Chapter 2
Literature Review

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2.1 An overview of neuron and action potential

A neuron is a nerve cell which transmits information via electric and chemical signals. The neuron signals are transmitted from one neuron to another through synapse which is the interconnection of neurons.

There are different types of neuron in a human body:

a) Sensory neurons which responds to touch, smell, sound and other stimuli that affects the sensory organs.
b) Motor neurons are those which respond to muscle contraction
c) Interneuron which connects one neuron to another

The structural diagram of neuron is given in Fig. 2.1. The body of the neuron is called soma and depicted in the figure below. The dendrites are the extensions from the soma which interconnects other neuron. Axon is a long fine fiber which elongates from the soma and ends in many branches. These branches are connected with other neurons for communication. The part of neuron from where axon extends from the soma is called axon hillock. The axon hillock contains most of the sodium channels and it is the most excitable part of the neuron. This region is known as the spike initiation part in the axon. The axon terminal is called synapse where chemicals are secreted for transmission of signals. The axon is covered with fatty substance for electrical insulation called myelin sheath.

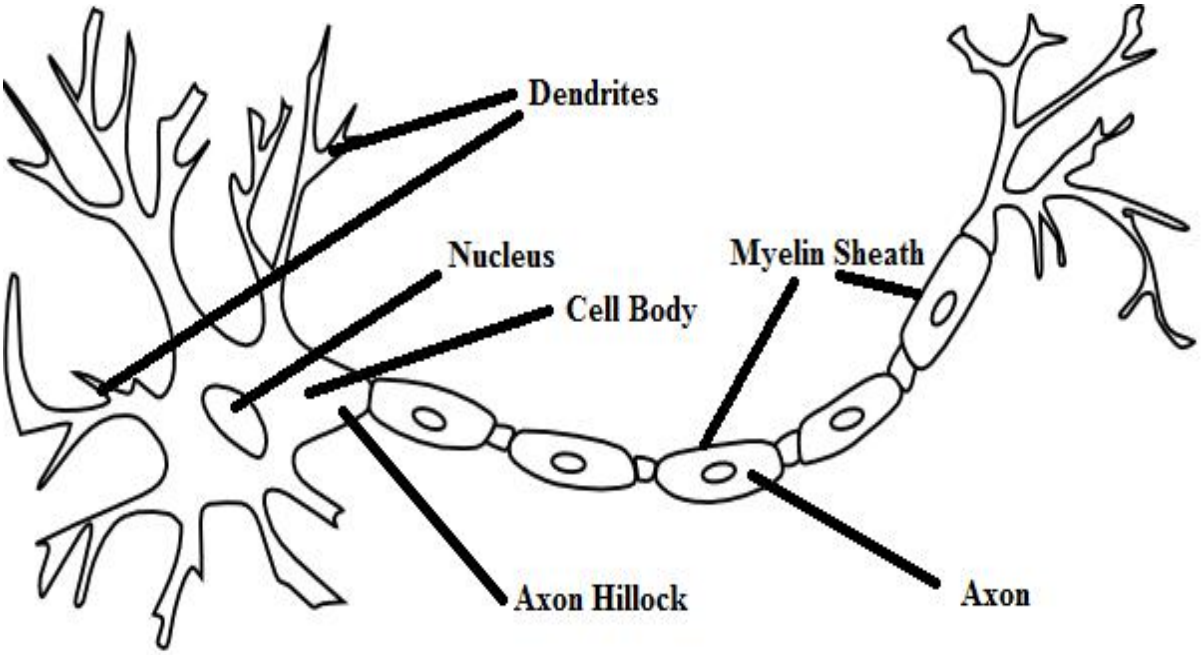


Fig. 2.1 Structure of neuron

There are many functions of neuron and many experiments have been carried out to study the properties of neuron. One of the most important functions of neuron is that it helps to communicate signals from body to brain and vice versa. This is possible through action potential signal which helps the neuron to communicate from one nerve to another and then to brain. Brain then sends the necessary signals to perform through nerve cells via action potential. Action potential occurs due to ionic current flow of potassium, sodium and leakage ions in the membrane. Hodgkin-Huxley had studied these currents with the help of voltage-clamp experiments and described the dynamics using differential equations.

The membrane of the neuron consists of three types of ions: sodium, potassium and leakage ions which mostly consist of chloride ions. Neuron possess a resting state where the sodium ions remain outside of the cell , potassium ions resides mostly inside the cell and leakage ions moves to and fro in the cell. Resting potential is the dynamic equilibrium of ionic flow between the channels. The membrane potential measured in

resting state is between -60mV to -70mV. When an external energy or any disturbance is exerted on the membrane , the sodium ions goes inside the cell and then the potassium ions comes outside the cell so as to maintain its charge neutrality. In exchange of every three sodium ions, two potassium ions are exchanged. The movement of sodium ions inside the cell produces a hump in the signal causing repolarization and then depolarization occurs when the potassium ions moves outside of the cell as shown in Fig.2.2. After the action potential occurs, a neuron goes to refractory period when no action potential can occur. Resting potentials of a membrane is that when there is no movement of ions in the cell and the inside of the membrane attains more negative charge than the outside. Action potential is generally of 20-30mV in human beings. If the external energy is more than the threshold point, action potential occurs and if the external energy is less than the threshold voltage, the action potential does not occur. This is an all or none response principle which is observed in a neuron.

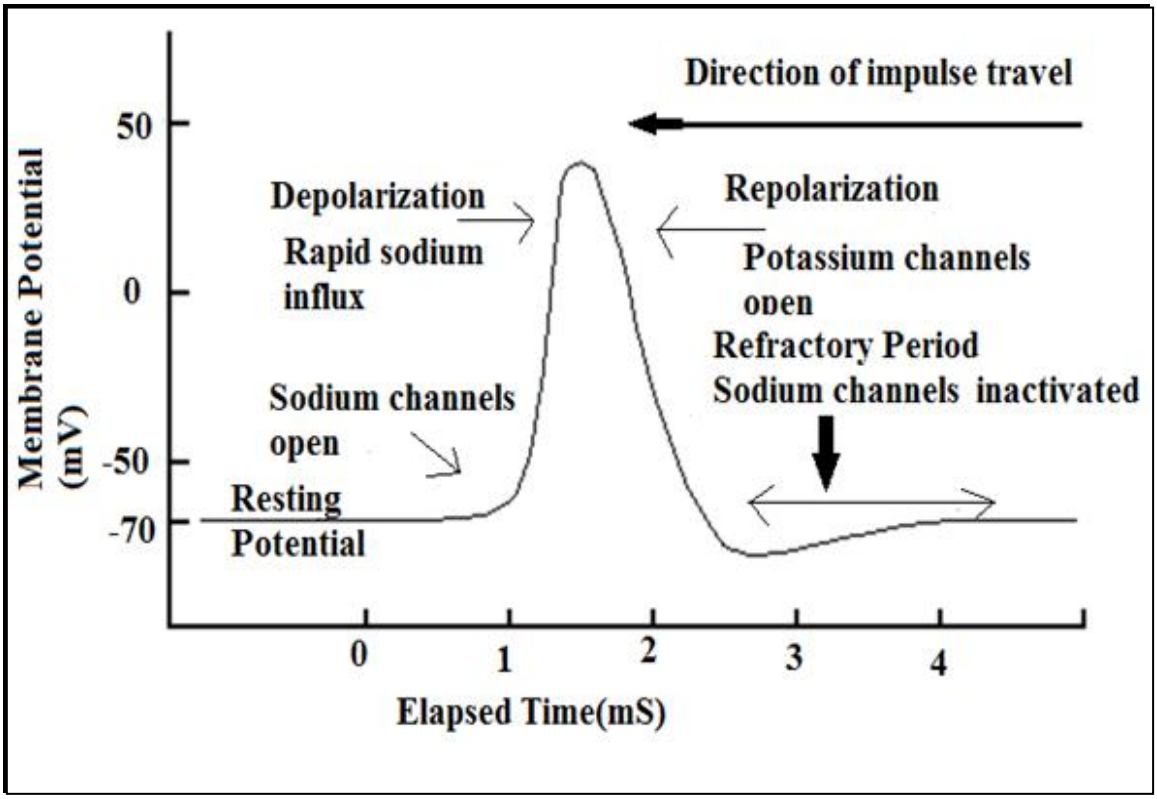


Fig.2.2: Action potential and its biophysical mechanism.

2.2 Overview of Nernst Potential

Neuron membrane is composed of ions both in and out of the cell possessing capacitance property [41]. When the ions move to and fro into the membrane, it produces current and it follows the Ohm's Law given in equation (2.1):

$$I = GV \tag{2.1}$$

In equation (2.1), ***I*** represent transmembrane current, ***V*** is the transmembrane potential and ***G*** is the conductance of ions.

Now the equilibrium potential for ion across the membrane can be written in Nernst form shown in equation (2.2):

$$E_{ion} = \frac{RT}{zF} ln \frac{[I_{on}]_e}{[I_{on}]_i} \tag{2.2}$$

Here, R is the universal gas constant(R=8.31J.mol^{-1}.K^{-1}), T is the absolute temperature, F is the Faraday's constant (F= 96,500 mol^{-1}), z is the number of electrons (valence of the ions), and $[I_{on}]_e$ is the extracelluar and $[I_{on}]_i$ is the intracellular ionic concentration respectively. In resting state, the equilibrium sodium potential outside the cell is 60 mV and equilibrium potassium potential inside the cell is around -90 mV. Goldman and Katz had simplified the above equation in generic form for ions moving to and fro in the membrane shown in equation (2.3):

$$E_M = \frac{RT}{F} \ln \left(\frac{\sum P_{ion}{}^{+}[ion^{+}]_e + P_{ion}{}^{-}[ion^{-}]_i}{\sum P_{ion}{}^{+}[ion^{+}]_i + P_{ion}{}^{-}[ion^{-}]_e} \right. \tag{2.3}$$

E_M is the membrane potential, P_{ion} is the permeability of a particular ion and similarly $[ion^{+}]_e$ and $[ion^{+}]_i$ is the permeability of individual ions. Therefore, it can be said that ions move to and fro throughout the cell under the influence of electric forces and ionic concentration.

In theoretical analysis, Nernst potential is applied to the steady state potential of the membrane when the membrane is permeable to more than one ion in different degrees. The equation given by Goldman is modified by Hodgkin for application in cell biology mostly and is called Goldman Hodgkin Katz equation. The Nernst potential can be better explained by the Fig.2.3.The Nernst equation is used for membrane voltage to

equilibrate the existing specified ion concentration

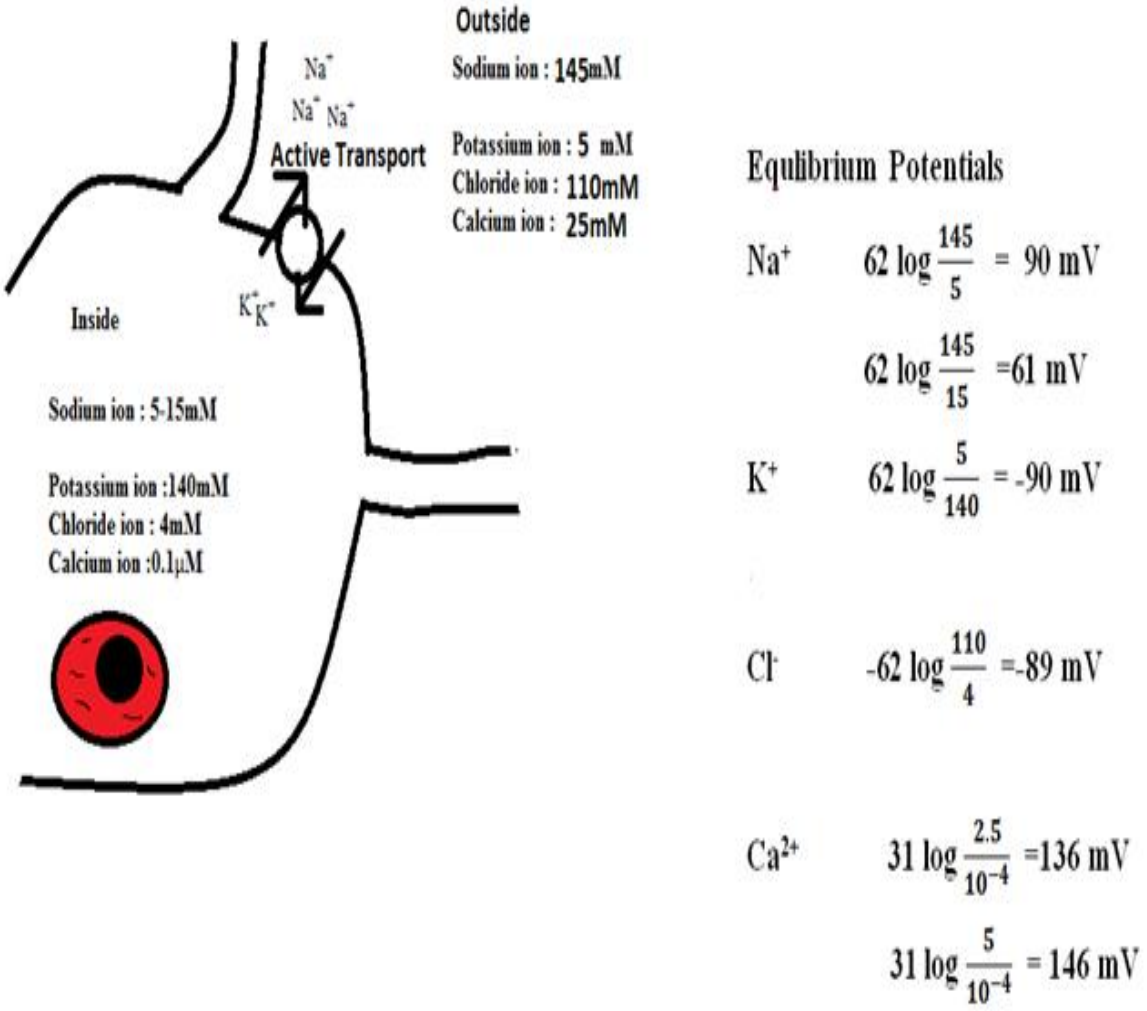


Fig.2.3: Nernst potentials of a neuron membrane for different ions

2.2.1 Reverse potential of sodium and potassium ions

Reversal potential is that potential where there is no ionic flow from either side of the membrane. Since the neuron membrane consists of ions inside and outside of the cell, the ion concentration is different in inside and outside the cell. If potential difference is less than the Nernst potential of sodium ions (E_{Na}), sodium ions enter into the cell to decrease the potential. If the potential difference is larger than the Nernst potential, the ions will move outward. Similarly, reverse potential for potassium (E_k) can be explained.

If the potential difference is higher than Nernst potential of potassium ions, the potassium ions will go outward.

2.3 Overview of conductance based model

Various neuron models had been developed to depict the biophysical nature of a neuron such as Hodgkin Huxley (H-H) conductance based neuron model in 1952,Fitghugh model(1961), Izhikevich model (2003), Morris Lecar (1949), Hindmarsh - Rose Model are among them[4,51,39,41].These models can mimic the neuronal signals occurring in neuron. Conductance based models are focused in this work since these models are the simplest representation of excitation and biophysical nature of a neuron is described in details. These models use minimum biophysical parameters and electronic components to depict the current flow in a membrane by charging of membrane capacitance and through ionic channels. Among the conductance based model, neuroscientists utilize Hodgkin-Huxley neuron model since it is a simple model describing all the biophysical parameters in a neuron and produces accurate neuron signals occurring in a neuron.

2.3.1 Overview of Hodgkin-Huxley model

A neuron is enclosed by a membrane which separates the intracellular and extracellular electrolytes containing various ions .Due to the concentration difference between the intracellular and extracellular medium, there exists a potential difference across the membrane. This potential difference across the membrane is called membrane potential and is generally denoted by V_m (or simply V). Hodgkin and Huxley had carried out many experiments on a giant squid axon to study the properties of action potential and ionic conductances[41-45]. They were awarded Nobel Prize in Physiology and Medicine in 1963 for their work. The first four papers describe the experimental work carried out by them and the fifth paper is a reconstruction of the experimental data into theoretical properties of the neuron known till day.

2.3.2 Brief description of experiment carried out by Hodgkin –Huxley in collaboration with Cole

Before Hodgkin and Huxley, it was known that the cell consists of cytoplasm, high membrane resistance outside the cell and membrane capacitance is associated with

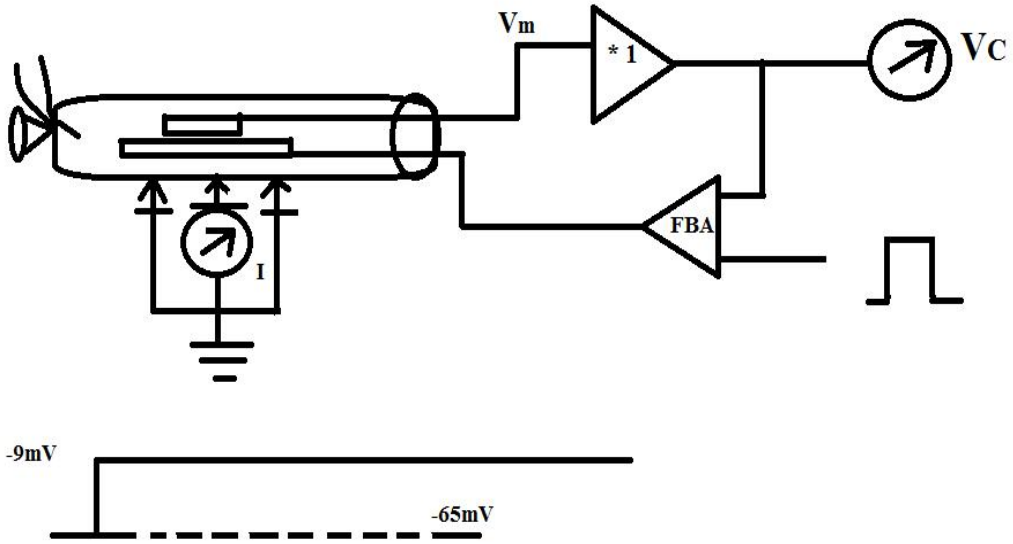
it. It was also known that there is a difference in potential between inside and outside of the cell. Prior to H-H, there was no proper equipment to measure action potential and observe its biophysical properties but the electrical signals of action potential were studied using external electrode. The electrophysiological properties remain unknown till that time. In 1939, Cole and Curtis used a wheatstone bridge to study the conductances of ion and they observed the transient increase of sodium and potassium conductance [17].

Later, in 1937-38, Hodgkin and Huxley collaborated with Cole to find the membrane voltage V_M by tunneling glass micropipette inside a giant squid axon. The membrane voltage obtained was much substantially overshoot than observed by Cole and Curtis. This led to the two very famous experiments known: Voltage clamp and Space clamp.

2.3.3 Space Clamp Method

Space clamp method was developed by Marmont and Cole in 1949 [41-45]. In this method, there is spatial uniform distribution between membrane voltage V_M, where the membrane current is to be measured. This is possible by intracellular capillary electrode which can be inserted directly inside the membrane to measure the current. The tip of the electrode flows into the axon for measurement of current in the membrane. To reduce resistance and achieve space clamp, a silver wire is tied and thus produces no voltage gradients.

Fig. 2.4 shows the space clamp set up and the current obtained for sodium and potassium by space clamp method. Hodgkin and Huxley had observed that both sodium and potassium ions contribute to total ionic current .They had also postulated that change in membrane permeability produces the action potential signal allowing the sodium ions to enter into the cell. In their view, V_M tends to be in the Nernst potential of the dominant ion and it tends to change according to the membrane permeability. Since at rest, potassium conductance is more dominant, therefore V_M approaches towards potassium (E_K) i.e. -60mV.



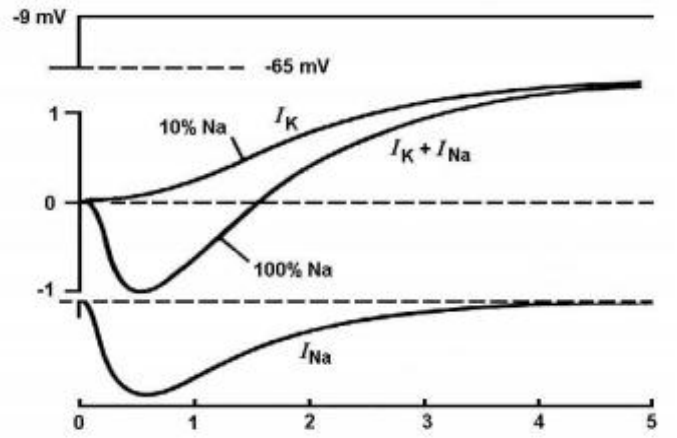


Fig.2.4: Space clamp method and the results obtained by Hodgkin-Huxley (From Hodgkin Huxley)

During action potential it tends towards sodium conductance(E_{Na}) i.e. V_M is approximately 30mV.Action potential mostly depend on sodium ions and decrease of sodium ions results in lowering of the peak. Until 1952, it was unknown how membrane permeability changes and the phenomenon behind the action potential. Hodgkin and Huxley had done this by measuring different concentration of ions separately and showed that they respond to V_M independently. They had achieved this by eliminating sodium in a bathing medium and then I_K is measured. Similarly, I_{Na} is calculated by subtracting from the total current measured. This experiment proved that sodium and

potassium current influence the membrane potential (V_M) [41-45].

2.3.4 Voltage Clamp Method

Similarly, voltage clamp is a method where the voltage inside the membrane is to be maintained constant or at a desired voltage level. This constant voltage cannot be obtained simply by connecting two electrodes in inside and outside of the cell because there may be voltage drop in the surrounding solution of electrodes. So, Hodgkin and Huxley had taken two pair of electrodes. One for measuring voltage inside the membrane

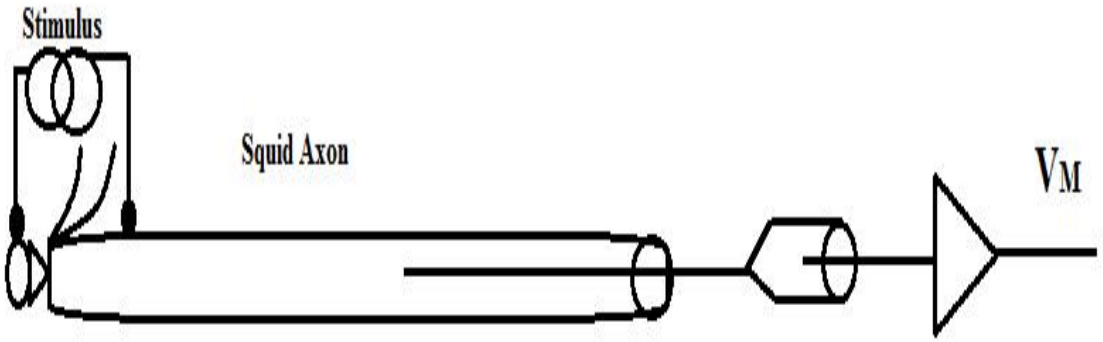


Fig.2.5: Voltage Clamp method to measure ionic current of membrane used by Hodgkin-Huxley

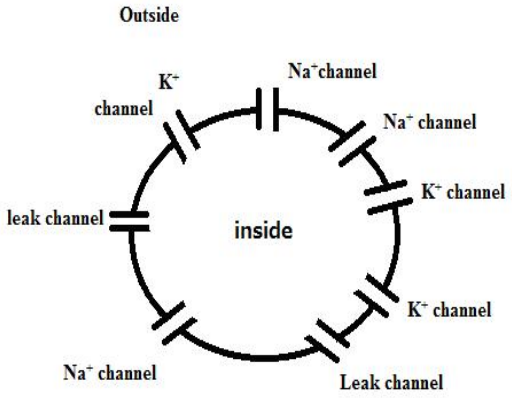
and the other for maintaining constant voltage inside the membrane. To maintain the permeability of membrane due to the injection of current, a feedback current amplifier circuit is connected as shown in Fig. 2.5.

Now, voltage clamp method was applied to measure individual current of potassium and sodium as described in space clamp method.

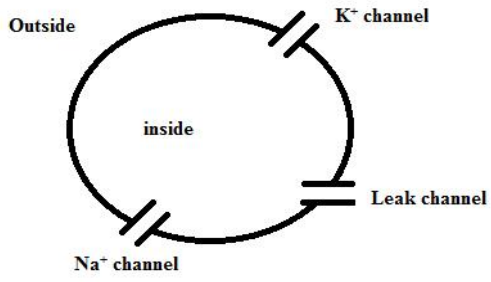
2.3.5 Hodgkin-Huxley's description of biophysical nature of neuron

Hodgkin and Huxley postulated that membrane consists of a lipid bilayer having a large number of microscopic ion selective channels embedded in it. For example, some channels are selective only for Na^+ ions , some are selective for K^+ ions and some are for other ions, called leakage ions as shown in Fig.2.6(a). The movement of ions through these channels is controlled by physical gates whose opening and closing depend on

membrane voltage and some other factors. Gates in each such channel may be of one type or of different types as shown in Fig.2.6 (b). The functions of different gate types in a particular type of ion channel are different. For example, if one type of gates activates the movement of ions through the channel, the other type may inactivate the movement, but activation and inactivation phases do not take place simultaneously. Na^+ channel has two types of gates namely m-type and h-type. m-type is responsible for activation of Na^+ ions and h type for inactivation of sodium ions. Similarly, K^+ channel has only one type of gate namely n-type responsible for activation of movement of K^+ ions, The number of gates in each type may be one or more as shown in Fig.2.7.



(a)



(b)

Fig.2.6: Neuron with different ion channels. (a) Large population of individual microscopic ion channels (b) Macroscopic individual ion channels which are considered to arise from the combined effects of large population of microscopic ion channels.

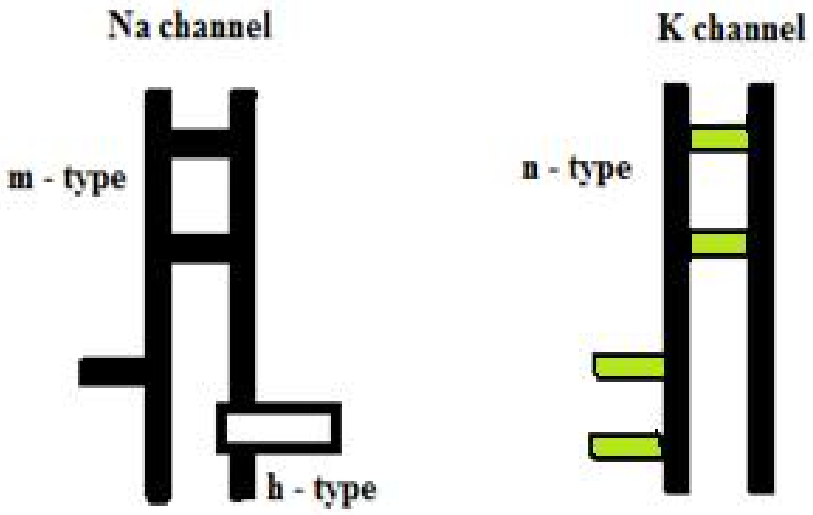


Fig. 2.7: Different- gate types of different ion channels

Since different ions move across the membrane, the membrane may be represented by a combination of circuit elements. This basic concept of electrical engineering is explored by Hodgkin-Huxley by performing a series of voltage clamp experiments. From these experiments, they had represented a short segment of axes by a

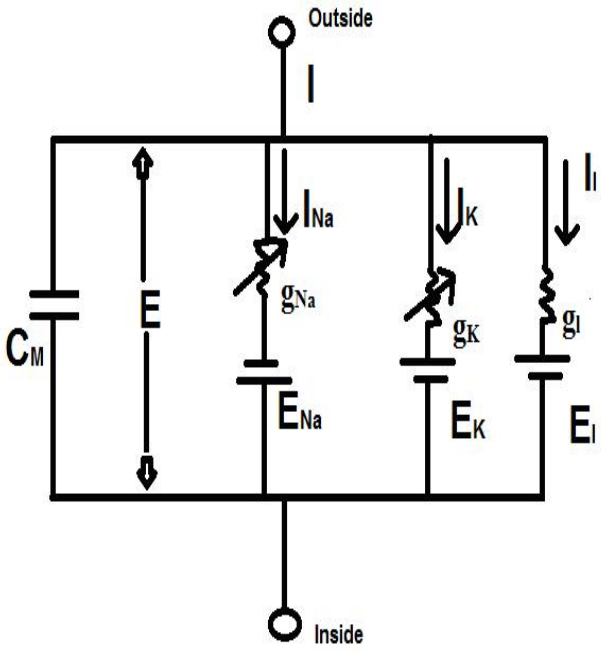


Fig.2.8: Hodgkin - Huxley model of a single neuron membrane showing membrane capacitance, sodium conductance, potassium conductance and leakage conductance.

parallel electrical circuit as shown in Fig. 2.8. This circuit is known as electrical equivalent circuit of neuron. C_M represents the capacitance of the cell membrane. Na^+ and K^+ channels are modeled by the conductances: g_{Na} and g_K respectively. These are voltage and time dependent. g_l represents the leakage conductance. The tendency of ions to move down their concentration gradients is denoted by the batteries with voltages E_{Na} , E_K and E_l. These voltages depend on the inside-outside concentration difference in each ion.

The ionic current is divided into sodium (I_{Na}), potassium (I_K) and leakage ions (I_l) and membrane capacitance(C_M). The total current can be expressed in differential form as [41]:

$$I = C_M \frac{dV}{dt} + I_{ion} \tag{2.4}$$

I is the total membrane current, C_M is the membrane capacitance, V is the voltage difference from resting potential, I_{ion} is the ionic current due to sodium, potassium and chloride ions.The total ionic current is represented by equation (2.5):

$$I_{ion} = I_{Na} + I_K + I_l \tag{2.5}$$

Total ionic current is the summation of all individual contributors. For example, for potassium ions, the current is shown in equation (2.6) [41-45]:

$$I_{ion} = \sum_k I_k = \sum_k G_k (V_m - E_k) \tag{2.6}$$

Each individual current is associated with conductance of the individual ion and equivalent potential of the ion. It is known that voltage dependency of the ions is due to membrane channels that allows the flow of ions across the membrane.

Hodgkin – Huxley (H-H) had postulated that conductance of each ions is possible due to the microscopic ion channels in the membrane. Movement of each ion is regulated by a number of physical gates. A physical gate may be open (permissive) or closed (non-permissive). Depending on the permissiveness and non-permissiveness of the gate, ions can pass through the membrane gate. The permissive state or in non-permissive state depends on the membrane voltage.

Suppose for a particular ion channel *i*, probability (p_i) is taken between 0 and 1 for permissive state. If a large number of channels is considered, p_i can be taken as fraction of gates for a particular channel to be in permissive state and (1-p_i) for non-permissive state. A gate being in permissive and in non-permissive state in Hodgkin-Huxley model obeys the first order kinetics shown in equation (2.7):

$$\frac{dp_i}{dt} = \alpha_i(V)(1 - p_I) - \beta_i(V)\,p_i \tag{2.7}$$

Where α_i is a rate constant for non-permissive to permissive and β_i is a rate constant for permissive to non-permissive state.

When membrane voltage (V_m) is clamped on a certain voltage (V), the permissive gates will be in a steady state value ($dp_i/dt = 0$) as t tends to infinity shown in equation (2.8):

$$p_{i,t\to\infty(V)} = \frac{\alpha_i(V)}{\alpha_i(V)+\beta_i(V)} \tag{2.8}$$

The time required to reach this equilibrium state is given by:

$$\zeta_i(V) = \frac{1}{\alpha_i(V) + \beta_i(V)} \tag{2.9}$$

Where $\zeta_i(V)$ is time constant.

If the individual channel is in permissive state, it contributes towards the total conductance. If it is in non permissive state, conductance is zero. Therefore, conductance is directly proportional to the gates in the permissive state or in open state. Thus, the conductance of potassium (G_K) due to channels of type *k* and gates *i* is proportional to the product of the gate probability (p_i) shown in equation (2.10) :

$$G_K = \bar{g}_K \prod_i p_i \tag{2.10}$$

$\bar{g}_K$ is the maximum conductance when the gates are in permissive state.

These equations can be explained for sodium ions and leakage ions as

well.Hodgkin-Huxley had modeled sodium conductance using three types of *m* gates for activation and one type of *h* gate (gating variables) for inactivation as depicted in equation (2.11).

$$G_{Na} = \overline{g_{Na}}\, p_m^3 p_h = \overline{g_{Na}}\, m^3 h \tag{2.11}$$

Potassium conductance is explained with four identical gate i.e.

$$G_K = \overline{g_K}\, p_n^4 = \overline{g_K} n^4 \tag{2.12}$$

Total ionic current can now be expressed as :

$$I = C_M \frac{dv}{dt} + \overline{g_K} n^4 (V - V_K) + \overline{g_{Na}} m^3 h (V - V_{Na}) + \overline{g_l}(V - V_l) \tag{2.13}$$

Where $V_K = E_K - E_R$, $V_{Na} = E_{Na} - E_R$, $V_l = E_l - E_R$. E_R is the resting potential.

The three rate constants defined by Hodgkin and Huxley are given in equation (2.14) to (2.16):

$$\frac{dm}{dt} = \alpha_m (V)(1 - m) - \beta_m (V) m \tag{2.14}$$

$$\frac{dh}{dt} = \alpha_h (V)(1 - h) - \beta_h (V) h \tag{2.15}$$

$$\frac{dn}{dt} = \alpha_n (V)(1 - n) - \beta_n (V) n \tag{2.16}$$

The rate constants at a particular voltage by curve fitting method are given in equations (2.17) to (2.22).

$$\alpha_n = 0.01 (V + 10) / (\exp \frac{V + 10}{10} - 1) \tag{2.17}$$

$$\beta_n = 0.125 \exp(V / 80) \tag{2.18}$$

$$\alpha_m = 0.1 (V + 25) / (\exp \frac{V + 25}{10} - 1) \tag{2.19}$$

$$\beta_m = 4 \exp(V / 18) \tag{2.20}$$

$$\alpha_h = 0.07 \ \exp(\ V/20\ ) \tag{2.21}$$

$$\beta_h = 1/(\exp\ \frac{V+30}{10} + 1) \tag{2.22}$$

The action potential and currents of sodium and potassium obtained by Hodgkin-Huxley is shown in Fig. 2.9 and Fig. 2.10.The currents of sodium and potassium ions were observed using voltage clamp method described in the previous section.

The conductance of potassium and sodium with respect to time is found by curve fitting equations described by Hodgkin-Huxley which is given in equations (2.23) and (2.24).

$$g_K = \{\left(gK_\infty)^t - [(gK_\infty)^t - (gK_0)^t\right] exp\left(-\frac{t}{\tau_n}\right)^4\ \}^4$$

(2.23)

$g_{K\infty}$ is the conductance of sodium which it attains and g_{Ko} is the conductance at time, t=0.

$$g_{Na} = g'_{Na}\left[1 - \exp\left(-\frac{t}{\zeta_m}\right)\right]^3 \exp\ (-\frac{t}{\zeta_h}) \tag{2.24}$$

g'_{Na} is the sodium conductance which it finally attains. The other values of equation (2.23) and (2.24) are taken from 1952 Hodgkin-Huxley [41-45]. Fig. 2.11 and Fig. 2. 12 shows the potassium and sodium conductance obtained from equation (2.23) and (2.24) at different depolarization [41-45].

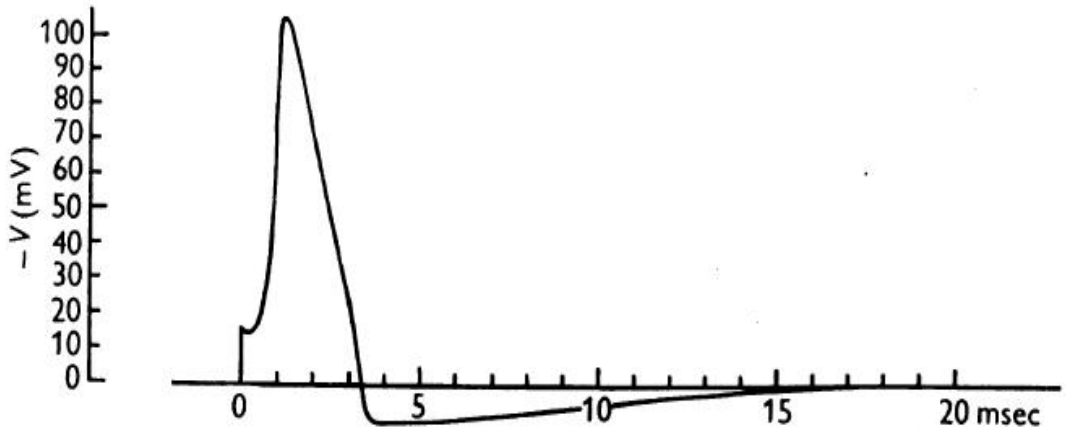


Fig.2.9: Action Potential generated by combining the equations given by H-H model

(From Hodgkin-Huxley)

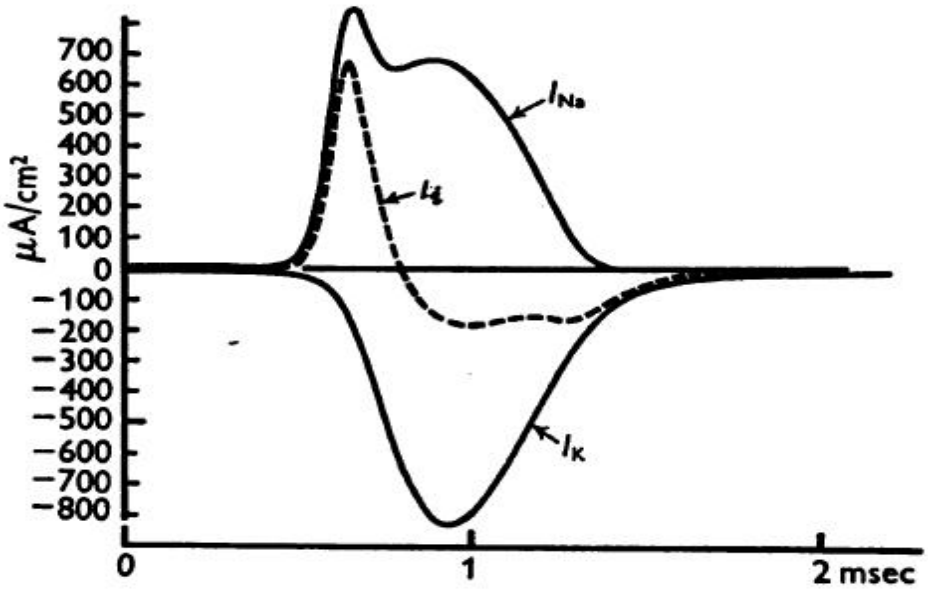


Fig.2.10: Total ionic current, sodium current and potassium current during action potential obtained by Hodgkin-Huxley

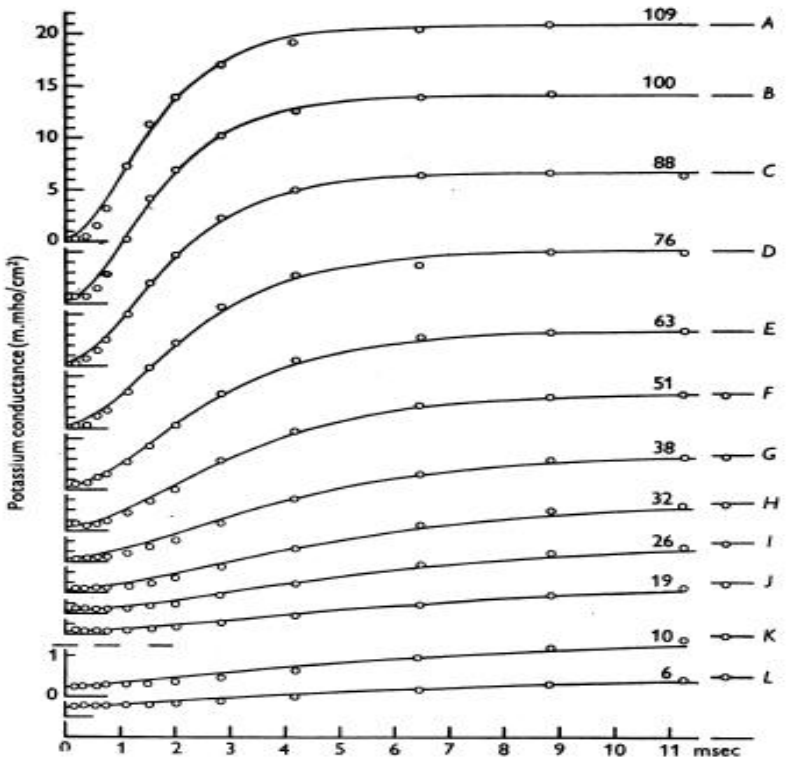


Fig. 2.11: Sodium conductance variance with time obtained by Hodgkin-Huxley at

different depolarization.

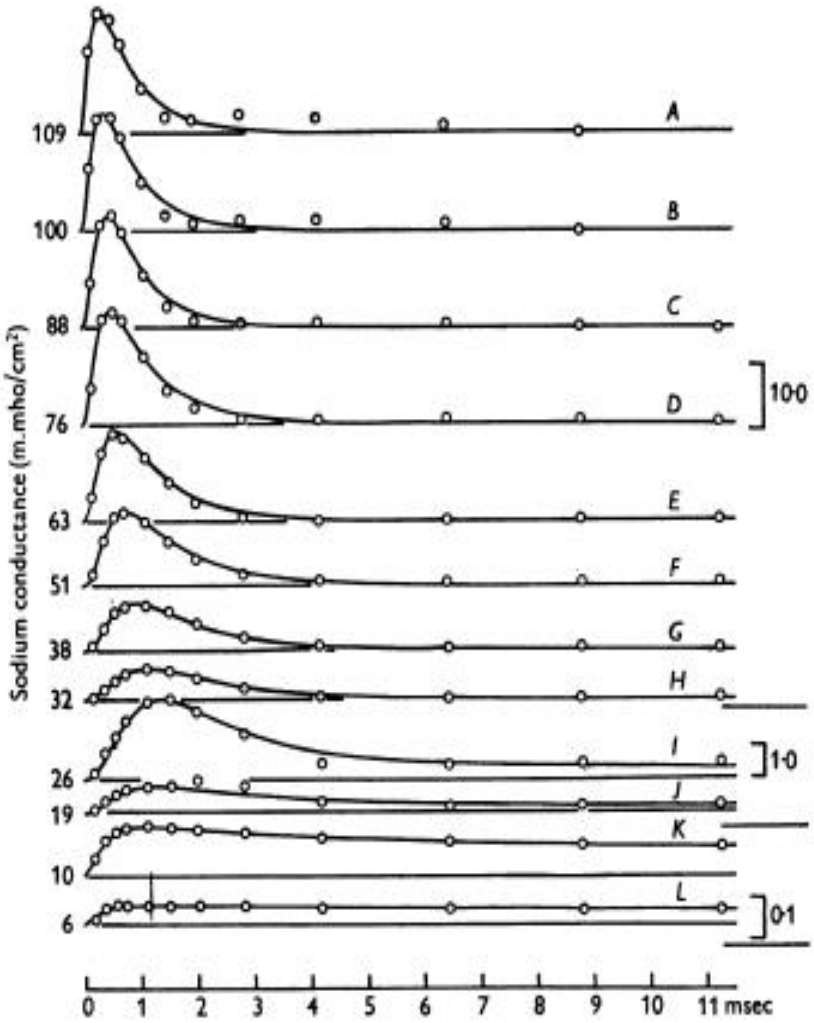


Fig. 2.12: Potassium conductance obtained by H-H model at different depolarization.

2.3.6 H-H Sign conventions

In this thesis, H-H sign convention is used for Chapter 4 and 5. Hodgkin-Huxley model used membrane potential as negative while modern sign convention takes membrane potential as positive. H-H chose resting intracellular potential as 0. Similarly inward current taken by them is positive i.e. sodium current is positive while potassium current is taken as negative.

2.4 An overview of other conductance based neuron models

Some neuron models are described below which were developed after H-H model and their strategies towards spike formation is briefly explained.

2.4.1 Bullock Model

Bullock in 1959 had described the neuron in a different perspective. He proposed that the spikes generated are localized in a small portion of neuron [65-66]. Several parts of neuron respond to impulses and all parts do not follow all or none conduction .Each of these impulses doesn't form spikes but several together determine the firing of impulses. The process occurring in synapse (neurotransmission) occurs in different parts of the neuron also. Synaptic transmission is of three different forms: It can be facilitating or antifacilitating, enhanced or diminished and excitatory or inhibiting. After going through the long train of pulses, the potential in the synapse may be positive or negative. There may be other complexity in the process due to which the spikes may be inhibitory. Bullock proposed a locus model on neuron where the spike initiation, synaptic transmission etc. occurs in localized part of neuron. Bullock assumed that spike formation depends on the loci of the spatial distribution of the neuron. This model did not describe the actual biophysical nature of a neuron.

2.4.2 Fitghugh model

Fitghugh had developed a mathematical model in 1961 describing the neuron functions which is a reduced form of H-H model [5,28,110]. It takes the principle of excitation and propagation. The mathematical description takes the form shown in equation (2.25) and (2.26). It is observed from H-H equation that *n* and *h* are slow and summed up to a very small value. Fitghugh had reduced the neuron model therefore in two dimensional model. *z* represents the external current stimulation in a neuron, *x* is the membrane potential, *y* is the recovery variable necessary to attain resting potential [5,28,110]. *V* and *m* in H-H model described are regarded as a single variable *x*. *n* and *h* from H-H model is denoted as *y*, the recovery variable. The waveforms obtained from Fitghugh model is shown in Fig. 2.13 at different z values.

$$\frac{dx}{dt} = c(x - y + z - \frac{x^3}{3}) \tag{2.25}$$

$$\frac{dy}{dt} = (x - by + a)/c \tag{2.26}$$

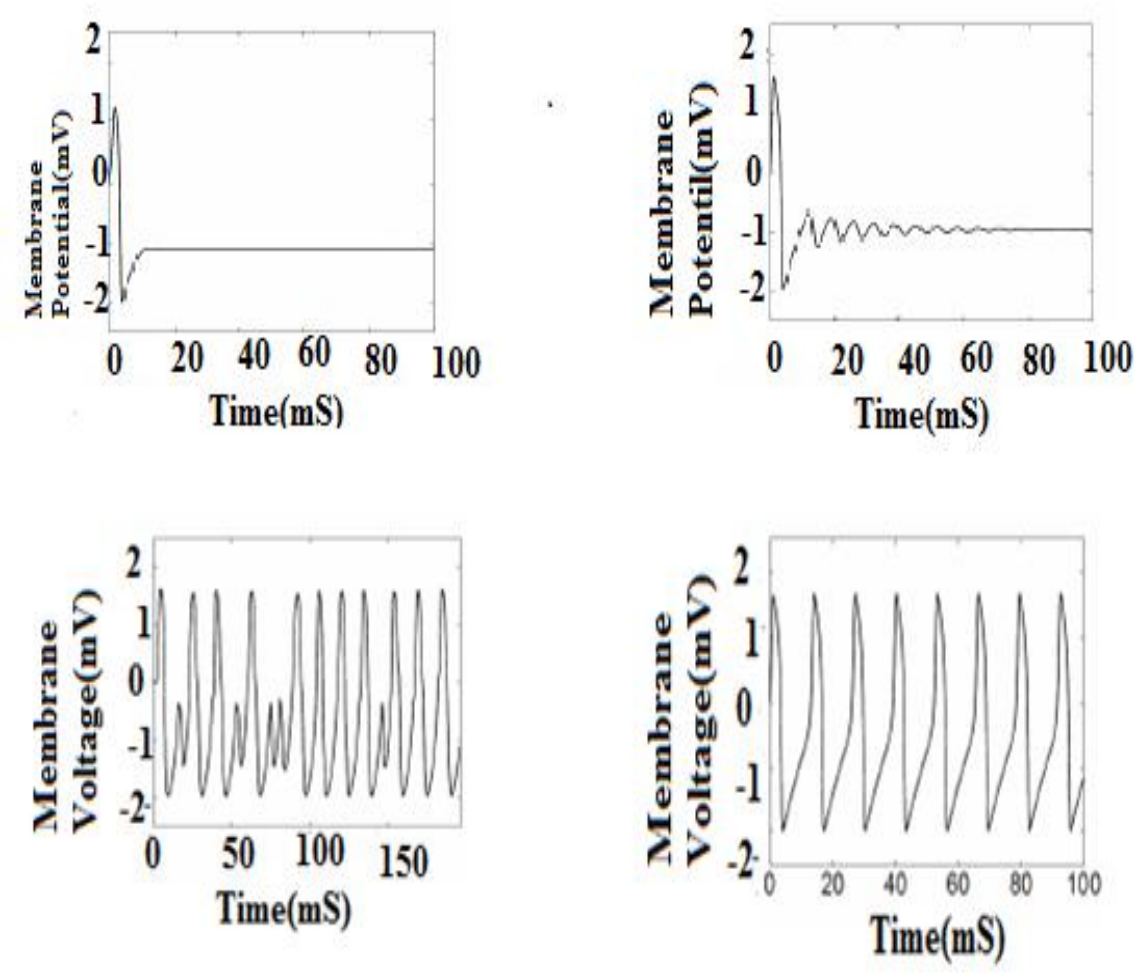


Fig.2.13: Fitghugh model spikes (a) z=0.1, an excitable membrane with a small spiral (b) z=0.33, an excitable membrane with a big spiral (c) z=0.337, bistable with a stable equilibrium point and (d) z=0.34, an oscillatory membrane. (From Fitghugh Model 1961)

The model developed by Fitghugh is simple and have less variables than H-H model but it fails to describe the physiological behavior of a neuron.

2.4.3 Morris and Lecar model

Morris and Lecar had developed a neuron model which describes the biophysical nature of a giant muscle fiber by mathematical equations [74-75]. It takes into account the calcium (Ca) current activation and potassium current. The equations (2.27) and (2.28) describe the model [62]:

$$C\frac{dV}{dt} = -(I_{Ca} + I_K + I_m) + I \tag{2.27}$$

$$\frac{dw}{dt} = \frac{w_\infty(v) - w}{\zeta_w(v)} \tag{2.28}$$

V is the membrane potential, C is the capacitance of the membrane, w is the activation variable for potassium. I_{Ca}, I_K and I_m are the ionic currents for calcium, potassium and leakage ions.

Calcium currents remains in equilibrium and the activation curve fitting equation is given by equation (2.29):

$$I_{Ca} = G_{Ca} m_\infty(V)(V - V_{Ca}) \tag{2.29}$$

Potassium and leakage current activation is given by equation(2.30) and (2.31) :

$$I_K = G_K w(V - V_K) \tag{2.30}$$

$$I_m = G_m(V - V_{rest}) \tag{2.31}$$

Activation curve of calcium current is calculated from the curve fitting equation given in equation (2.32):

$$m_\infty(v) = 0.5\left(1 + tanh\frac{V+1}{15}\right)$$

(2.32)

Potassium current activation is given in the following first order equation:

$$w_\infty(v) = 0.5\left(1 + tanh\frac{V+1}{30}\right) \tag{2.33}$$

and the time constant is given by:

$$\zeta_w(v) = \frac{5}{\cosh\frac{V}{60}}$$

(2.34)

Where G_{Ca} =1.1mS/cm^2, G_K =2 mS/cm^2, G_m =0.5 mS/cm^2, V_{Ca}=100mV, E_K= -70mV, V_{rest}= -50mV.

Simulating the equations given by Morris Lecar, an action potential can be generated.

2.4.4 Wilson Cowan model

Wilson Cowan model (1973) describes the biophysical nature of a neuron in two non linear differential equations (2.35 and 2.36) [74]. *f(u)* is a sigmoid function. This model also represents the interaction between inhibitory and excitation of a neuron :

$$\frac{dV}{dt} = -V + f + \beta_v + aV - bR \quad (2.35)$$

$$\frac{dR}{dt} = -R + f + \beta_R + CV - dR \quad (2.36)$$

$$f(u) = \frac{1}{1+e^{-u}} \quad (2.37)$$

Where a,b,c,d ,β_v and β_R are curve fitting parameters.

2.4.5 Hindmarsh Rose

Hindmarsh Rose (1980) model describe the spiking behavior of a neuron mathematically and shown in equations below [39-41]. Membrane potential is represented by *x(t)*, sodium conductance and potassium conductance is represented as *y(t)* and *z(t)*. *y(t)* is the fast moving variable used for sodium ions and *z(t)* is a slow moving variable for potassium and leakage ions. The spiking nature of neuron is described in ordinary differential equations in equation (2.38) to (2.42).

$$\frac{dx}{dt} = y + \varphi(x) - z + I \quad (2.38)$$

$$\frac{dy}{dt} = (x) - y \quad (2.39)$$

$$\frac{dz}{dt} = r[s(x - x_r) - z] \quad (2.40)$$

where

$$\varphi(x) = -ax^3 + bx^2 \quad (2.41)$$

$$\varphi(x) = c - dx^2 \quad (2.42)$$

The model has parameters namely *a, b, c, d ,r, s,* x_r and *I*. *I* is the total ionic current. Some parameters are taken as control parameter and some are fixed values. When s and x_r are taken as fixed parameter, then s = 4 and x_r = -8/5 is taken. When *a*, *b*, *c*, *d* are fixed , the values given is taken as a = 1, b = 3,c = 1, and d = 5. The parameter *r* is in the order of 10^{-3}. Equation (2.36) reveals many interesting and dynamic pattern of the neuron. This model is quite simple and shows many unpredictable behavior of a neuron.

2.4.6 Izekevich Model

A neuron model was developed by Izekevich (2003) to reproduce spiking nature of a neuron. This model can produce tens of thousands of spikes generated in a neuron by using the mathematical equations. Izhekevich had mathematically described the spike initiation by the following equations 2.43 and 2.44 [18, 51]:

$$V' = 0.04v^2 + 5v + 140 - u + 1 \quad (2.43)$$

$$u' = a(bv - u) \quad (2.44)$$

If v= 30 mV then $v \leftarrow c$, $u \leftarrow u+d$

a,b,c,d are the constant parameter and are dimensionless. *v* is the membrane voltage and *u* is the recovery variable which is constantly changed to get the required signal. *a* is the timing variable recovery, *b* determines the sensitivity of the recovery variable and the oscillations of the spikes to *v*, c is the recovery variable for *v* due to potassium ions after the spike and *d* represents reset of variable *u* due to conductance of sodium and potassium .The two simple equation can produce different type of spikes by varying the variables unlike H-H model which takes into account many differential equations to produce the action potential. By changing the values of *a,b,c,d* one can generate different types of spikes such as regular spikes, fast spikes, slow threshold spikes etc shown in Fig. 2.14.These equations were established using curve fitting

method such that membrane voltage is in mV and time is in ms. Resting potential is between -60 mV to -70mV and the threshold potential can be from -50mV to -40mV.Value of *a* is generally taken as 0.02, *b*=0.2,*c*= - 65mV,*d*=2.

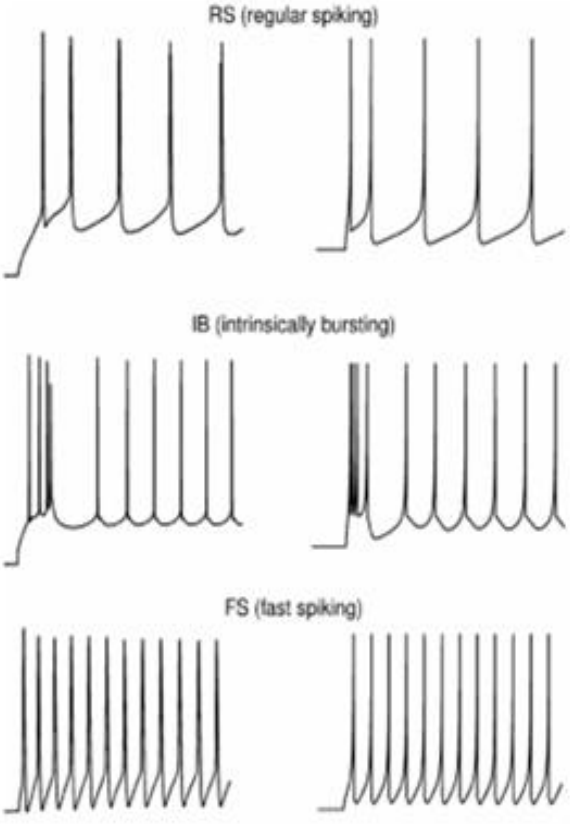


Fig. 2.14: Electric spikes of neuron obtained by Izekevich model

Regular spiking involve singular spike in a particular interval of time which gets increased eventually. For RS spiking type, *c* is taken as -65mVand *b* is equal to 8. For Intrinsic bursting, *c* = -55mV and *d*=4. Similarly for fast spiking, *a*=0.1is taken.

All the neuron models developed after H-H did not consider the biophysical nature of a neuron and all the parameters were not taken into account.

2.5 An overview of Electronic neuron models

Referring to conductance based models; electronic based models have advantages of obtaining neuron signals in real time. Electronic based models also helps to observe neuron signals in direct waveform, time dependent parameters with external stimulus and the change of parameters effect etc [32,62,64,67,87].

2.5.1 Lewis Model

Edwin R. Lewis (1964) [65-66] had published several papers to establish the H-H membrane properties. Lewis had developed an electronic neuron circuits using discrete transistors for realization of H-H neuron spikes. This model is an accurate representation of Hodgkin-Huxley model and can reproduce membrane potential depicted by H-H expressions. The sodium, potassium and leakage conductances are realized using discrete transistors connected in parallel between nodes showing inside and outside of the membrane. Each potassium and sodium conductance was represented separately using active filters and is shown in Fig. 2.15 and Fig. 2.16. When simulated, the circuit produces membrane potential as realized by Hodgkin and Huxley. While realizing the membrane properties in electronic circuit, V_M is multiplied by 100 to fit the electronic circuit. The circuit simulating potassium conductance (G_K) variance is similar to the Hodgkin-Huxley model and is measured from non-linear active filter. The three variable resistors provide the necessary delay, rise and fall time. Amplitude of the potassium conductance can be varied by using potentiometer. Potentiometer is used as an amplitude generator for multiplier. Multiplier circuit produces the function G_K (V_M,t).V_k where V_K is the potential difference between potassium potential and membrane potential (V_M).Multiplier output is based on quadratic equation of two diodes.

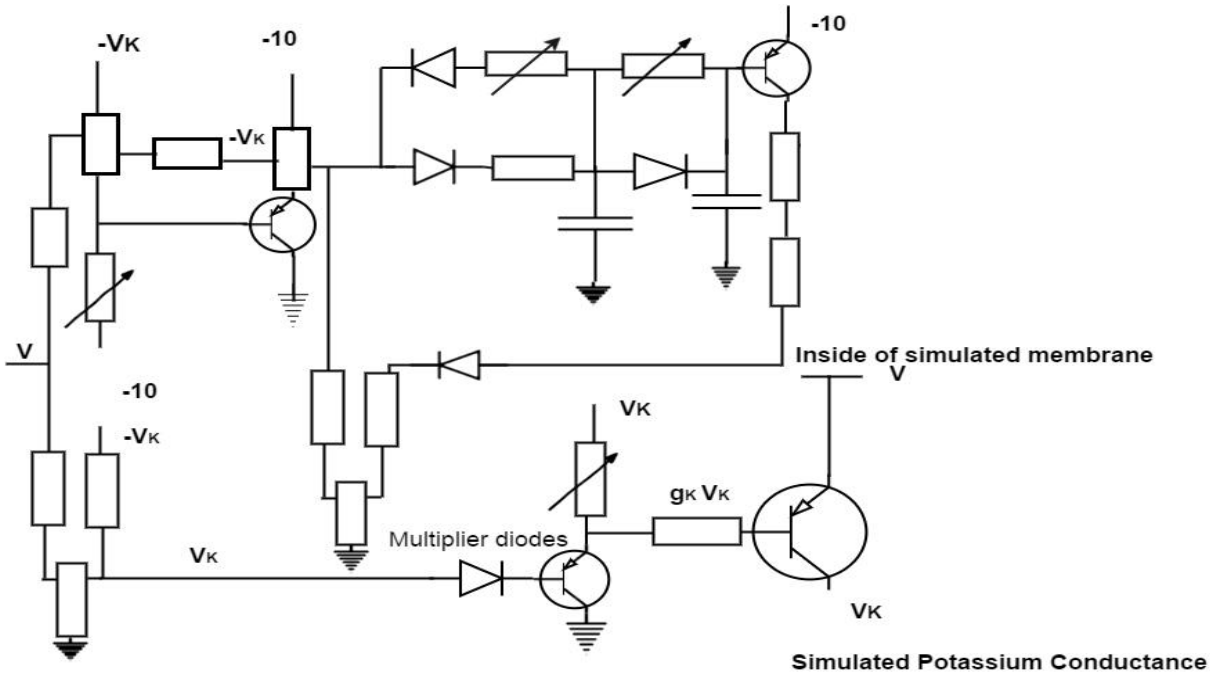


Fig. 2.15: Lewis model for potassium conductance

The simulating circuit for the sodium conductance is shown in Fig.2.16. In this circuit, the multiplier is not connected since sodium conductance is 120 mV more than resting potential. Sodium conductance can be taken as constant for a small membrane voltage change. Inactivation and activation of sodium conductance is varied by the varistor used in the circuit. The circuit generates the activation of sodium conductance variance with depolarization exactly like H-H model.

While connecting all the components of neuron membrane, an action potential is generated. This circuit is shown in Fig.2.17. The action potential generated is similar to the natural action potential [65-66].

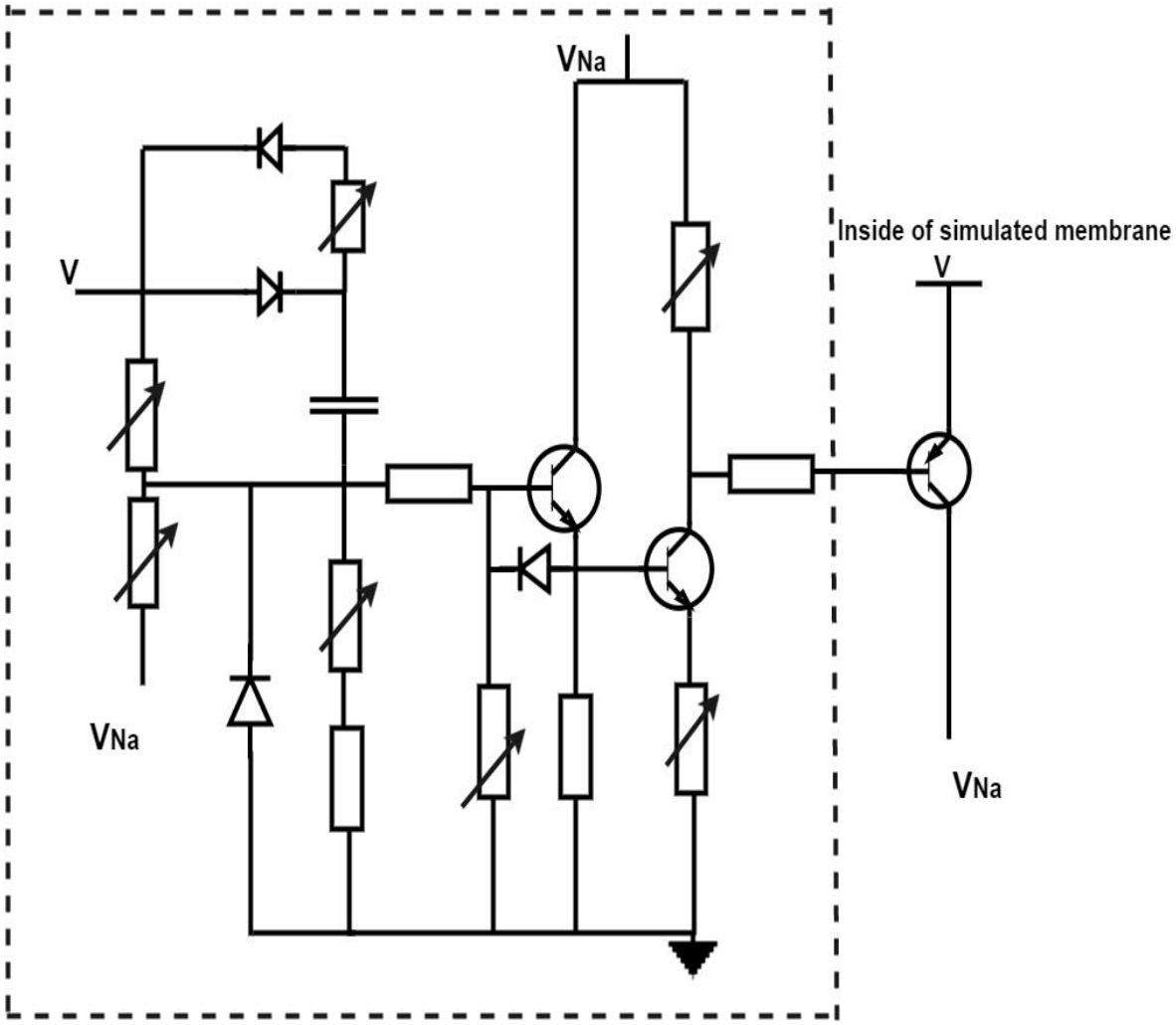


Fig.2.16: Lewis circuit for sodium conductance

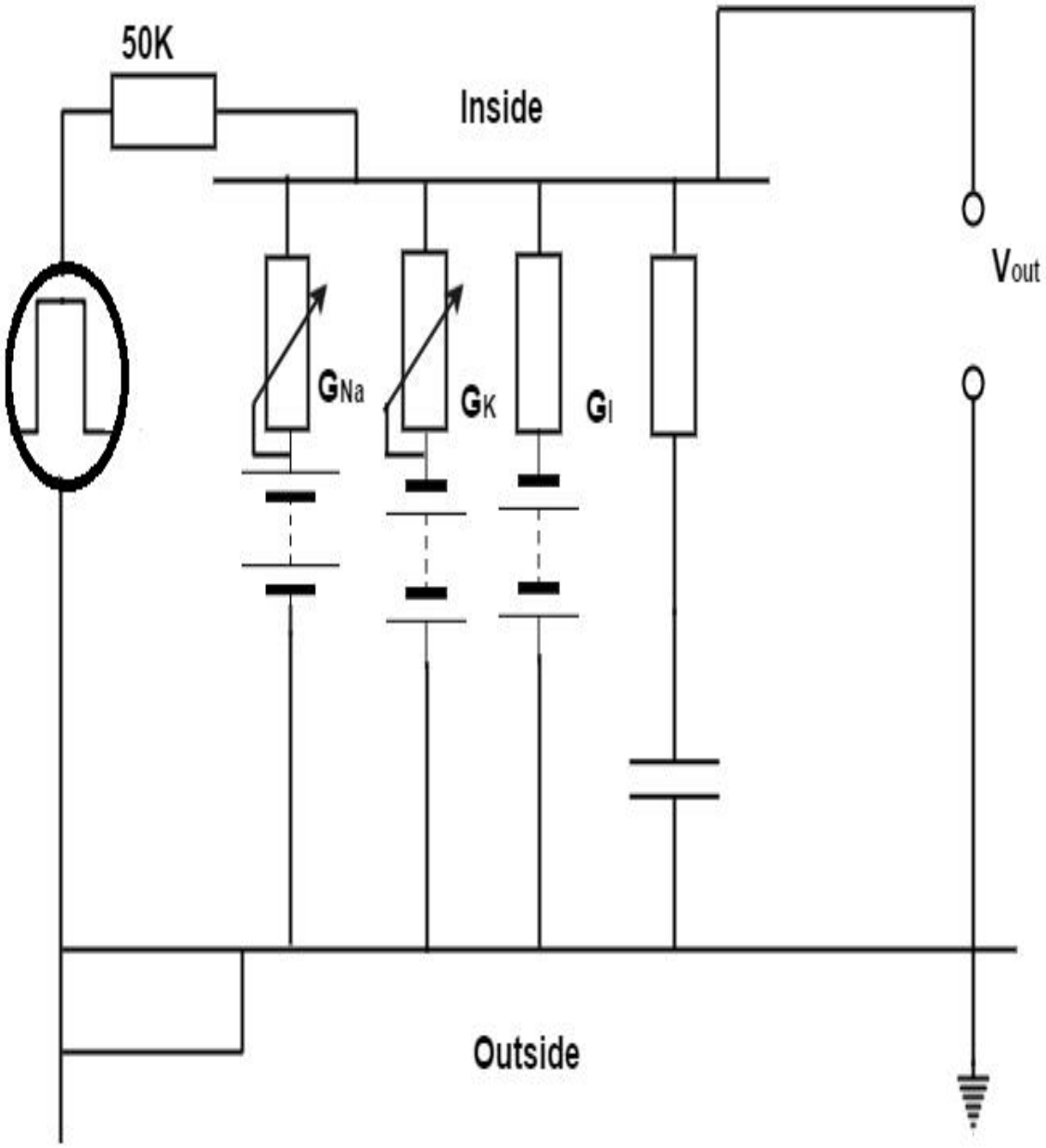


Fig. 2.17: Combined Lewis circuit of potassium, sodium and leakage conductance to obtain action potential

The spiking nature obtained by Lewis is shown in Fig. 2.18. This circuit produces electric spikes of a neuron but it is quite complex and costly to build.

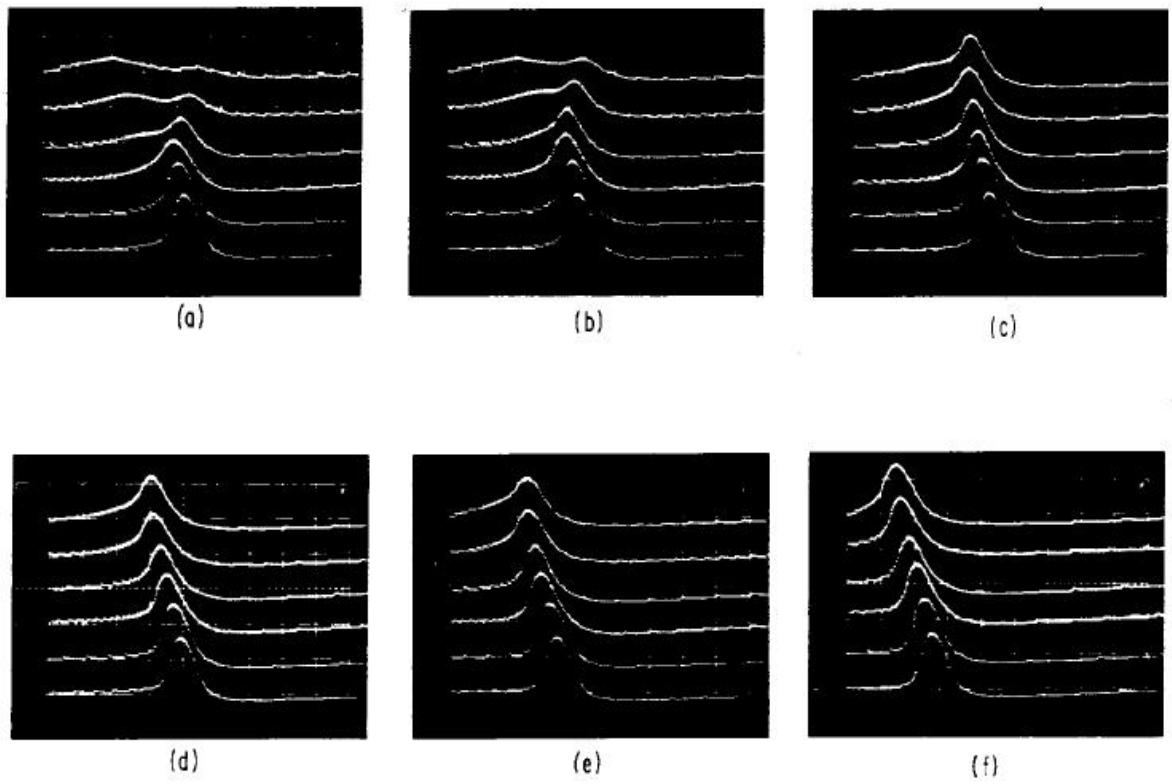


Fig.2.18: Spikes obtained from Lewis circuit at different depolarization (From Lewis 1964)

2.5.2 Johnson and Hanna model

Johnson and Hanna (1969) had developed a circuit using pnp transistor which produces the electric synapse generated in a neuron. In this circuit, transistor is used for conductances of potassium and sodium [55]. Transistor and the membrane are quite similar in structure as shown in Fig.2.19. The mobile charge carrier in the emitter represents the outside membrane (p region), the base as a membrane (n region) and the collector (p region) as an intracellular fluid. The polarity and the static charge are similar to a membrane ignoring the fact that they are made of different materials. Using a transistor, Johnson and Hanna had developed a neuron model within the constraints of Hodgkin-Huxley model. The emitter to base junction is taken as the outside of the membrane and collector to base junction is considered as inside of the membrane. The polarities of the batteries in Hodgkin -Huxley are maintained. Therefore, the transistor is reverse biased because in forward bias, the circuit will not operate as required.

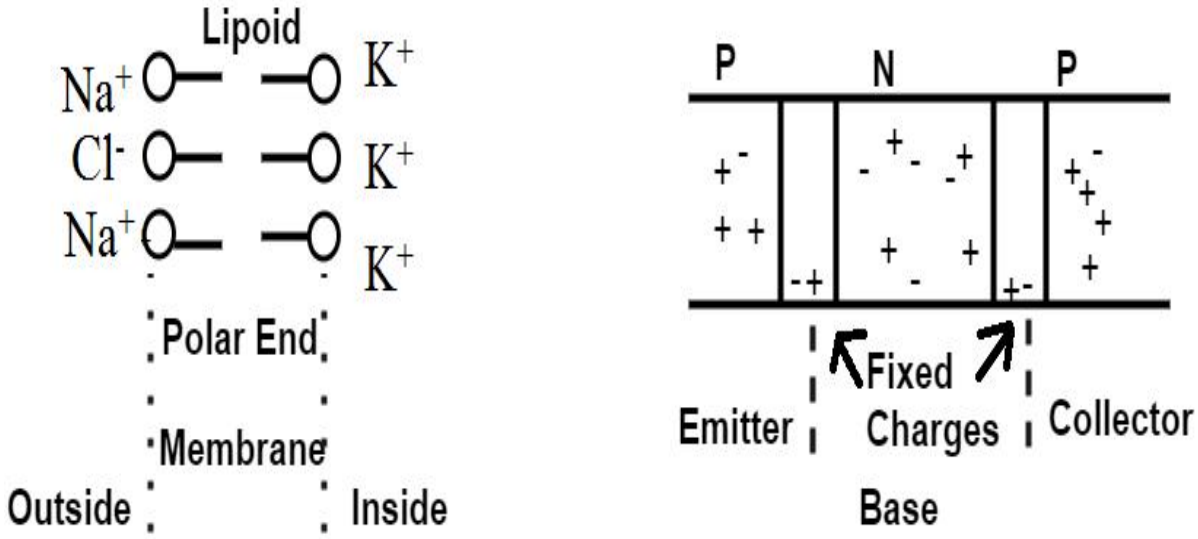


Fig.2.19: Similarity of neuron membrane and a bipolar transistor.

If capacitor current is sufficient to forward bias the transistor, avalanche will be reached and an action potential (depolarization) will be produced. When the capacitor voltage is increased by charging the capacitor, hyperpolarization occurs. In the circuit shown in Fig.2.20, when a positive pulse is triggered between resistors and ground, it produces a response of an axon. A simulated action potential can be produced using alternating active and passive sections. One active section acts as a trigger for the next section. Velocity is controlled by the resistors and capacitors in the circuit used. The resting potential in a neuron is -85V measured between point 1 and ground. In this circuit 1V is scaled to 1mV. All or none response can be attained if the value of R1 is made small or E_{Na} is made more negative. It will require greater stimulus to overcome the threshold. This circuit can be used in various purposes such as to produce different excitable membrane, pacemaker, to produce variation in frequency of pacemaker and bidirectional operation can be achieved when extra elements are connected. But the model cannot describe the biological phenomenon in a neuron. Fig.2.21 shows the response obtained from Johnson and Hanna's circuit.

Fig.2.20: Johnson and Hanna's practical model of membrane for reproduction of spikes

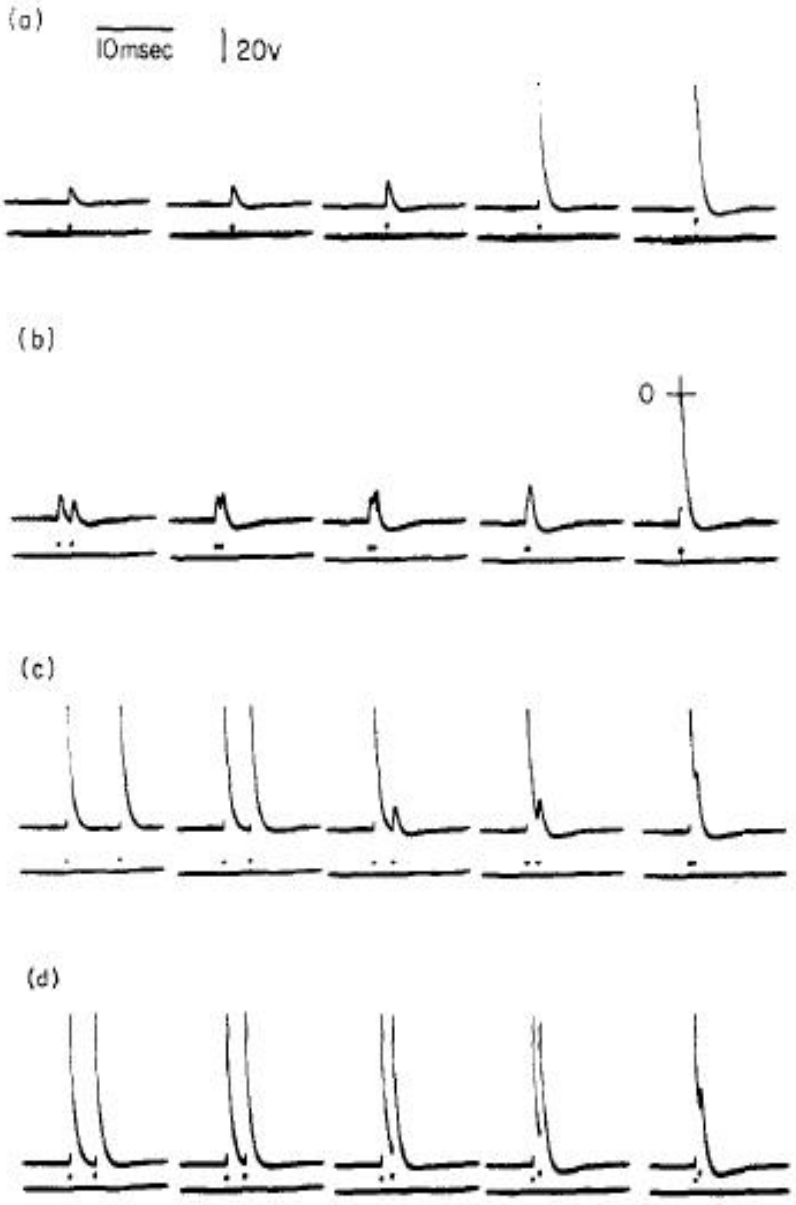


Fig.2.21: Spikes generated from Johnson and Hanna circuit (From Johnson 1969)

2.5.3 Guy Roy model

Guy Roy developed a simple electronic model to reproduce potassium and sodium conductances satisfactorily [83]. The circuits proposed for simulating the sodium

and potassium currents are shown in Fig. 2.22 and Fig.2.23. This circuit model of neuron used two JFETs. One is used for sodium conductance and other is used for potassium conductance. The voltage across the drain to source of JFET represents the membrane voltage and drain to source current of the JFET represents the membrane current. The voltage and time dependent characteristics of the two conductances were realized by introducing a feed back and RC circuits respectively [83, 33-35].

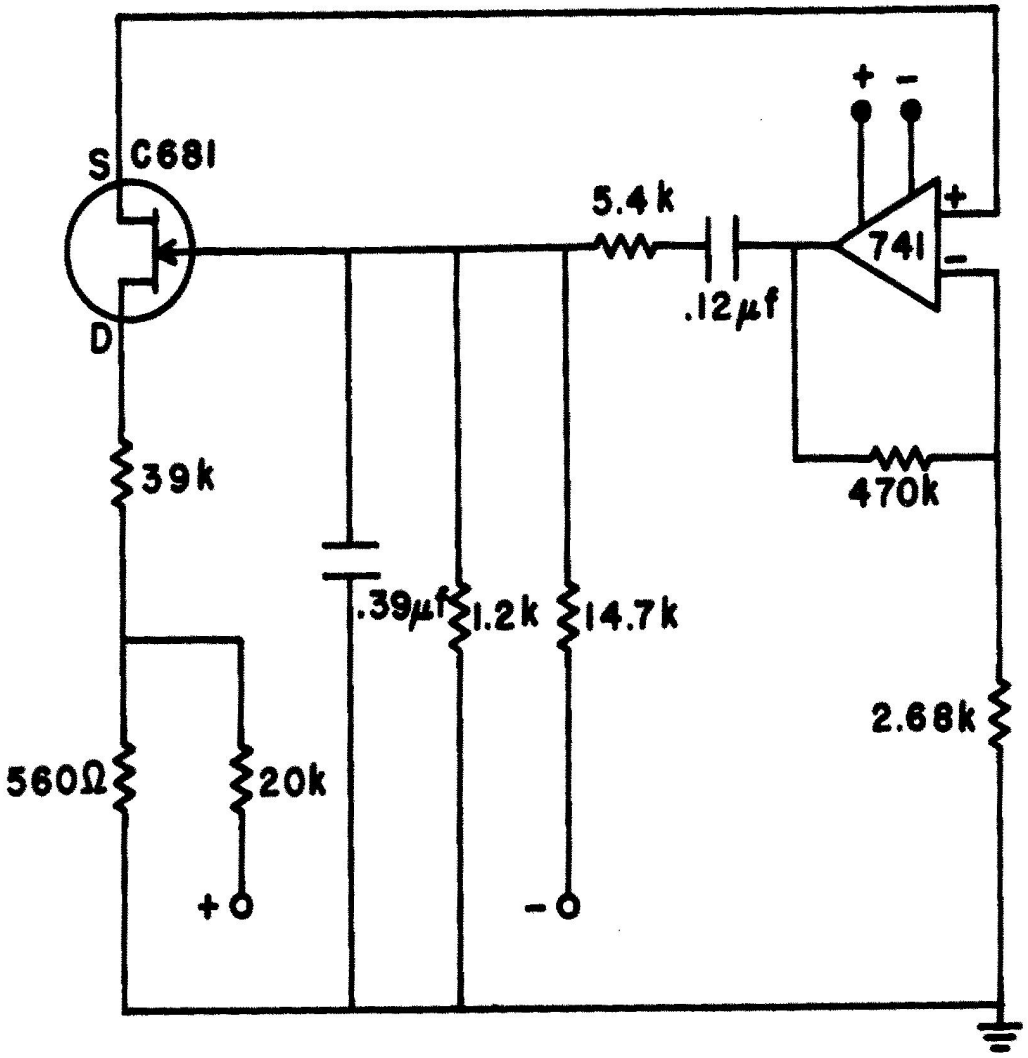


Fig. 2.22: Roy model for simulating sodium current (From G.Roy 1972)

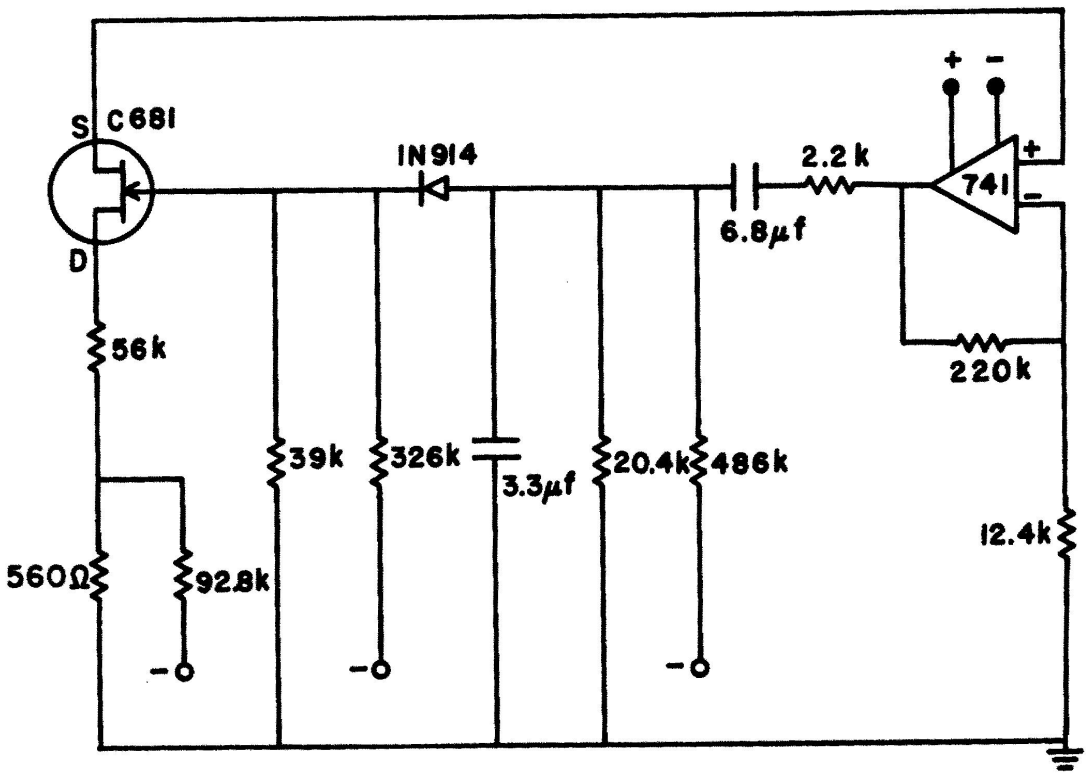


Fig.2.23: Guy Roy model for simulating potassium current (From G.Roy 1972)

2.5.4 William Brockman Model

William H Brockman (1979) developed a model for a membrane patch. In this model, the neuron membrane is divided into parts and represents each resistance by R_A, extracellular resistance by R_E, transmembrane resistance by R_M, membrane capacitance by C_M [6]. The conductance of ions is taken as fixed appropriate values while the transmembrane elements are provided by the active membrane patch circuit shown in Fig.2.24. The active circuit consists of a collector coupled astable multivibrator with a large base resistance. The transmembrane voltage V_M should rise to an appropriate voltage so that Q_1 conducts to produce action potential .V_{CE1} falls and Q_2 gets off, V_{CE2} rises and thus increasing V_M. When R_SC_1 discharges, Q_2 goes in saturation condition. This produces a pulse similar to neuron impulse. The width of the pulse is maintained by R_SC_1 and pulse repetition rate is maintained by R_MC_M. Rate of firing also depends on the voltage applied to V_M for proper depolarization. Positive dc current in point A produces linearly depolarized current.

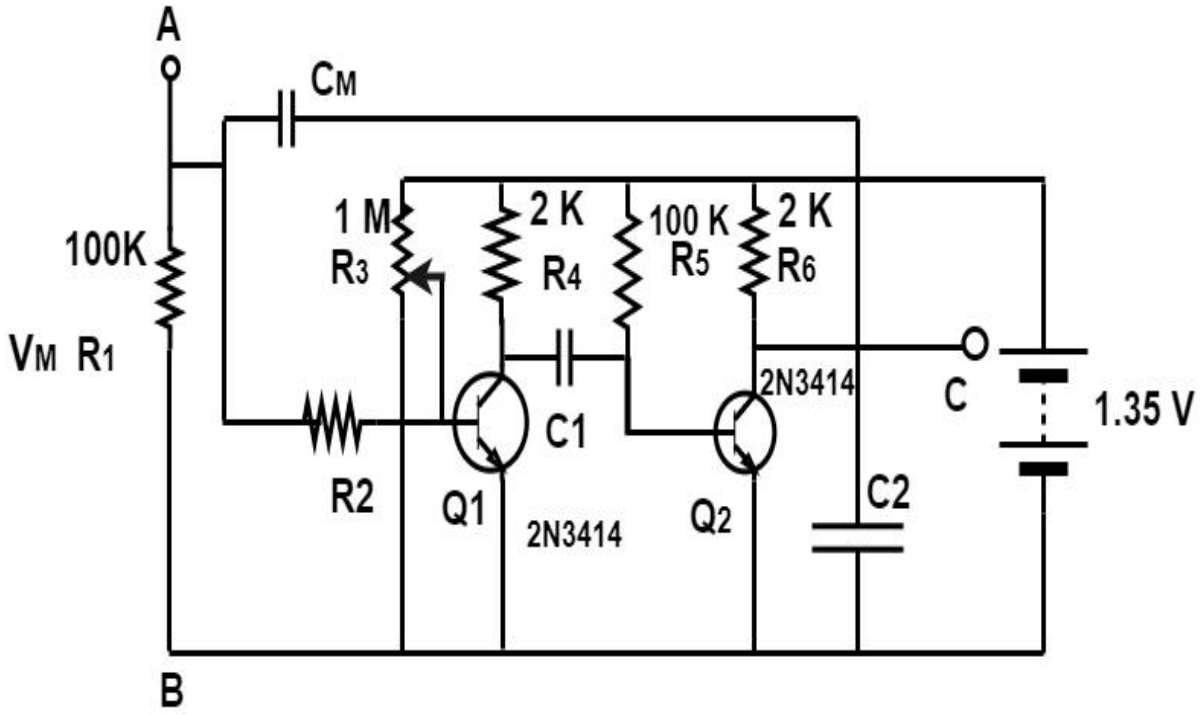


Fig.2.24: William H Brockman neuron model

2.5.5 Maeda Makino Model

Maeda and Makino (2000) had developed one electronic circuit using three transistors to develop Fitghugh Nagumo circuit. Fitghugh Nagumo model replaces fast activation process of sodium current by a simple fast depolarizing process [68]. Similarly, slow inactivation of sodium current and repolarization of potassium current is replaced by simple inactivation process. In this circuit, transistors are modeled to get the required repolarizing process and a neuron with bursting behavior is obtained. The circuit of Maeda and Makino representing the Fitghugh Nagumo model is shown below in Fig. 2.25. The V_M obtained is large due to realization of neuron impulse by electronic circuit, approximately of 5V. If the required amount of stimulus (constant current) is given to the circuit, it produces train of simulated action potential. The model can be made to oscillate without the current source by adding RL to the circuit. The resistor will act as inward current leak and is in the range of 25K to 250K.

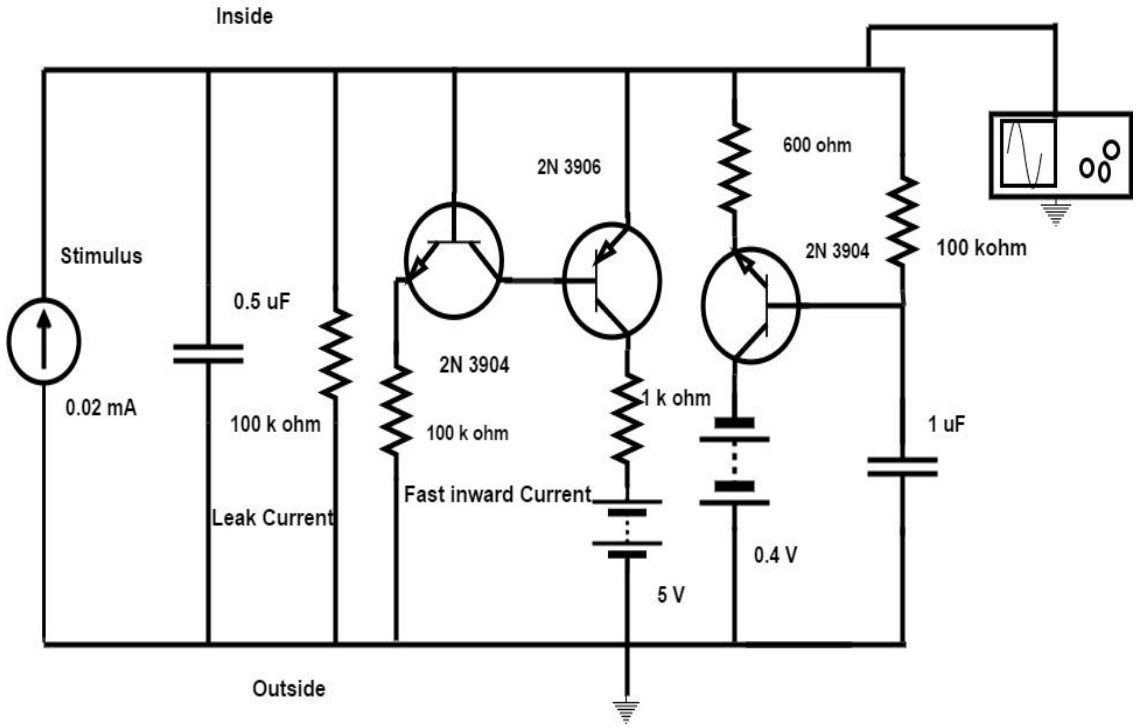


Fig. 2.25: Maeda Makino circuit representing Fitghugh Nagumo model

Robert B. Szlavik(2006) *et al*. had developed a neuron model with electronic devices such as OPAMP and they had simulated the circuit with the help of PSpice software [90]. The circuit developed by them is an improved electronic circuit of H-H model. Xiafung Hu *et al*, Jiu Yang *et al*. in 2017 had modeled a neuron using memristor for synaptic response and simulated in Pspice [102-103]. Massino Gattaro *et al*., Xoshifumi Sekine *et al*, Ying Xu *et al*, Xinyi Wu *et al* had also developed electronic neuron model to simulate different types of neuronal signals using PSpice [70-71,80,99-102].

The electronic neuron models developed so far have high complexity and do not explain neurotransmitter initiated conductances [6, 36, 65-66]. These drawbacks can be eroded by using biologically motivated field effect transistor (BIOFET).

2.6 Effect of sodium and potassium conductance on action potential and currents carried by sodium and potassium

Curtis and Cole (1942) had studied the effect of potassium ions and Hodgkin and Katz (1949) studied the effects of sodium using microelectrode in action potential of giant axon of squid. Ling and Gerald (1950) and Nastuk and Hodgkin (1950) studied the effects of potassium and sodium in muscle fibres of the frog [38, 41-45, 82].

Hodgkin-Huxley had studied the effect of sodium and potassium on myelinated nerve fibres of the frog in 1951 using Ringers solution. They had changed the concentration of sodium and potassium in Ringers solution to study the effects on action potential and resting potential. Solutions of higher concentration of potassium was made by replacing sodium chloride with potassium chloride and lowered by replacing potassium chloride with sodium chloride. Similarly, sodium concentration was lowered by replacing sodium chloride with choline chloride in the Ringers solution. Higher concentration of sodium was made by adding 1.65 of sodium chloride per litre of Ringer solution. Normal sodium concentration of Ringer solution was made by adding choline chloride. By adding and replacing the required concentration of ions, the effects of potassium and sodium was studied on action potential [41-45].

It was observed that lowering of potassium concentration causes an overshoot in action potential and resting potential. On the other hand, increasing potassium concentration results in decrease of action potential and resting potential [5].

When sodium concentration was decreased, it was observed that the overshoot of action potential decreases and resting potential increases. Increase of overshoot in action potential occurs when the sodium concentration was increased while resting potential decreases. Fig. 2.26 and Fig. 2.27 illustrate the effect of sodium and potassium on action potential and resting potential respectively. It shows the change in resting potential and action potential with sodium and potassium concentration. Change in action potential is calculated from the difference of actual action potential to change in action potential due to concentration change. Similarly the change in resting potential is calculated.

When sodium concentration was made zero, the inward current reduces but outward current increases causing a hump. If sodium concentration was 15-20% less, outward current slightly alters. The reverse happens when sea water was replaced. Sodium ion current reverses it sign when sodium concentration was reduced. This is due to potassium ion current which flows from inside. Sodium current rises rapidly during depolarization while potassium current rises slowly and takes the shape of S. Potassium current was prolong but sodium current drops rapidly. Fig. 2.28 shows effect on ionic current of sodium and potassium ions due to sodium and potassium conductance. The experiment to observe the effects on action potential and ionic current is not easy to perform; time consuming and highly trained persons are required. This can be made easy when effects can be observed in a simple electronic circuit by varying the conductance.

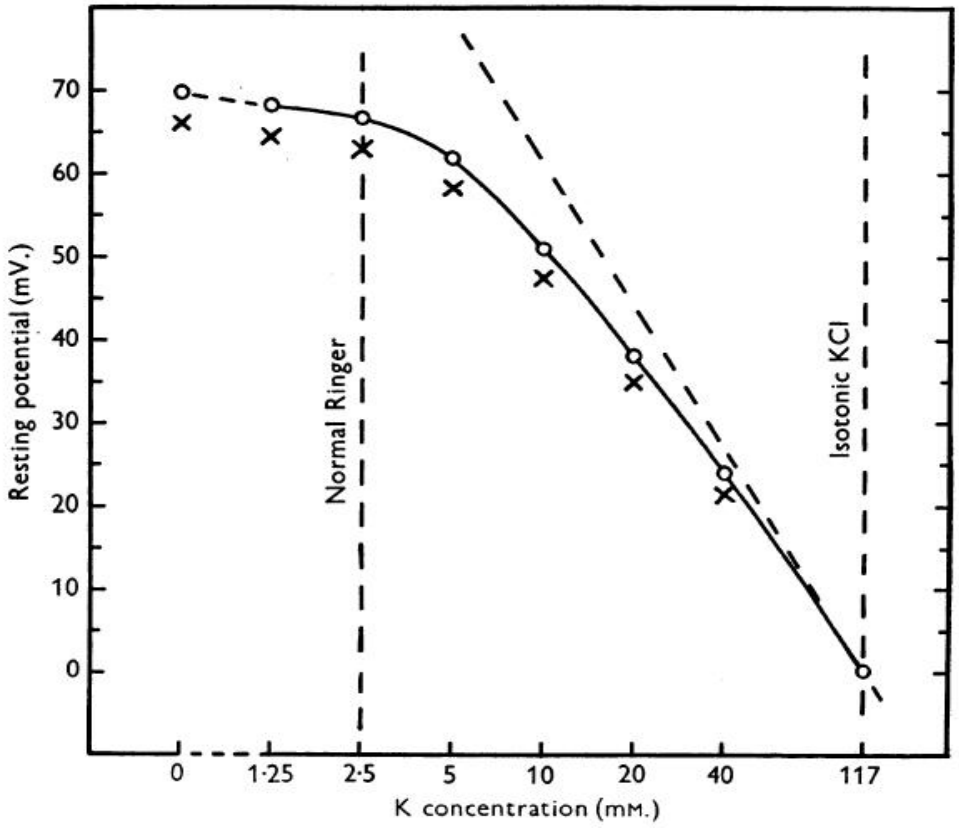


Fig.2.26: Change of resting potential with potassium concentration (logarithmic scale). (Huxley 1951)

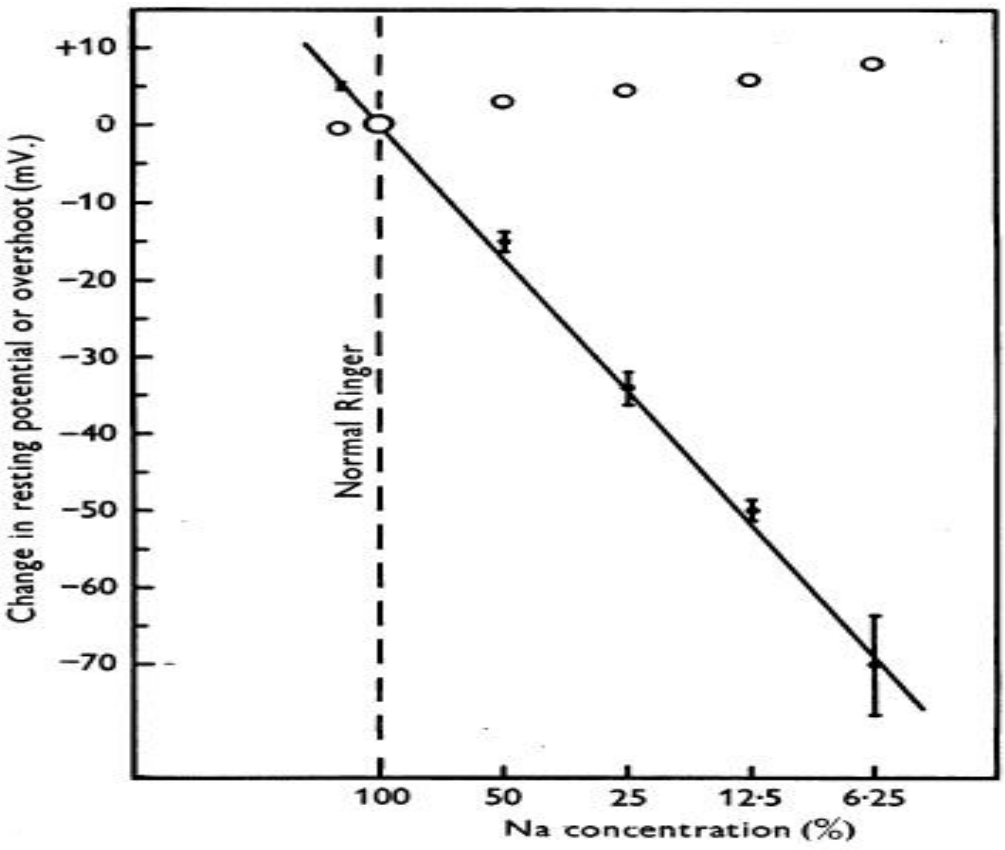


Fig.2.27: Change of resting potential with sodium concentration (logarithmic scale). Circles showing resting potential increase due to lowering of sodium concentration. Vertical lines showing action potential change with sodium concentration**.**

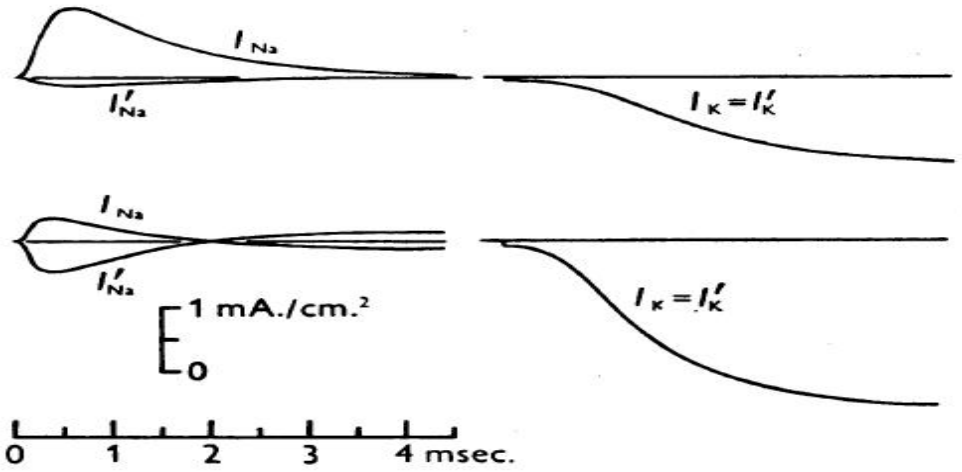


Fig.2.28: Effect of sodium and potassium concentration in ionic current of sodium and potassium .I_{Na} is the sodium current at sea water and I_{Na}' is the sodium at 10% sea water.I_K is the potassium current at sea water and I_K' is the potassium at 10% sea water in different depolarization.

2.7 An overview of parameter estimation and related work

Nowadays, data driven neuron models have hold an eminent position in studying the electrical activity of neuron [52, 92-93, 95-99]. There are various parameters associated with a neuron model and it is vital to know the values of the parameter for proper neuron modeling [15, 79, and 81,103,108-109]. Moreover, the parameters cannot be estimated with great precise for small currents and noise in voltage clamp data. These modern days estimation methods can simultaneously estimate all the parameters and is less time consuming [46-50, 53].Parameter estimation is an essential part of neuron modeling. By estimation of the parameters, one can validate a neuron model and parameters related to abnormality can be found. Neurocomputing is a significant area in neuron modeling. Below are some neuron models developed and the estimation methods used by them [18-24, 60, 63, 69, 72-73].

David Csercsik, Gabor Szederkenyi, Katalin M. Hangos and Imre Farkas(2008) had estimated the parameters of GnRH neurons which is responsible for proper functioning of endocrine system [21]. David *et al*. had developed a neuron model for GnRH neuron. GnRH neurons are the important element for reproductive neuroendocrine system. It plays an important role in the hormonal cycle. They had developed one mathematical neuron model similar to H-H conductance based model as a fitness function taking into account simple ordinary differential equation. A fitness function is a function which helps to find how close the solution is to achieve the set goal. This model can take up-to-date data for estimation purpose. The experimental data were taken from the Institute of Experimental Medicine of the Hugarian Academy of Sciences for reference. They had taken the conductance based model of Hodgkin-Huxley and used asynchronous parallel pattern search (APPS) procedure for estimation of updated values in GnRH neurons. APPS is a gradient free optimization method and convergence to the solution is fast. This work was a first of its kind to develop a GnRH neuron model. It can also handle linear and non-linear inequalities of constraints. The GnRh neuron model is constructed and the parameters related to the characteristic features of that particular neuron had been estimated. But the APPS method is a complex method and time consuming.

In 2011, Crotty and Sangey had observed that neuron model parameters can operate in broader parameter range [19] .They had investigated the optimized values of reverse potentials of sodium and potassium. Reversal potential of sodium and potassium has large effect on action potential and energy consumption on the neuron rates. They had taken H-H model to investigate whether the reverse potential of sodium and potassium maximizes or minimizes the energy consumption of action potential, maximum firing frequency, energy efficiency and velocity of action potential. They had observed that the velocity of action potential was maximized and the energy consumption of action potential and in rest was minimized for a certain value of E_K. The third optimization was done for the ratio of action potential and firing frequency which was maximized for a certain value of E_K.

In 2012, Dimitrios V. Vavoulis ,Volko A. Straub,John A. D. Aston, Jianfeng Feng had used Kitagawa's self organizing state space model on H-H conductance based data of simulated and experimental data for estimation of parameters. Vavoulis *et al.* had taken hidden dynamical systems formalism to estimate the parameters. This algorithm can predict correctly the parameters like maximum conductance, ionic currents, reverse potentials etc. by imposing pre-defined constraints on the model. This method even can work in noisy environments and can yield accurate results. When this method was combined with Covariance Matrix Adaptation Evolution strategy, there is an enormous reduction in variance of estimation of the parameters. The algorithm did not require a formulated cost function and it can be directly applied to compartmental model like H-H. Therefore, the method can be used in noisy environment for high inference problem [94]. They had estimated many parameters such as maximum conductances, velocity of ionic currents, reverse potentials, noise measurements and much other information regarding action potential. By combining self space algorithm and adaptive algorithm, there is a huge reduction in the parameter estimates complicacy. This method works in high noisy environment in biophysical model and it doesn't require any explicit formulation of cost function [94].Kitagawa's method is very complex in nature due to its hidden dynamical systems formalism to estimate the parameters.

L. Buhry (2012) [7- 11] had estimated parameters of conductance based model using different metaheuristic methods such as differential evolution, particle swarm optimization and simulated annealing. Lauhre Buhry had implemented these estimation

methods to Hodgkin -Huxley conductance based model for estimating parameters in fast spiking, regular spiking, and intrinsically bursting neurons and found an alternative method to voltage clamp method[7 -11]. Estimated parameters from differential evolution algorithm produce the optimized solutions and many features of neurons are observed. They had also developed one analog circuit which produces the spiking nature of a neuron. They had used differential evolution method to estimate the parameters taking H-H signal as a reference. This work was implemented in IC but it can be used in biological cell needing intracellular measurement. Software implemented estimation method is finding its place in neuroengineering since voltage clamp experiment is difficult to perform. The estimated parameters and their values are shown in Table 2.1. Fig.2.29 shows the estimated signal for action potential using H-H signal as reference. Fig. 2.30 shows estimation of sodium and potassium conductance. But these methods takes large number of iterations for estimation and is complex

Table 2.1: Theoretical and estimated parameters by the tuning system for analog integrated circuit developed by L.Buhry. (From L. Buhry)

Parameters	Sodium		Potassium		Leak	
	Theoretical	Estimated	Theoretical	Estimated	Theoretical	Estimated
g_{ion} ($\mu s/cm^2$)	44.00	34.65	10.00	9.17	0.150	0.151
g_{ion2} ($\mu s/cm^2$)					0.150	0.166
E_{equ}(mV)	50.00	45.14	-90.00	-117.0	-70.00	-91.00
τ_m(ms)	0.0700	0.0722	1.00	0.958		
V_{offset_m}(mV)	-34.42	-44.74	-29.08	37.80		
V_{pente_m}(mV)	6.47	6.99	7.854	7.483		
τ_h(ms)	0.462	0.3143				
V_{offset_h}(mV)	39.07	-50.79				
V_{pente_h}(mV)	3.932	4.44				

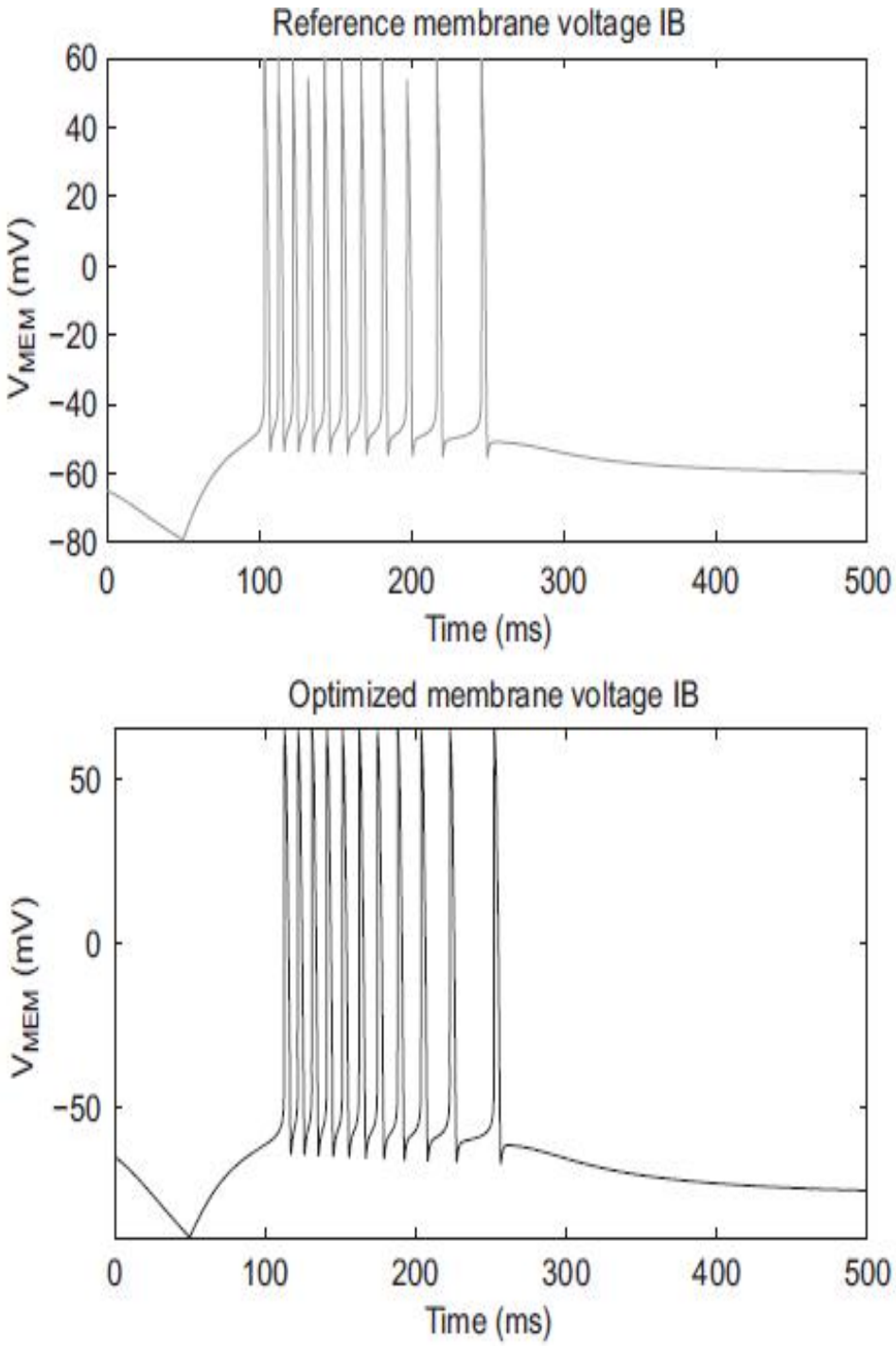


Fig.2.29: Reference signal taken from H-H model and the optimized signal for estimation of parameters in action potential by L. Buhry.

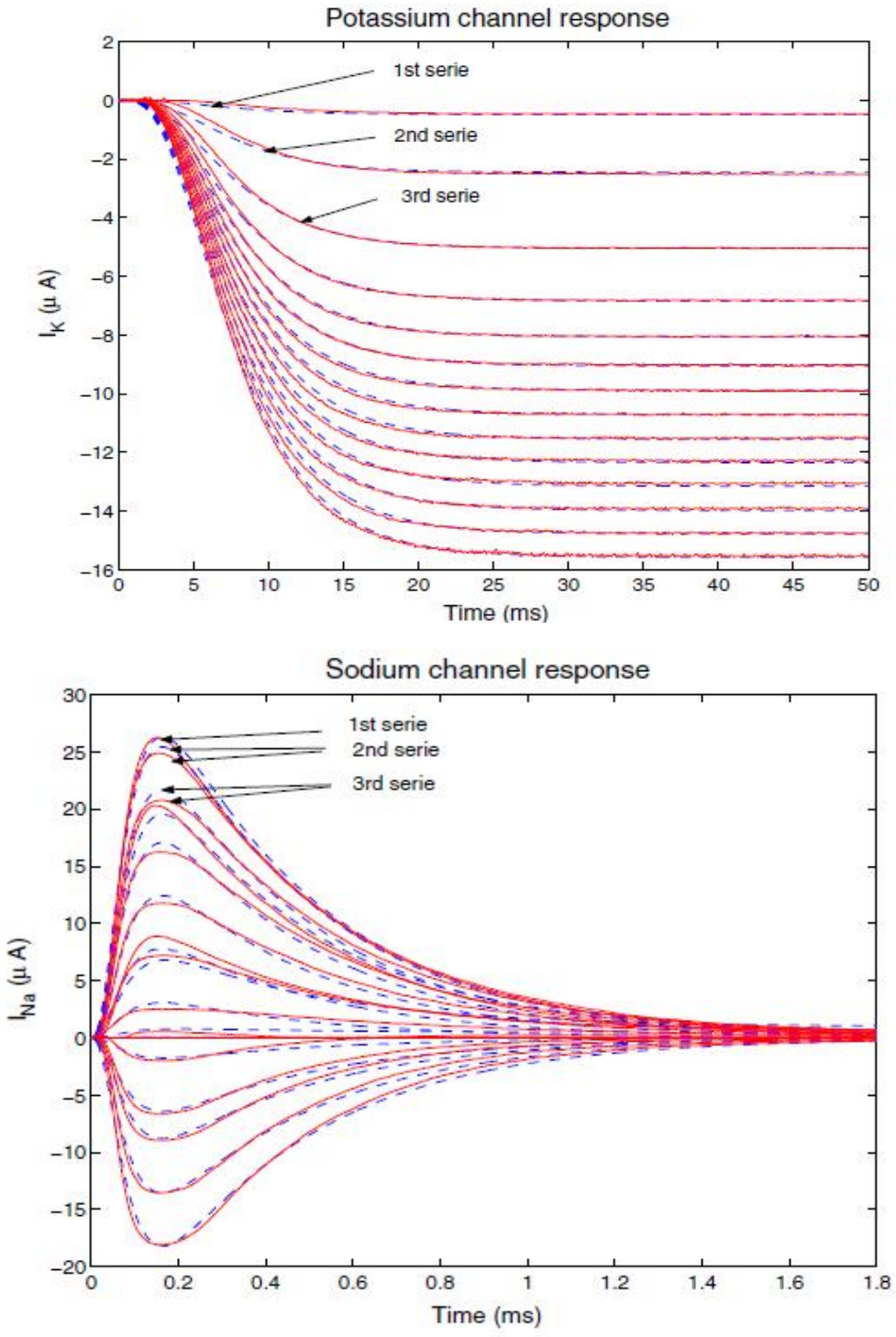


Fig.2.30: Estimation of sodium conductance and potassium conductance by Differential evolution method [From L. Buhry].

2.8 Nature inspired Estimation algorithms

Nature inspired metaheuristic algorithms are becoming powerful in modern optimization such as ant and bee algorithms, simulated annealing, cuckoo search, bat algorithm, flower algorithm, harmony search, Differential evolution (DE), Particle Swarm Optimization methods (PSO), Genetic Algorithm (GA) etc. Among biologically - derived algorithms, the metaheuristic algorithms such as Genetic algorithm (GA), particle swarm optimization (PSO) and Firefly Algorithm (FA) form hot research topics in the state–of-the-art algorithm development in optimization and other applications. These methods are simple, convergence to solution is more accurate and efficient [14,30,56,59,85-86].

2.8.1 Genetic Algorithm

Genetic algorithm is an optimization method which drives on the principle of natural selection and Darwin's principle of evolution. It repeatedly modifies the solution space by natural selection, mating, crossover and mutation process [29, 37].

2.8.2 Particle Swarm Optimization

PSO is another optimization method which search for a better solution on the base of measured quality [16,78]. This method is inspired from flocking of birds, insects in search of food and developed by James Kennedy & Russell Eberhart. Each solution (particle) will move over the search space according to their position and velocity. The particles will move towards the best position (local best) and the position gets updated by other particles in the surrounding searching in the search space. This process is repeated to get the best solution [57].

2.8.3 Firefly Algorithm

Firefly algorithm was proposed by Xin –She Yang. It was based on the principle of flashing of fireflies. The algorithm follows that the fireflies are unisex and are attracted by the flashing pattern of fireflies. The more is the brightness, the more is the attractiveness. However, the attractiveness decreases with distance. If there is no brighter firefly, the firefly will move randomly [27, 88,104-107].

2.9 An overview of Acetylcholine and its detection methods

Acetylcholine (Ach) is a neurotransmitter found in many organisms. This neurotransmitter helps to initiate action potential and its propagation. When acetylcholine is released in the synapse (neuron terminal), it initiates the opening of gates for the sodium and potassium ions for depolarization which leads to generation of action potential. Structure of the acetylcholine is shown in Fig.2.31. Its chemical formula is $CH_3COO\ (CH_2)_2N^+(CH_3)_3$.

Fig.2.31: Structure of Acetylcholine

Many industrial methods such as gas diffusion flow, injection analysis, chromatographic method etc. are used for detection purpose [31]. But these methods are complex, costly and require trained persons. The above said problems can be eradicated by using biosensors. Biosensors can detect biomolecules with great precise and it is cost effective. It has the property of high sensitivity, good selectivity, compact in size, good compatibility.

2.10 An overview on Enzyme modified field effect transistor (ENFET)

An ENFET is a bioelectronic device which is fabricated in combination of biomolecules with Ion sensitive Field Effect Transistor (ISFET) [26]. The biocatalytic reaction stimulated by enzyme in the ENFET changes the pH in the gate surface either

by consuming or generating protons. This changes the surface potential and which in turns changes the drain current. An ENFET can convert biological signal into an electric signal which is detectable by ISFET.

There are many ENFET developed so far. Berveld had developed ISFET which was used for measurements of ions in biological environment [77]. Janata *et al*. had developed penicillin sensitive ENFET layering co-cross-linked penicillinase-albumin. Its response time was 25 s and 2 months of lifetime [13]. In 1997, Jiminez *et al.* had developed ENFET to detect glucose, citric acid and ascorbic acid [54]. But these fabrications methods were quite time consuming. Kharitonov (2000) had made an ENFET using alumin as gate insulator using 3 aminopropyltriethoxysilane [58]. This device was affected by pH and has low sensitivity. In 2006, Temple Boyer *et al*. had developed ph-sensitive ENFET for creatinine detection [91]. Rafiq in 2013 had developed an ENFET using solution based technique but the device is not sensitive enough and fabrication technique is complex [1]. All the biosensors developed had less sensitivity, non-linearity , costly and complex technique of manufacture [1,13,54,77].

The drain current of ENFET depends on the interaction of biomolecules (enzyme) on the gate insulator and products of enzymatic reaction with the analytes. When biomolecules are immobilized in the surface, the biomolecules gets attached to particular sites and then reacts with the analytes to form a product which in turn changes the potential in the gate surface. This modulates the drain current proportional to the biomolecules concentration. The drain current increases when positive biomolecules gets binded to the insulating surface and decreases when negative biomolecules gets binded. Similarly for p type ENFET, drain current increases when negative biomolecules are added and decreases when positive molecules are added. Depending upon the FET principle, the biomolecules are chosen.

ENFET is analogous to ISFET. ISFET is basically a MOSFET whose metal electrode is replaced by an electrolyte solution under examination and a reference electrode. Only difference is that ENFET has an additional sensing membrane on the top of the gate insulator of the ISFET for immobilizing biomolecules.

The threshold voltage V_{TH} and the Drain current (I_D) of ENFET are given by equation (2.45) and (2.46) respectively [89]:

$$V_{TH(EN)} = (V_{\mathrm{Re}f} - \Psi_0 + \chi^{\mathrm{sol}} - \frac{\Phi_{Si}}{q} - \frac{Q_{OX} + Q_{SS} + Q_B}{C_{ox}} + 2\phi_f) \tag{2.45}$$

$$I_{DS,total} = C_{ox}\mu(\frac{W}{L})\{[V_{GS} - (V_{\mathrm{Re}f} - \Psi_0 + \chi^{\mathrm{sol}} - \frac{\Phi_{Si}}{q} - \frac{Q_{OX} + Q_{SS} + Q_B}{C_{ox}} + 2\phi_f)]V_{DS} + \frac{V_{DS}^2}{2}\} \tag{2.46}$$

Where V_{Ref} is the constant potential of the reference electrode, Ψ_0 is the surface potential which is a function of pH of the solution and χ^{sol} is the surface dipole potential of the solvent, ϕ_{si} is the work function of silicone, Q_{ox} is the oxide charge, Q_{ss} is the surface state charge, Q_B is the bulk charge, V_{GS} is the gate source voltage, V_{DS} is the drain source voltage, W is the width of the channel, L is the length of the channel, C_{OX} is the capacitance of gate oxide and μ is the mobility of electron.

2.11 An overview of Carbon nanotube (CNT) based ENFET (CNT-ENFET)

CNT-ENFET works in enhancement mode. Therefore, for working of the CNTFET, positive biomolecules are used. Electron transfer in CNT occurs in ballistic or diffusion process. Ballistic process occurs when the CNT's mean free path is longer than the length of the CNT through which electron travels, otherwise ballistic process occurs. CNTFET have only one type of carrier and there is no p or n substrate like in MOS FET. When positive voltage is applied, the electrons get accumulated beneath the oxide layer and n-channel is produced. If negative voltage is applied, the electrons are repelled and pushed back. Thus, the CNT- ENFET doesn't work. Similarly, p-type of CNTFET works. For making n-type CNTFET, potassium, polyethyleneimine (PEI) is doped. Generally PEI doped CNT is used since potassium dope CNT is unstable. Now, the drain current (I_D) of CNT-ENFET from the knowledge of the I_D of ENFET becomes [84, 89]:

$$I_{DS,total} = C_{ox}\mu(\frac{W}{L})\{[V_{GS} - (V_{\mathrm{Re}f} - \Psi_0 + \chi^{\mathrm{sol}} - \frac{\Phi_{CNT}}{q} - \frac{Q_{OX} + Q_{SS} + Q_B}{C_{ox}})]V_{DS} + \frac{V_{DS}^2}{2}\} \quad (2.47)$$

Where ϕ_{CNT} is the work function of CNT. Here $2\phi_f$ appeared in equation (2.47) is removed since surface inversion potential is not required in CNTFET due to the existence of n channel (doping of CNT by n-type dopants).

CNT-ENFET has got many advantages such as high mobility, high conductivity, less affected by temperature, pH, and negligible interference by other biomolecules etc. on the response of the device [2 -3].