenden Photorezeptors und der nachfolgenden bipolaren Zellen in einer beschädigten Retina zu reproduzieren. Retinale Implantate können epiretinal sein, wenn das Implantat auf der Oberfläche der Retina insertiert wird, wobei sich Elektroden in die inneren Schichten der Retina erstrecken, um entweder die bipolaren oder Ganglienzellen zu stimulieren. Bei subretinalen Implantaten wird das Implantat innerhalb der Retina im Photorezeptorbereich eingesetzt. Diese subretinalen Implantate erzeugen abgestufte Potentiale in bipolaren Zellen. Künstliche Erzeugung von synaptischer Freisetzung durch depolarisierende spannungsempfindliche Calciumkanäle hat die Hauptrolle bei der subretinalen Stimulation.

Retinale Bipolarzellen bilden einen Teil der direkten visuellen Information von einer Lichtquelle zum Gehirn. Sie befinden sich in der äußeren und inneren plexiformen Schicht der Netzhaut, zwischen den Photorezeptor- und Ganglienzellschichten. Photorezeptoren können Licht empfangen und Ganglienzellen können Aktionspotentiale an den Sehnerv abgeben.

Die bipolaren Zellen, horizontalen und amakrinen Zellen kommunizieren über synaptische Kontakte mit den Ganglienzellen. Bipolare Zellen haben zusammen mit amakrinen und horizontalen Zellen die Aufgabe, den Nachweis von Licht durch abgestufte Impulsantworten von Glutamat zu steuern, die von den Photorezeptoren freigesetzt werden. Die Ganglienzellen werden durch synaptische glutamaterge Freisetzung aus bipolaren Zellen depolarisiert. Die synaptische Übertragung hängt von der Höhe der intrazellulären Calciumkonzentration in den bipolaren Zellen ab.

In dieser Arbeit wird der Einfluss des Membranpotentials auf die Dynamik der Calciumkanäle und den Calciumstrom simuliert und analysiert. Der Calciumionenfluss ändert seine Richtung von innen nach außen in Bereichen, in denen die Spannungsmembran das Gleichgewichtspotential von Calcium übersteigt. Eine Computerstudie wird durchgeführt, um einen besseren Einblick in die Rolle der bipolaren Zelle im transduzierten Signal zu erhalten. Darüber hinaus soll die intrazelluläre Calciumkonzentration in den bipolaren Zellterminals mittels „Voltage-Clamp“- oder „Space-Clamp“-Technik untersucht werden. Die intrazelluläre Calciumkonzentration hat die Hauptrolle beim Auslösen der Neurotransmitterfreisetzung und in diesem Zusammenhang wird analysiert, wie sich diese während eines Stimulus verschiedener Wellenformen mit unterschiedlichen Amplituden und Zeitdauer ändert. Im letzten Abschnitt wird der Einfluss verschiedener intrazellulärer Calciumkonzentrationen auf die Vesikelfreisetzung

untersucht. Die diskrete synaptische Vesikelfreisetzung wird mit einem „Vier-Pool-Modell“ simuliert und enthält keinen Endozytosemechanismus, da die Simulationen in kurzen Zeitabständen durchgeführt wurden. In dieser Studie wurden L-Typ spannungsgesteuerte Calciumkanäle verwendet, um den Calciumstrom in ON-Bipolarzellen vom Typ 9 ON in der Rattenretina zu erklären.

Die Ergebnisse dieser Arbeit können einen Einblick geben, wie verschiedene Computersimulationen dem physiologischen Prozess in retinalen bipolaren Zellen ähneln können. In diesem Zusammenhang wurde der Einfluss sinusförmiger Stimuli unterschiedlicher Frequenzen (von 20 Hz bis 5 kHz) und Amplituden (30 μA bis 50 μA) oder wiederholende Rechteckimpulse untersucht.

Eine konstant hohe intrazelluläre Calciumkonzentration führt zu einer schnellen und anhaltenden Freisetzung von Vesikeln.

Im Falle einer Rechteckwelle folgt die Depolarisationsmembran unmittelbar dem zeitlichen Verlauf des Impulses und wird eingeklemmt, bis der Impuls ausgeschaltet ist. In dieser Situation wird die intrazelluläre Calciumkonzentration schnell ansteigen und die Vesikelfreisetzung wird eine große transiente Komponente haben. Dies beschreibt allerdings nicht richtig den Prozess, der durch Lichteingaben geschieht. Die Spannungsmembran reagiert auf Lichtreize in einem viel größeren Zeitintervall (bis zu 100ms).

Die Impulsform als auch die Stimulusamplitude und Stimulusfrequenz sind sehr wichtige Parameter bei der weiteren Untersuchung des Einflusses der subretinalen Stimulation bei der Wiederherstellung des Sehvermögens.

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Glossary

$\mathbf{J_{diffusion}}$	flow of ions across the cell membrane due to diffusion
$\mathbf{J_{drift}}$	flow of ions across the cell membrane due to drift in an electrical field
$\mathbf{V_m}$	voltage across the cell membrane
$\mathbf{I_c}$	capacitive current
$\mathbf{I_i}$	ionic current
$\mathbf{I_L}$	leak current
$\mathbf{I_{ca}}$	calcium current
$\mathbf{C_m}$	capacity of the cell
$\mathbf{g_{Camax}}$	maximum conductance of calcium per 1 cm^2 of cell membrane
$\mathbf{I_{stimulation}}$	stimulus current
$\mathbf{[Ca^{2+}]_i}$	intracellular calcium ion concentration
$\mathbf{[Ca^{2+}]_e}$	extracellular calcium ion concentration
$\mathbf{[Ca^{2+}]_{res}}$	residual level of the intracellular calcium ion concentration
$\boldsymbol{\tau_{dec}}$	time constant of the passive extrude of calcium from the cell
d	distance between the calcium channels and the location of the calcium sensor of the vesicular release
$\mathbf{E_{ca}}$	calcium equilibrium potential
$\mathbf{E_L}$	leak equilibrium potential
$\mathbf{J_{cc}}$	calcium flux due to a ionic calcium current
c	open gate probability of calcium channel
$\mathbf{c_0}$	open gate probability of calcium channel at rest
$\mathbf{c_\infty}$	open gate probability of calcium channel at steady state
$\boldsymbol{\alpha}$	rate constant of the close-to-open state
$\boldsymbol{\beta}$	rate constant of the open-to-close state
$\boldsymbol{\rho_{RP}}$,$\boldsymbol{\rho_{RRP}}$	refill rates for the releasable pool respectively rapid releasable pool
$\boldsymbol{\alpha_{RP}}$,$\boldsymbol{\alpha_{RRP}}$	release rate constants of the releasable pool respectively rapid releasable pool
$\mathbf{RP_0}$,$\mathbf{RRP_0}$	releasable pool respectively rapid releasable pool initial state
$\mathbf{C_0}$	cytoplasmatic pool initial state
HH	Hodgkin-Huxley
FCM	Fohlmeister-Coleman-Miller
R	resistor
C	capacitor

Chapter 1 Visual System and anatomy of the eye

In this chapter it is intended to describe the function of the retinal neurons and their roles on converting the visual image into an array of neural activity.

With this introductory section, it is aim to get a better understanding on how the visual information is transmitted to the brain. Visual processing starts by light entering the retina which is absorbed by the photoreceptor neurons after passing through the pupil. The absorption of light initiates the process of phototransduction which in return triggers changes in membrane potential. Furthermore these signals are passed to the bipolar cells layer and in the end to the ganglion cells.

There are regional differences in visual perception: the central visual field is color-sensitive, has high acuity vision and operates at high levels of illumination (i.e., operates with the photopic, light-adapted subsystem). In contrast, the visual field periphery is more sensitive at low levels of illumination, so relatively color insensitive and has poor visual acuity (i.e., operates with the scotopic, dark-adapted, subsystem).

The image projected onto the retina is distributed over photoreceptors. Light energy projected onto each photoreceptor is converted into receptor membrane potential changes by a process that involves photosensitive pigments and cyclic nucleotide-gated ion channels in the photoreceptor outer segment. The phototransduction process converts light energy into photoreceptor membrane potential changes that produce a chemical signal (the release of glutamate), which results in membrane potential changes in the postsynaptic bipolar and horizontal cells. (Fig.1.1)

The retina is made up of three main functional neural cell layers

- photoreceptor cells consisting two types, cone and rod
- bipolar cells
- ganglion cells

Between the layers the horizontal and amacrine cells were spread.

Retina

The retina is part of the central nervous system and consists of two main parts:

- the retinal pigment epithelium, absorb light passing through the receptor layer, thus reducing light scatter and image distortion within the eye.
- neural retina, contains five types of neurons: the visual receptor cells (the rods and cones), the horizontal cells, the bipolar cells, the amacrine cells, and the retinal ganglion cells.

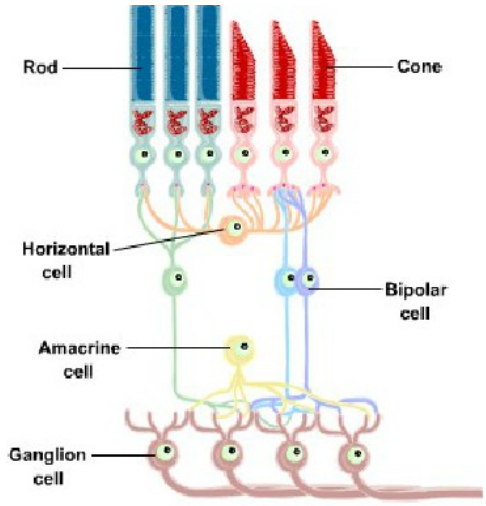

Fig. 1.1 The structure of the retina, (from Valentin Dragoi, 1997).

Retina has a laminated structure and consists of alternating layers, (Fig. 1.2).

- retinal pigment epithelium, supports the metabolic functions of the photoreceptors
- receptor layer, consists the light sensitive outer segments of the photoreceptors
- outer nuclear layer, consists of the photoreceptors cell bodies
- outer plexiform layer, consists of the photoreceptor cells, bipolar cells and horizontal cells synapse
- inner nuclear layer, consists the horizontal cell, bipolar cell, amacrine cell bodies
- inner plexiform layer, consists of the bipolar cells, amacrine, ganglion cells synapse
- retinal ganglion cell layer, contains the retinal ganglion cell body
- optic nerve layer, contains the ganglion cell axons which form the optic nerve

The light entering the eye, need to pass through the cornea, lens and vitreous, some of the retinal layers, and reach the light sensitive portion of the photoreceptor.

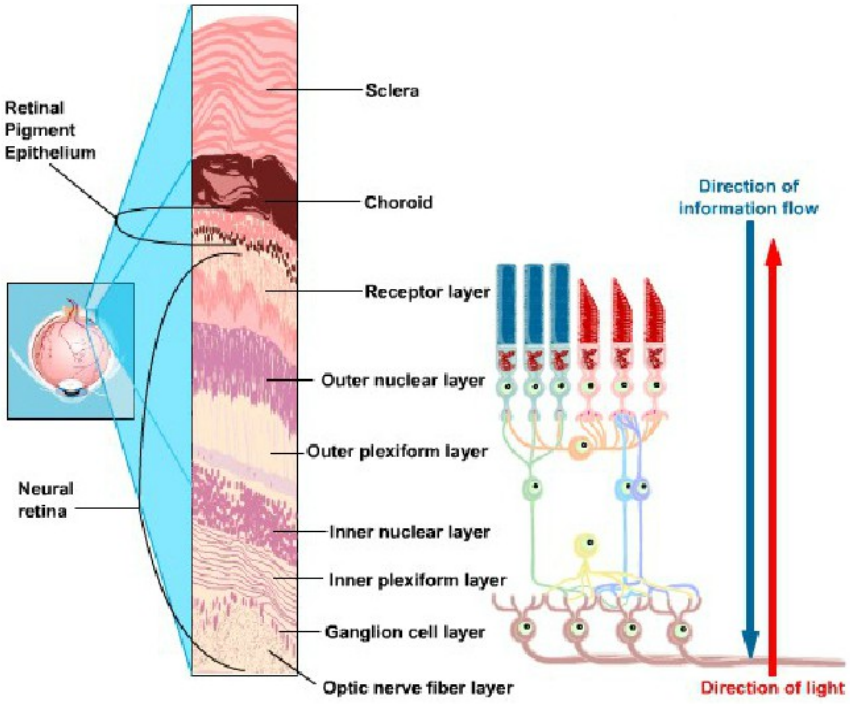

Fig. 1.2 Retina layers. The retina is formed by different layers. The receptor cells synapse with bipolar and horizontal cells in the outer plexiform layer. The bipolar cells synapse with amacrine and ganglion cells in the inner plexiform layer. The axons of the retinal ganglion cells exit the eye to form the optic nerve, (from Valentin Dragoi, 1997).

The photoreceptors

The photoreceptor cells which are at the back of the retina, transduce the light energy into electrical signals which is transmitted and processed by the bipolar cells and ganglion cells. The axon of the ganglion cells form the optic nerve which leads the visual information to the processing centers in the brain.

The photoreceptors cells are of two types, rods and cones. The rods have the role in light detection and are sensitive to low light level. The cones detect colors and are sensitive to bright light.

There are three types of cones, red, green, blue and each cone type responds to only one type of cone photo-pigment. Each cone responds best to a specific color of light and the rods responds to white light.

The rods are more sensitive to light and function at low level of illumination, dominate in the peripheral retina which is color insensitive. But the cones are color sensitive, and less sensitive to light.

The process of adaptation in dark and in light is taken place in the photoreceptors. Dark adaptation is supposed to be slow increase in light sensitivity and is related to the intracellular calcium concentration.

The adaptation process in high levels of illumination is much faster and the cone response dominates over rod response.

A photoreceptor cell have a resting potential between 30mV to 40 mV. The photoreceptor cells have embedded sodium and potassium ion channels in the outer membrane. In the absence of light, a continuous flow of positively charged sodium ions into the cell and potassium ions out of the cell is taken place which leads to releasing the neurotransmitters (glutamate) from the synaptic terminals of the photoreceptor cell. By the light absorption, the photoreceptor axon terminals release a reduced amount of synaptic neurotransmitters.

The bipolar cells

The retina is organized in three nuclear and two synaptic layers. Light enters the eye and passes the tissue to reach the light sensitive outer segments of the rod and cone photoreceptor cells where the signal is transduced into an electrical signal. At the first synaptic layer, the signal is shaped by 1-3 types of horizontal cells and subsequently distributed onto more than 13 types of bipolar cells. The bipolar cells and ganglion cells are interpolated with the horizontal and amacrine cells. The visual signal from the photoreceptors is directly or indirectly received by the bipolar cells through the horizontal cells. The signal further is recast directly or indirectly via other adjacent horizontal cells by other bipolar cells. In a similarly way, the bipolar cells interface the ganglion cells directly or indirectly through the amacrine cells.

Around 125 million photoreceptor cells synapse with approximately 10 million bipolar cells and only a reduced number of horizontal cells synapse with the photoreceptor cells in the outer plexiform layer.

Characteristics of the bipolar cells:

- most of them do not generate action potentials
- respond to the release of neurotransmitter vesicles from the photoreceptors (glutamate) with graded potentials (by hyperpolarizing or depolarizing)

The bipolar cells can be classified into:

- OFF bipolar cells
- ON bipolar cells

OFF bipolar cells have the role to detect objects in a lighter background. ON bipolar cells have the role to detect objects in a darker background.

The light has an induced effect of reducing the amount of the released glutamate from the photoreceptor cells.

The OFF bipolar cells depolarize and ON bipolar cells hyperpolarize when the environment is dark while ON bipolar cells depolarize and OFF bipolar cells hyperpolarize when the environment is bright.

The different types of bipolar cells capture and process the photoreceptor signals in a way that link the outer and the inner retina.

Bipolar cells form parallel information pathways representing different transformations of the photoreceptor signal and provide the inner retina with highly pre-processed excitatory input. The bipolar cells axon terminals synapse onto amacrine cells and retinal ganglion cells.

Amacrine cells are the diverse cell class in the retina and provide bipolar cells and ganglion cells with mostly inhibitory or neuromodulatory input. In the end, the retinal ganglion cells integrate the input from distinct sets of bipolar cells and amacrine cells, and encode the results as trains of spikes which are sent to higher visual centers via their axons, which form the optic nerve.

The response of the bipolar cells is determined by a series of factors like size of dendritic field, the set of photoreceptors types contacted, the glutamate receptors, the distribution of ion channels and the cell's morphology. The bipolar cell is releasing glutamate onto postsynaptic retinal ganglion cells and amacrine cells.

The bipolar cells provide a sensory input to the ganglion cells through chemical axon terminal synapse. Some points involved in the signal transformation are:

- depolarization which starts a calcium influx
- the calcium influx cause triggering of the neurotransmitter release
- the axon of the bipolar cells has a low pass filtering effect over the voltage signal; fast voltage transient are attenuated as a function of axon length and diameter. Simulations based on passive cable theory show that a typical bipolar cell with an axon length of about 30 μm and a diameter of 200nm filter frequency higher than 20 Hz
- OFF bipolar cells have shorter axons which in return detect the fast changes in the visual scene
- a stronger attenuation due to the higher length of the axon can signal a high influence of the amacrine cell input over the bipolar cell terminal in the neurotransmitter release.
- usually the calcium which enters bipolar cells is clustered around synaptic ribbons
- the operating voltage range of a bipolar cell is around -30mV to - 40mV
- the calcium concentration is subjected to a series of processes like influx of calcium through voltage-gated channels, diffusion in the intracellular space, buffering and extrusion from the cytoplasm

The role of ganglion cells is related to the perception of color vision as a function of time. The ganglion cells are sensitive to speed of movement. The output synapses of the ganglion cells transmit the neural image further to the visualcortex for decoding the visual signal and get a perception of the image.

The horizontal cells

The photoreceptor cells communicate with the horizontal cells in a presynaptic or postsynaptic formalism in the outer plexiform layer.
The horizontal cells have a large area of signal reception, namely an axonal presynaptic contact with a reduced number of photoreceptor cells and a dendritic postsynaptic contact with an increased number of photoreceptor cells.
The role of horizontal cells is to create a sharp image and to maintain a contrast under different illumination condition.

The ganglion cells

In the inner plexiform layer, the axon of the bipolar cells make synaptic contact with the dendrites of the amacrine and ganglion cells, (Fig. 1.2). The processing visual signal results in the release of neurotransmitter (glutamate) by the axon terminals of the bipolar cells to the ganglion cells which leads to depolarizing the ganglion cells.
Some of the amacrine cells may have a synaptic contact with bipolar cells, other amacrine cells or ganglion cells.
The ganglion cells deliver the visual information to the thalamus and other diencephalic and midbrain structures.
The ganglion cells have the role to deliver information in order to detect shape and movements in the visual signal.
There are two types of ganglion cells in the primate eye:

- the P type
  - is color sensitive
  - is suited to mark the presence, color, duration of a visual stimulus
- the M type
  - is color insensitive
  - is suited to mark temporal variations and movements of a visual stimulus

The amacrine cells

Their role is to ensure lateral connections, and synapse with bipolar cells and ganglion cells.
They are movement sensitive, allow ganglion cells to respond to a big range of light levels and they also connect the rod bipolar cells to cone bipolar cells.

Chapter 2 Introduction to human nervous system

The human nervous system has around 90 billion nerve cells where their variety depends on their morphology and their response to different neurotransmitters.

2.1 Neuron model

The fundamental building block of this neural communication network is the individual nerve cell, neuron. It consists of three major components, the soma (cell body), dendrites (receptor zone) and axon (which carries electrical signals from the main body of the cell to the muscles, gland or other neurons).

Soma

Soma consists of cytoplasm and cytoskeleton and within the cytoplasm there are other organelles, found also in other cells, like nucleus, nucleolus, endoplasmic reticulum, Golgi apparatus, mitochondria, ribosomes, lysosomes, endosomes, peroxixisomes. Its function is to regulate the metabolism of the neuron, to receive signals for synaptic inputs from other cells, (Fig. 2.1).

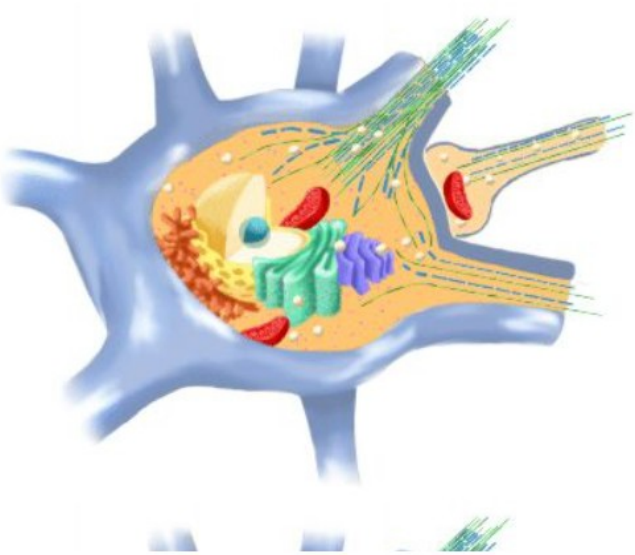

Fig. 2.1. Neuron cell body, (from Jack Waymire, 1997).

Dendrites

The dendrites have the function of receiving signals from other neurons through an array of receptors available on dendrite surface. These receptors react to released transmitters from the axon terminal of other neurons. Dendrites are capable to receive signals from thousands of other cells. The dendrites have a tree structure and conduct the input signals forward into the soma where they are computed, (Fig. 2.2).

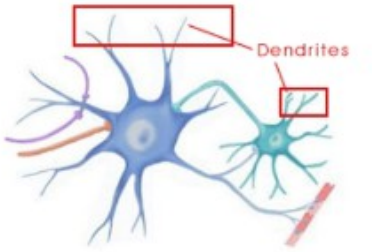

Fig. 2.2. Dendrites, (from Jack Waymire, 1997).

Initial segment and axon hillock

Axon Hillock is the region of the soma where the axon initially begins. The region between the axon hillock and the beginning of the myelin sheath is termed as the initial segment. This is the area where the actions potential are usually initialized, (Fig. 2.3).

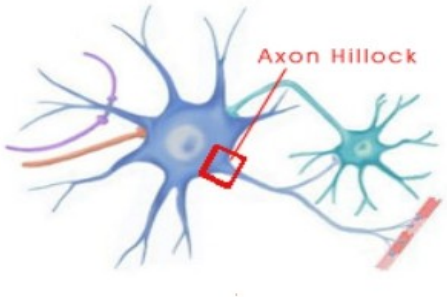

Fig. 2.3. Initial segment and axon Hillock, (from Jack Waymire, 1997).

Axon

The neuron has only one axon which contains microtubules and neurofilaments and mitochondria. The axon is often surrounded by a myelin sheath (formed by glia cells). In the myelinated neurons the action potentials jump from one Ranvier node to another because of the insulated axon parts. This myelin sheath insulate the plasmalemma of the axon so that the depolarization of plasmalemma is much faster and increase the speed of conduction of the nerve impulse. Conduction in a non-myelinated neuron happens continuous because of the depolarization of the surrounded region, (Fig. 2.4).

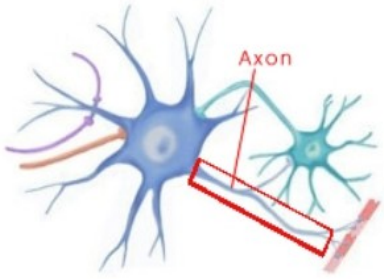

Fig.2.4 Axon, (from Jack Waymire, 1997).

Nerve ending and the cellular elements

The nerve ending that forms contacts to other cells is called the synapse (Fig. 2.5). When a nerve ending synapses on a dendrite or soma of a second neuron, it is named axo-dendritic or an axo-somatic synapse.
Neurons are anatomically distinct units with no physical continuity between them. The transmitting portion of a neuron, its axon, ends in a series of synapses, thereby making contact with other neurons. The synaptic terminals are separated by a gap, synaptic cleft. Each of these synaptic endings contains a large number of synaptic vesicles. These synaptic vesicles are “chemical carriers“ containing transmitter substance that is released into the synaptic clefts in the case of excitation.
The following sequence of events that enables one neuron to communicate with another:

- a neuron is excited, then an electrical signal is transmitted along its axon towards branches
- if the electrical pulse arrives at the synapse, it triggers the release of an amount of transmitter substance
- the chemical carrier floats across the synaptic cleft, altering the status of the receiving neuron.

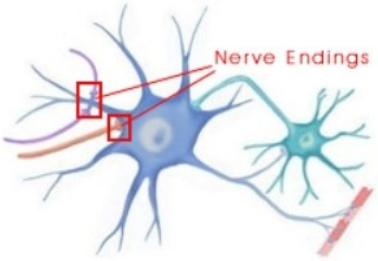

Fig. 2.5 Nerve Endings, (from Jack Waymire, 1997).

2.2 Neurons classification

There are different neuron classifications depending on:

- the neuron structure (Fig. 2.6, Fig. 2.7)
  - Bipolar neurons, the soma is between dendrite and axon. They are found in retina and the olfactory bulb
  - Unipolar neurons or pseudo-unipolar neurons, the axon and the dendrite have the same origin and have one cell process. These neurons can be found in sensory organs
  - Multipolar neurons, are found in central nervous system and have more dendrites and one axon
  - Pyramidal cells, are found in the cerebral cortex and hippocampus

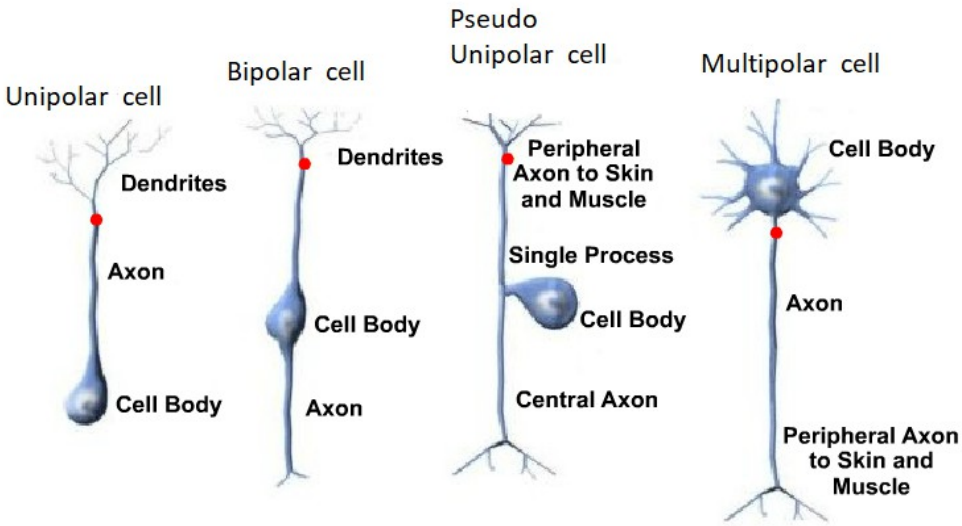

Fig. 2.6 Neuron cell types, (from Jack Waymire, 1997).

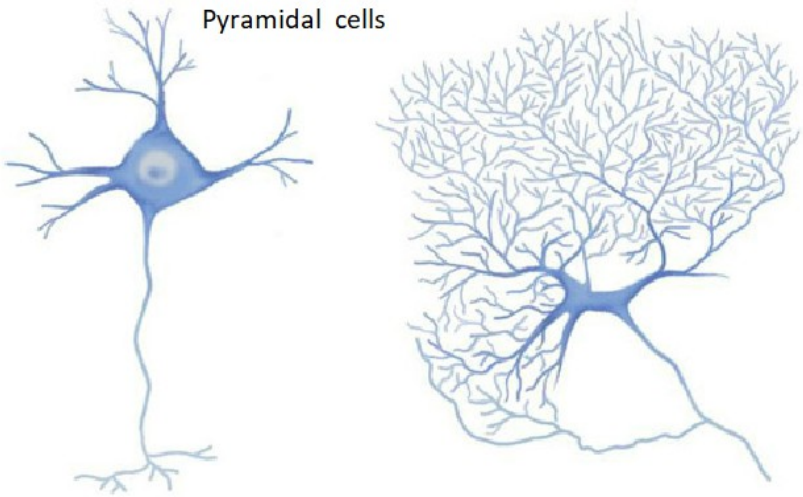

Fig. 2.7 Neuron cell type, (from Jack Waymire, 1997).

- the neurons function
  - afferent neurons which deliver information from sensory receptors to the central nervous system
  - efferent neurons which deliver information from the central nervous system to organs
  - interneurons, ensure the connectivity between sensory and kinetic information, located in the central nervous system

2.3 Functionality of the cell

2.3.1 Membrane potentials

The neuron, like other cells in the body, has a separation of charge across its external membrane. The cell membrane is positively charged on the outside and negatively charged on the inside. The separation of charges, because of the selective permeability of the membrane to ions leads to a membrane potential.
Permeability describes the state of the membrane for a particular ion and it is not variable with the concentration gradient.
The potential difference across the cell membrane, in most of the neurons, is between 60 mV and 90 mV. Taking this into consideration it been convenient the outside of the cell as 0 mV, the ground, and the resting potential of the neuron it been considered $V_m = V_i - V_o = -60mV$.
This resting membrane voltage is important in our case due to the fact that changes of charges concentrations affect the resting potential. The resting potential of the membrane can be explained by the existence of the different distribution of ions in and around the membrane of the nerve cell. This different ionic concentrations are kept through the cell by using a selectively permeable membrane and an active ion pump.
The ions are allowed to pass across the membrane only through channels which are selective, passive or active. Passive channels are always opened and are ion specific, allowing only one type of ion to pass through the membrane and prevents all other ions from crossing the membrane through that channel.
Signals like action potentials result from the perturbations of the membrane. If the membrane is more negative than the resting potential it is called, hyperpolarization and the increase in the membrane potential from the resting potential is called, depolarization. These two phases of the membrane potential which is caused by the ions traveling across the membrane through ion selective channels.
There are two laws to analyses the resting potential:

- Ficks's Law of Diffusion, that states „The flow of particles due to diffusion is along the concentration gradient, with particles moving from high concentration areas to low ones.“

$$Jdiffusion = -D\frac{d[I]}{dx} \quad \text{(Eq. 2.1)}$$

Where J: flow of ions due to diffusion
[I]: the ion concentration
dx: membrane thickness
D: diffusion constant m^2/s

- Ohm's Law, that states “Charged particles in a solution experience a force resulting from other charged particles and electric field present.“

$$Jdrift = \mu Z[I]\frac{dv}{dx} \quad \text{(Eq. 2.2)}$$

where, J: the flow of ions due to drift in an electric field E
μ:mobility m^2/sV
Z: ionic valence
[I]: ion concentration
V: voltage across the membrane

The resting membrane potential is obtained when the two forces are balanced. Goldman Equation describes the relationship between voltage membrane and permeable ions and can be calculated when the membrane potential is constant. When voltage membrane is influenced by two or more ions, voltage membrane is determined by its concentration and membrane permeability.
The Goldman equation for calculation of the membrane potential at rest is explained by:

$$V_m = \frac{KT}{q} \ln\left(\frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}\right) \quad \text{(Eq. 2.2)}$$

where, K is Boltzmann constant
T is the absolute temperature in Kelvin
q is the magnitude of the electric charge
$\frac{KT}{q} \sim 25\text{mV}\ (\textit{for room temperature})$
P is the permeability of different ions(Na^+, K^+, Cl^-)
$[K^+]_o$, $[Na^+]_o$, $[Cl^-]_i$ are the concentration outside the cell of the K^+, Na^+ and the concentration inside the cell of the Cl^- respectively.
$[K^+]_i$, $[Na^+]_i$, $[Cl^-]_o$ are the concentration inside the cell of the K^+, Na^+ and the concentration outside the cell of the Cl^-, respectively.

The active channels are selective and allow only one type or some types of ion to pass through the membrane. Their functionality is determined by an external chemical or electrical stimulation. So, the active channels open if there is a change in the membrane potential or neurotransmitter.
The role of the passive channels is to ensure the resting potential of the membrane and the active channels are responsible for the graded response and action potentials.

2.3.2Graded response and action potentials

A neuron has the capacity of changing the membrane potential of another neuron to which it is connected by releasing its neurotransmitter.
The neurotransmitter has to pass the following stages to change the membrane potential of the receptor neuron:

- crosses the synaptic cleft
- interacts with the receptors in the postsynaptic membrane of the adjacent neuron

The change in membrane potential at the post synaptic membrane is due to the transformation of the neurotransmitter chemical energy to electrical energy.
The change in membrane potential can be depolarizing /hyperpolarizing and depends also on the amount of the received neurotransmitter. This type of change in the membrane potential is called "graded response " because is a function of the amount of neurotransmitter. Because a synapse can be excitatory or inhibitory, according to the received signal from a neuron, it provides the nervous system the ability to perform complex tasks. An "action potential " is the net activity of a cell. An action potential, which in fact is a fast membrane depolarization, travels along the axon without decreasing in amplitude and last between 1ms and up to 5ms. When the action potential is at the end of the axon; at the presynaptic terminal, the change in membrane potential causes the release of neurotransmitter.
Signals can be transmitted over long distances in the nervous system due to the fact that nerve cells have the ability to propagate action potentials. An action potential do not decrease in amplitude with increasing the covered distance. An action potential occurs when membrane voltage, Vm, reaches to a certain value called „threshold potential". If voltage membrane reaches to the threshold, the conductance, which is a function of voltage and time, changes in the active gates (Na^+ and K^+) which leads to change in the voltage membrane.

2.3.3Ion channels

There are three types of ion channels, voltage activated, calcium activated and transmitter activated ion channels.
The transmitter activated ion channels are involved in synaptic transmission and the activation of a presynaptic neuron results in a release of neurotransmitters into the synaptic cleft. The neurotransmitter molecules diffuse to the postsynaptic membrane and activate postsynaptic receptors, (Fig. 2.8). The activation of the receptor leads to opening of certain ion channels which excite or inhibit the ceel.

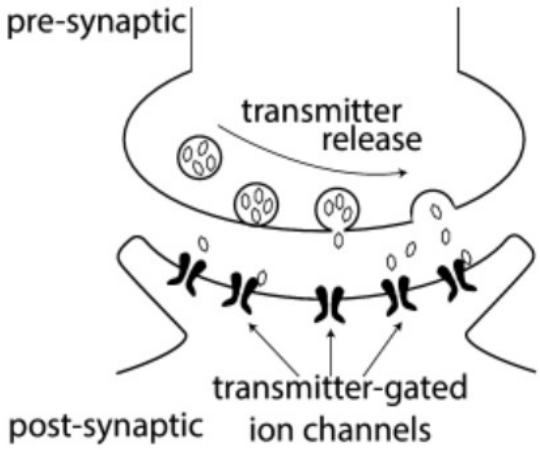

Fig 2.8 Synaptic transmission diagram. By arrival of a presynaptic spike, the neurotransmitters are spreading into the synaptic cleft and afterwards are captured by postsynaptic receptors, (from Gerstner et al., 2014).

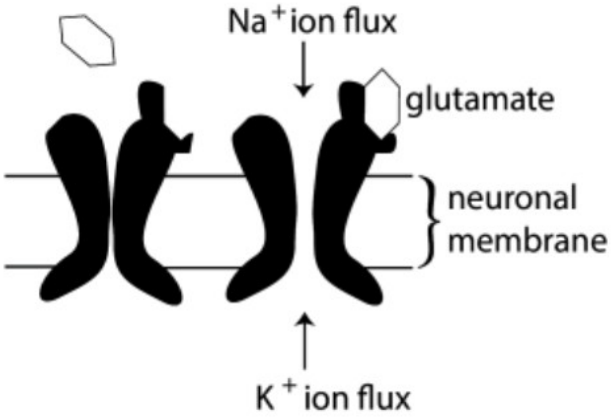

Fig. 2.9 An example of the postsynaptic AMPA receptor of an excitatory synapse. By the bounding of the glutamate to the receptor, Na^+ and K^+ ions can flow through the membrane, (from Gerstner et al., 2014).

By classification on their gating nature, the ion channels are voltage-gated or ligand gated ion channels. In most cases, the receptors in the ligand gated ion channels presented at the postsynaptic membrane are NMDA, AMPA or GABA, (Fig. 2.9).
The voltage-gated ion channels undergoes conformation changes by changes in membrane voltage of the cell and these voltage changes are due to different selectivity for Na^+, K^+ or Ca^{2+}.
To maintain a constant voltage membrane, it is necessary to maintain separation of charges and ionic concentrations. In consequence, the flow of charge into the cell must be balanced by the flow of charge out of the cell. An example in this way is the Na-K pump that prevents any change in the concentration gradient of K^+ and Na^+. For Na^+, the concentration and electric gradient creates a force that pushes the Na^+ into the cell at rest while the K^+ flow out of the cell due to the fact that the diffusion force is greater than the drift force. Removal of Na^+ from the cell is against its concentration and electric gradient and it is accomplished with the active ion pumps.
At rest, the active and passive ion flows are balanced and a differential potential across the membrane exists if the membrane is impermeable to some ions and there is an active pump. The role of an active pump is to ensure a certain resting voltage membrane, Vm, based on the concentrations. Other ion concentrations that move across the membrane through passive channels are determined by the voltage membrane.

2.3.4 Equivalent circuit model for the cell membrane (Fig. 2.10)

- **Electromotive Force Properties**: the major ions (K^+, Na^+, Cl^-) have different distribution across the cell membrane at rest that creates an electromotive force (force that drives that ion through that channel) and a gradient concentration. The Nernst potential for each ion is the equilibrium between electrical and diffuse potential which can be modeled as a battery.
- **Resistive Properties**: each channel type have its own resistance that is the opposition to the movement of specific ion types through the channel. It is usually referred as the conductance, measured in Siemens. Because the channels are in parallel, the total conductance is the number of channel times the conductance for each channel. An equivalent circuit for a single ion type is represented by a resistor in series with a battery. The conductance of a channel is direct proportional to the probability that the channel is in the open state.
- **Capacitive properties**: the structure of a cell has the intracellular (cytoplasm) and extracellular fluid as electrical conductors which are separated by the lipid bilayer which is an insulator. Typically the capacitance of a neuron membrane is 1μF. The membrane capacitance ensure also that ions do not move through the membrane except through ion channels. Another role of the membrane capacitance is that change in the membrane voltage is not immediate but is described by an exponential function. As steady state of the capacitor acts as an open circuit and the battery equivalent voltage is equal to the resting voltage membrane.

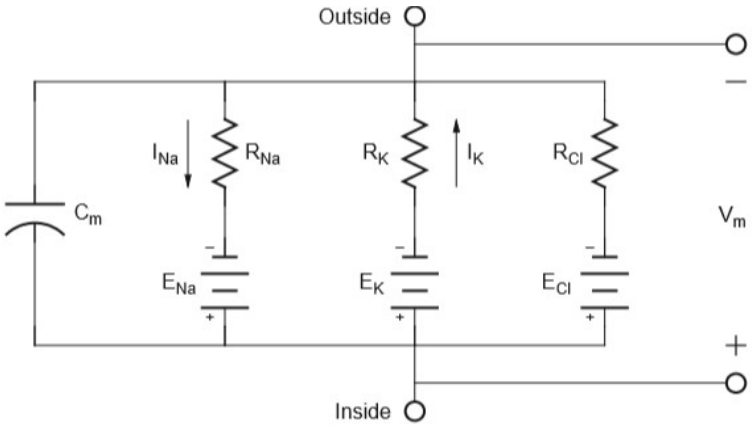

Fig. 2.10 Equivalent circuit model for the cell membrane with passive electrical properties.

Chapter3 Electrophisiology

In order to communicate, the neurons send both chemical and electrical signals. The dendrites of neurons receive information as input from other neurons, the information is passed to the cell body and further to the axon. Once the signal reached to the axon area, it will propagate as an action potential down the length of the axon. A depolarization of the cell membrane needs to overcome a certain threshold in order to generate an action potential. Once the electrical signal traveled till the end of the axon, the information must be further transmitted to the dendrites of the adjacent neurons via a synaptic gap.
The neurotransmitters are chemical messengers released from the axon terminals and reach the receptor sites of the neurons from the near vicinity.

3.1 The synapse and its functionality

A synapse is the way of communication between two or more nerve cells. A synapse uses chemical transmitters to excite the postsynaptic neuron so that an action potential could propagate from the presynaptic neuron to the postsynaptic neuron.
A synapse has a size of about 1 μm both in length and diameter and consists of:

- a presynaptic terminal at the end of the axon that forms at the end a presynaptic membrane
- a synaptic cleft which is a gap of 10 nm to 50nm spread
- an adjacent postsynaptic receptor. The postsynaptic receptor is usually situated on the dendrites or on the soma

A presynaptic terminal contains neurotransmitter vesicles in size of 50 nm. The presynaptic membrane has a region named active zone which contains vesicles docked on its internal side and is rich in Ca^{2+} channels. If an action potential arrives to the presynaptic terminal, the presynaptic membrane becomes depolarized which leads to opening of the voltage-gated Ca^{2+} channels. Once the concentration of Ca^{2+} in the active zone increases, the Ca^{2+} ions fuse with the vesicle membrane. While they fuse, the neurotransmitters vesicles are released into the synaptic cleft and the remaining vesicles are returned back into the cell.
The next step in the signal processing is that the neurotransmitter diffuse through the synaptic cleft and bind to the specific receptors at the postsynaptic membrane.
At the bounding receptor sites, the channels are opening, allowing the Na^{+} ion to enter the postsynaptic nerve cell and K^{+} to leave it. Because the outflow of K^{+} is much more reduced than the inflow of Na^{+}, this will cause the intracellular voltage to be less negative and the postsynaptic membrane gets depolarized, (Fig. 3.1).

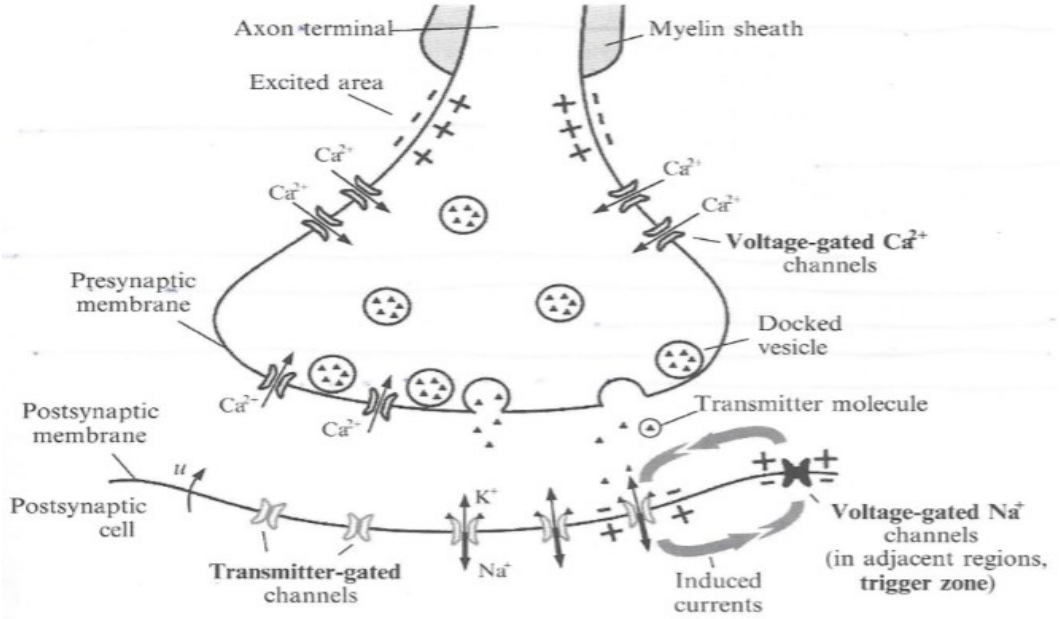

Fig 3.1Excitatory electrochemical synapse. Action potential reach the axon terminal bouton, the membrane depolarizes and the voltage-gated Ca^{2+} channels open. The increased Ca^{2+} intracellular ionic concentration activates vesicle fusion with the presynaptic membrane. The fusion releases transmitter molecules into the synaptic cleft. In the postsynaptic membrane, the molecules open transmitter-gated channels making the inflow of Na^{+} ions and an outflow of K^{+} ions possible, (from Kaniusas 2012).

The depolarization voltage decreases with the distance. In excitatory neural synapses the potential is graded which shows that a single excitatory postsynaptic potential is not sufficient to trigger an action potential. The local electrical potential tends to reach the threshold at the trigger zone after which an action potential is generated.

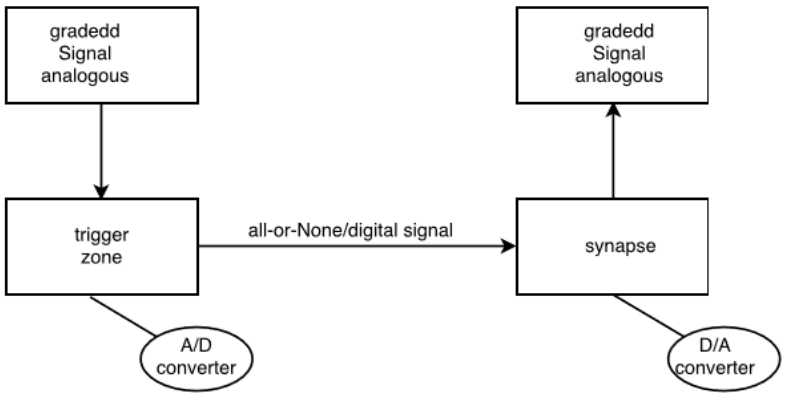

Fig. 3.2. Signal conversion in a sensory neuron. A graded input signal is encoded into all-or-none signals at the trigger zone (series of action potentials) and the signal is re-encoded into a graded signal in the synapse (amount of transmitter released).

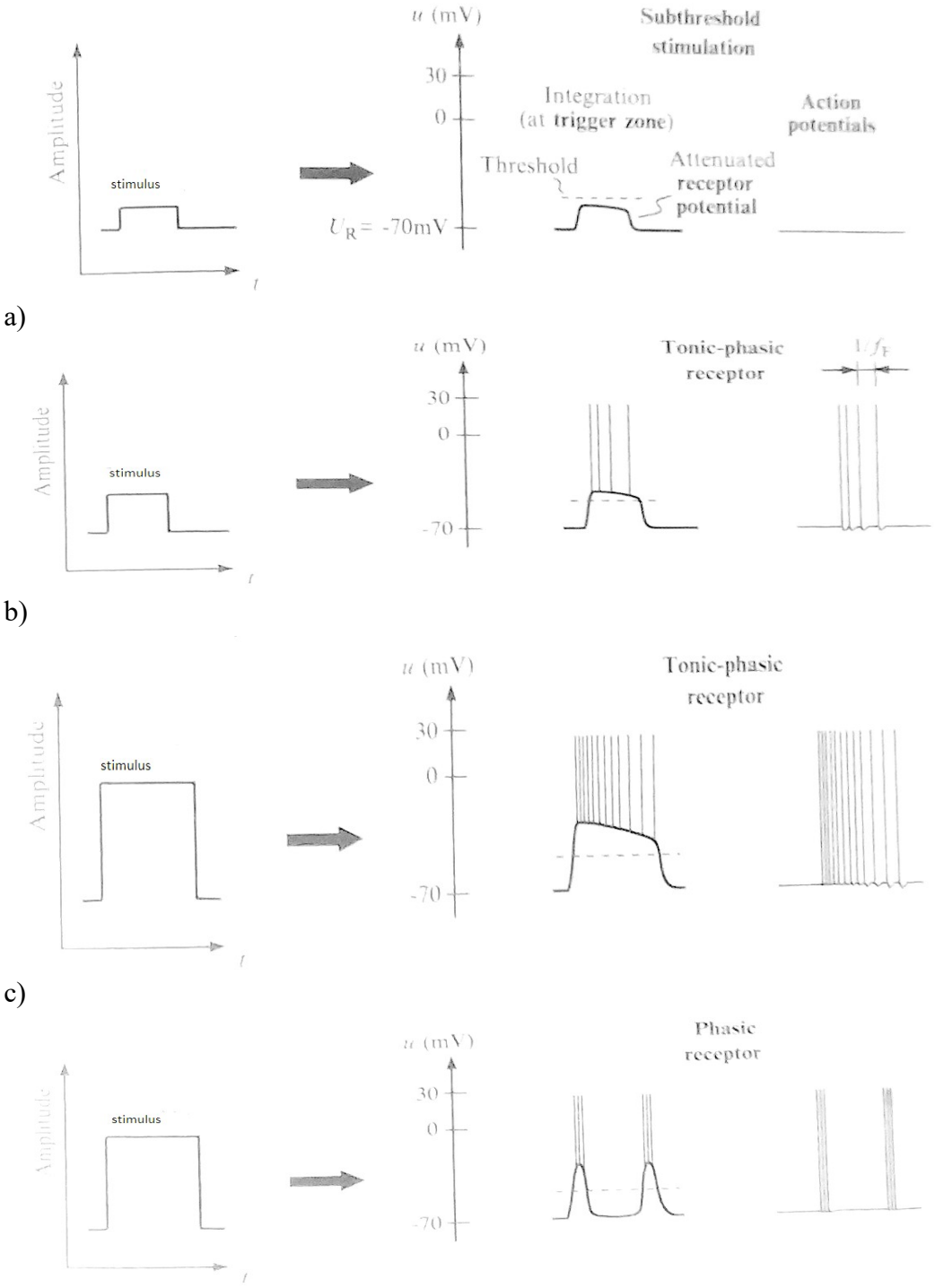

Fig. 3.3 Membrane voltage in case of different receptors. Cases a) In case of a threshold stimulus, there is no action potential generated in the trigger zone b) The tonic phasic receptor under weak stimulation outputs action potentials with a low firing rate c) The tonic phasic receptor under strong stimulation outputs action potentials with high firing rate. In case of a prolonged and constant excitatory stimulus the receptor adapts to this stimulus by rising its excitation threshold and the neuron reduces its firing rate d) Only the stimulus changes (increases or decreases) is coded by a train of action potentials and the receptor potentials is independent of the stimulus duration, (from Kaniusas, 2012).

The amount of neurotransmitter from the synaptic cleft is proportional to the number of action potentials entering the presynaptic terminal. The signal generated from the presynaptic terminal carries the physiological information encoded in action potentials over the synaptic gap towards postsynaptic terminal in which graded potentials are induced.

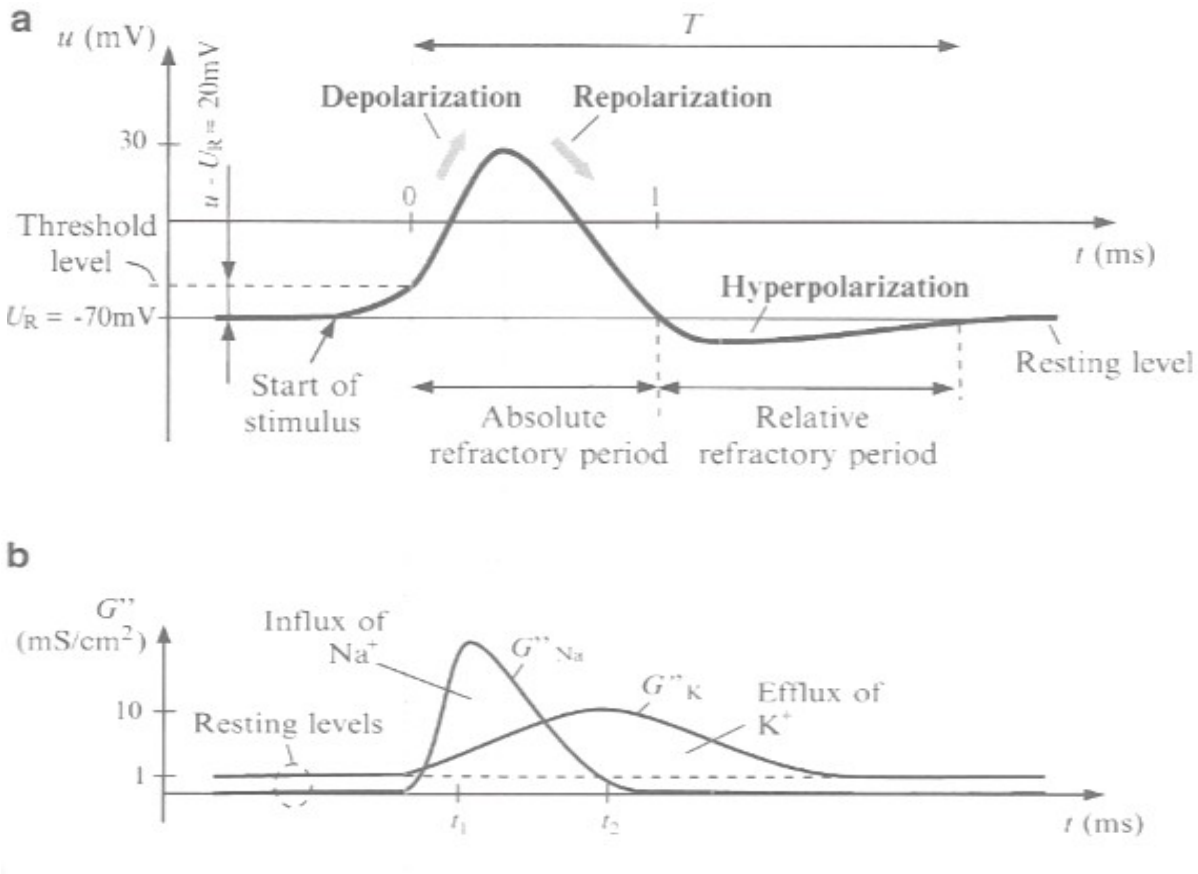

Fig. 3.4. a) Action potential membrane. By injecting a stimulus current if the threshold is reached by the depolarization cell (the conductance of the voltage-gated Na^+ increases due to the increased number of opened channels; outflow of K^+ and inflow of Cl^-). During the stimulus voltage-gated Na^+ channels are not allowed to close (if the stimulus is low, it implies slow depolarization and therefore the threshold level increases because of the number of the inactivated voltage-gated Na^+ channles). An absolute refractory period, during which no other stimulus can trigger an action potential (duration about 1ms). A relative refractory period, during which only a large stimulus could trigger an action potential (duration about 2-4 ms). During the relative refractory period, the threshold is higher than when the membrane would be at rest and the action potentials generated in this period time have a low peak voltage amplitude. b) Membrane conductance for K^+ and Na^+ ions, (from Kaniusas 2012).

If the action potentials arrive at the trigger zone and overcome the threshold then the action potential can be propagate along the axon without any attenuation. After releasing an action potential an “absolute refractory period“ follows in which no action potential can be generated, (Fig. 3.4).

The trigger zone has more features:

- place of origin of action potentials
- integrates the input signal from other neuronal cells into one neuronal response
- has a high density of voltage-gated Na^+ channels with low threshold (around -50mV)
- is the place in which graded potentials which pass the threshold are transformed into a digital train of action potentials (similar to A/D converter). In the synapse, the digital train is transformed into a graded signal of release neurotransmitters (similar to D/A converter.), (Fig. 3.2).

The action potentials have different physiologic information like:

- the stimulus strength is coded by the frequency of action potential
- stimulus duration is coded by the number of action potential
- the stimulus type is coded by distinct receptors which respond to only a stimulus type, (Fig.3.3).

Chemical synapses allow the communication between neurons by exciting or inhibiting the activity of another cell. A single action potential can generate from 10000 to 100000 of transmitters release into the synaptic cleft. Transmitters are released in packets called quanta. Concentrating the transmission in synaptic vesicles (around 50nm in diameter) and docking these vesicles at active zones (where the Ca^{2+} channels are co-localized) along the presynaptic membrane, allow a fast transmission across synapses.
By an action potential arriving in the nerve terminal, synaptic vesicles fuse with the plasma membrane of the presynaptic terminal. The vesicles are docked near the membrane and fuse with the plasma membrane at the active zone by Ca^{2+} entry, (Fig. 3.5, Fig. 3.6, Fig. 3.7).

At fast synapses:

- Ca^{2+} enters the presynaptic terminal near the docked vesicles in the active zones
- Ca^{2+} acts at short distances (10 nm) and very fast (200µs) and at very high local concentrations (10 µM to 100 µM)
- Ca^{2+} triggers exocytosis

At slow synapses:

- release depends linearly of Ca^{2+} intracellular concentration
- release of some transmitters, stored in larger, dense vesicle which are not docked at the plasma membrane in active zones, is much slower than in case of fast synapses
- release of slow transmitters may be governed by a Ca^{2+} step different from that triggering exocytosis of docked vesicles at fast synapses

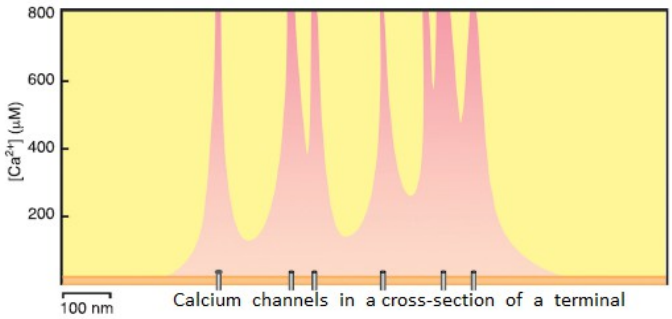

Fig. 3.5. Model of $[Ca^{2+}]$ dynamics in the terminal. By opening the Ca^{2+} channels, the influx of Ca^{2+} ions leads to an increase of 800 µM within 50nm and then drops down, (from Byrne et al., 2014).

According to Schoch and Gundelfinger (2006), voltage-gated calcium channels are not uniformly distributed in the presynaptic terminal, but concentrated in nanodomain. These calcium channels are close enough so that their calcium inflow can overlap and sum. These clusters of overlapping nanodomains form the microdomain. Active zones are important in concentrating Ca^{2+} microdomains by tethering presynaptic Ca^{2+}channels to release sites at the active zone.

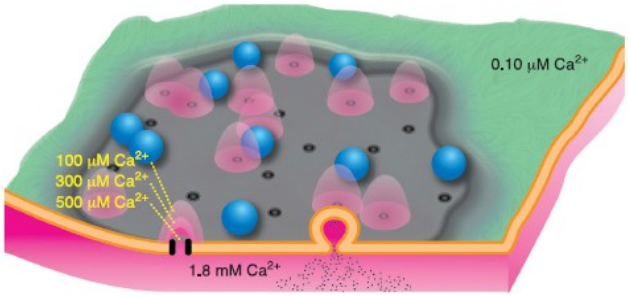

Fig. 3.6. Microdomain of high [Ca $^{2+}$]. In the active zone (gray), an action potential opens a number of calcium channels and form microdomains (pink) around these opened channels as Ca^{2+} ions are flown into the cells. In the rest of the cytoplasm, the $[Ca^{2+}]_i$ is at the resting level except within these microdomains where the $[Ca^{2+}]_i$ arise. Some vesicles (blue) which are primed and docked can be triggered by these microdomains to fuse with the membrane, (from Byrne et. al, 2014).

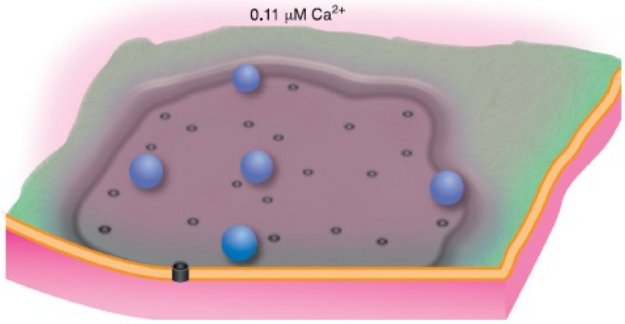

Fig. 3.7. Rest of the cell membrane. After the action potential, the calcium channels have closed and the microdomains have dispersed. The overall intracellular calcium concentration arise very slightly than before the action potential. If no other action potentials occur, the cell pumps the extra Ca^{2+} ions out the cell membrane and restore the resting level of $[Ca^{2+}]_i$ after several hundred milliseconds, (from Byrne et. al, 2014).

3.2 Definition and role of the ribbon synapses

Ribbon synapses are special structures that can be found in the retina by photoreceptors and bipolar cells and in the inner ear. The ribbon synapses hold the vesicle close to the active zone and are characterized by a tight vesicle calcium channel that promote rapid neurotransmitter release and sustain signal transmission.
These features of the ribbon synapses enables it to process information extremely quickly is critical for the perception of complex senses such as vision.
The ribbon is an electron dense bars perpendicular to the membrane at the active zone and are surrounded by synaptic vesicles that are tethered to the ribbon by short filaments.
The main difference between a conventional synapse and a ribbon synapse is that the ribbon synapse are tonically active, they release neurotransmitters continuously. The ribbon synapse has the function to capture synaptic vesicles from the cytosol and transport them to the active zone.
Up to 100 vesicles tether to a ribbon. Each presynaptic cell can have between 10 to 100 ribbons tethered at the membrane which lead to a totally number of 1000 to 10000 vesicles in the active zone.
A conventional synapse is releasing neurotransmitters at an action potential generation (principle all-or-none).
When an action potential reaches to the cell terminal, it triggers exocytosis. The exocytosis is the process where the vesicles dock and fuse with the cell membrane and release the neurotransmitters into the synaptic cleft. The active zone of the cell membrane is the place where the docking and the fusion is taking place.
A similarity between the conventional synapse and the ribbon synapse is that in both cases the neurotransmitter release is mediated by the calcium dependent vesicle fusion with the presynaptic membrane. If the cell membrane terminal is depolarized this would lead to the opening of the voltage-gated Ca^{2+} channels allowing the Ca^{2+} ions to enter the terminal.
The neurotransmitter, glutamate, is released continuously (tonically) from these ribbon synapses and the rate of release is modulated. Ribbon synapses release the neurotransmitter continuously (tonically) at a rate modulated in response to graded changes in the membrane potential. In contrast, the conventional synapses release its neurotransmitter when an action potential arrives to the presynaptic terminal and depolarize the membrane.
Synaptic terminals of photoreceptors and bipolar cells contain a higher number of vesicles compared with conventional synaptic terminals; in the bipolar cell terminals in the goldfish retina it have been estimated between 400000 to 1000000 vesicles. Based on these measurements made with an electron micrographs, the assumptions were made that the ribbon synapse support tonic transmitter release, (Fig. 3.3).
The visual information is conveyed from the photoreceptor cells to the bipolar cells by releasing glutamate from the ribbon synapses. Tonic glutamate release from photoreceptors and bipolar cells is provided by non-inactivating calcium channels that remain opened for prolonged depolarizations.
The vesicle which are located in the close proximity to the presynaptic plasma membrane, at the base of the ribbon, form the rapid releasable vesicle pool. On the other hand the remained vesicles tethered to the ribbon form the releasable vesicle pool, which is much slower and have bigger dimensions. On the docking site of the ribbon synapse there are L type voltage-gated Ca^{2+} channels which enable triggering neurotransmitter release, (Fig. 3.8, Fig. 3.9).
L-type Ca^{2+} channels are activated by large depolarizations (around -20mV) and can remain open for long time before getting inactivated.
There are four sub-types of L-channels: $Ca_v1.1$ sub-unit founds in skeletal muscle; $Ca_v1.2$ sub-units found in cardiac muscle, smooth muscle, brain; $Ca_v1.3$ sub-units found predominant in cochlear hair cells, endocrine and kidney cells, in brain; $Ca_v1.4$ sub-units found only in the retina.

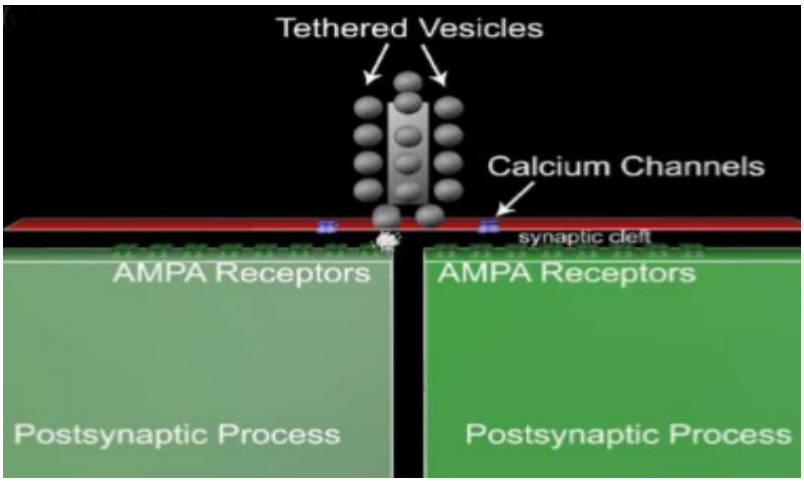

Fig.3.8. Ribbon synapse. A vesicle fuse at the base of the ribbon and releases its contents into the synaptic cleft, (from Sikora et. al, 2004).

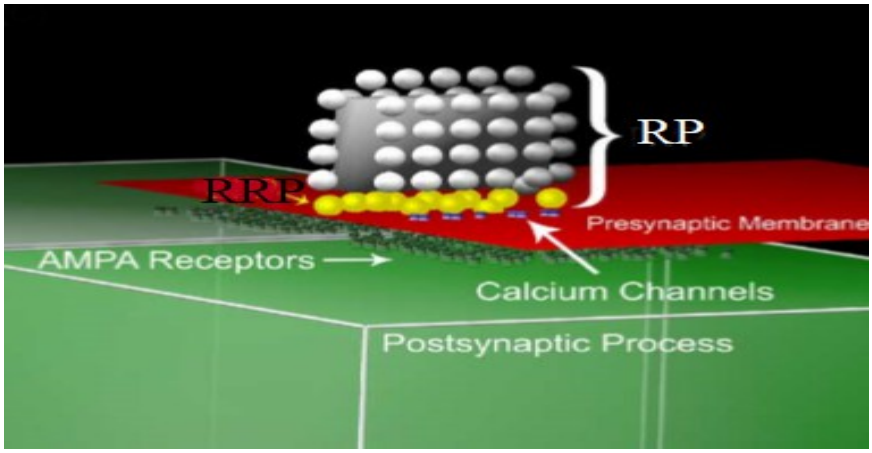

Fig.3.9. A rotated view of the ribbon with more release sites. The vesicles at the base of the ribbon are docked to the presynaptic membrane and form the RRP (rapid releasable pool); the column of vesicles above each site of vesicle fusion (or release site) forms the RP (releasable pool), (from Sikora et. al, 2004).

3.3 Visual Processing in the retina

The vision involves the transduction of light into membrane potential changes as a result of enzymatic reactions occurred in the outer segments of rod and cone photoreceptors. The changes in the membrane voltage is transmitted to second order bipolar and horizontal cells by changes in release of neurotransmitter; glutamate, from the synaptic terminals of rods and cones.
The photoreceptors show a high glutamate release. When the light reaches to the photoreceptors, it starts a biochemical process that reduces the release of glutamate. The inhibitory effect over the glutamate release affects further the functionality of the bipolar and horizontal cells. The bipolar cells communicate with the amacrine and retinal ganglion cells. Furthermore, the retinal ganglion cell axons exit the eye as an optic nerve and transmit the visual information to the brain. The transmission line of the light perception is made through the receptor cell than bipolar cell and ganglion cells. The amacrine and horizontal cells influence indirectly the transmission. The horizontal cells modulate the synaptic activity of the photoreceptors and therefore affect the transmission of the bipolar cells and ganglion cells. The amacrine cells modulate the synaptic activity of the bipolar cells and ganglion cells and so it affects the transmission of the ganglion cells. Bipolar cells receive glutamatergic inputs from the photoreceptors and GABAergic from horizontal cells and in return the bipolar cells send glutamatergic excitatory inputs to retinal ganglion cells and glutamatergic inhibitory inputs to amacrine cells. The major classes of neurotransmitters are amino acid transmitters such as glutamate and GABA.

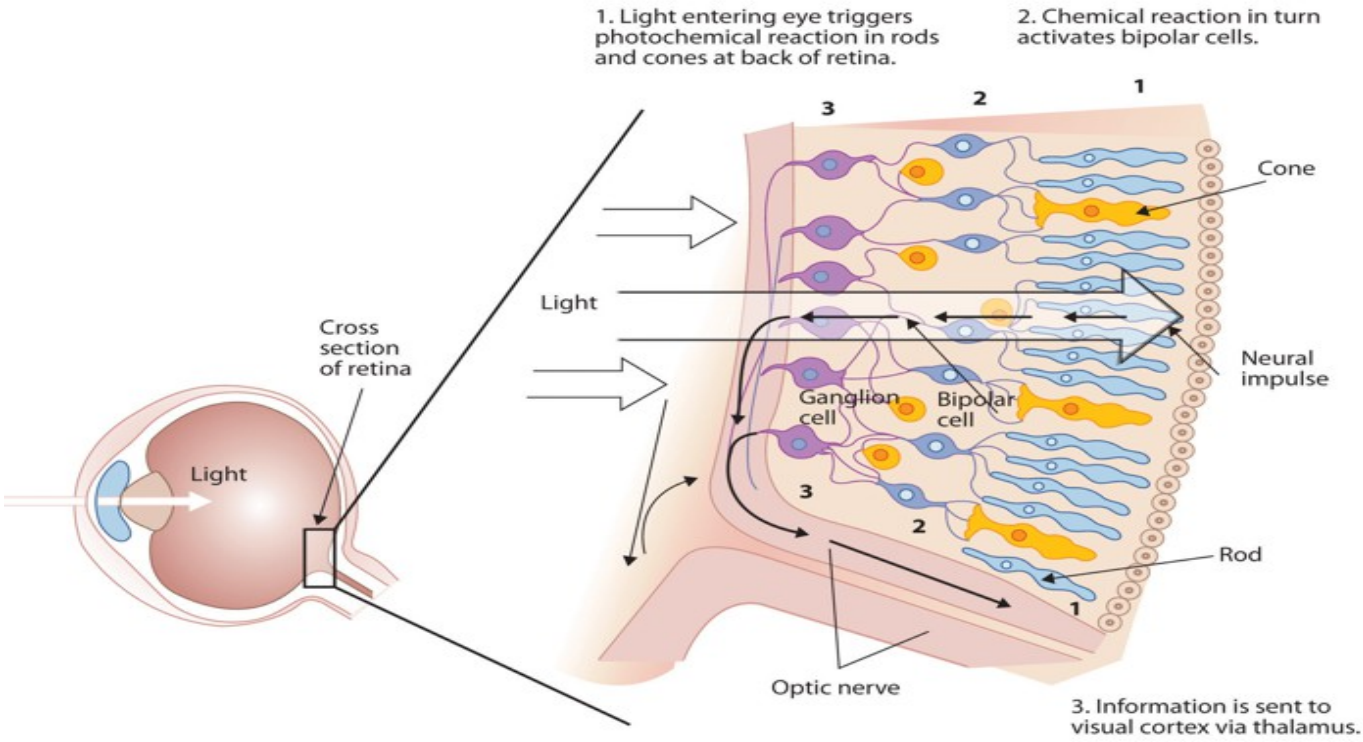

Fig. 3.10. Visual processing in the retina diagram. When light falls on the retina, it creates a photochemical reaction in the rods and cones, in the bipolar cells, and in the ganglion cells, and eventually to the optic nerve, (Introduction to Psychology, 2012).

The neurotransmitters are stored in synaptic vesicles that fuse with the presynaptic membrane and release their contents when an action potential arrives the terminal and causes an increase in the Ca^{2+} concentration by activation of the voltage dependent Ca^{2+} channels, (Fig. 3.10).
Rod and cones photoreceptors are tonically depolarized in darkness with a voltage membrane between -35mV to -45 mV which enable a continuous release of the neurotransmitter, glutamate. Light on will cause a hyperpolarization of the photoreceptors which determinate further a reduction in glutamate release while the light off will cause a depolarization of the cell membrane and an increase in glutamate release. The ribbon structure presented in the photoreceptors synaptic terminals allows a fast respond to the changes in illumination. Different factors influence the kinetics of synaptic transmission. Hyperpolarization of the membrane of the photoreceptors reduces the open probability of Ca^{2+} channels which leads to reduce the rate of glutamate vesicles release rate.
The depolarization leads to increase of Ca^{2+} influx and stimulates the exocytosis of glutamate.
The steps that follow the signal processing at the level of the bipolar cells are:

- terminal membrane depolarization
- calcium influx
- neurotransmitter release

Increase of intracellular Ca^{2+} concentration can lead to triggering of neurotransmitter release from a nerve axon terminal. L channels are activated by large depolarization (around -20mV) and can remain open for a long time before inactivating. The sub-type Cav1.4 is found only in the retina.
An OFF bipolar cell depolarizes when the photoreceptors are in dark and an ON bipolar cells depolarizes when the photoreceptors are in light, (Fig. 3.11).
Cone bipolar cells have a synaptic contact with cone receptor cells and are color sensitive. The cone bipolar cells can be hyperpolarized or depolarized depending on the amount of the glutamate release and so they could be ON or OFF bipolar cells.
Rod bipolar cells have synaptic contact with rod receptors cells and are color insensitive. The rod bipolar cells are hyperpolarized by the glutamate neurotransmitter and are only ON bipolar cells.

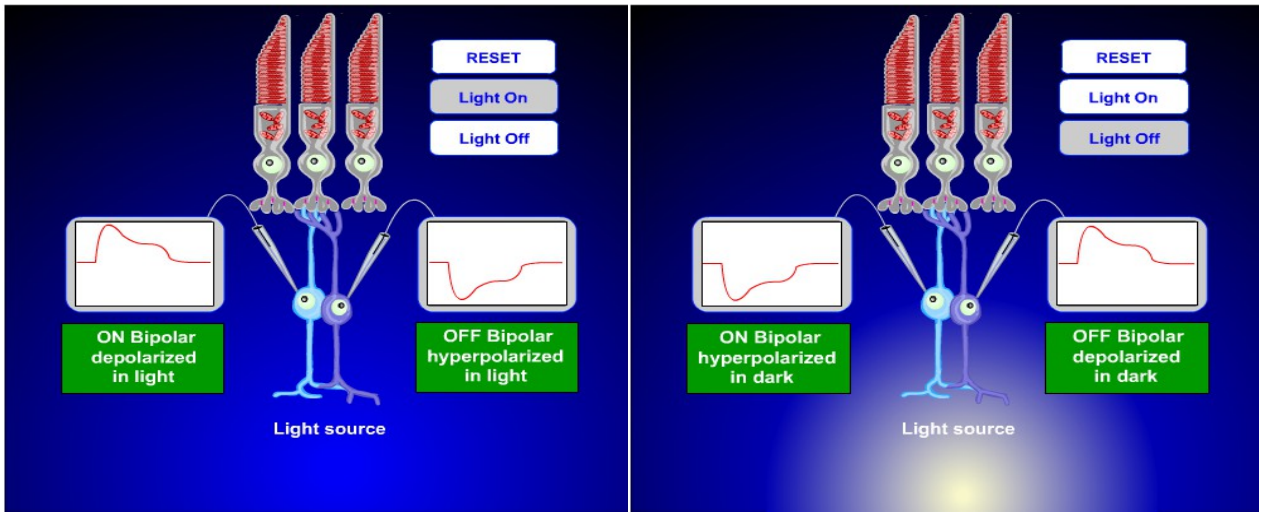

Fig. 3.11. (left) the photoreceptors cells are in light which cause for the on biploar cells depolarization and OFF bipolar cells hyperpolarization. (right) the photoreceptor cells are in the dark which causes ON bipolar cells and OFF bipolar cells hyperpolarized and depolarization, respectively. (Neuroscience online, 1997).

Most of the bipolar cells do not generate action potential but in return generate graded potentials (depolarization or hyperpolarization) depending on the inputs. Fast voltage transients have an attenuation depending on the length and diameter of the axon. According to T.Euler and T.Baden(2014) some simulations based on passive cable theory show that a bipolar cell with an axon length of cc. 30µm and diameter of 200nm, filter frequencies above 20Hz in a percentage more than 50% and on the other hand OFF bipolar cells with shorter axons can render these frequencies in a better way with the scope to signal fast changes in the visual scene.
Bipolar cells have concentric receptive field (a receptive field is the position where they make with the receptor cells synaptic contact). If the light is directed on the photoreceptors, which make synaptic contact with the bipolar cells, then the response produced by the bipolar cells is called the center response. The light directed over the surrounding receptors produce the inverse response, (Fig. 3.12).

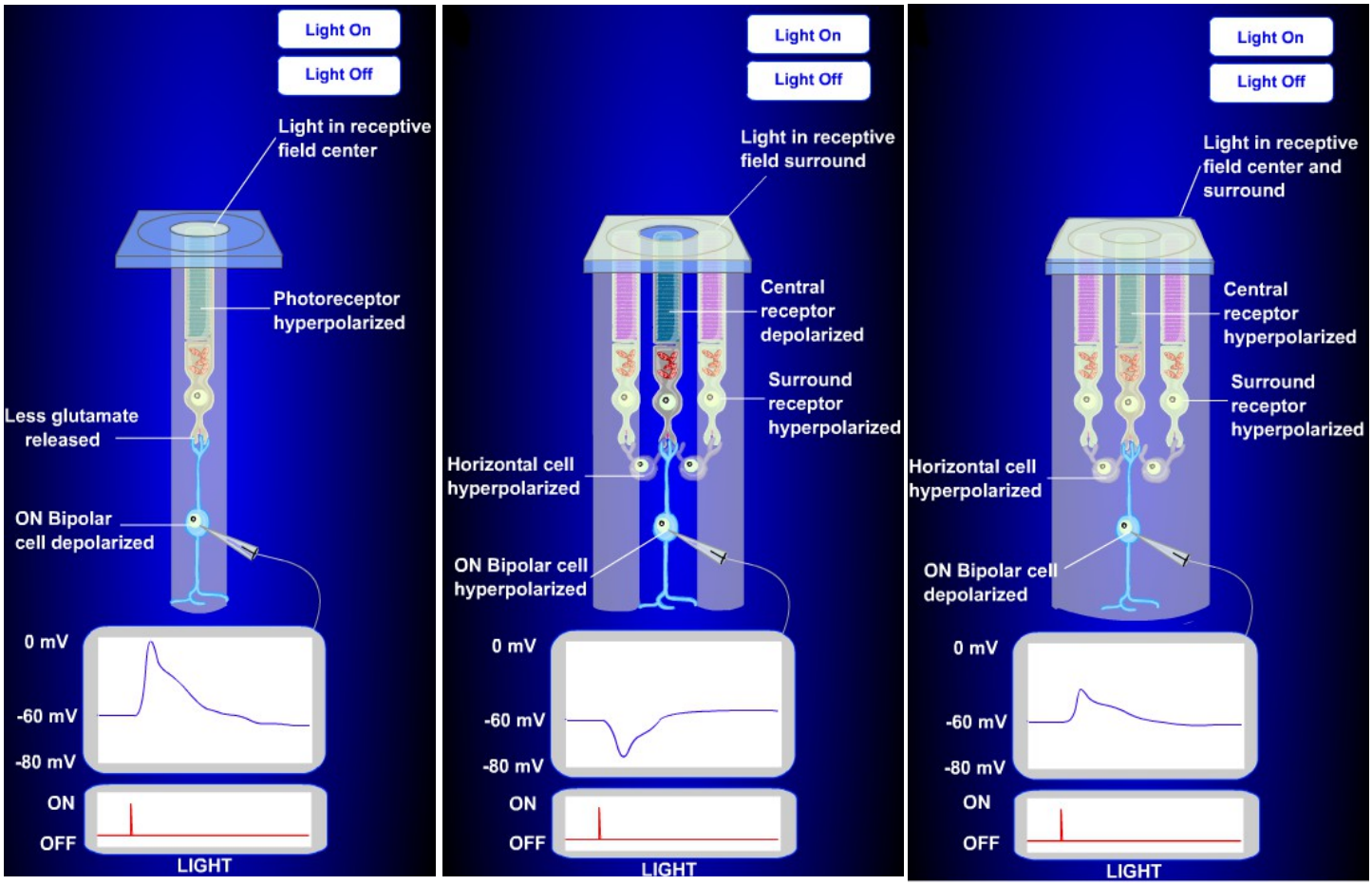

Fig. 3.12 (left) the center receptors cells (the ones that makes direct synaptic contact with the bipolar cell) are direct lighted and the ON bipolar cell is depolarized. (Middle) the receptors surrounding the center receptors of the ON bipolar receptive field receive light and the center photoreceptors are prohibited to receive light, then the ON bipolar cell is hyperpolarized. (Right) both the center and surrounding receptors of the ON bipolar cell receptive fields are lighted, then the ON bipolar cell depolarizes but is lower in magnitude, (Neuroscience online, 1997).

As a result, the ON bipolar cells have the strongest depolarization when the stimulus is a light spot encircled by a dark ring and for the OFF bipolar cells the strongest depolarization is when the stimulus is a dark spot encircled by a light ring.

The bipolar cells will control the transmission of the visual information towards the brain by depolarizing the amacrine cells by releasing glutamate.

Ca^{2+} enters bipolar cells through L - type Ca^{+} channels and gather around synaptic ribbons.
L type channels show little inactivation over time and can deliver a linear relationship between voltage and calcium influx.
L-type Ca^{2+} channels with slow inactivation would be especially suitable to control graded and sustained neurotransmitter release in bipolar cells. The opening of voltage-gated Ca^{2+} channels in response to depolarization causes a rapid increase in the intracellular concentration of Ca^{2+}, which in turn causes the release of neurotransmitter.
By the time the axon terminal is depolarized, the vesicles clustered in the active zone of the terminal will be released. The amount of transmitter in each vesicle is called a quantum. Because transmitter release proceeds through the fusion of individual vesicles with the presynaptic terminal plasma membrane, the release process is known as quantal release. Exocytosis is determined by the influx of Ca^{2+} induced by depolarization of the nerve terminal. Terminals must be capable of sustained firing and neurotransmitter release because communication between neurons often involves repeated trains of stimuli.
There are some steps to accomplish the exocytosis process, (Fig. 3.13):

- docking, vesicles must be specifically targeted to the active zone through a process referred to as docking
- priming, active maturation process of the vesicle which requires ATP hydrolysis. During priming, SNARE proteins embedded on the plasma membrane and on the vesicle membrane form a complex that brings the vesicle into close apposition with the plasma membrane. Primed vesicles release neurotransmitter by the Ca^{2+} influx.
- fusion/exocytosis, extrusion of vesicle contents into the synaptic cleft by a rise in intracellular calcium

Intracellular calcium concentration triggers vesicle fusion with the plasma membrane and the neurotransmitter is released into the synaptic cleft.

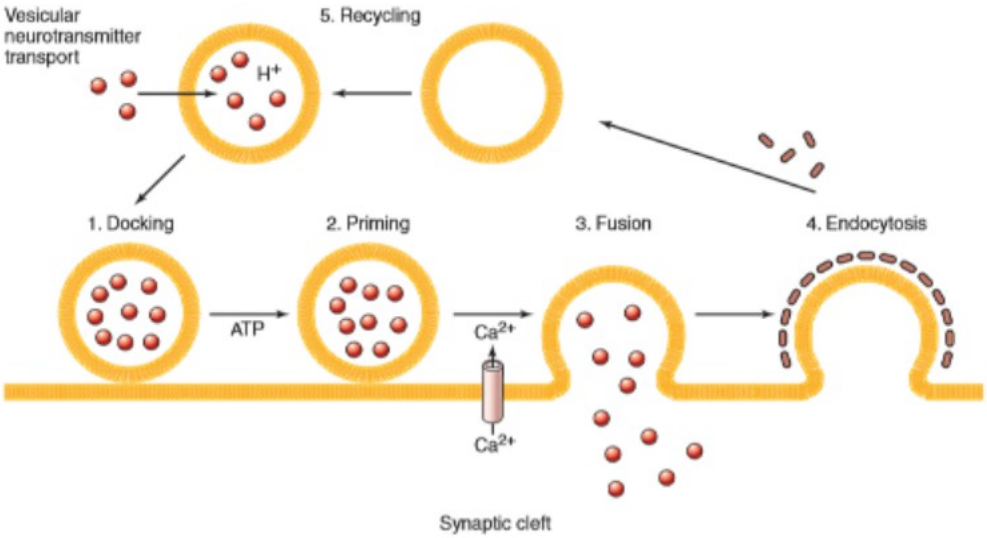

Fig. 3.12 The exocytotic cycle and the different steps involved: 1) docking of synaptic vesicles at the plasma membrane of the active zone 2) priming requests ATP and prepares each vesicle for release 3) fusion/exocytosis neurotransmitter releases are triggered by the Ca^{2+} influx in the plasma membrane, (from Nestler et. al, 2009).

Chapter 4 Methods

4.1. The Hodgkin-Huxley and Fohlmeister -Coleman-Miller model of the action potential

To characterize the electrical properties of the membrane cell, the HH and FCM models were used. Hodgkin and Huxley used the Voltage Clamped techniques for the study of the action potential generation in a giant axon of squid.
Hodgkin and Huxley considered that the ionic transport in membrane of a giant squid axon is due to a gating mechanism and sodium and potassium ions are responsible for axon excitation.

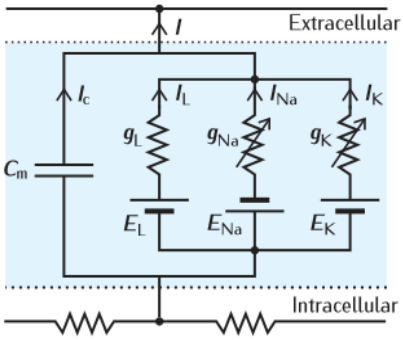

Fig. 4.1. The Hodgkin-Huxley equivalent circuit, (from D.Sterratt et. al, 2011).

The electrical properties of a neuron are represented by capacitors, used to model the charge storage of the cell membrane, resistors used to model different ion channels and batteries used to model electrochemical potentials obtained due to different intracellular and extracellular ion concentrations.
In the HH model the equivalent electrical circuit, (Fig. 4.1.) of a compartment shows three types of ionic currents, sodium, potassium and leak currents; the potassium and sodium conductances depend on voltage.
HH equations describe the voltage-current relationship for the membrane cell in a squid axon.
The equation corresponding the electrical circuit is:

$$I = I_c + I_i = C_m \frac{dV}{dt} + I_i \quad \text{(Eq. 4.1)}$$

where I is the membrane current
I_c is the capacitive current
I_i is the ionic current
C_m is the membrane capacitance and usually equal to 1μF cm^{-2}

$$I_i = I_{Na} + I_K + I_L \quad \text{(Eq. 4.2)}$$

$$I_{Na} = g_{Na}(V - E_{Na}) \quad \text{(Eq. 4.3)}$$

$$I_K = g_K(V - E_K) \quad \text{(Eq. 4.4)}$$

$$I_L = g_L(V - E_L) \quad \text{(Eq. 4.5)}$$

where g is the conductance
E is the equilibrium potentials
g_L is the conductance of the leak current

The magnitude of each ionic current type is calculated as a difference between the membrane potential and the equilibrium potential. The HH model can be extended to include the calcium currents.
Calcium channels allow the Ca^{2+} influx through selective pores which open in response to cell membrane depolarization. The calcium influx creates an accumulation of Ca^{2+} ions in the cytoplasm and can act as a trigger for secretion of neurotransmitters. The calcium channel have a particularity of dual functionality in the cell membrane:

- generation of an electrical signal
- creating an intracellular chemical messenger

Voltage-gated calcium channels were discovered by Fatt and Katz (1953) as all the excitable cell show a voltage dependence calcium channels. The voltage-gated calcium channels mediate calcium influx in response to cell membrane depolarization and regulate neurotransmission. The main calcium channels are divided in three main groups, L, T, N type.
L-Type, is the main calcium channel type and require a strong depolarization for activation; they are also found in muscle and endocrine cells.
T-type channels are activated by weak depolarization and are contributing to action potential shape and controlling patterns of repetitive firing. They are inactive except in the hyperpolarization that follows a burst of action potentials and this conductance controls the duration of the inter-burst interval. A burst of spikes is terminated when the accumulation of intracellular calcium activates a calcium dependent potassium conductance.
In this thesis the used kinetics model is for the ribbon synapse of a rat type 9 cell. However the relations used for the simulations were extracted from the paper „A computational model of the ribbon synapse“ by Sikora and Miller 2005. The calcium channel kinetics were fitted to tiger salamander data experiments.

$$I_{Ca} = g_{Ca} * c^3 * (V - E_{Ca}) \quad \text{(Eq. 4.6)}$$

Ion	K^+	Na^+	Cl-	Ca^{2+}
Concentration inside(mM)	400	50	50	10^{-4}
Concentration outside(mM)	20	440	560	10
Nernst Potential(mV)	-72	52	-64	139

Table 4.1.Generalized form of the HH model. The concentration of various ionic concentration in the squid axon and outside the axon (Baker et al., 1971).

Voltage clamp, the technique used in this thesis, set the voltage membrane to a constant value and in this case there is no capacitive current to flow as the change in membrane potential, dV/dt, is zero. The voltage-clamp current is equal to the ionic current.
Space clamp technique, allow to inject a current into the cell and simultaneously measure the voltage membrane.

$$I_{stimulation} = I_{ionic} + C\frac{dV}{dt} \quad \text{(Eq. 4.7)}$$

By applying Kirchoffs's Law in a certain compartment using the extended HH model , (Table 4.1).

$$I_{stimulation} = C_m\frac{dV}{dt} + g_{Na\,max}m^3h(V - V_{Na}) + g_{Ca\,max}c^3(V - V_{Ca}) + g_{K\,max}n^4(V - V_K) + g_L(V - V_L)$$

(Eq. 4.8)

The probability of open or close state, determine the ionic currents and further the voltage across the membrane, can be calculated as:

$$\frac{dx}{dt} = -(\alpha + \beta)x + \alpha \quad \text{(Eq. 4.9)}$$

where x states for the m,h,c,n

$$\frac{dV}{dt} = -g_{Na\,max}m^3h(V - V_{Na}) - g_{Ca\,max}c^3(V - V_{Ca}) - g_{K\,max}n^4(V - V_K) - g_L(V - V_L) \quad \text{(Eq. 4.10)}$$

The aim of the thesis is to make an overview of the calcium current in the presynaptic membrane of the retinal bipolar cell by simulating the voltage or space clamp techniques. Therefore only the calcium ionic currents and the leakage current will be simulated as the potassium and sodium doesn't influence the vesicle release.
Fohlmeister-Coleman-Miller model describes the experimentally data obtained for retinal ganglion cells in salamander, (fohlmeister- coleman-miller 1990, f&m 1997).

The circuit contains five types of ionic currents contributing to action potential in these cells; sodium current, calcium current, leak current, potassium current, calcium activated potassium current, potassium type A current.

$$I_i = I_{Na} + I_K + I_L + I_{K,A} + I_{K,Ca} \quad \text{(Eq. 4.11)}$$

$$I_i = I_{Na} + I_K + I_L \quad \text{(Eq. 4.12)}$$

$$I_K = g_K (V - E_K) \quad \text{(Eq. 4.13)}$$

$$I_L = g_L (V - E_L) \quad \text{(Eq. 4.14)}$$

$$I_{Ca} = g_{Ca} (V - E_{Ca}) \quad \text{(Eq. 4.15)}$$

$$I_{K,A} = g_A (V - E_K) \quad \text{(Eq. 4.16)}$$

$$I_{K,Ca} = g_{K,Ca} (V - E_K) \quad \text{(Eq. 4.17)}$$

where E is the reversal potential for different ionic currents
g is the conductance for different ion currents

Most of the ionic currents are modeled with HH model except the calcium current.

$$g_{K,Ca} = g_{K,Ca\,max} \frac{([Ca^{2+}]_i / [Ca^{2+}]_{diss})^2}{1 + ([Ca^{2+}]_i / [Ca^{2+}]_{diss})^2} \quad \text{(Eq. 4.18)}$$

where $g_{K,Ca\,max}$ is the maximum conductance of calcium activated potassium
$Ca^{2+}{}_{diss}$ is a calcium dissociation constant equal to 10^{-3} mM/dm^3

$$\frac{d[Ca^{2+}]_i}{dt} = \frac{I_{Ca}}{2 * F * d} - \frac{[Ca^{2+}]_i - [Ca^{2+}]_{res}}{\tau_{dec}} \quad \text{(Eq. 4.19)}$$

where $[Ca^{2+}]_i$ is the Ca^{2+} concentration at the depth d inside the presynaptic membrane
I_{ca} is the Ca^{2+} current through the L type channel
F is the Faraday constant
d is the distance from the synaptic membrane of the calcium channels
$[Ca^{2+}]_{res}$ is the resting level of Ca^{2+} in the terminal
τ_{dec} is time constant of removal Ca^{2+}

$$E_{Ca} = \frac{RT}{2F} \ln \frac{[Ca^{2+}]_e}{[Ca^{2+}]_i} + 50 \quad \text{(Eq. 4.20)}$$

where E_{Ca} is the reversal potential calculated with Nernst equation
R is the gas constant equal to 8.314J/(MK)
F is Faraday constant
$[Ca^{2+}]_e$ is the extracellular calcium concentration equal to 1.8mM/dm^3

The intracellular calcium concentration varies with calcium current.
According to the observations made by Fohlmeister and Miller (1997), the calcium channel has no inactivation kinetics. The reduction in calcium current in the voltage clamp records is due to the Ca^{2+} concentration dependence. The equilibrium potential should be each time recalculated with the Eq. 4.20 because intracellular calcium depends on calcium current.
Although these considerations make a more accurate simulation of the calcium current, for simplification reasons it will be considered for the further results a reversal calcium currents E_{Ca} in the interval 10mV to 40mV.
Fohlmeister and Miller in their work considered the equilibrium potential for Ca^{2+} to 130mV. Because the calcium channels are also selective to potassium ions and the E_K is in the range of -60 mV to-90 mV it has been concluded by Protti and Llano (1998) that the variation range of the E_{Ca} can be approximated with the voltage range between 10mV to 40 mV.

4.2. Intracellular calcium concentration

As we want to analyze the neurotransmission at the level of the retinal bipolar cells, it is been simulated by the synaptic input. By doing this, a pulse of current is injected to a synapse and the membrane potential is calculated at different points.
The calcium concentration in a cellular compartment is determined by the influx of Ca^{2+} through voltage-gated channels, extrusion by membrane bound calcium pumps, buffering entry through voltage and ligand gated Ca^{2+}, uptake and release from intracellular stores

$$\frac{dCa^{2+}}{dt} = J_{diff} - J_{buff} + J_{cc} - J_{pump} - J_{up} + J_{rel} - J_{leak} \quad \text{(Eq. 4.21)}$$

where J_{diff} is diffusion flux
J_{buff} is buffering flux
J_{cc} is ionic current flux
J_{pump} is extrusion by the membrane bound ionic pumps
J_{up} is extrusion by uptake
J_{rel} is extrusion by release
J_{leak} is influx through the voltage-gated Ca^{2+} channels at rest membrane potential

Each flux specifies the rate of change of the number of molecules of Ca^{2+} per unit volume (rate of change of the concentration measured usually in μMs^{-1}). Flux leakage J_{leak} ensures the total flux is zero at rest.
The intracellular Ca^{2+} concentration variation is due to the transmembrane fluxes into and out of a cell's compartment. The input of the Ca^{2+} into the cell membrane of a specific compartment is due to the voltage or ligand gated calcium channels. The intracellular Ca^{2+} concentration dynamics is equilibrated by the extrusion of the Ca^{2+} back into the cell membrane which is accomplished by the active ionic pumps and by the uptake.

In this thesis it is aimed to study the intracellular Ca^{2+} concentration dynamics determinated by the ionic Ca^{2+} currents.

$$J_{cc} = \frac{-I_{Ca^{2+}} * A}{2FV} \quad \text{(Eq. 4.22)}$$

where F is a Faraday constant set to 96485.33Cmol^{-1}
A is the compartment surface
V is the volume compartment
I_{Ca}^{2+} is the calcium current across the cell membrane

The ionic Ca^{2+} currents will cause a rise in the intracellular Ca^{2+} concentration. On the other hand, the buffering flux or the flux due to the membrane bound pump restore the intracellular Ca^{2+} concentration at the resting level.

$$\frac{d[Ca^{2+}]}{dt} = J_{cc} - \frac{[Ca^{2+}]_i - [Ca^{2+}]_{res}}{\tau_{dec}} \quad \text{(Eq. 4.23)}$$

where J_{cc} is the flux due to the Ca^{2+} ionic current entered through the voltage-gated Ca^{2+} ion channels
$[Ca^{2+}]_{res}$ is the resting Ca^{2+} level, usual of a concentration of 0.34 μM
τ_{dec} is time constant of the passive extrusion process, usually 10ms

This model describes more complex models of extrusion of intracellular Ca^{2+} concentration from a cellular compartment due to the flux pump or buffering and diffusion by a single exponential decay model.
This formula was used in the thesis to assess the intracellular Ca^{2+} concentration dynamics. The model will describe the Ca^{2+} decay.
The two main factors which determine the $[Ca^{2+}]_i$ are:

- influx of Ca^{2+} into the cell
- passive extrusion mechanism governed by the time constant τ_{dec} and the $[Ca^{2+}]_{res}$

The rise of $[Ca^{2+}]_i$ represents the trigger for the fusion of the vesicles with presynaptic membrane. The influx of the $[Ca^{2+}]_i$ is due to the fact that Ca^{2+} channels open by the depolarization of the presynaptic membrane.

4.3Synapse neurotransmission model

A chemical synapse is the transduction signal that produces a postsynaptic response by entering an action potential into the presynaptic terminal.
In this thesis a four vesicle pools model resemble the neurotransmitter vesicles at the retinal bipolar cells. The cytoplasmic pool C refills the two pools RRP and RP with the two constant rates $\rho_{RRP,}$ ρ_{RP}, respectively.The RP pool will characterize the vesicles located in the upper rows of the ribbon and the RRP pool contains vesicles tethered at the ribbon close to the fusion site and are primed for release. The two pools, the one has a slow release rate but constant (RP) and the other one has a fast release rate (RRP) and both of them release their content into the exocytosis pool E,(Fig. 4.2).

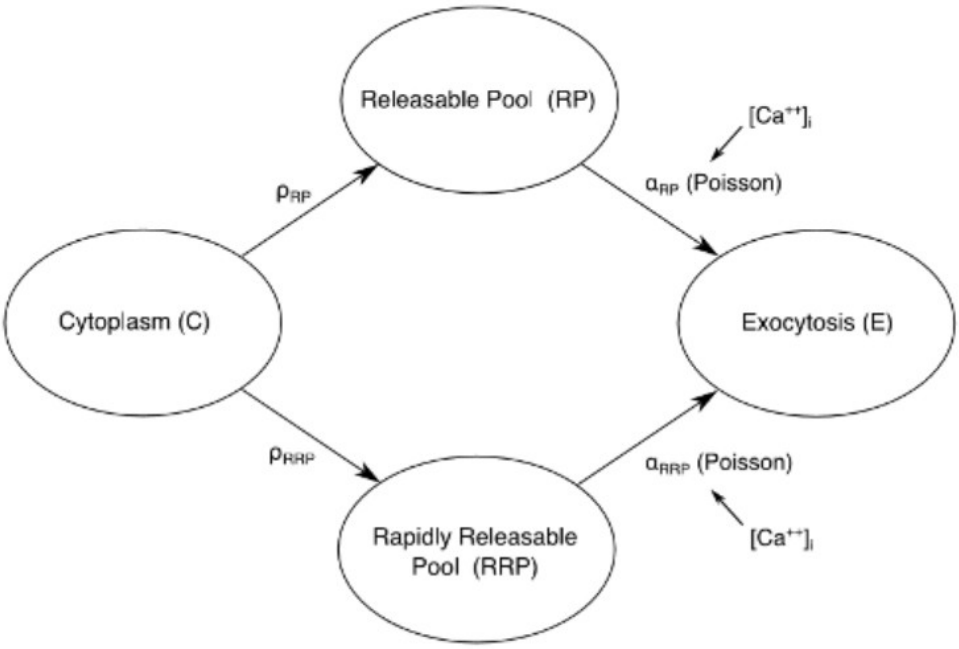

Fig. 4.2. Diagram of the synapse model, (from Werginz and Rattay, 2016).

A chemical presynaptic neuron contains an active zone which is formed by two pools RRP (rapid releasable vesicle pool) and RP (releasable vesicle pool) which are replenished from a single reserve vesicle pool. In a normal chemical synapse, a presynaptic action potential determinate Ca^{2+} entering the presynaptic terminal through the voltage-gated Ca^{2+} channels which may result in a vesicle from the RRP or RP fusing with the presynaptic membrane and releasing neurotransmitter into the synaptic cleft.
The retinal bipolar cell synapses have special structures; synaptic ribbons which are localized in the presynaptic terminal membrane. Ribbon synapses are surrounded by tethered synaptic vesicles and release vesicle in response to graded changes in the membrane potential.
The postsynaptic response depends on the releasing of the neurotransmitters from the presynaptic terminal which depends on voltage membrane state.
The transmitter activated by ion channels can be described by the time dependent conductivity that will open whenever a presynaptic spike arrives. The current that passes through a channels depends on the difference between its reversal potential and the actual value of the membrane potential. It is aimed in this thesis to evaluate the importance of intracellular calcium concentration over the vesicle release.
The ion channel is simulated with an opened/closed gate and by applying Ohm's law. The current that enters the cell can be calculated with the following relations:

$$V = I * R \quad \text{(Eq. 4.24)}$$

$$I = g * V \quad \text{(Eq. 4.25)}$$

$$I = g_{max} * c * V \quad \text{(Eq. 4.26)}$$

where g_{max} is the maximum conductance of the channel
c is the probability that a channel is opened

$$I(t) = g(t) * (V_m - E_{reversal}) \quad \text{(Eq. 4.27)}$$

where I is the ionic current channel
V_m is the voltage membrane at a certain time
$E_{reversal}$ is the reversal potential
g(t) is the probability over time that a channel is opened
In this study it was considered the morphology of a rat type 9 ON BC. The ion currents of a bipolar cell were calculated using the Eq. 4.28:

$$I_{ion,n} = (I_{Ca,n} + I_{L,n}) * A_n \quad \text{(Eq. 4.28)}$$

where A_n is the surface area of the n-th compartment
I_{Ca} is the Ca^{2+} current through a L-type calcium channel (Ca_v 1.4)
I_L is the leak current

$$I_{Ca,n} = g_{Ca} * c_n^3 * (V_m - E_{Ca}) \quad \text{(Eq. 4.29)}$$

where g_{Ca} is the cell membrane conductivity for calcium. It is set to 0 mS cm^{-2} in all non-terminal compartments and to 1mS cm^{-2} in terminal compartments
c is the probability that the channel is in the open conformation
V_m is the voltage membrane in a specific compartment
E_{Ca} is the Nernst potential (equilibrium potential) and is set in the range of 10mV to 40 mV

$$E_{Ca} = \frac{R * T}{(+2)F} \ln \frac{[Ca^{2+}]_o}{[Ca^{2+}]_i} \quad \text{(Eq. 4.30)}$$

where R is the universal gas constant and is set to 8.314J K^{-1} mol^{-1}
T is the Kelvin temperature
z is the valence of Ca^{2+} ions
$[Ca^{2+}]_o$ is the extracellular calcium concentrations
$[Ca^{2+}]_i$ is the intracellular calcium concentrations

The ionic concentrations and the equilibrium potential are assumed constant during the electrical activity of the cell. These assumptions are true in case of Na^+ and K^+ ion concentration and it is less true in case of Ca^{2+}. The intracellular calcium concentration changes relative to the extracellular concentration and it should be recalculated the equilibrium potential.

$$\frac{d[Ca^{2+}]_i}{dt} = \frac{I_{Ca}}{2 * F * d} - \frac{[Ca^{2+}]_i - [Ca^{2+}]_{res}}{\tau_{dec}} \quad \text{(Eq. 4.31)}$$

where $[Ca^{2+}]_i$ is the Ca^{2+} concentration at the depth d inside the presynaptic membrane
I_{ca} is the Ca^{2+} current through the L type channel
F is the Faraday constant
d is the distance from the synaptic membrane of the calcium channels
$[Ca^{2+}]_{res}$ is the resting level of Ca^{2+} in the terminal
τ_{dec} time constant of removal Ca^{2+}

The Ca^{2+}concentration which drives the exocytosis will be calculated near the presynaptic membrane.
A transient increase in Ca^{2+} concentration have different consequences. Some secondary effects as:

- there are some voltage-gated Ca^{2+} channels which are cross-sensitive themselves to the level of intracellular Ca^{2+} concentration. These Ca^{2+} channels close when the excessive Ca^{2+} ions bind to their intracellular receptive surface.
- depolarization of the membrane (Ca^{2+} ions carry positive charge into the cell). The K^+ channels activate and increase the outward ionic current (composed of out flowing K^+ ions) and cause the cell to repolarize.

These two consequences oppose to further Ca^{2+} inflow so the influx of Ca^{2+} ions is self-limited and contributes to the repolarization of the cell.

The open channel probability can be described with the following kinetics relations:

$$\frac{dc}{dt} = (1-c)*\alpha - c*\beta \qquad \text{(Eq. 4.32)}$$

$$Ca_V 1.4_{closed} \underset{\beta}{\overset{\alpha}{\leftrightarrow}} Ca_V 1.4_{opened} \qquad \text{(Eq. 4.33)}$$

where α is the rate that the channel goes from the close state to open state
β is the rate that the channel goes from the open state to close state
c is the probability that the channel is in open conformation

In our case, the ion channels are voltage-gated types which involve a membrane voltage dependent gating via open and close rates α and β.

According to Hodgkin and Huxley voltage clamp experiments, the following relations are used to calculate the open-close rates.

$$\alpha = \frac{-0.3*(V_m+70)}{\exp(-0.1*(V_m+70))-1} \quad \text{(Eq. 4.34)}$$

$$\beta = 10*\exp(-(V_m+38)/9) \quad \text{(Eq. 4.35)}$$

In Chapter 5 the model equations will be solved using backward Euler with a fixed step size of 0.1 ms

$$I_L = g_L*(V_m - E_L) \quad \text{(Eq. 4.36)}$$

where g_L is the cell membrane conductivity. It is set to 1mS cm^{-2} in all non-terminal compartments and to 2mS cm^{-2} in terminal compartments
V_m is the voltage membrane in a specific compartment
E_L is the resting potential set to -50 mV

In a normal chemical synapse, a vesicle releases its content when an action potential arrives at the presynaptic terminal.

Vesicle release can be modeled by assuming two variable:

- p, probability of vesicle release
- n, the number of releasable vesicle

The probability of vesicle release depends on the intracellular Ca^{2+} concentration in the presynaptic terminal. A Ca^{2+} influx through the voltage-gated Ca^{2+} channels will increase the probability (according to a study made by Zucker and Fogelson,1980 with a power of 3- 4).

In case of the bipolar cells, the intracellular Ca^{2+} concentration regulates the release of vesicle transmitter.
The ribbon synapse can release neurotransmitters continuously:

- from the rapid releasable vesicle pool (RRP)
- from releasable vesicle pool (RP)

The filling of these pools determinates by:

- their current pool state
- their rate constants for refill and release

$$\rho_{RP} = \rho_{RPconstant} * \frac{RP_0 - RP_t}{C_0} \quad \text{(Eq. 4.37)}$$

$$\rho_{RRP} = \rho_{RRPconstant} * \frac{RRP_0 - RRP_t}{C_0} \quad \text{(Eq. 4.38)}$$

where $\rho_{RP\ and}$ ρ_{RRP} are the refilling rates for the two pools

$C_{0,}$ RP_0, RRP_0 are the initial state of the cytoplasmatic pool, releasable pool and rapidly releasable pool, respectively

RRP_0 is set to 6 vesicles, RP_0 is set to 30 vesicles (RP_0 =5*RRP_0) and C_0=360 vesicles (10*(RP_0 + RRP_0) per ribbon

$\rho_{RP\ constant,}$ $\rho_{RP\ constant}$ are the refill constant rates and set to 0.25 s^{-1}

RP_t, RRP_t are current releasable pool and rapidly releasable pool state, respectively

According to a study made by Sterling and Matthews "Structure and function of ribbon synapses", the ratio between the total number of vesicles tethered to the retinal synaptic ribbon and those tethered vesicles docked also at the plasma membrane is 5:1.
The release vesicle from either releasable or rapidly releasable pool is modeled mathematically with a Poisson process.

The filling of the exocytosis pool determinates by:

- the releasable pool and rapidly releasable pool state
- their rate constants for refill and release
- accomplishment of the condition of Gaussian (0,1) <=$rate_{RP}$ and Gaussian (0,1) <=$rate_{RRP,}$

$$rate_{RP} = 1 - \exp\left(-\alpha_{RP} * [Ca^{2+}]_i * RP_t * \Delta t\right) \quad \text{(Eq. 4.39)}$$

$$rate_{RRP} = 1 - \exp\left(-\alpha_{RRP} * [Ca^{2+}]_i * RRP_t * \Delta t\right) \quad \text{(Eq. 4.40)}$$

where α_{RP} is set to 60 (vesicle*M*ms) $^{-1}$ and α_{RRP} is set to 5000 (vesicle*M*ms) $^{-1}$. They are the release rate constant into the exocytosis pool

$[Ca^{2+}]_i$ is the intracellular calcium concentration in the synaptic terminal

RP_t and RRP_t are the pool state at time t

Δt is the simulation step

The Ca^{2+} influx controls the vesicle release from the RRP and RP. In the synaptic terminals, the graded membrane potential changes and influence the number of vesicle which undergo exocytosis.

Chapter 5.Computations

The basic function of the action potentials in synaptic terminals is to open voltage-gated calcium channels locating near the vesicles containing neurotransmitters. The calcium inflow triggers the release of these vesicles and is the main factor of releasing. In consequence, the shape of the action potential and the gating properties of the calcium channels will influence the amount of the vesicle release.
Augustine 1990, stated that if the action potentials open calcium channels with a low probability, there will be closed channels in between open calcium channels. This means that the distance between open calcium channels is high and the intracellular calcium concentration needed for the vesicle release would be obtained from an adjacent open calcium channel.
If action potentials open calcium channels with a high probability, according to Zucker (1996), open calcium channels are close to each other and the calcium influx from adjacent channels enlarge the region with high calcium concentration and lead to the release vesicles.
In this chapter, the simulations results are presented. The tool used for the computations was Matlab 2016.

5.1. Gating variable

The membrane conductance describe the probability of a certain channel to be in an open, ion conducting state. This probability depends on the time course of the membrane potential.
The calcium gate can be controlled by a number of independent gating particles. In case of calcium, the gating particles which describe the model is „c“ (according to other experiments from Byerly et al 1984, Kay and Wong 1987, Zidanic and Fuchs 1995). The gating variable c, is the probability of a single calcium gating particle to be open; the probability of the calcium gate to be open is c^3 (3 comes from the number of gating particles in the gate). These assumptions were made in the HH experiment in a squid giant axon but it is still used in modeling.
The membrane conductance is calculated with the formula:

$$g_{Ca} = g_{Ca\,max}\, c^3 \quad \text{(Eq.5.1)}$$

where $g_{Ca\,max}$ =1mS/cm^2 for terminal compartments, (from Werginz and Rattay, 2016).

$$I_{Ca} = g_{Ca}(V_m - E_{Ca}) \quad \text{(Eq.5.2)}$$

where V_m=-50mV
E_{ca}=20mV

The state variable of the calcium voltage-gated channel „c“ follows the first order kinetics equation of the Hodgkin-Huxley formalism.

$$\frac{dc}{dt} = -(\alpha + \beta)c + \alpha \quad \text{(Eq.5.3)}$$

and the rate constants are:

$$\alpha = \frac{-0.3*(V_m+70)}{\exp(-0.1*(V_m+70))-1} \quad \text{(Eq.5.4)}$$

$$\beta = 10*\exp(-(V_m+38)/9) \quad \text{(Eq.5.5)}$$

The time course of c(t) will be obtained as:

$$c(t) = c_\infty(v) - (c_\infty(v) - c_0)e^{-(\frac{t}{\tau(v)})} \quad \text{(Eq.5.6)}$$

$$c_\infty = \frac{\alpha}{\alpha+\beta} \quad \text{(Eq.5.7)}$$

$$\tau(v) = \frac{1}{\alpha+\beta} \quad \text{(Eq.5.8)}$$

The calcium channel has no inactivation (inactivation is a decay of current magnitude during the membrane depolarization, and a decreased probability for channel to open at more depolarized membrane potentials).
According to Fatt and Katz (1953), all excitable cells have voltage-gated Ca^{2+} channels. Their characteristics are that they do not inactivate during a depolarization but close immediately after repolarization. The calcium ionic magnitude current is calculated as a product of the difference between the membrane potential and the equilibrium potential multiplied by the membrane conductance.
For V_m=-20mV the probability of the calcium channel to be open is 0.9. it means that almost all of voltage-gated channels open which lead to entering an inward depolarizing calcium current, (Fig. 5.1). Accumulation of the Ca^{2+} ions in the cytoplasm stops for E_{Ca}= 20 mV since I_{ca} becomes positive which causes an outward current. This observations denote that a high depolarization membrane doesn't contribute to a higher intracellular calcium concentration.

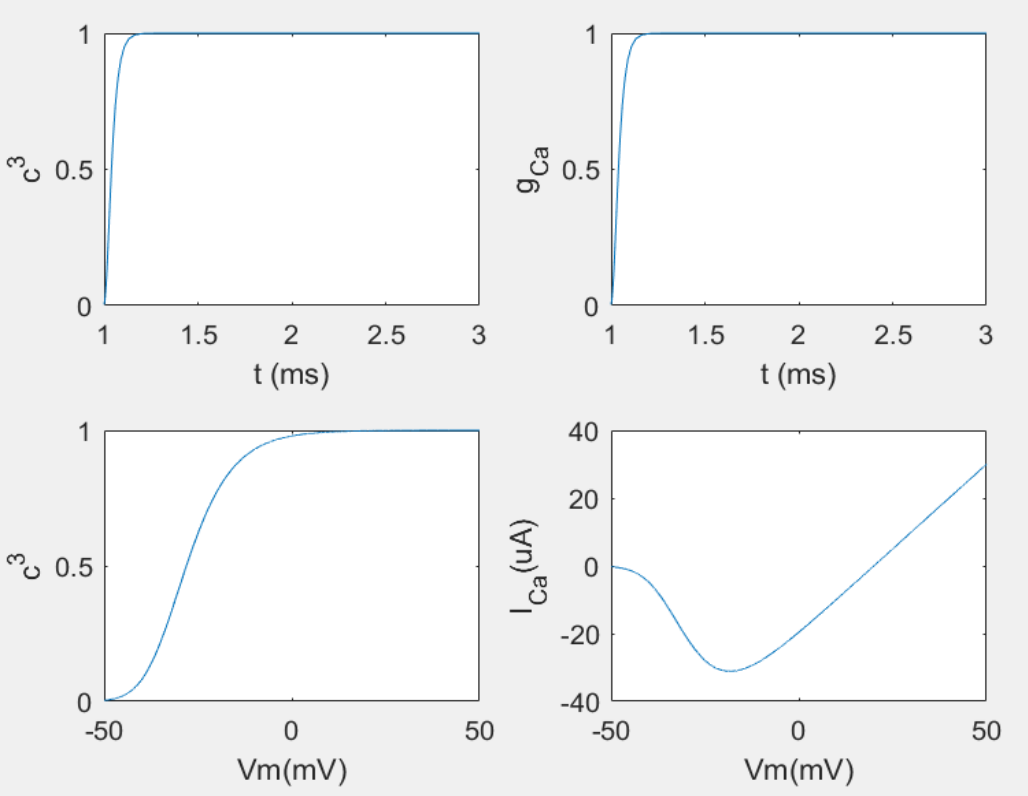

Fig. 5.1 (Up-left) open probability of calcium gating particle vs time. (Down-left) open probability of calcium gating particle vs voltage membrane. (Up-right) maximum conductance vs time. (Down-right) calcium current as a function of voltage membrane.

If V_m <-50 mV, the c^3 is approx.0 which states that all calcium voltage-gated channels are closed, the lower the probability is, the hardly any calcium ion will cross the membrane.
The variable „c“ (in Eq.5.3) is calculated by the ode23 Matlab.

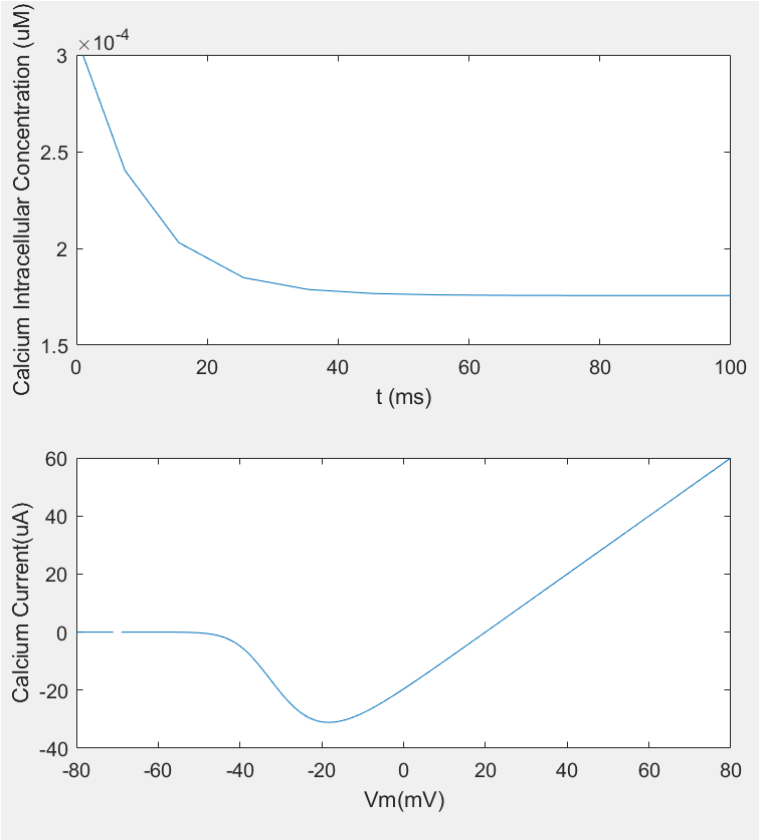

Fig.5.2 (Top) Intracellular calcium concentration vs time. (Bottom) Calcium current vs voltage membrane.

The intracellular calcium concentration is calculated in special depth inside the presynaptic membrane. Calcium influx is calculated as a function of calcium current across the membrane, surface area and volume of the compartment. In our case and according to Sikora et al. (2005), for distances very close to the membrane, the ratio can be approximated with the reciprocal of the depth.
In these simulations:

- depth=25nm
- E_{ca}=20mV
- Calcium concentration at the resting level =0.34μM
- τ, the time constant of removal calcium from the terminal=10ms

The simulations below show that without a sustained depolarization the intracellular calcium concentration decreases versus time exponentially due to the voltage-dependent conductance. At voltage membrane V_m = -50 mV, all calcium channels are closed and if the membrane depolarizes, calcium channels begin opening and an inward calcium current comes into the cell.

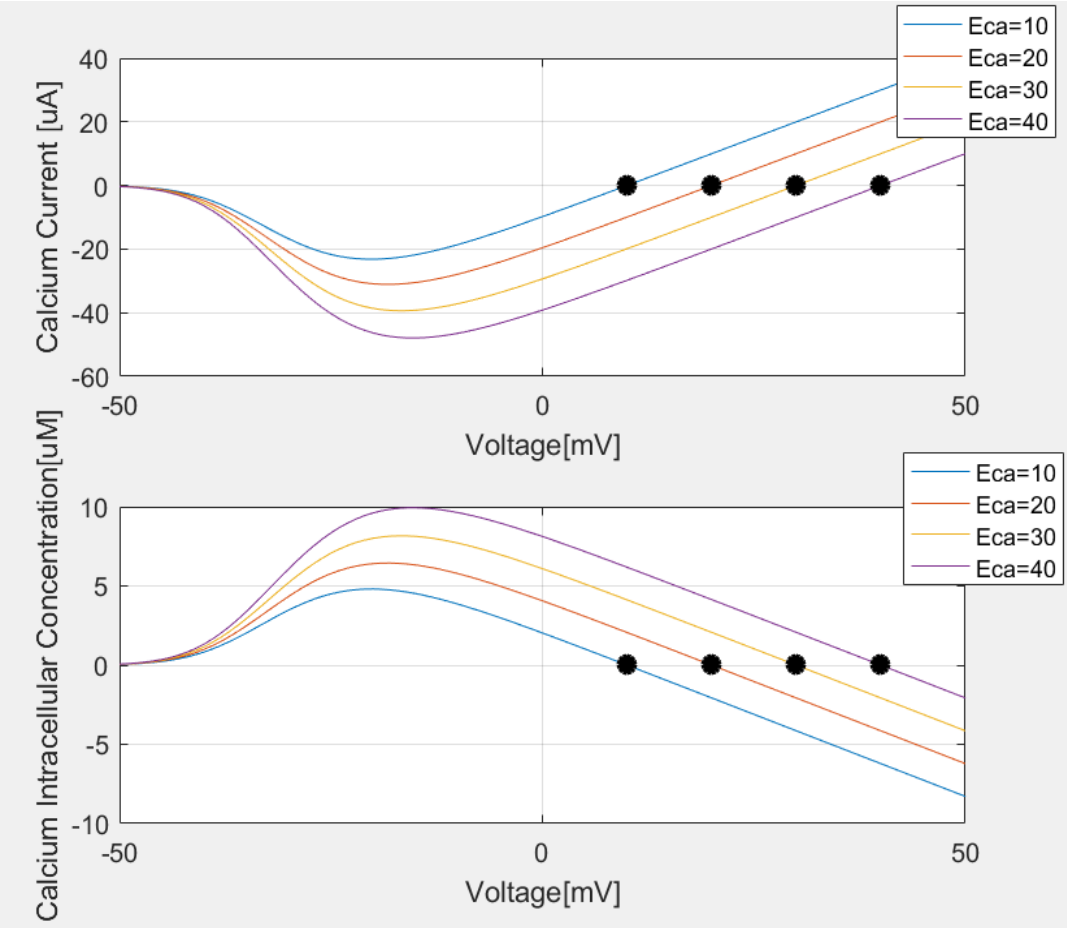

Fig. 5.3 a) (Top)Calcium current as a function of the voltage membrane state. b)(Bottom) Intracellular calcium concentration as a function of the voltage membrane state.

Entering calcium ions begins after reaching the peak of the action potential to the terminal and ends before the end of repolarization stage, (Borst,Sakmann,1996).
Calcium channels are activated near the peak of the action potential and calcium currents are largest during the falling phase and have higher values in the last stage of the repolarization, (Sharma, Garg, 2014).
The voltage membrane is stepped in the interval between -50mV to 50 mV. For different reversal calcium potentials leads to the unwanted out flux of Ca^{2+} ions at V_m =10mV, V_m =20mV, V_m =30mV, V_m =40mV, (Fig5.3. a).
For E_{Ca}=20mV the amplitude current is around -23.19 uA.
According to Kanaeda and Kanaeko (1991), the calcium current diminishes after the negative peak because the calcium reversal potential is dependent on concentration. Voltage-gated calcium channels mediate intracellular calcium concentration in response to membrane depolarization.
Fig 5.3. b shows the intracellular calcium concentration at depth d=25nm at different calcium reversal potentials when the voltage ramp from -50mV to 50 mV is applied. The intracellular concentration exhibit a peak near the position where the current calcium exhibit a negative peak and then the concentration decreases until it reaches to the resting level of 0.34μM at V_m=10mV, V_m =20mV, V_m=30 mV, V_m=40mV.

5.2Calcium intracellular concentration in case of voltage clamp

In order to measure the influx of calcium, the voltage membrane is clamped using action potential waveforms as command voltages.
Voltage clamp implies the clamping of the membrane potential to a steady value or to a time varying profile and measure only the ionic currents, in our case only the calcium current.
Voltage clamp set the membrane potential to a constant value, in our case V_m = -20mV and no capacitive current will flow because the rate of change in the membrane potential, dV/dt, is zero.
The voltage clamp current will measure directly only the calcium current.

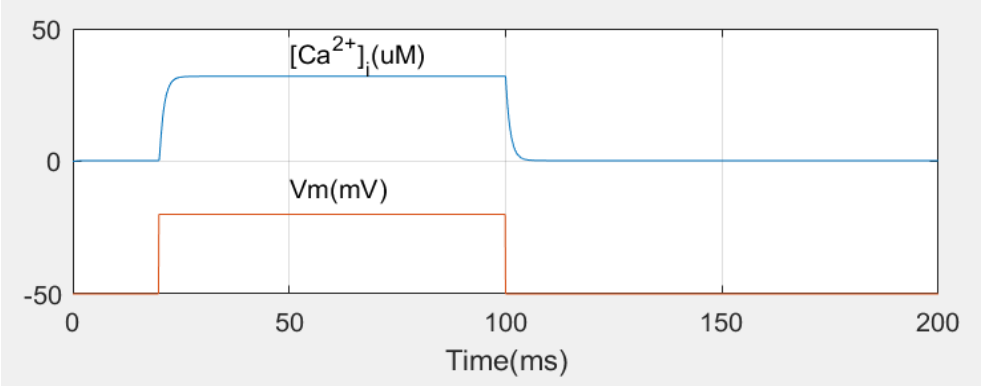

Fig. 5.4. Intracellular calcium concentration in case of a voltage membrane step -50mV to -20mV.

In Fig.5.4, the voltage membrane was clamped from its resting potential from -50 mV amplitude to -20mV with the duration of 80ms. The intracellular calcium concentration arises up to around 32 μM and drops down to its resting level, in our case 0.34 μM, after the clamp finishes.
The equations involved in determining the intracellular calcium concentration are Eq.5.2, Eq.5.3, Eq.5.4, Eq.5.7 and Eq.5.9.

$$\frac{d[Ca^{2+}]_i}{dt} = \frac{I_{Ca}}{2*F*d} - \frac{[Ca^{2+}]_i - [Ca^{2+}]_{res}}{\tau_{dec}} \quad \text{(Eq.5.9)}$$

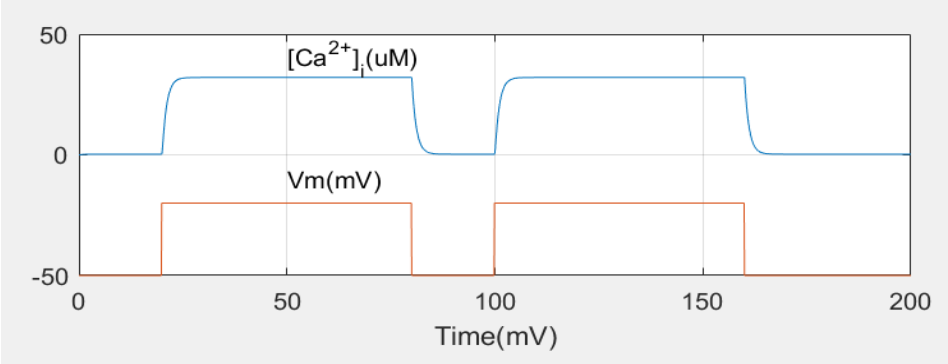

Fig. 5.5 The intracellular calcium concentration by applying two step voltage, using the same amplitude -50mV to -20mV and the same duration pulse of 60ms.

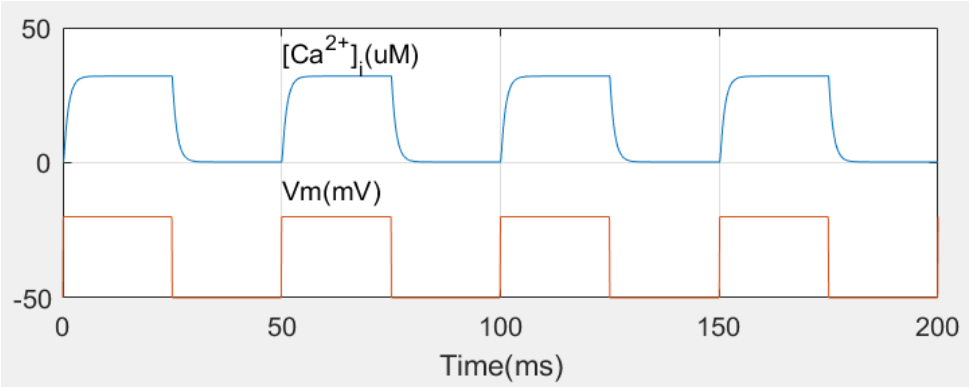

Fig. 5.6 The intracellular calcium concentration by applying a train of step voltages from -50mV to -20mV and frequency of 20Hz.

The calcium concentration characteristics follows the voltage. According to Zucker (1996), the calcium channel gate is a fast one which enables an action potential to achieve a short and large calcium influx. The shape of the calcium current will generate a large increase in the calcium concentration in the membrane.
Borst and Sakmann (1998) stated that calcium channels could present a faster gating in presynaptic terminals than in the soma.
At rest, the probability of open gate is according to the equation

$$c_0 = \frac{\alpha(V_0)}{\alpha(V_0)+\beta(V_0)} \quad \text{(Eq.5.10)}$$

where V_0 = -50 mV (in our case)

The probability „c“ increases exponentially to a steady state c_∞. The equations which describe the probability evolution during a voltage step are Eq.5.3, Eq.5.7 and Eq.5. 8 which describe how fast the gate will achieve to a steady state.

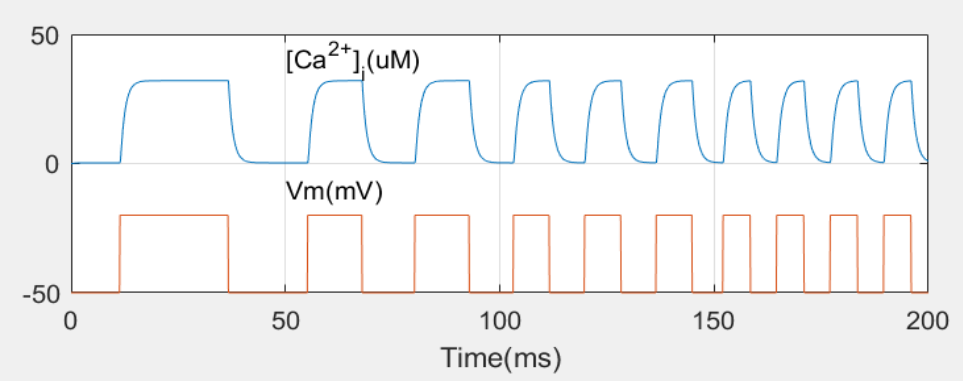

Fig. 5.7 The intracellular calcium concentration by applying a train of step voltages, using the same amplitude but different frequencies.

It can be seen from the simulation (Fig. 5.7) that increasing the frequency would keep the intracellular calcium concentration relative constant. The frequencies of the voltage step are f=20 Hz, 40 Hz, 60 Hz, 80 Hz.

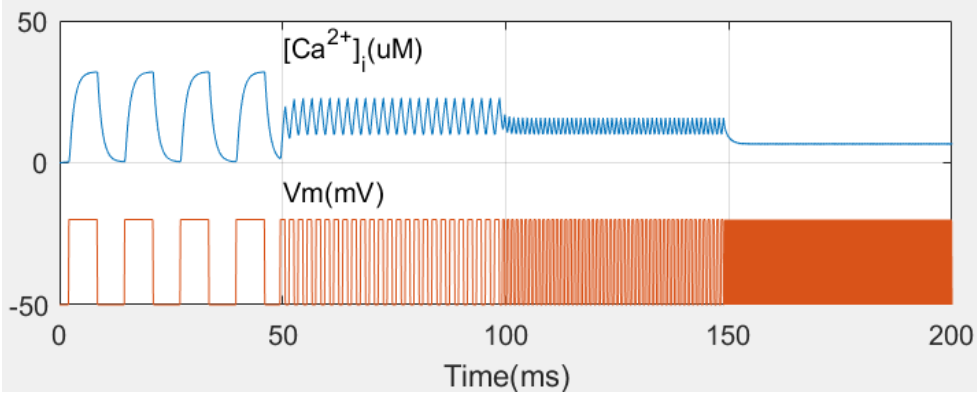

Fig. 5.8 The intracellular calcium concentration by applying a train of step voltages, using the same amplitude but different frequencies (80Hz, 500Hz, 1kHz, 5kHz).

If the frequency of the voltage potential increases even more, the observations shows that from a certain frequency, f =5kHz, the kinetics gate cannot follow the change in the voltage membrane, (Fig. 5.8).

By prolonging presynaptic depolarization, it is more likely that calcium channels open asynchronous and determine a linear relationship between increase influx of Ca^{2+} and transmitter release, Zucker et. al (1991).

Increasing frequency of successive voltage potentials leads to increasing the amount of intracellular Ca^{2+} concentration if another action potential occurs before all Ca^{2+} ions have been removed from the cytoplasm.
The threshold level depends on the preceding firing. After release of an action potential, there is a refractory period, so there is a maximum firing frequency which theoretically is less than 1KHz but in our case it is around 5kHz. During the relative refractory period, if the amplitude of the voltage potential is very strong it can fire another action potential.

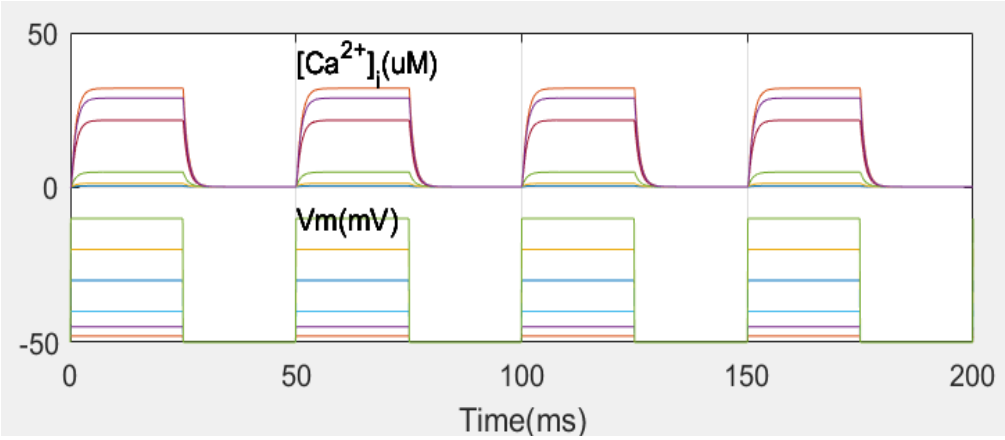

Fig. 5.9 The intracellular calcium concentration by applying a train of step voltages, using the different amplitudes with the same frequency.

According to Augustine and Charlton (1986), if transmitter release increases using voltage clamp of different increased amplitudes, then a third order power law relationship exists between presynaptic Ca^{2+} current and post synaptic response.
If we consider that with increasing the depolarization, the calcium concentrations overlap at the active zone, then the calcium concentration that triggers the vesicles release will increase because more calcium channels are recruited, (Zucker and Fogelson, 1986).

Voltage amplitude(mV)	Caclium Intracellular Concentration(μM)
-48	0,533
-45	1,36
-40	4,98
-30	21,71
-20	32,05
-10	28,89

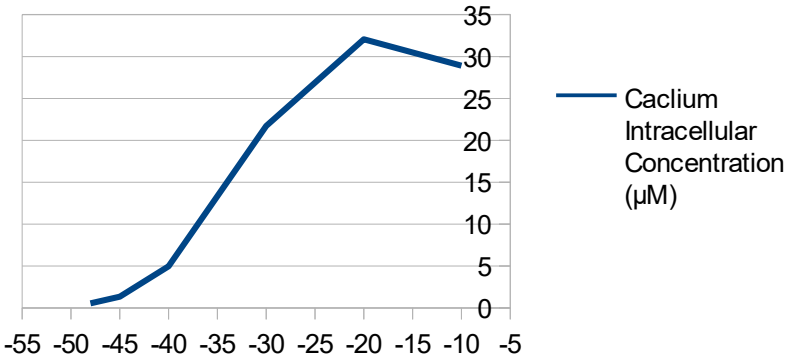

Fig. 5.10 The graphic of the calcium concentration at different amplitudes of the voltage clamp.

As Fig. 5.9 shows, if the amplitude of the voltage clamp increases as in the Table Fig. 5.10, -48mV, -45mV, -40mV, -30 mV, -20 mV,-10mV, and the frequency remains constant to f=20Hz, the calcium current peak increases, so the calcium ion inflow increases. The greater the depolarization level of the membrane, the higher calcium influx and thus the more voltage-gated calcium channels will open.
It can be seen that above an amplitude of -20mV the intracellular calcium concentration starts decreasing and up to this amplitude the concentration has a linear growing. Around 20mV, the amplitude of the voltage clamp, the calcium concentration inside the cell is almost zero due to the reversal calcium potential, in our case E_{ca}=20mV.

5.3 Intracellular calcium concentration in case of an current injected via an intracellular micro-electrode

In this section the intracellular calcium concentration is calculated by applying an intracellular current stimulus of rectangular waveform.
The following equations describe the relation between the injected current, voltage membrane and intracellular calcium concentration.

$$I_{stimulus} = \frac{I_{injected}}{2\Pi rl} \quad \text{(Eq.5.11)}$$

where A=2πrl is area of the cell, considered in these simulations 1cm^2

$$C_m \frac{dV}{dt} = I_{stimulus} - I_{ionic} \quad \text{(Eq.5.12)}$$

$$I_{ionic} = I_{Ca} + I_L \quad \text{(Eq.5.13)}$$

and Eq. 5.2 and Eq.5.9

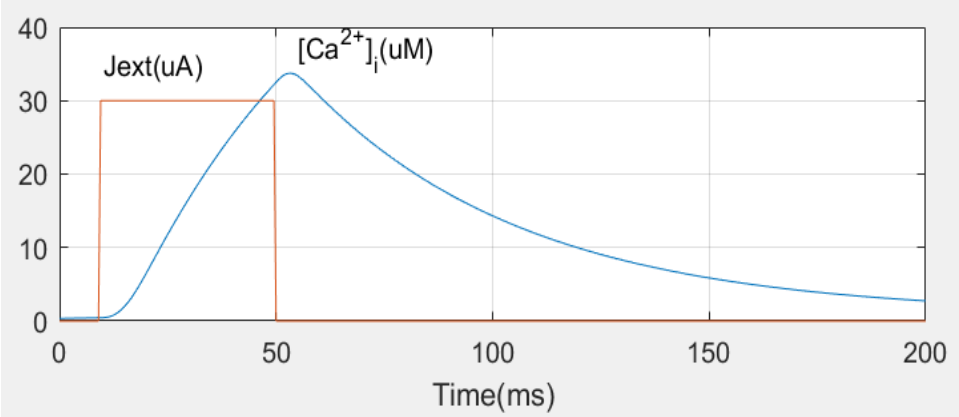

Fig. 5.11 The intracellular calcium concentration by applying an intracellular current stimulation.

The intracellular current stimulus has an amplitude of 30 μA with duration of 50 ms. The duration of the stimulus is important since the calcium concentration falls down when the current pulse returns to zero, (Fig. 5.11).
The time constant is responsible for synaptic release. By the time the current reverses its polarity, the calcium will go out of the intracellular space and intracellular calcium concentration drops to the resting level.
For small time constants, the variable probability „c“ of the voltage-gated calcium channel decreases fast so that the channel become inactivated and therefore not enough calcium influx passes the channels for synaptic release.
Large time constants will determine a slow inactivation of the variable „c“ and the voltage-gated calcium channels will remain for longer time open which leads to a prolonged inward calcium current and so a sustained synaptic release.

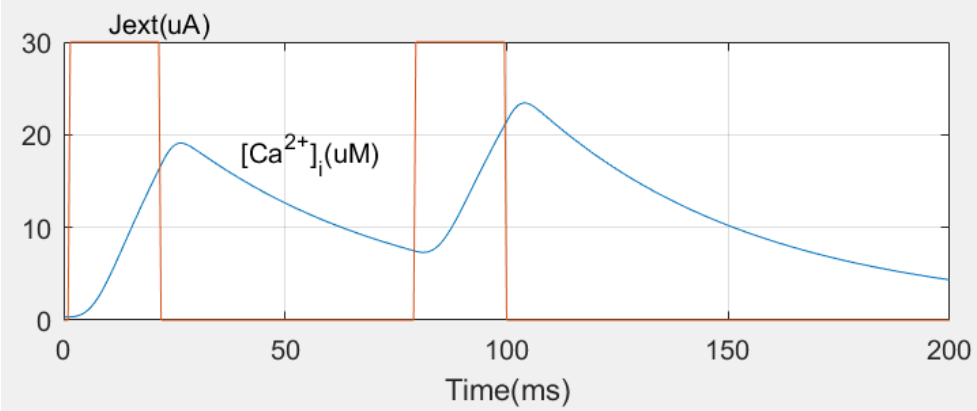

Fig. 5.12 The intracellular calcium concentration by applying a two intracellular current stimulus with the same amplitude of 30µA with duration pulse of 40ms.

By applying an intracellular current stimulus with amplitude of 30µA and pulse duration of 40 ms, the intracellular calcium concentration is relatively constant and decreases as the intracellular current stimulus falls down, (Fig. 5. 12) (Fig. 5.16).

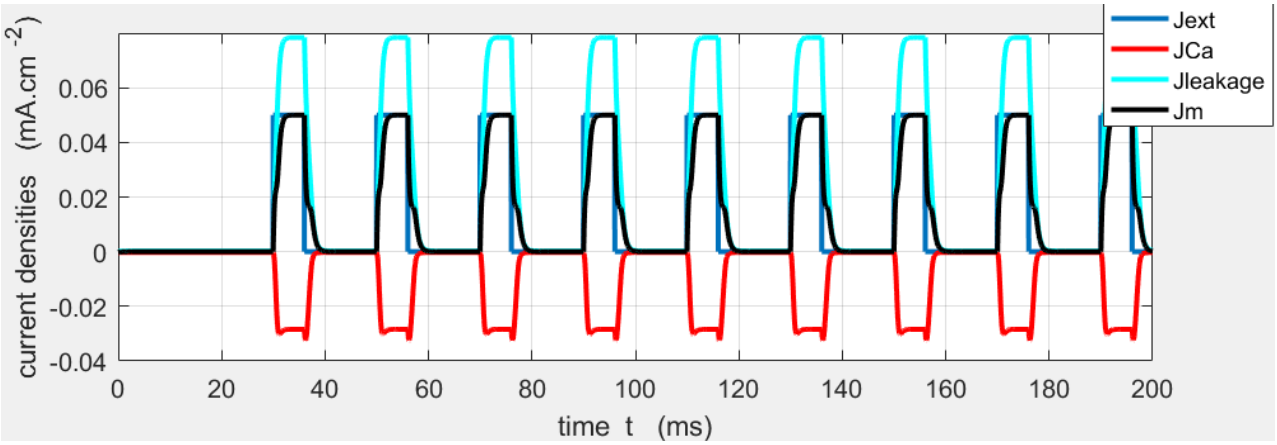

Fig. 5.13 Current densities across the membrane by applying a train of intracellular current stimulus with the same amplitude (50µA) and frequency (50Hz).

By applying an intracellular current stimulus with current density of 50µAcm^{-2}, we get a negative calcium current which shows the influx of calcium ions, (Fig. 5.13). Current density of the membrane can be determined with Eq.5.13.

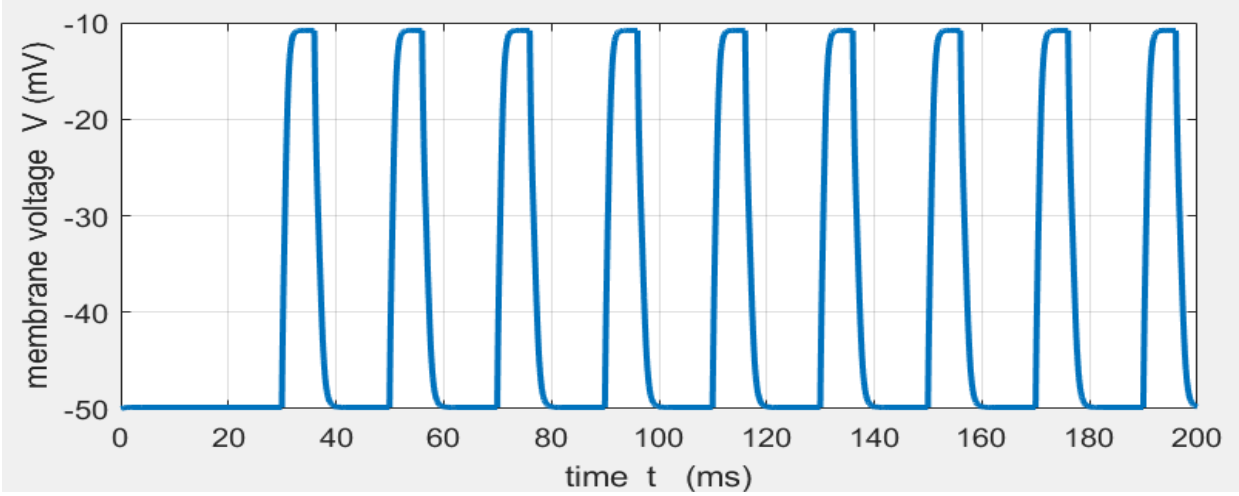

Fig. 5.14 Voltage membrane by applying a train of intracellular current stimulus with the same amplitude and frequency.

By applying an intracellular current stimulus of 50μA, the membrane is depolarized up to -10mV, (Fig. 5.14).

The voltage membrane is calculated by using the Forward Euler to determine the calcium conductance, calcium current density and leak current density, (Eq.5.12 and Eq.5.13).

A second action potential can be generated only after the refractory period so that the voltage membrane should be in the resting state.

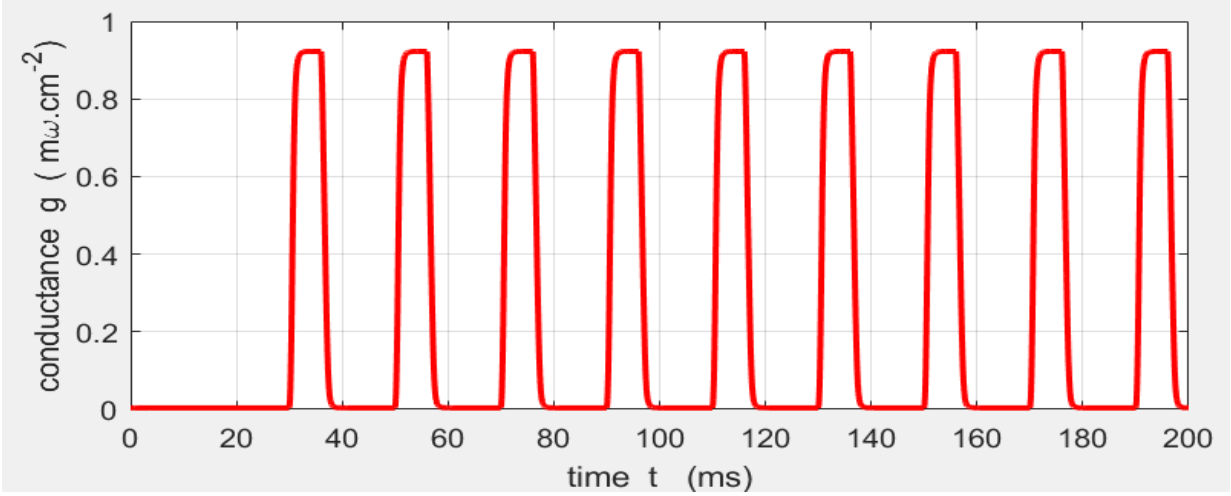

Fig. 5.15 The conductance of the calcium channel by applying a train of intracellular current stimulus with the same amplitude and frequency.

Eq.5.3 describes the open probability of the channels where the variables α and β are rate coefficients and dependent on the membrane potential.

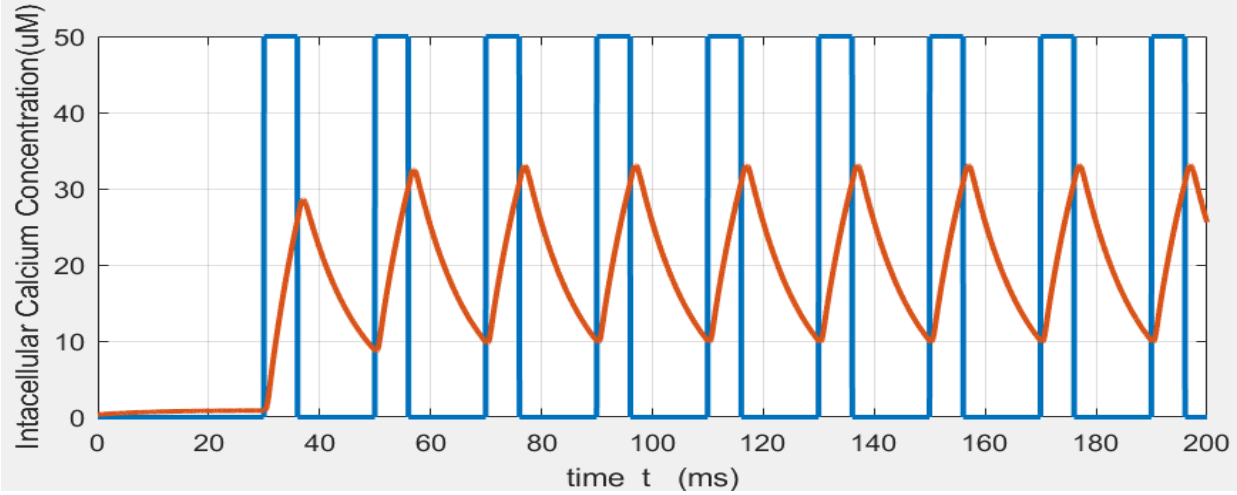

Fig. 5.16 The intracellular calcium concentration by applying a train of intracellular current stimulus with the same amplitude (50μA) and frequency (50Hz).

The intracellular current stimulus is positive which causes the membrane to depolarize. If no stimulus is applied and the membrane potential is at rest level, the current across the membrane will be zero and the potential across membrane will not change among time. It is necessary to apply a stimulus in order to create a pulse. If the current stimulus is negative then the calcium current will reverse and flow from inside towards outside and the membrane becomes hyperpolarized. If the current stimulus is positive, the membrane will be depolarized and will generate an inward calcium current.

In the Fig.5.16, by applying a train of pulses, the intracellular calcium concentration will be around 30μM.

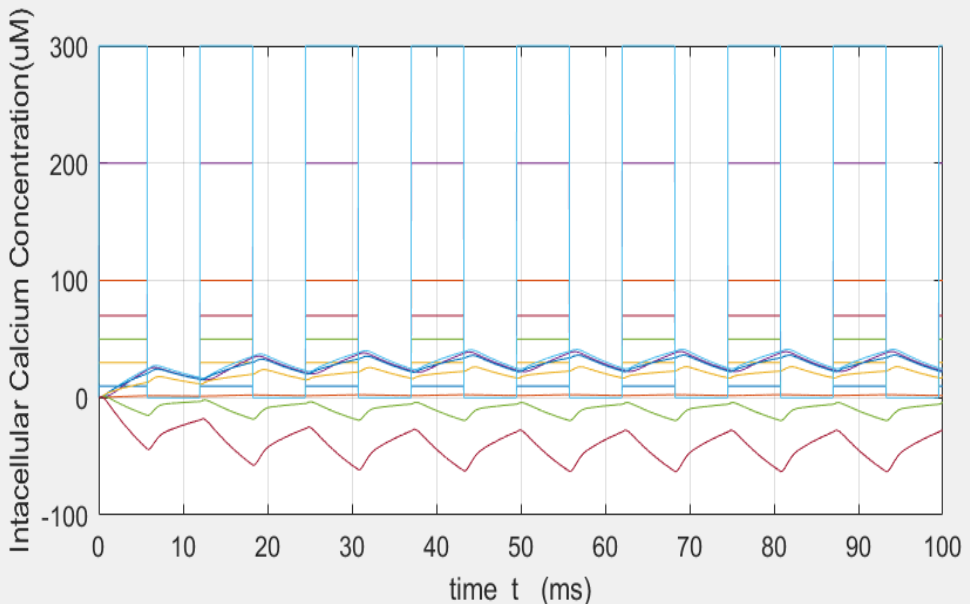

Fig. 5.17 The intracellular calcium concentration by applying a train of intracellular current stimulus with the different amplitudes but the same frequency. J_{ext}=1e-5, 3e-5, 5e-5, 7e-5, 10e-5, 20e-5, 30e-5.

External Stimulus(μA)	Caclium Intracellular Concentration(μM)
10	2,6
30	39
50	41
70	36
100	26
200	-3,4
300	-24,9

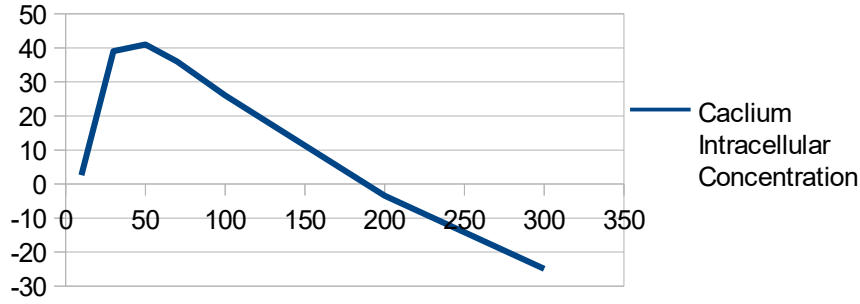

Fig. 5.18 Calcium concentration at different amplitudes of the intracellular current stimulus. The amplitudes changes from 10μA to 300μA and frequency is 40Hz.

As it can be seen in the graph above, (Fig. 5.18) the calcium concentration begins decreasing above a certain stimulus amplitude. The highest intracellular concentration is obtained for an amplitude of 50 μA. Amplitudes higher than 50μA do not contribute to increase of calcium concentration and so a stronger stimulus wouldn't be efficient. Amplitudes lower than 50 μA lead to a linear increase of the calcium concentration and also amplitudes higher than 50 μA lead to a linear decreasing.

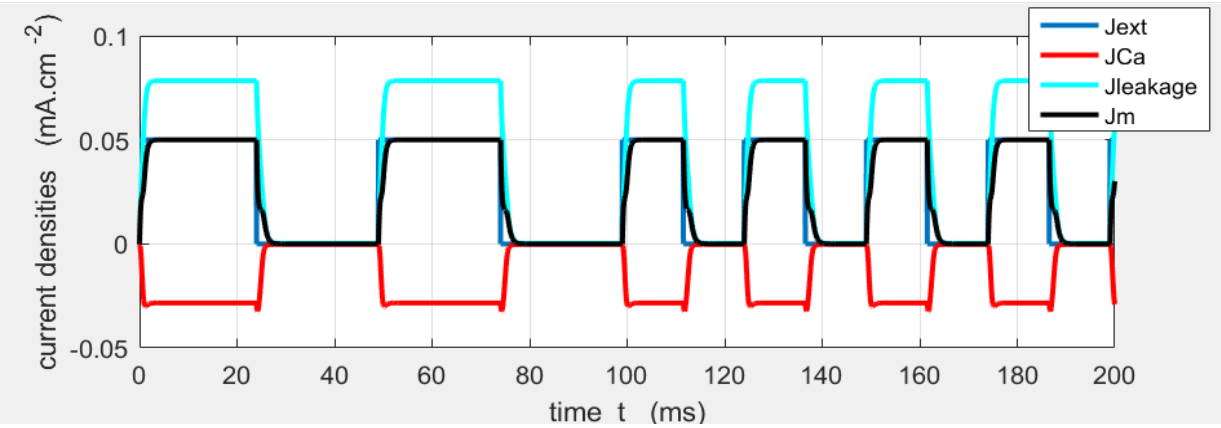

Fig. 5. 19 The currents across the membrane by applying a train of intracellular current stimulus with the same amplitude (50μA) and an increasing frequency from 20Hz to 40 Hz.

By injecting a series of pulses, number of generated action potentials are limited since after releasing an action potential there is an absolute refractory period which no other action potential would be generated.
Fig. 5.19 shows injecting an intracellular current stimulus with amplitude of 50μA with different frequencies; 20 Hz the first 100ms and 40 Hz for the last 100ms, the ionic current density across the cell membrane, calcium and leak current densities.

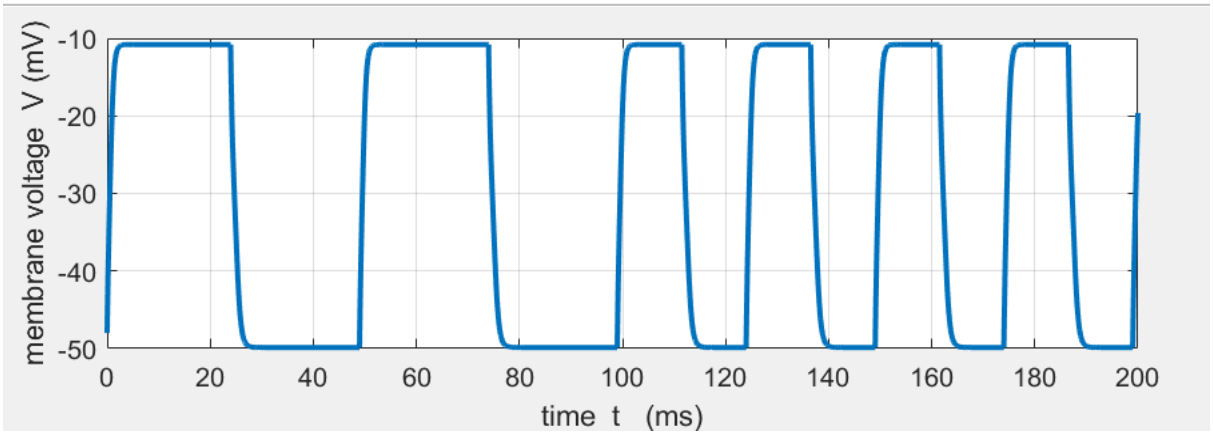

Fig. 5. 20 Voltage membrane versus time by applying a train of intracellular current stimulus with the same amplitude and different frequencies (20 Hz and 40Hz).

Fig 5.20 shows the voltage membrane doesn't decreases by increasing the frequency. It is important that with a frequency around 200Hz, the voltage membrane decreases and the kinetics of the calcium gate will not follow the changes in the intracellular current stimulus.

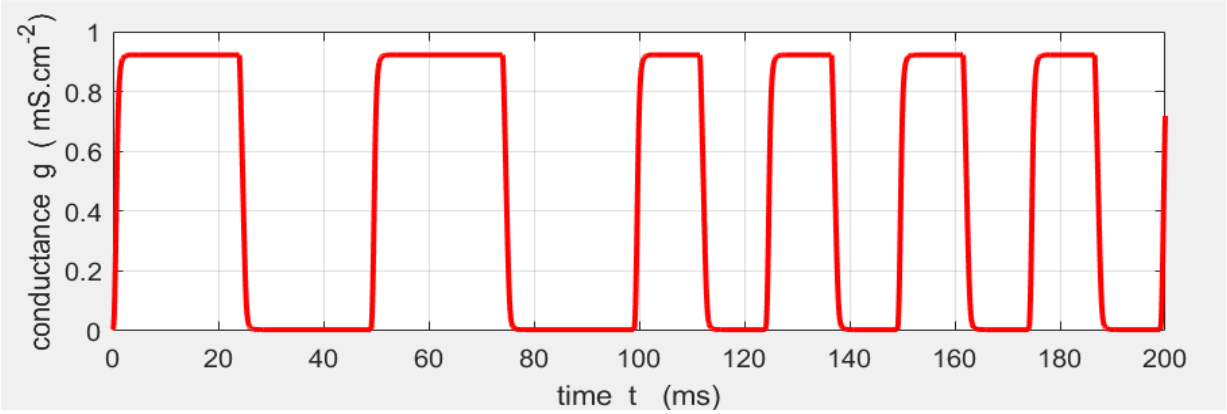

Fig. 5. 21 The conductance of the calcium channel by applying a train of intracellular current stimulus with the same amplitude (50µA) and frequency (from 20Hz to 40 Hz).

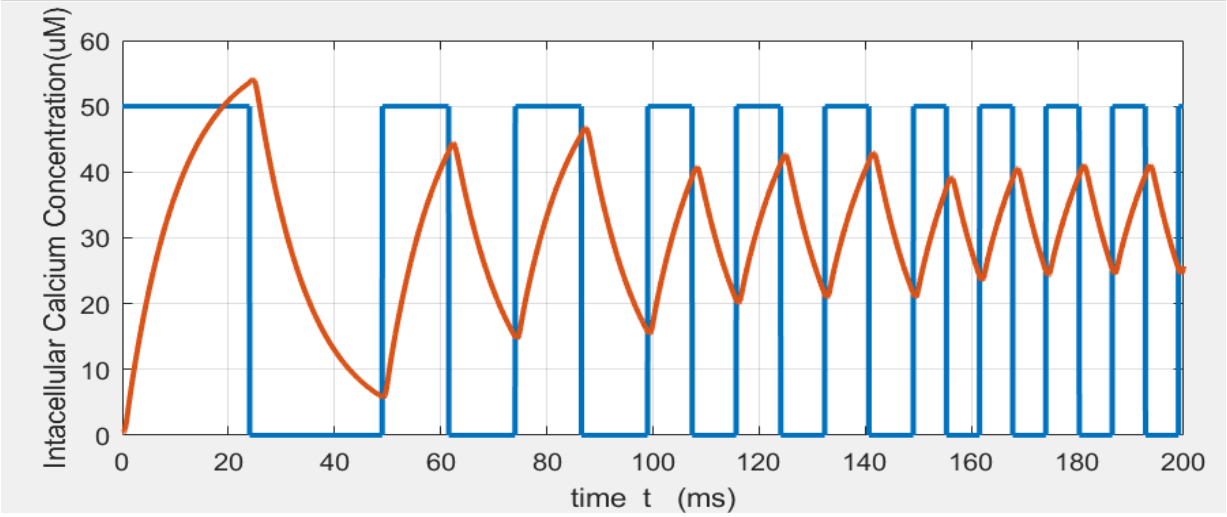

Fig. 5. 22 The intracellular calcium concentration by applying a train of intracellular current stimulus with the same amplitude (50µA) and different frequencies (20Hz, 40Hz, 60Hz, 80Hz).

Calcium channels begin to open after the peak of the action potential. For a rectangular pulse stimulus, the threshold to depolarize the membrane can be reached either by a low and high stimulus or by a long and short stimulus. If the frequency of the stimulus increases, the threshold will increases

External Stimulus Frequency(Hz)	Caclium Intracellular Concentration(μM)
20	54,19
40	44,42
60	42,98
80	41,1

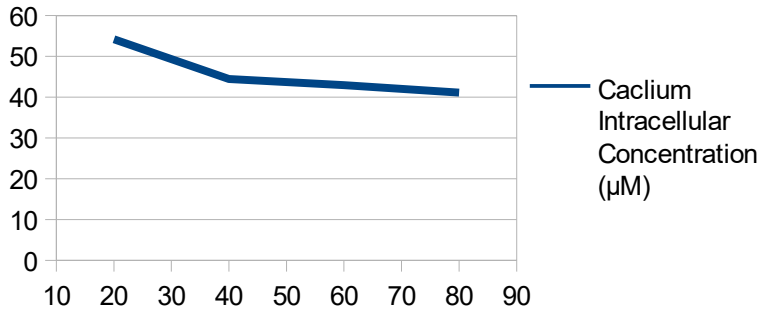

Fig. 5. 23 The intracellular calcium concentration by applying a train of intracellular current stimulus with the same amplitude and different frequencies.

As Fig. 5.23 shows, by increasing frequency, the calcium concentration decreases.

5.4 Intracellular calcium concentration using as waveform command a cosine function

The injected current stimulus that crosses the membrane which has an area of $1cm^2$ would make the current stimulation as:

$$C_m = 1\,\mu F \quad \text{(Eq.5.14)}$$

$$J_{stimulation} = \frac{I_{injected}}{A} \quad \text{(Eq.5.15)}$$

5.4.1 Calcium intracellular in case of using the voltage clamp as a cosine function

a) b)

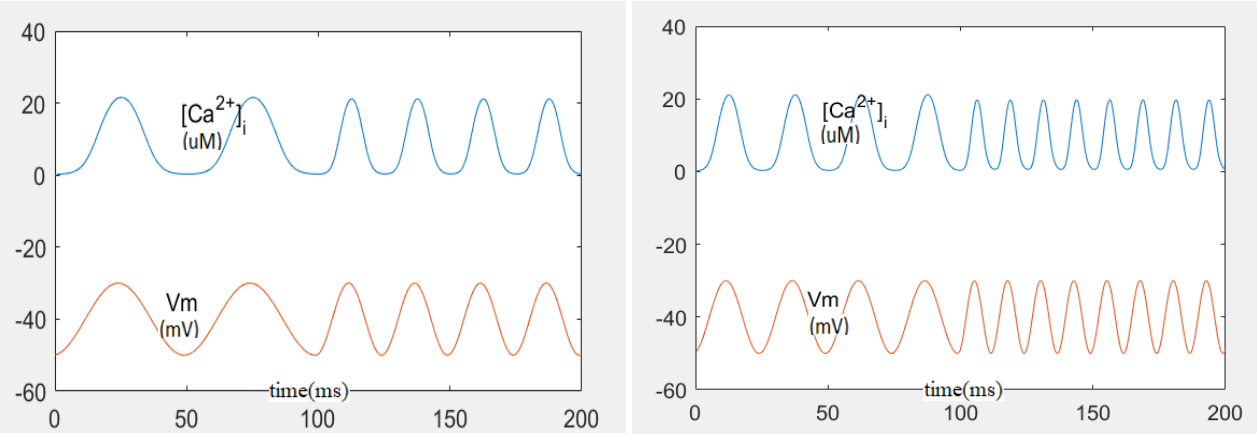

Fig.5.24. Intracellular calcium concentration by applying a voltage clamp with cosine shape containing two different frequencies, 20Hz and 40Hz in a) and 40Hz and 80Hz in case b). The voltage clamp has an amplitude between -50mV to -30mV.

Using the voltage clamp, the capacitive current will become zero (dV/dt=0) and only the ionic currents will be calculated.
The depolarization caused by the clamp will generate an inflow of calcium current and the rate of increasing calcium current will increase with the size of the voltage clamp.
In the simulations above it can be observed that an increased in the voltage frequency leads to slightly decreasing the calcium concentration, (Fig. 5.24). One of the reason is that more time is needed in order to open calcium channels. According to the experiments by Fohlmeister and Miller (1997), the kinetics of the calcium channels are similar to the sodium channels but the calcium channels activate at a more depolarized level.

a) b)

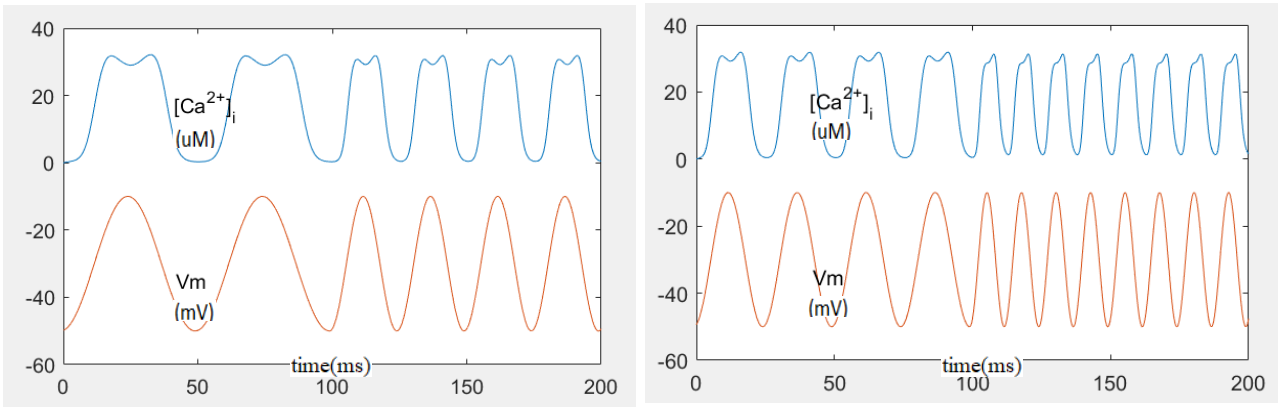

Fig.5.25 Intracellular calcium concentration by applying a voltage clamp with cosine shape containing two different frequencies, 20Hz and 40Hz in a) and 40Hz and 80Hz in b). The voltage clamp has an amplitude between -50mV to -10mV.

If the voltage clamp amplitude is increasing also calcium concentration will be increased and with increasing frequency, the amplitude of the calcium concentration inside the cell will decrease very little, (Fig. 5.25).

a) b)

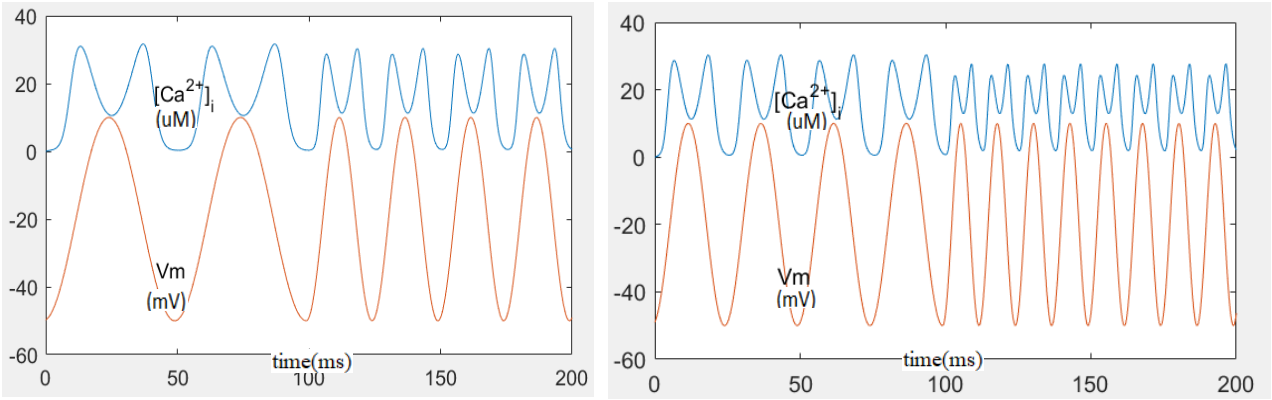

Fig. 5.26 Intracellular calcium concentration by applying a voltage clamp with cosine shape containing two different frequencies, 20Hz and 40Hz in a) and 40Hz and 80Hz in b). The voltage clamp has an amplitude between -50mV to 10mV.

a) b)

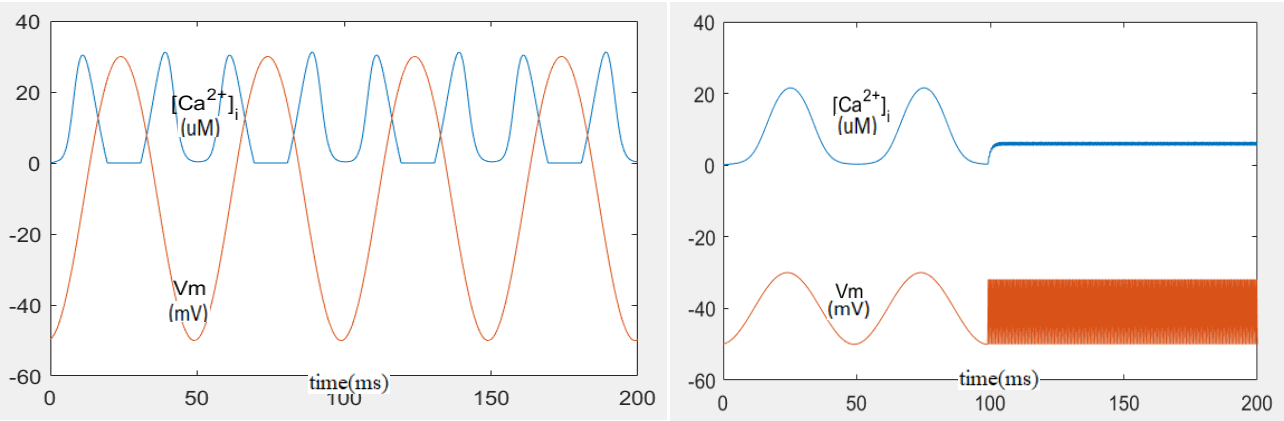

Fig. 5.27 Intracellular calcium concentration by applying a voltage clamp with cosine shape. a) a frequency of 20Hz and voltage between -50mV and 30mV. b) the voltage clamp has an amplitude between -50mV and -30mV and the frequency increases up to 2kHz for the period of 100 ms suddenly from 20Hz.

In Fig 5.26 and Fig. 5.27, both amplitude and frequency increase in order to see the cut frequency which determines the maximal depolarization of the membrane that can affect the calcium concentration inside the cell. As Fig. 5.26 shows, at a frequency of 20Hz with an amplitude up to 10mV, the calcium concentration is slightly higher than in Fig. 5.27 a) where the frequency is the same but the amplitude is 30mV. A reason for it can be that when calcium current direction changes, the calcium concentration decreases, so a positive voltage membrane will affect the calcium current that further decreases the calcium concentration inside the cell.

In Fig.5.26 b), frequency as well as amplitude diminish the intracellular calcium concentration and in Fig.5.27 b), by using an amplitude voltage clamp between -50mV to -30mV and frequency of 2kHz, the intracellular calcium concentration tends to become zero. An explanation is that the kinetics of voltage-gated calcium channels are not so fast.

Large depolarizations allow few Ca^{2+} ions come inside the cell because of the getting close of the voltage membrane to calcium equilibrium potential. So high depolarizations would suppose reduced I_{ca} and therefore reduced transmitter release. These considerations were made by Augustine and Llinas with the remark that large depolarizations can release more transmitter than small depolarizations.

Apparently,the transmitters release is voltage dependent and this can be explained by the fact that in the active zone, during high depolarization, the calcium concentration has different spatial distributions due to the overlapping, (Zucker and Fogelson, 1986).

5.4.2 Calcium intracellular concentration in case of using an intracellular current stimulus with a cosine shape

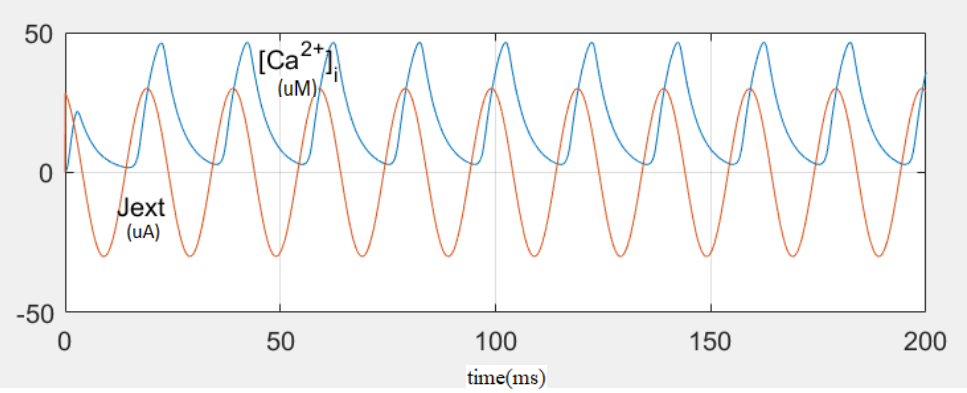

Fig. 5.28 Intracellular calcium concentration by applying an intracellular current stimulus with cosine shape. Frequency of the intracellular current stimulus is of 50Hz and of 30µA amplitude.

By applying a cosine-shaped intracellular current stimulus, the membrane would be depolarized so the intracellular calcium concentration increases. Where the half positive part depolarizes the cell and the negative part hyperpolarizes the membrane, (Fig. 5.28).

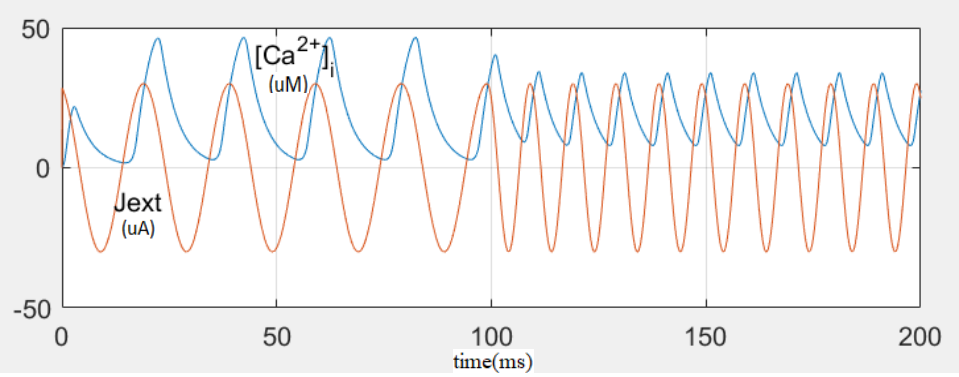

Fig. 5.29 Intracellular calcium concentration by applying an intracellular current stimulus with cosine-shaped. Frequency of the intracellular current stimulus increases from 50Hz to 100Hz but the amplitude of 30µA remains unchanged.

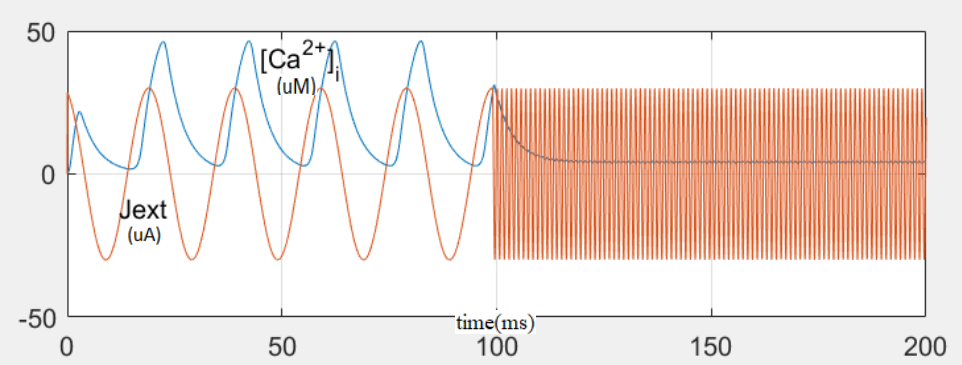

Fig. 5.30 Intracellular calcium concentration by applying an intracellular current stimulus with cosine-shaped. Frequency of the intracellular current stimulus increases from 50Hz to 900Hz and the amplitude of 30μA remains the same.

If the frequency of the stimulus increases, the threshold level will increase because the depolarizing half period begins to overlap with the relative refractory period from a previous action potential. If the frequency is even more increased, in our case up to 900Hz, the depolarizing half period starts to overlap with the absolute refractory period and so no action potential would be generated, (Fig. 5.30).
In the range of 1 kHz and more, the membrane cell acts as a short circuit where the capacitive currents is dominated and the ionic currents tend to become zero.

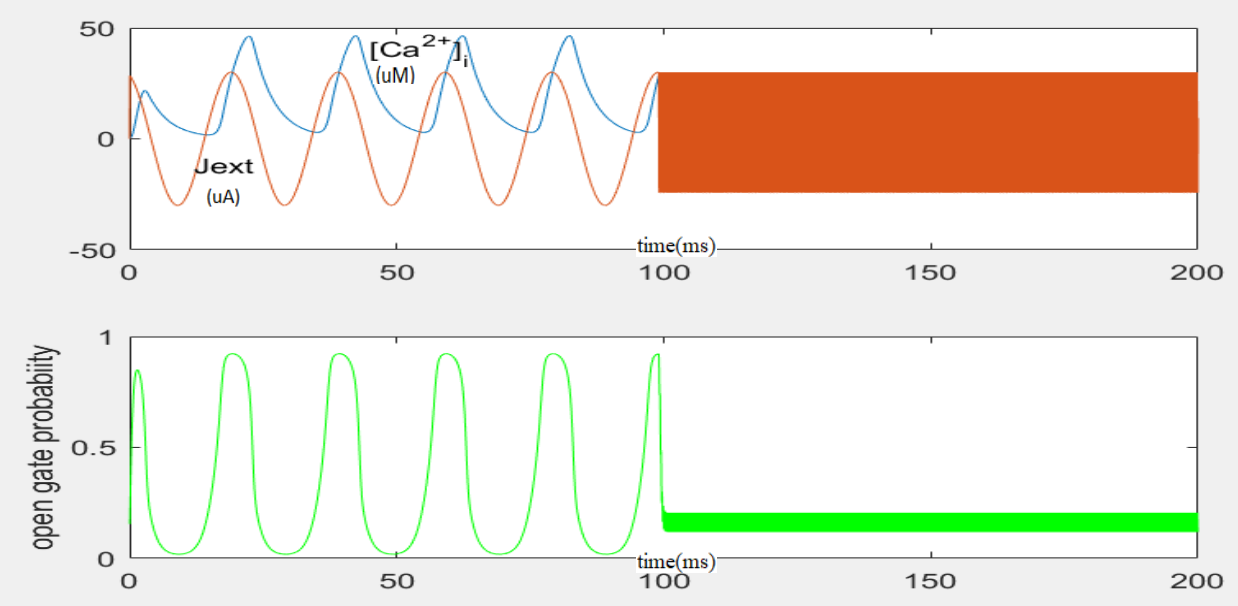

Fig. 5.31(Top) Intracellular calcium concentration by applying an intracellular current stimulus with cosine-shaped. Frequency of the intracellular current stimulus is increased from 50Hz to 5kHz and the amplitude is 50μA.(Bottom)Open calcium channel probability.

As Fig. 5.31 shows, the open probability of voltage-gated calcium channel, marked with the green line, tends to become zero above the frequency in range of kHz while in the range of Hz the probability, has the value of 0.9. With high frequency of intracellular current stimulus, there is not sufficient electric charge to depolarize the membrane before the current calcium reverses its direction so then acts to repolarize the membrane.

5.5 Neurotransmitter release

A high number of presynaptic terminals may converge on the dendrite of a postsynaptic cell. Each neurotransmitter causes a change in the membrane potential. Most excitations are not synchronous but because of the time constant, some portions of the stimulus are added together. In case of conventional synapses, the majority of release occurs synchronously with action potential triggering Ca^{2+} influx. Delayed release or asynchronously release follow the peak Ca^{2+} transient. If to the membrane is applied a train of stimulation signal, synchronous release decreases whereas asynchronous release increases as a response to the increases of intracellular calcium concentration during repetitive stimulation.
The value of the time constant of the calcium concentration is important in the integration of neurotransmitters packets at the synapse. Each packet of neurotransmitter acts as a current pulse.

In the following simulations the equations used are

$$\rho_{RP} = \rho_{RPconstant} * \frac{RP_0 - RP_t}{C_0} \quad \text{(Eq. 16)}$$

$$\rho_{RRP} = \rho_{RRPconstant} * \frac{RRP_0 - RRP_t}{C_0} \quad \text{(Eq17)}$$

where ρ_{RP} and ρ_{RRP} are the refilling rates for the two pools

C_0, RP_0, RRP_0 are the initial state of the cytoplasmatic pool, releasable pool and rapidly releasable pool, respectively.

RRP_0 is set to 6 vesicles, RP_0 is set to 30 vesicles ($RP_0 = 5*RRP_0$) and C_0=360 vesicles ($10*(RP_0 + RRP_0)$ per ribbon

$\rho_{RP\ constant}$, $\rho_{RP\ constant}$ are the refill constant rates and set to 0.25 s^{-1}

RP_t, RRP_t are current releasable pool and rapidly releasable pool state

$$rate_{RP} = 1 - \exp(-\alpha_{RP} * [Ca^{2+}]_i * RP_t * \Delta t) \quad \text{(Eq. 18)}$$

$$rate_{RRP} = 1 - \exp(-\alpha_{RRP} * [Ca^{2+}]_i * RRP_t * \Delta t) \quad \text{(Eq19)}$$

where $rate_{RP}$ respectively $rate_{RRP}$ release vesicles rate to exocytosis if Gaussian(0,1) ≤ $rate_{RP}$ respectively and if Gaussian(0,1) ≤ $rate_{RRP}$

α_{RP} is set 60 $(vesicle*M*ms)^{-1}$ and α_{RRP} is set 5000 $(vesicle*M*ms)^{-1}$. They are the release rate constant into the exocitosis pool

$[Ca^{2+}]_i$ is the intracellular calcium concentration in the synaptic terminal

RP_t and RRP_t are the current pool state at time t

Δt is the simulation step

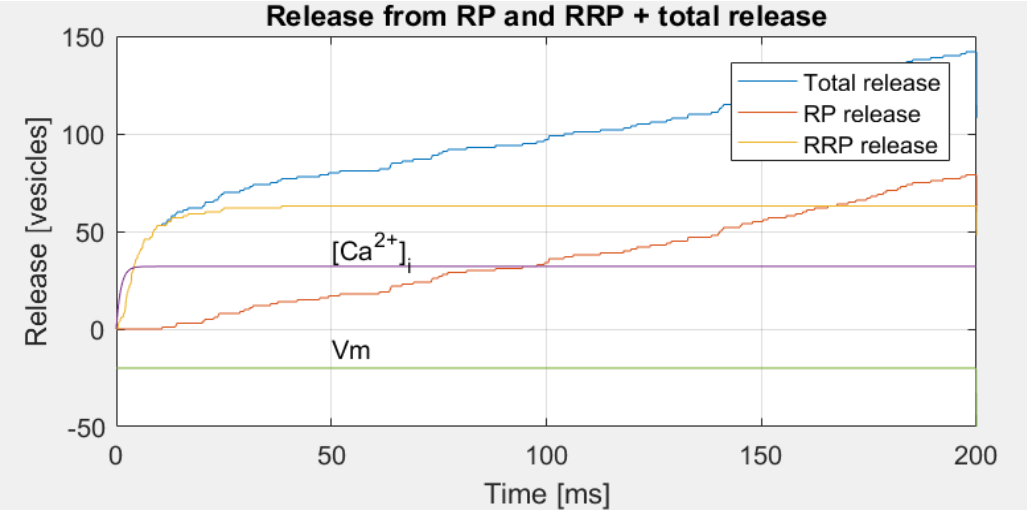

Fig. 5.32 Release of the vesicles from the two pools RP and RRP by applying a single voltage clamp. The voltage membrane is clamped to -20mV for a period of 200ms and the calcium concentration of around 30µM is obtained.

In Fig. 5.32 it is observed that at a continuous calcium inflow for a period of 200ms, the RRP will empty its content in the first 35ms while the RP will release its vesicle while the calcium concentration is maintained at an special level.

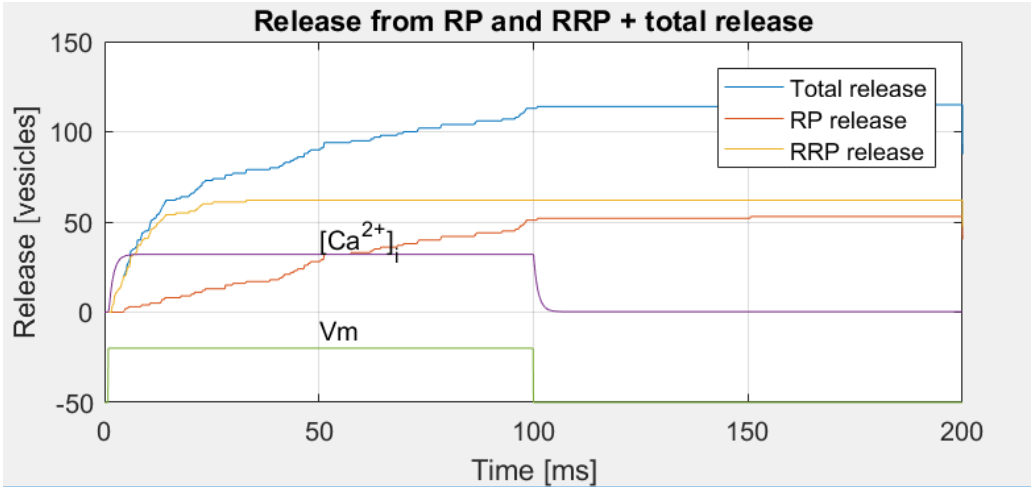

Fig. 5.33 Release of the vesicles from the two pools RP and RRP by applying a single voltage clamp. The voltage membrane is clamped to -20mV for a period of 100ms and a calcium concentration around 30µM is obtained.

In the simulation above, a voltage clamp used for 100ms in order to get a calcium concentration level for the period of 100ms. It is noticed that RRP doesn't change its releasing rate and the RP pool will release its content for a period of 100ms, (Fig. 5.33).

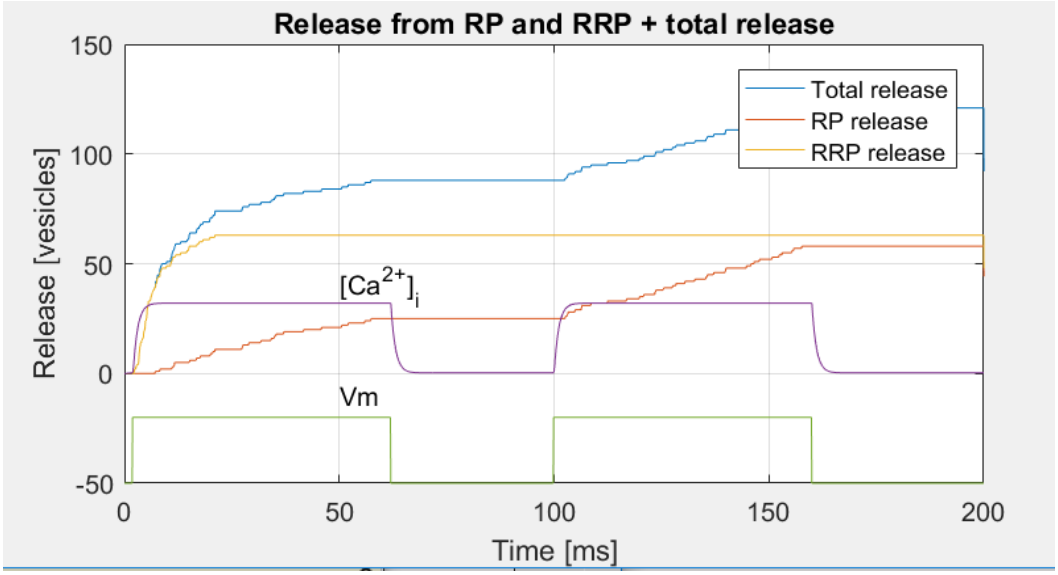

Fig. 5.34 Release of the vesicles from the two pools RP and RRP by applying two signals voltage clamp. The voltage membrane is clamped to -20mV for a period of 60ms and a concentration calcium around 30µM is obtained.

In case, more command signals are used in the predetermined time period of 200 ms, the extrusion time of calcium has a big effect on vesicle release. In case of a long time release vesicle, it is needed a high intracellular calcium concentration near the vesicles and it will fall to its resting level after the command signal turns off. As Fig.5.34 and Fig.5.35 show, the RP releases vesicles only when the signal is on. The intracellular calcium concentration will fall back to the resting level in the resting signal time, 1ms, and elevate again when another signal will increases the inflow of calcium ions. This will lead to another RP vesicle release. RRP releases starts at the beginning of the clamp and no more afterward.

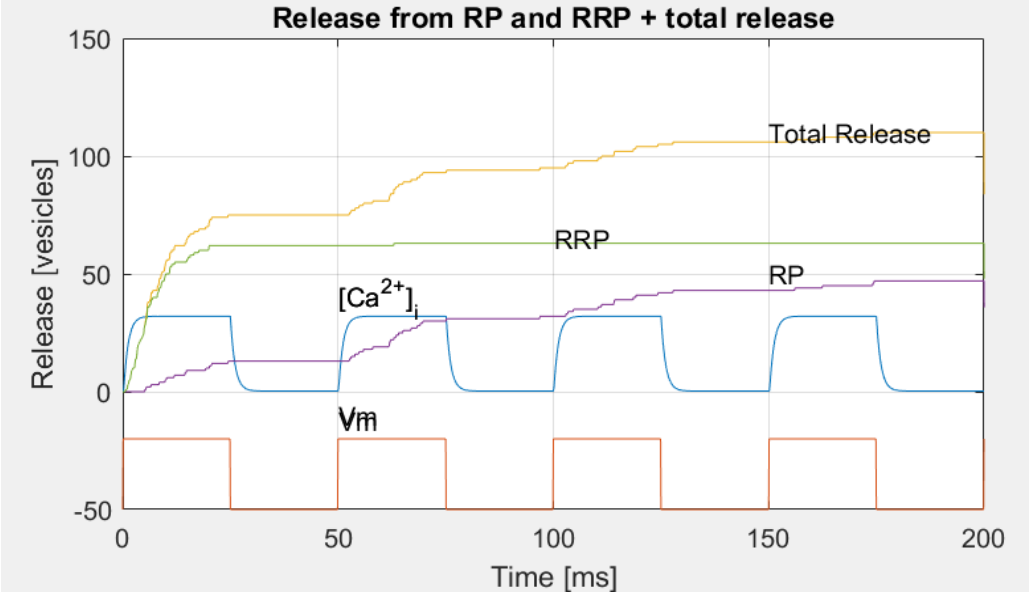

Fig. 5.35 Release of the vesicles from the two pools RP and RRP by applying a train signal with the same frequency 20Hz and amplitude of -20mV. A calcium concentration around 30µM is obtained.

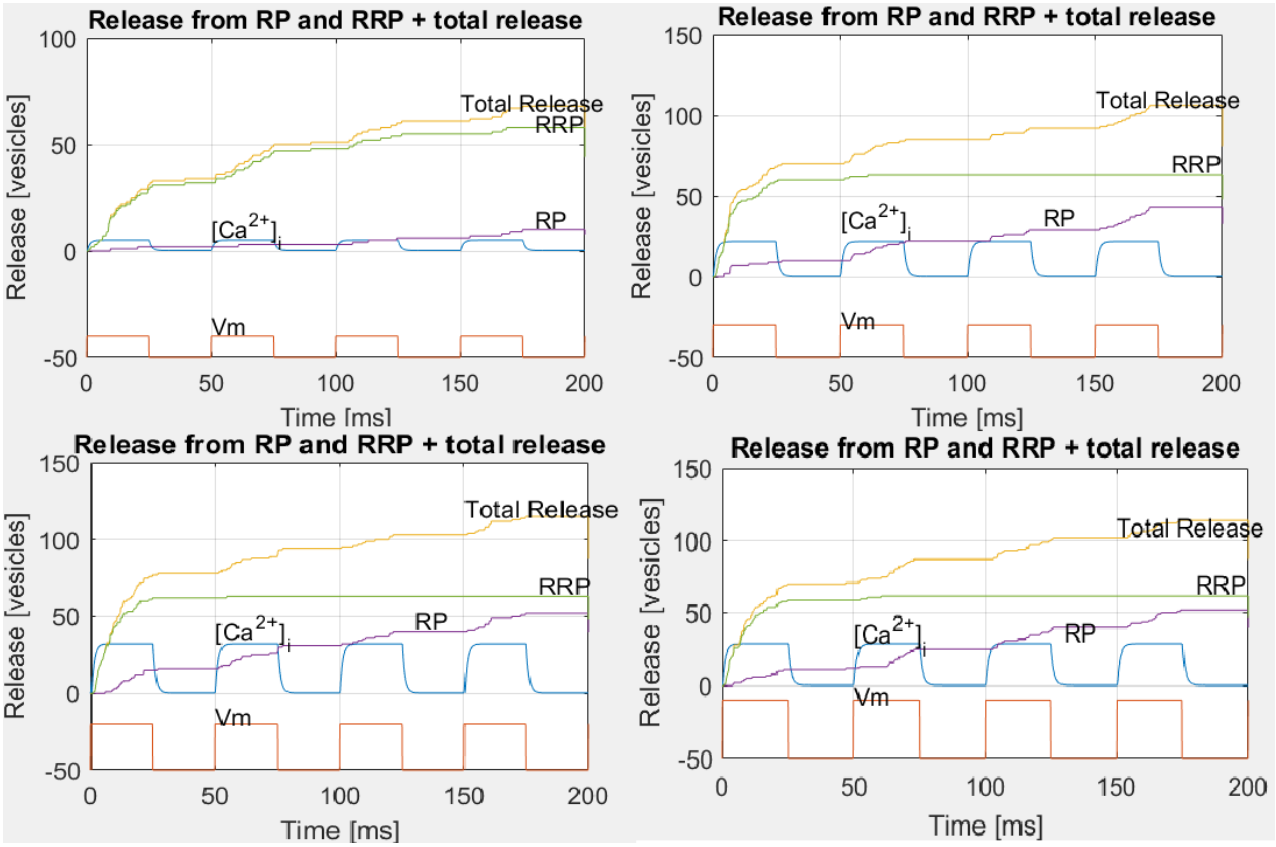

Fig. 5.36 Release of the vesicles from the two pools RP and RRP by applying a train pulses with different amplitudes and same frequency of 20Hz.

Simon and Llinas (1986) stated that if we consider the vesicle release occurs by entering the Ca^{2+} ions through a single calcium channel then the depolarization would be increased, this would lead to opening of more calcium channels and therefore proportionally more vesicles release.
In the simulations above the voltage membrane increases from its resting level, -50mV to -40mV, -30mV, -20mV, -10mV to see the effect over the neurotransmitter release.
Low membrane depolarization leads to low calcium concentration inside the cell, low RP vesicle release and a continuous RRP vesicle release. With increasing voltage clamp amplitude, the RP release vesicle increases and the RRP starts to release at the beginning of the train pulses and afterward become constant, (Fig. 5.36).

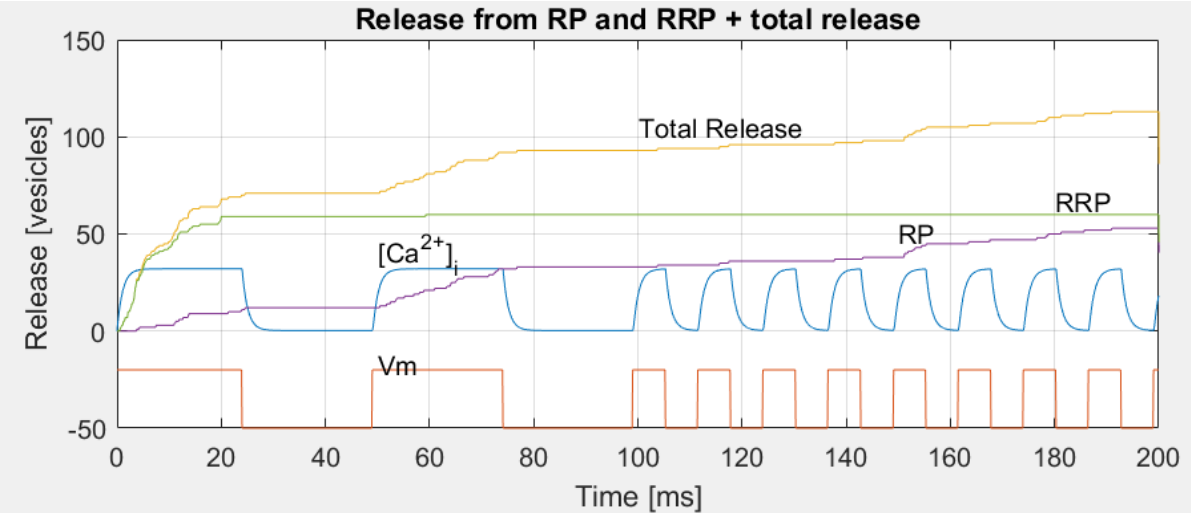

Fig. 5.37 Release of the vesicles from the two pools RP and RRP by applying a train signals with the same amplitude of -20mV and different frequencies.

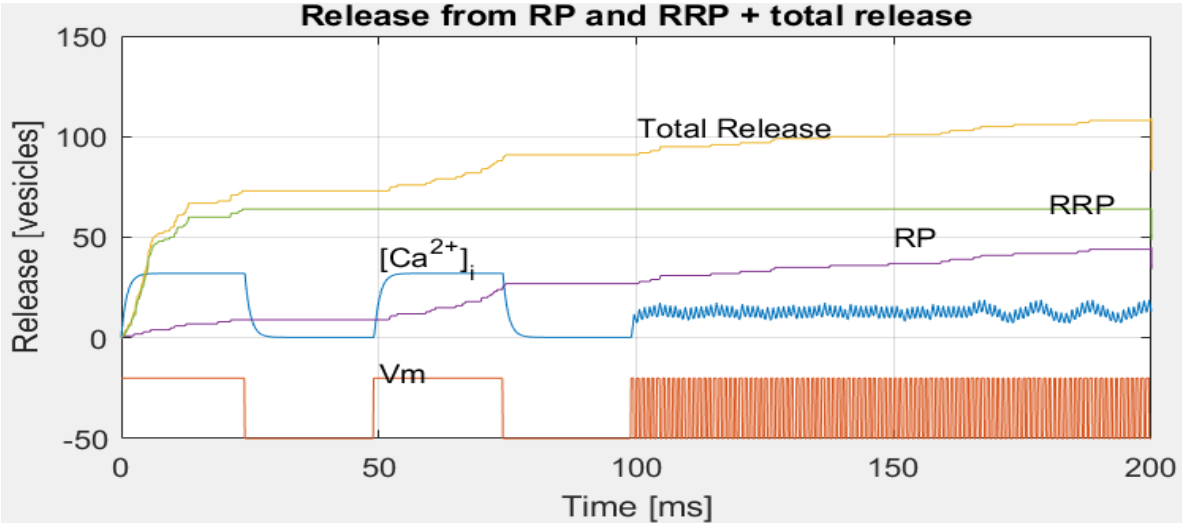

Fig. 5.38 Release of the vesicles from the two pools RP and RRP by applying a train signals with the same amplitude of -20mV and different frequencies.

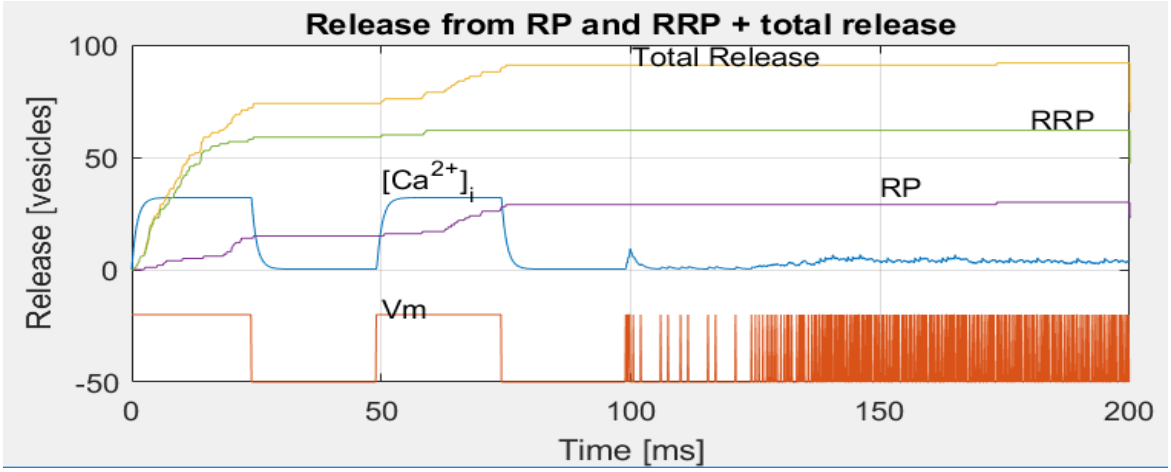

Fig. 5.39 Release of the vesicles from the two pools RP and RRP by applying a train signals with the same amplitude of -20mV and different frequencies.

In Fig. 5.37, Fig. 5.38, Fig. 5.39, the frequency is increased in order to see the effect on the vesicle release. In the Fig.5.37, the frequency of 80Hz will determine RP to release its vesicles during the high level of the calcium concentration and to stop when the calcium concentration decreases to its resting level. The RRP will release its content at the beginning of the train pulse.
In the Fig.5.38, increasing the frequency to 1kHz, the release vesicle from the RP is continuous while in the Fig.5.39 the frequency increases up to 10kHz which stop the release of vesicles.
An extended synaptic firing could lead to diminishing the amount of vesicle released per action potential. This conclusion is explained by the fact that the vesicle pools are limited and a new transmitter supply need a special time to be replenished.
A temporally synaptic firing, with an increased frequency, leads to an accumulation of Ca^{2+} ions in the terminal and an increase of the vesicles released neurotransmitters for a short term.
A high frequency train of action potential leads to saturation and temporal excess of Ca^{2+} concentration in the presynaptic terminal. As a result, more vesicles will be released because the resting level of Ca^{2+} concentration increases.
By increasing frequency of successive stimulus, it leads to a sustained and prolonged vesicle release. The amount of calcium ion inside the cell increases if at a previous current stimulus not all the calcium ions have been removed and another stimulus have taken place.

Chapter 6. Conclusions

The present study purpose was to demonstrate the dependence of the synaptic release to the intracellular calcium concentration.
The two main factors that determine the intracellular calcium concentration near the fusion of the vesicles with the membrane are the calcium ionic current and a passive extrusion mechanism which is governed by a time constant and a calcium residual level.
The simulations in Chapter 5 show that a prolonged high level calcium concentration leads also to a prolonged vesicles release. In case of pulses with different frequencies, the calcium concentration will have similar shapes, elevating during the pulses and fall back to its resting level after pulse offset. The time extrusion of the calcium is important in synaptic release during repetitive pulses of different amplitudes and frequencies.
An intracellular current stimulus above 50μA will determine a decrease in calcium concentration inside the cell; also an increased frequency has a decreasing effect over the intracellular calcium concentration.
Also the reversal potential of calcium has an important role in synaptic release since it will determine the magnitude of the calcium current inside the cell.
The techniques used to investigate the intracellular calcium concentration, voltage clamp and space clamp, are used in different waveforms like square or cosine-shaped. Because retinal implants use stimulus bursts to get visual perceptions for blind people, these simulations are important in order to get a feedback of the effect over the calcium concentration inside the cell that is the promoter in synaptic release.
In case of a train stimulus of square wave, the $[Ca^{2+}]_i$ decreases with increasing frequency (20Hz to 80 Hz); if the frequency remain constant then also $[Ca^{2+}]_i$ would remain constant. The amplitude stimulus influences also the $[Ca^{2+}]_i$, so that $[Ca^{2+}]_i$ increases with the amplitude up to a critical value. At a 50 μA amplitude, $[Ca^{2+}]_i$ is around 40μM and above this amplitude the $[Ca^{2+}]_i$ begin decreasing. Also at a frequency of 20 Hz the $[Ca^{2+}]_i$ is around 50 μM and with higher frequency, the $[Ca^{2+}]_i$ begin decreasing.
In case of cosine-shaped stimulus, at a frequency of 100 Hz and 30μA amplitude, the $[Ca^{2+}]_i$ would be around 30μM, where at a frequency of 50Hz and 30μA amplitude, $[Ca^{2+}]_i$ would be around 45μM. By increasing stimulus amplitude and at a frequency of 50Hz, $[Ca^{2+}]_i$ is 20μM at an amplitude of 20μA, at an amplitude of 40μA the $[Ca^{2+}]_i$ is 50μM and at an amplitude of 50μA the $[Ca^{2+}]_i$ is above 50μM. With increasing the amplitude more, the $[Ca^{2+}]_i$ begins decreasing.

Chapter 7. Appendix

```
%get the square waveform pulse train with amplitude and frequency as arguments
function[pwm]=VoltageClamp7_pwm_Train(AmpPwm,f)
%f=0.02;
a0=0;AmpSin=5; %sample wave values
t0=0;tf=200;Ts=1; %time vector and resolution
v=0;
%AmpPwm=30; %Pwm options, increase v for lower dutty-cycle
t=t0:Ts:tf; %create the time vector
fun=AmpSin*sin(2*pi*f*t+a0); %create the function
pwm=-50 + AmpPwm*(fun>v); %get the pwm
end
*********************************************************************************
function [pwm]=pwm_generation_differentF()
a0=0;AmpSin=40; %sample wave values
t=1:1:201;
i=max(size(t));
%period of time divided in four time intervals with different frequency
for i=1:201
 if((t(i)<=50)&(t(i)>0))
 fun(i)=1-AmpSin*cos(2*pi*0.02*t(i)); %20Hz
 end
 if((t(i)<=100)&(t(i)>50))
 fun(i)=1-AmpSin*cos(2*pi*0.04*t(i));%40Hz
 end
 if((t(i)<=150)&(t(i)>100))
 fun(i)=1-AmpSin*cos(2*pi*0.06*t(i));%60Hz
 end
 if((t(i)<=201)&(t(i)>150))
 fun(i)=1-AmpSin*cos(2*pi*0.08*t(i));%80Hz
 end
end
%set up the amplitude of the pwm
v=0;AmpPwm=30; %Pwm options, increase v for lower dutty-cycle
pwm=-50 + AmpPwm*(fun>v); %get the pwm
end

*********************************************************************************
%VoltageClamp rectangular waveform
function[Cai]=VoltageClamp()
tmin = 0; tmax =200;
num =201; num1 = 20; num2 = 80;num3=100;num4=160;
t = linspace(tmin, tmax,num);
dt = 0.1;
F=96485.33;%Faraday constant
Eca=20;%equilibrium potential for calcium
gca=1;%calcium conductance
tau=1;%extrusion time
d=0.025;% 25nm; the distance from the membrane
V=pwm_generation_differentF_1();%get the pwm with different frequencies
%V=VoltageClamp7_pwm_Train(40,0.02);
%initialization calcium current vector,constant rates of the channel to open/close and intracellular calcium concentration
Cai =zeros(1,num);
c=zeros(1,num);
alpha=zeros(1,num);
beta=zeros(1,num);
I=zeros(num,1);
Cai(1)=0.3e-3;
```

```
Ca_res=0.3e-3;%calcium resting level,set to 0.3μM
alpha(1)=(-0.3.*(V(1)+70))./(exp(-0.1.*(V(1)+70))-1);%constant rate from close to open state
beta(1)=10*exp(-(38+V(1))/9);%constant rate from open to close rate
c(1)=alpha(1)/(alpha(1)+beta(1));
I(1)=gca*c(1).^3.*(V(1)-Eca);_%calculating calcium current
%calculating with backward Euler the differential equation in order to calculate the open probability of the channel
%calcium current and then the intracellular calcium concentration
for j=1:num-1
        alpha(j)=(-0.3.*(V(j)+70))./(exp(-0.1.*(V(j)+70))-1);%constant rate of the channel to open
        beta(j)=10*exp(-(38+V(j))/9);%constant rate of the channel to close
        c(j+1)=(c(j)+alpha(j)*dt)/(1+dt*(alpha(j)+beta(j)));%calculating calcium current
        %c(j)=c_inf*(1-exp(-t(j)/taux(j))) +c_o*(exp(-t(j)/taux(j)));
        I(j+1)=gca*c(j+1).^3.*(V(j)-Eca);%calculating calcium current
        Cai(j+1)=(Cai(j)-(I(j+1).*dt*10000)./(2*F*d) +(Ca_res*dt)/tau)./(1+dt/tau);%calculating calcium
concentration
end
for j=1:num-1
 if(Cai(j)<0.1)
 Cai(j)=0.34e-3;
 end
end
subplot(2,1,1)
plot(t,Cai)
hold on
plot(t,V)
box on
hold all
grid on
text(50, 40, '[Ca^2^+]_{i}', 'Color', 'k')
text(50,-10, 'Vm', 'Color', 'k')
end
*********************************************************************************
%generate intracellular current stimulus of different frequencies and amplitude 50μA
function[Jstimulation]=Jext_generation_differentF()
a0=0;AmpSin=5; %sample wave values
t=1:0.04:201;
M=max(size(t));
for i=1:M
 if((t(i)<=50)&(t(i)>0))
 fun(i)=AmpSin*sin(2*pi*0.02*t(i)+a0);%20Hz
 end
 if((t(i)<=100)&(t(i)>50))
 fun(i)=AmpSin*sin(2*pi*0.04*t(i)+a0);%40Hz
 end
 if((t(i)<=150)&(t(i)>100))
 fun(i)=AmpSin*sin(2*pi*0.06*t(i)+a0);%60Hz
 end
 if((t(i)<=201)&(t(i)>150))
 fun(i)=AmpSin*sin(2*pi*0.08*t(i)+a0);%80Hz
 end
end
 v=0;AmpPwm=5e-5; %Pwm options, increase v for lower dutty-cycle
 Jstimulation= AmpPwm*(fun>v); %get the pwm
 end
*********************************************************************************
%get intracellular current stimulus of a fix frequency and a given amplitude
function[Jstimulation]=Jext_generation_sameF(Amp)
a0=0;AmpSin=5; %sample wave values
t=1:0.04:201;
M=max(size(t));
```

```
for i=1:M
 if((t(i)<=100)&(t(i)>1))
 fun(i)=AmpSin*sin(2*pi*0.04*t(i)+a0);%40Hz
 end
 if((t(i)<=201)&(t(i)>100))
 fun(i)=AmpSin*sin(2*pi*0.04*t(i)+a0);%40Hz
 end
end
v=0;
%Amp=5e-5; %Pwm options, increase v for lower dutty-cycle
Jstimulation= Amp*(fun>v); %get the pwm
end
*********************************************************************************
 % intracellular current stimulus rectangular waveform
close all
clear all
clc
global Vr
VR = -50e-3; % resting voltage (V)
VCa = 20e-3; %equilibrium potential calcium
Cm = 1e-6;  % membrane capacitance/area (F.cm^-2)
Cai(1)=0.34e-6;
Ca_res=0.34e-6;%resting level calcium
tau=10e-3;%extrude calcium time constant
d=25e-9;% 25nm; the distance from the membrane
tmin = 0;  % starting time
tmax = 100e-3; % finishing time (s) default 5e-3
F=96485;%Faraday constant
gLmax = 2e-3; % max leakage conductance (ohm-1.cm-2)
gCamax=1e-3; %Ca conductance(ohm-1.cm-2)
Jext_max = 5e-5; % max current density for ext stimulus (A.cm^-2);%50uA.cm-2
sf = 1e3;  % scale factor for conversion V to mV and s to ms
num = 5000;%number samples of the vectors calcium current,voltage membrane and calcium intracellular
%concentration
t = linspace(tmin,tmax,num);
dt = t(2)-t(1);%fixed step size backward Euler Integrator
%i%initialization voltage membrane,calcium current vector,leak current vector,constant rates of the channel to
open/close and intracellular calcium concentration
Jext = zeros(num,1); % intracellular  current density (A.cm^-2)
JL = zeros(num,1); % leakage current density (A.cm^-2)
Jm = zeros(num,1); % membrane current (A.cm^-2)
JCa = zeros(num,1);
V = zeros(num,1); % membrane potential (V)
gCa = zeros(num,1); %calcium conductance
gL = ones(num,1); % gL conductance
c = zeros(num,1);
V(1) = VR;   % initial value for membrane potential
Jexternal=Jext_generation_sameF(5e-5);
%Jexternal=Jext_generation_differentF();
Jext=Jexternal(1:5000);
%initialization calcium and leak current density vector,constant rates of the channel to open/close and intracellular
calcium concentration,voltage membrane
[ Ac ] = alpha1(V(1)*1000); % rate constant from close to open state
[ Bc ] = beta1(V(1)*1000);% rate constant from open to close state
c(1)=Ac/(Ac+Bc);
gL = gLmax.* gL;
gCa(1)=gCamax.*c(1)^3;
JCa(1)=gCa(1) * (V(1) – Vca);%calculate the calcium current
JL(1) = gL(1) * (V(1) - VR );%calculate the leak current
Jm(1) = JCa(1) + JL(1);%total ionic current
```

```
V(1) = VR + (dt/Cm) * (-JCa(1)-JL(1) + Jext(1));%calculate the voltage membrane
%using backward Euler to calculate the ionic current(calcium and leak current),the voltage membrane and the
intracellular calcium concentration
for cc = 1: num-1
        [Ac] =alpha1(V(cc)*1000);%calculating constant rate from close to open state
        [Bc] =beta1(V(cc)*1000);%calculating constant rate from open to close rate
        Ac=sf*Ac;Bc=sf*Bc;
        c(cc+1)=(c(cc)+Ac*dt)/(1+dt*(Ac+Bc));%calculating the probability of the channel to open
        gCa(cc+1)=c(cc+1)^3*gCamax;%calculating calcium conductance
        Jca(cc+1)=gCa(cc+1)*(V(cc)-VCa);%calculating calcium current
        JL(cc+1) = gL(cc+1) * (V(cc) +50e-3);%calculating leak current
        Jm(cc+1) = JCa(cc+1) + JL(cc+1);%calculating ionic current across cell membrane
        V(cc+1) = V(cc) + (dt/Cm) * (-JCa(cc+1) - JL(cc+1) + Jext(cc+1));
        Cai(cc+1)=(Cai(cc)-(JCa(cc+1).*dt)./(2*F*d) +(Ca_res*dt)/tau)./(1+dt/tau);%calculating calcium
concentration
end
 figure(1) % current ---------------------------------------------
title_x = 'time t (ms)'; title_y = 'current densities (mA.cm ^{-2})';
tt1 = 'time constant \tau =';
tt2 = num2str(1e3*tau,3); tt3 = ' ms';
tt = [tt1 tt2 tt3];
x = t.*sf; y = Jext.*sf;
plot(x,y,'linewidth',2); % Current - ext
hold on
x = t.*sf; y = JCa.*sf;
plot(x,y,'r','linewidth',2); % Current - Ca2+
x = t.*sf; y = JL.*sf;
plot(x,y,'c','linewidth',2); % Current - leakage
x = t.*sf; y = Jm.*sf;
plot(x,y,'k','linewidth',2); % Current - Leakage+ Calcium
xlabel(title_x); ylabel(title_y);
title(title_main);
grid on
legend('Jca','JL','Jm')
figure(4) % Intracellular calcium concentration ------------------------------------------------
box on
hold all
set(gcf,'units','normalized');
set(gcf,'position',[0.3 0.65 0.25 0.25]);
title_x = 'time t (ms)'; title_y = 'Calcium Intracellular';
x = t.*sf; y = Jext.*1e6;
plot(x,y,'linewidth',2);
xlabel(title_x); ylabel(title_y);
hold all
x = t.*sf; y = Cai.*1e6;
plot(x,y,'linewidth',2);
xlabel(title_x); ylabel(title_y);
grid on
figure(2) % voltage ------------------------------------------------
set(gcf,'units','normalized');
set(gcf,'position',[0.3 0.65 0.25 0.25]);
title_x = 'time t (ms)'; title_y = 'membrane voltage V (mV)';
x = t.*sf; y = V.*sf;
plot(x,y,'linewidth',2); % membrane voltage
xlabel(title_x); ylabel(title_y);
grid on
figure(3) % conductance ---------------------------------------------
set(gcf,'units','normalized');
set(gcf,'position',[0.58 0.65 0.25 0.25]);
title_x = 'time t (ms)'; title_y = 'conductance g ';
```

```
x = t.*sf; y = gCa.*sf;
plot(x,y,'r','linewidth',2); % conductance Ca2+
xlabel(title_x); ylabel(title_y);
grid on
legend('g_{Ca}')
*********************************************************************************
%generate co sinus function of different frequencies
function [fun]=sin_cos_generation_different_Thesis_1()
AmpSin=30; %sample wave values
t=1:1:201;
i=max(size(t));
for i=1:201
 if((t(i)<=100)&(t(i)>0))
  fun(i)=-20-AmpSin*cos(2*pi*0.02*t(i));%20Hz
 end
 if((t(i)<=201)&(t(i)>100))
  fun(i)=-20-AmpSin*cos(2*pi*0.04*t(i));%40Hz
 end
end
v=0;AmpPwm=30; %Pwm options, increase v for lower dutty-cycle
pwm=-50 + AmpPwm*(fun>v); %get the pwm
end
*********************************************************************************
%Main function
%Voltage Clamp co-sinus waveform
num =201; num1 = 20; num2 = 100;
t=1:1:201;
i=max(size(t));
V=sin_cos_generation_differentF_Thesis_1();
dt = 0.1;%fixed step size backward Euler integrator
F=96485.33;%Faraday constant
Eca=20;%equilibrium potential calcium
gca=1;%conductance calcium
tau=1;%extrude calcium time constant
d=0.025;% 25nm; the distance from the membrane
%initialization calcium current vector,constant rates of the channel to open/close and intracellular calcium concentration
c=zeros(1,num);
alpha=zeros(1,num);
beta=zeros(1,num);
I=zeros(num,1);
Cai(1)=0.3e-3;
Ca_res=0.3e-3;
alpha(1)=(-0.3.*(V(1)+70))./(exp(-0.1.*(V(1)+70))-1);% constant rate from close to open state
beta(1)=10*exp(-(38+V(1))/9);%constant rate from open to close state
c(1)=alpha(1)/(alpha(1)+beta(1));% calculating open calcium channel probability
I(1)=gca*c(1).^3.*(V(1)-Eca);
%calculate with backward Euler the
for j=1:num-1
        alpha(j)=(-0.3.*(V(j)+70))./(exp(-0.1.*(V(j)+70))-1)% constant rate from close to open state
        beta(j)=10*exp(-(38+V(j))/9);%constant rate from open to close state
        c(j+1)=(c(j)+alpha(j)*dt)/(1+dt*(alpha(j)+beta(j)));%probability of the channel to open
        I(j+1)=gca*c(j+1).^3.*(V(j)-Eca);%calcium current
        Cai(j+1)=(Cai(j)-(I(j+1).*dt*10000)./(2*F*d) +(Ca_res*dt)/tau)./(1+dt/tau);%intracellular calcium
concentration
end
```

```
for j=1:num-1
 if(Cai(j)<0.1)
 Cai(j)=0.34e-3;
 end
end
grid on
plot(t,Cai)
hold on
plot(t,V)
hold on
text(48, 17, '[Ca^2^+]_{i}', 'Color', 'k')
text(42,-6, 'Vm', 'Color', 'k')
*****************************************************************************************
function [fun]=sin_cos_generation_differentF_Thesis_3_2()
AmpSin=50; %sample wave values
t=1:0.04:201;
M=max(size(t));
for i=1:M
 if((t(i)<=100)&(t(i)>1))
  fun(i)=AmpSin*cos(2*pi*0.05*t(i));%50Hz
 end
 if((t(i)<=201)&(t(i)>100))
  fun(i)=AmpSin*cos(2*pi*5*t(i));%5KHz
 end
end
v=0;AmpPwm=30; %Pwm options, increase v for lower dutty-cycle
pwm=-50 + AmpPwm*(fun>v); %get the pwm
end
*****************************************************************************************
%intracellular  stimulus co-sinus function
clear all
tmin = 0; tmax =200;
num =5000; num1 = 20; num2 = 100;
t = linspace(tmin, tmax,num);
dt = 0.1;%fixed step size backward Euler integrator
F=96485;%Faraday constant
Eca=20;%calcium equilibrium potential
El=-50;%leak equilibrium potential
gca=1;%calcium conductance
gl=2;%leak conductance
tau=10;%calcium extrusion time
d=25e-6;% 25nm; the distance from the membrane
Vr=-50;%resting membrane voltage
Jext = zeros(num,1); % intracellular  stimulus
Je=sin_cos_generation_differentF_Thesis_3_2();% intracellular  stimulus co sinus function
Jext=Je(1:5000);
%initialization voltage membrane vector,calcium current vector,constant rates of the channel to open/close and
intracellular calcium concentration,voltage membrane
c=zeros(num,1);
alpha=zeros(num,1);
beta=zeros(num,1);
I=zeros(num,1);
Cai=zeros(num,1);
V=zeros(num,1);
Ica=zeros(num,1);
Cm = 1; %uF.cm-2;capacity membrane over the surface
Cai(1)=0.34;
Ca_res=0.34;%resting level of the intracellular calcium concentration
V(1)=Vr;
alpha(1)=(-0.3.*(V(1)+70))./(exp(-0.1.*(V(1)+70))-1);%constant rate from close to open state
```

```
beta(1)=10*exp(-(38+V(1))/9);%constant rate from open to close state
c(1)=alpha(1)/(alpha(1)+beta(1));
Ica(1)=gca*c(1).^3.*(V(1)-Eca);%calcium current
I(1)=(gca*c(1).^3.*(V(1)-Eca)+gl*(V(1)-El));%ionic current across the cell membrane
V(1)= Vr + (dt/Cm)*(-I(1)+Jext(1));
for j=1:num-1
alpha(j)=(-0.3.*(V(j)+70))./(exp(-0.1.*(V(j)+70))-1);%constant rate of the channel to open
beta(j)=10*exp(-(38+V(j))/9);%constant rate of the channel to close
c(j+1)=(c(j)+alpha(j)*dt)/(1+dt*(alpha(j)+beta(j)));%probability of the channel to open
%c(j)=c_inf*(1-exp(-t(j)/taux(j))) +c_o*(exp(-t(j)/taux(j)));
Ica(j+1)=gca*c(j+1).^3.*(V(j)-Eca);%calcium current
I(j+1)=gca*c(j+1).^3.*(V(j)-Eca)+ gl*(V(j)-El);%ionic current
V(j+1)=V(j) + (dt/Cm)*(-I(j+1)+Jext(j+1)) ;%voltage membrane
Cai(j+1)=(Cai(j)-(Ica(j+1).*dt)./(2*F*d) +(Ca_res*dt)/tau)./(1+dt/tau);%intracellular calcium concentration
end
subplot(2,1,1)
plot(t,Cai)
hold on
plot(t,Jext)
box on
hold all
grid on
plot(t,c)
text(45, 40, '[Ca^2^+]_{i}', 'Color', 'k')
text(12,-12, 'Jext', 'Color', 'k')
text(10,-1, 'open gate', 'Color', 'k')

*******************************************************************************************
 %Neurotransmitters vesicles
 nSyn=10;
 %caI=ones(length(caI))*30
%% ------------- Step 1: PARAMETERS -------------
dt = 0.1; % Time step [ms]
rhoRPMax = 0.00025; % Refill rate for RP [1/ms]
rhoRRPMax = 0.00025; % Refill rate for RRP [1/ms]
alphaRP = 60; % Release rate for RP [1/(M*ms*vesicles)]
alphaRRP = 5000; % Release rate for RRP [1/(M*ms*vesicles)]
RRP0 = 6; % Inital pool size of the RRP [vesicles]
RP0 = RRP0*5; % Inital pool size of the RP [vesicles]
C0 = (RP0+RRP0)*10; % Inital pool size of the cytoplasm [vesicles]
% Temporal parameters
stop=200;
steps = stop/dt+1; % Number of points in time
time = 0:dt:stop+dt; % Points in time vector
 % Initialize matrices and set initial values
C = zeros(nSyn,length(time));
RP = zeros(nSyn,length(time));
RRP = zeros(nSyn,length(time));
E = zeros(nSyn,length(time));
tempRefillRP = zeros(nSyn,length(time));
tempRefillRRP = zeros(nSyn,length(time));
releaseRP = zeros(nSyn,length(time));
releaseRP1 = zeros(nSyn,length(time));
releaseRRP1 = zeros(nSyn,length(time));
releaseRRP = zeros(nSyn,length(time));
release = zeros(1,length(time));
caI=VoltageClamp();
C(:,1) = C0;
RP(:,1) = RP0;
```

```
RRP(:,1) = RRP0;
%% --------- Step 2: SOLVE ODE ---------
% Backward Euler method
for t=1:steps -1
  % Loop over all synapses
 for q=1:nSyn
   % REFILL
  rhoRP = rhoRPMax*((RP0-RP(q,t))/C0);
  rhoRRP = rhoRRPMax*((RRP0-RRP(q,t))/C0);

  C(q,t+1)=C(q,t)/(1 + rhoRP*dt + rhoRRP*dt);
  tempRefillRP(q,t+1)=tempRefillRP(q,t) + C(q,t+1)*rhoRP*dt;
  tempRefillRRP(q,t+1)=tempRefillRRP(q,t) + C(q,t+1)*rhoRRP*dt;
  % Refill releasable pool (RP)
  if tempRefillRP(q,t+1)>=1
   RP(q,t+1) = RP(q,t)+1;
   tempRefillRP(q,t+1) = tempRefillRP(q,t+1)-1;
  else
   RP(q,t+1) = RP(q,t);
  end
  % Refill rapidly releasable pool (RRP)
  if tempRefillRRP(q,t+1)>=1
   RRP(q,t+1) = RRP(q,t)+1;
   tempRefillRRP(q,t+1) = tempRefillRRP(q,t+1)-1;
  else
   RRP(q,t+1) = RRP(q,t);
  end
  % RELEASE
  % Releasable pool (RP)
  if RP(q,t)>=1
   pS = 1-exp(-alphaRP*caI(t)*1e-6*dt*RP(q,t));
   rS = rand;
   if rS<pS
    RP(q,t+1) = RP(q,t)-1;
    releaseRP(q,t+1) = releaseRP(q,t)+1;
    release(t+1) = release(t+1)+1;
   end

  end
  % Rapidly releasable pool (RRP)
  if RRP(q,t)>=1
   pF = 1-exp(-alphaRRP*caI(t)*1e-6*dt*RRP(q,t));
   rF = rand;
   if rF<pF
    RRP(q,t+1) = RRP(q,t)-1;
    releaseRRP(q,t+1) = releaseRRP(q,t)+1;
    release(t+1) = release(t+1)+1;
   end
  end

  % Total release
  E(q,t+1) = E(q,t)+releaseRP(q,t+1)+releaseRRP(q,t+1);
  end
 end
 releaseRP1(1:nSyn,1:2001)=cumsum(releaseRP(1:nSyn,1:2001),2);
 releaseRRP1(1:nSyn,1:2001)=cumsum(releaseRRP(1:nSyn,1:2001),2);
% Release from RP and RRP + total release
figure
title('Release from RP and RRP + total release')
```

```
box on
hold all
plot(time,sum(E,1))
if nSyn>1
 plot(time,sum(releaseRP1))
 plot(time,sum(releaseRRP1))
else
 plot(time,cumsum(releaseRP1,2))
 plot(time,cumsum(releaseRRP1,2))
end
legend('Total release','RP release','RRP release')
xlim([0 stop])
xlabel('Time [ms]')
ylabel('Release [vesicles]')
```

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