

NILE UNIVERSITY

**The set of intelligence**  
**(Cardiovascular System)**

Physiology (BMD-103) Project Report

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## 1. Abstract

This experience examined the effect of contraction on the blood flow rate or action potential of the heart. Contraction of the heart means the flow of blood in the heart. When the SA node sends an electrical impulse, it triggers. that control blood flows in the heart. the blood transfer from one cell to another according to change in action potential membrane of the cell. In our report, we focus on the transition of ions through the membrane. that related to Hodgkin and Huxley model which is a mathematical model that describes how action potentials in neurons are initiated and propagated. Moreover, the aim of the computations detailed in this paper is to see if Hodgkin and Huxley's description of the properties of excitable membranes can also be used to characterize the long-lasting action and pace-maker potentials of the Purkinje fibers of the heart, with some adjustments. These fibers differ from squid nerve in that depolarization reduces the membrane's potassium permeability (Hodgkin & Huxley, 1939, 1945; Curtis & Cole, 1940, 1942). Part of this drop appears to be temporary during significant depolarizations, and the potassium permeability slowly increases during the passage of the depolarizing current. We conclude that our human SAN cell model can be a useful tool in the design of experiments and the development of drugs that aim to modulate heart rate.

## 2. Introduction

The Huxley model of muscular contraction, which was developed more than fifty years ago, is still the most widely utilized by researchers. This reflects both the model's simplicity and the difficulty of more complex models to duplicate its precision on basic experimental tests and to apply successfully to more than one extended test at the same time.

Hodgkin and Katz demonstrated in 1949 that the amplitude and rate of rise of the action potential of the squid nerve vary with extracellular sodium concentration, implying that the rising phase of the nerve impulse is caused by a large and specific increase in the membrane's permeability to sodium ions. The reversal of the membrane potential that had already been observed was easily explained by this concept, because the sodium equilibrium potential is generally opposite in sign to that of potassium. Separated the sodium and potassium components of the membrane current using the voltage-clamp technique, and constructed equations explaining how these currents change with membrane potential and time. They demonstrated that their equations, when paired with cable theory equations, could accurately duplicate many of the electrical features of the squid nerve, including the form and size of the action potential, impedance variations, conduction velocity, and ionic exchanges. Since then, the variety of phenomena to which they've been demonstrated to apply has grown significantly.

Cole, Antosiewicz, and Rabinowitz (1955), who were the first to place the equations on an electronic computer, validated the initial hand calculations. Using the experimental data acquired by Frankenhaeuser & Hodgkin, Huxley (1959) applied the equations to the influence of temperature on the propagating response and to the recurrent firing found in low calcium concentrations (1957). The hyperpolarizing responses obtained at high extracellular potassium concentrations can be described, at least qualitatively, by introducing the appropriate change in the potassium equilibrium potential. The prolonged action potentials produced by treating squid nerve with tetraethylammonium ions can be largely accounted for by greatly slowing the rise in potassium permeability.

The aim of the computations detailed in this paper is to see if Hodgkin and Huxley's description of the properties of excitable membranes can also be used to characterize the long-lasting action and pace-maker potentials of the Purkinje fibers of the heart, with some adjustments. These fibers differ from squid nerve in that depolarization reduces the membrane's potassium permeability. Part of this drop appears to be temporary during significant depolarizations, and the potassium permeability slowly increases during the passage of the depolarizing current.

The cardiac conduction system is collection of nodes and specialized cells that initiate and co-ordinate contraction of the heart muscle such as Sinoatrial node, Atrioventricular node, and

Purkinje fibers. The SA node sends an electrical impulse through muscle cells in the right and left atria, the atria contract and then it is pumping blood into the left and right ventricles. The atrioventricular node is primarily an electrical gatekeeper between the atria and ventricles and introduces a delay between atrial and ventricular excitation, allowing for efficient ventricular filling. The Purkinje fibers carry the contraction impulse to the myocardium of the ventricle. This generates force to eject blood out of the heart, which is then released into the lungs.

There are specialized muscle cells called cardio myocytes and the contraction of these cells is initiated by electrical impulses; known as action potentials. It occurs in the depolarized cells when  $Na^+$ ,  $Ca^{2+}$  channels open and  $K^+$  is closed. The action potential in cardio myocytes is composed of 5 phases (0-4): Phase 4: The resting phase, Phase 0: Depolarization, Phase 1: Early repolarization, Phase 2: The plateau phase, and Phase 3: Repolarization. The action potential in typical cardio myocytes begins and ends with phase 4 (the resting membrane), and it will differ in all the 5 phases as the following: the resting phase which known as (Phase 4), The resting membrane potential is at around -90 mV. At this phase, the concentration of  $Na^+$ ,  $Ca^{2+}$ , and  $Cl^-$  ions on the outside is higher than inside the cell. On the other hand, concentration of  $K^+$  is higher than on the inside. Then the depolarization occurs in (Phase 0) When the stimulus reaches the threshold value,  $Na^+$  voltage gated channels on the cell membrane open rapidly. The movement of  $Na^+$  ions into the cell causes depolarization and about -40mV, L-type  $Ca^{2+}$  channels will be opened. After that there is an early repolarization in the (Phase 1) When the voltage reaches to +30mv, the  $Na^+$  channels will be closed quickly. The  $K^+$  voltage gated channels open slowly. Also, the inside become less positive because of the movement of positive ions out of the cell. Then The plateau phase (Phase 2) When the voltage difference is approximately 0 mV, the influx of  $Ca^{2+}$  will be equal to efflux of  $K^+$ . The last event is the repolarization which occurs in (Phase 3) since  $Ca^{2+}$  voltage gated channels is closed, the efflux of  $K^+$  exceeds the influx of  $Ca^{2+}$ .  $K^+$  voltage gated channels open and make the membrane potential to return back to the normal.

### 3. Methods

Our study analyzed contraction of the heart using action potential. We used Hodgkin and Huxley paper to know about action potential which occurs in cardiac myocytes, explaining the voltage clamp, and analyzing of potassium and sodium currents. We obtained that voltage sensitive gates in membrane that will open and close in response to membrane voltage and gives us an additional set of differential equations to model (M, N, H) to represent the probability that those gates open or close and the probability of gates being opened is determined by forward and backward rate constant of alpha and beta. To clarify this, we utilized the software “OpenCOR” (cellml file) to examine when K<sup>+</sup> channels open and when Na, Ca channels open, and we got some main results in the results section.

**Materials:** CellML file

**Tools:** DOI & OpenCOR

**Methods:** We used DOI to get the Mathematical model, then we collect the data from the equations.

After that we use open COR and start to enter the data we collect from the equations and the results appear the action potential and which channel opens during polarization & repolarization

#### 3.1 Hodgkin and Huxley model

1. Action potentials occur in cardiac myocytes.
2. Voltage clamp.
3. Analyzing of potassium current using voltage clamp.
4. Analyzing of sodium current.
5. The collapse of sodium current.

##### 3.1.1. Action potential

There are specialized muscle cells called cardio myocytes and the contraction of these cells is initiated by electrical impulses; known as action potentials. It occurs in the depolarized cells when  $Na^+$ ,  $Ca^{2+}$  channels open and  $K^+$  is closed. The action potential in cardio myocytes is composed of 5 phases (0-4): Phase 4: The resting phase, Phase 0: Depolarization, Phase 1: Early repolarization, Phase 2: The plateau phase, and Phase 3: Repolarization.

##### 3.1.2. Voltage clamp in Hodgkin and Huxley model

It is an experimental method used by electrophysiologists to measure the ion currents through the membranes of excitable cells, such as neurons, while holding the membrane voltage at a set level. Hodgkin and Huxley showed that the membrane's voltage is controlled by injecting

different amounts of current. In Hodgkin and Huxley model the potassium current was analyzed with equations and graphs.

### 3.1.3. Potassium current in Hodgkin and Huxley model

scientists Hodgkin and Huxley were able to examine using voltage clamp how the current changed over time at different fixed voltages and they found the potassium currents vary over time as the membrane voltage is stepped to different potentials which make them able to find voltage at which no current flowed through the membrane and measure the value of  $E_{K+}$ . They observed after stepping the membrane potential to increasingly positive potentials that the potassium current increased, and it grew larger with increasingly positive voltage steps. After measuring many of potassium currents as a function of different voltages, Hodgkin and Huxley finally, could then estimate the potassium conductance using the equation is given by:

$$g_{K+} = \frac{I_{K+}}{V_m - E_{K+}}$$

where is  $g_K$  is the potassium conductance,  $I_{K+}$  is potassium current.

- The absolute values of the conductance have been adjusted to give a resting conductance (slope conductance at  $E_m = -90$  mV) of about  $1 \text{ mmho/cm}^2$ . The potassium equilibrium potential will be set at  $-100$  mV so that the total potassium current is given by:  $I_K = (g_{k1} + g_{k2})(E_m + 100)$ .

### 3.1.4. Sodium current in Hodgkin and Huxley model

To evaluate the sodium currents, suppose that the capacitive and leak currents have been removed and that the potassium current has been blocked. Negative values are used to indicate the bottom voltage step from resting potential (approximately  $-61$  mV) to  $0$  mV, which causes a significant downward deflection inward current measured under voltage clamp. As a result, the downward deflection indicates a depolarizing negative inward current. The voltage-dependent sodium channels will open and enable sodium ions to flow down their concentration gradient into the cell, delivering positive charges into the cell and depolarizing the neuron if the membrane is stepped to a depolarizing potential. Unlike the potassium current, the sodium current eventually collapses to zero. This means that the channel is inactive.

It is concluded from the paper that when The results of the middle voltage step to  $+50$  mV, There is a small inward current, but the overall current is very close to zero. because equilibrium potential for sodium is approximately  $+55$  mV.



Another conclusion, the results of largest voltage step, to +100 mV, The membrane has been stepped beyond the sodium ion equilibrium potential; as a result, the electrical gradient now strongly opposes and exceeds the sodium concentration gradient, causing the net flow of sodium ions to shift outward. As a result, the sodium current switches from negative to positive. This is also evident that driving force, changes sign.

Since we know the current and the voltage, we can solve for the sodium conductance using the equation

$$g_{\text{Na}^+} = \frac{I_{\text{Na}^+}}{(V_m - E_{\text{Na}^+})}$$

Hodgkin and Huxley found that the rising phase could be fit using a gate that rapidly opened in response to depolarizing voltage, but that three such gates needed to become conducting for the ion channel to open and conduct current. They named this gate *m*, and the need that three gates open for current to flow meant that the chances of this happening were slim to none.

$$m \times m \times m = m^3$$

### 3.1.5. The collapse of the sodium conductance

To describe this portion of the sodium conductance, Hodgkin and Huxley first stepped the membrane to a depolarized potential (e.g., 0 mv), then stepped the membrane to the same depolarized potential after varying durations of time.

- The current was substantially reduced after only 10 minutes of waiting.
- They increased the current in response to the second voltage pulse after waiting 20 minutes, but it was still decreased.
- The current in reaction to the second voltage pulse was virtually as large as it had been when they waited 40 minutes.

They were able to calculate the rate at which the inactivation gate, dubbed *h*, altered in response to voltage using these data.

Thus, their data suggested that the best fit for the sodium conductance would be based on the probability of three *m* gates being open, and the *h* gate also being opened, or  $m^3h$ .

## 4. Results

Our study analyzed the improvement of what channels opens during polarization and depolarization. We used software “OpenCOR” (cellml file) to explain that, and to see when  $K^+$  channels open and when  $Na^+$  &  $Ca^{2+}$  channels open, so we found that when depolarization occur the curve sloping up from resting point  $-90\text{mV}$  until it reaches  $+ve$  number. we found that Alpha and Beta have a great effect on potassium and sodium channels, and they are inversely proportional.

According to Richard E. klabunde “Many cells in the body have the ability to undergo a transient depolarization and repolarization that is either triggered by external mechanisms (e.g., motor nerve stimulation of skeletal muscle or cell-to-cell depolarization in the heart) or by intracellular, spontaneous mechanisms ”2021.

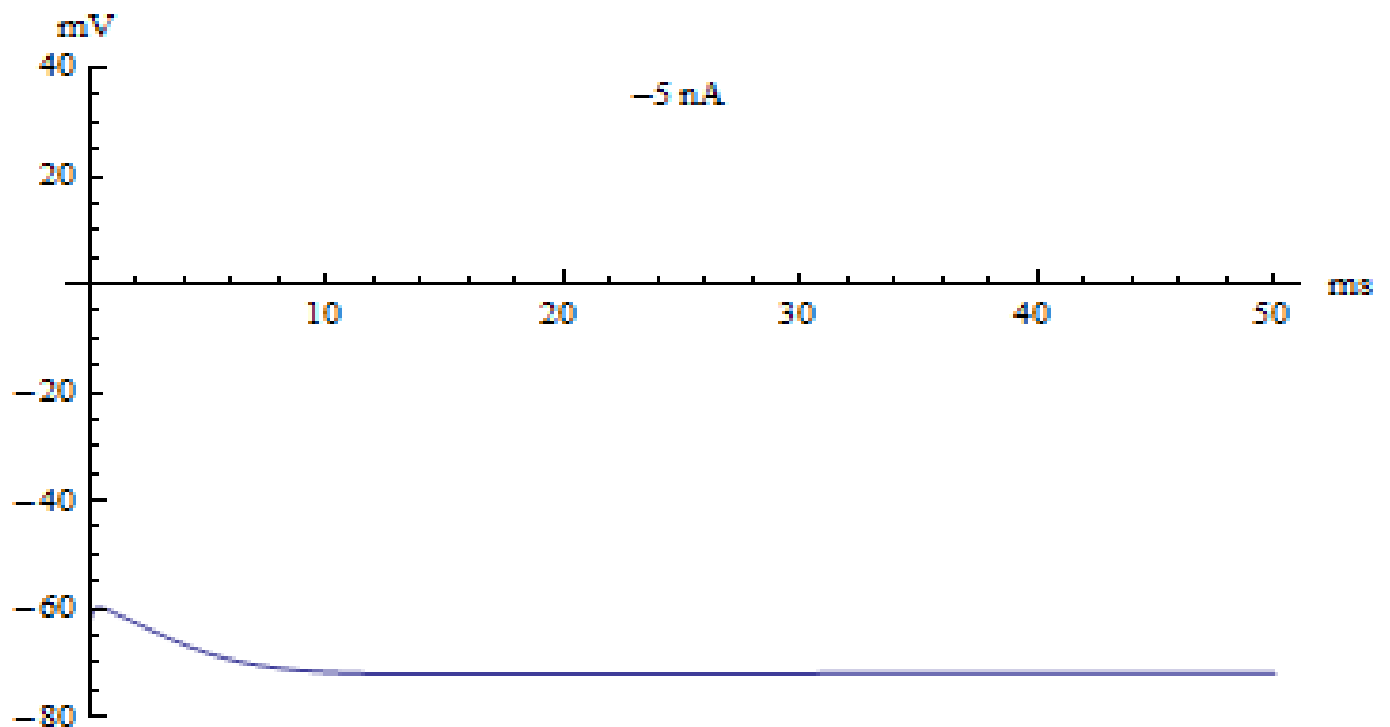


Figure 1: The voltage  $v(t)$  (in millivolts) of the Hodgkin–Huxley model

The voltage  $v(t)$  (in millivolts) of the Hodgkin–Huxley model, graphed over 50 milliseconds. The injected current varies from  $-5$  nanoamps to  $12$  nanoamps. The graph passes through three stages: an equilibrium stage, a single-spike stage, and a limit cycle stage.

## 1. The sodium and potassium conductance.

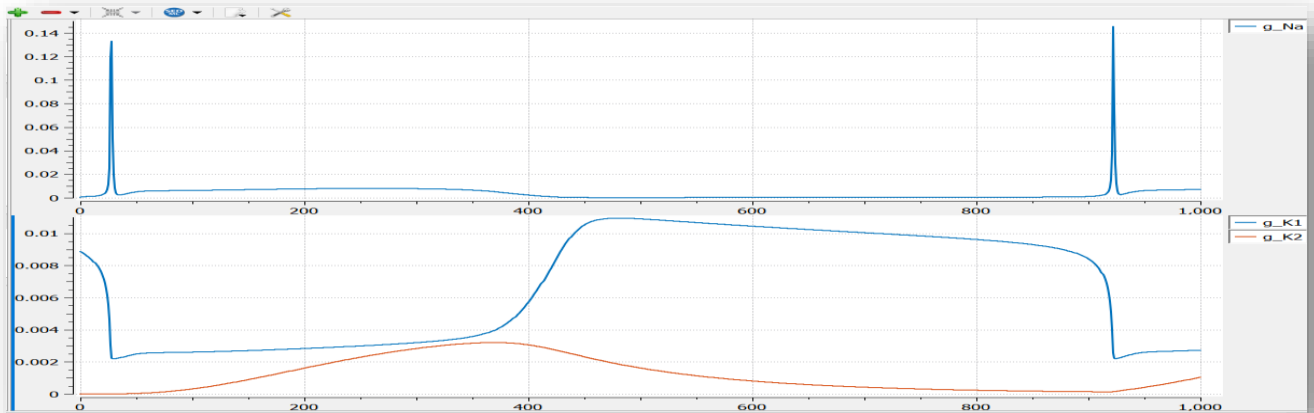


Figure 2:  $G_{K1}$  Potassium conductance &  $G_{Na}$  Sodium conductance

- Equation:

Equation 1

$$g_{Na} = I_{Na} / (E_m - E_{Na}), \quad (1)$$

$$g_K = I_K / (E_m - E_K), \quad (2)$$

• $g_{Na}, g_K$	• They are the sodium and potassium conductances respectively in mmho/cm <sup>2</sup> .
• $I_{Na}$ and $I_K$	• They are the ionic currents in $\mu A/cm^2$
• $E_{Na}$ and $E_K$	• They are the equilibrium potentials in mV
• $E_m$	• It is the membrane potential in mV expressed as the inside potential minus the outside potential.

- **Explanation:**

Hodgkin & Huxley (1952a) showed that for squid nerve in sea water the permeability of the membrane to Na and K ions is best described in terms of the contributions which these ions make to the membrane conductance. The individual ionic conductance is defined by these equations 1.

- A method for turning a membrane potential control system on and off in less than 10  $\mu\text{sec}$  is described. This method was used to record membrane currents in perfused giant axons from *Dosidicus gigas* and *Loligo forbesi* after turning on the voltage clamp system at various times during a membrane action potential.
- The membrane current measured just after the capacity charging transient was found to have an almost linear relation to the controlled membrane potential.
- The total membrane conductance taken from these current—voltage curves was found to have a time course during the action potential similar to that found by Cole & Curtis (1939).
- The instantaneous current voltage curves were linear enough to make it possible to obtain a good estimate of the individual sodium and potassium channel conductances, either algebraically or by clamping to the sodium, or potassium, reversal potentials. Good general agreement was obtained with the predictions of the Hodgkin—Huxley equations.
- We consider these results to constitute the first direct experimental demonstration of the conductance changes to sodium and potassium during the course of an action potential.

## 2. sodium gate $h(t)$ , $m(t)$

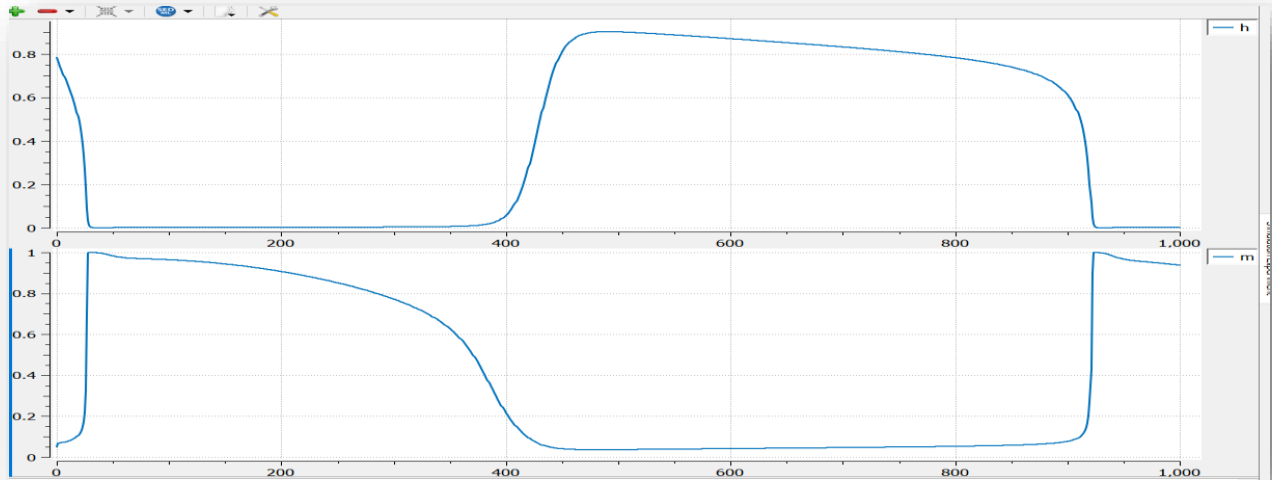


Figure 3:  $h$  response to time,  $m$ (sodium) response to time

- **Equation:**

Equation 2

$$g_{\text{Na}} = m^3 h \bar{g}_{\text{Na}},$$

Equation 3

$$\begin{aligned} \frac{dm}{dt} &= \alpha_m(V_m)(1 - m) - \beta_m(V_m)m \\ \frac{dh}{dt} &= \alpha_h(V_m)(1 - h) - \beta_h(V_m)h \end{aligned}$$

$g_{\text{Na}}$	a constant and $m$ and $h$ obey the equations:
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H and M	They are sodium gate ,where H in activation gate inside membrane.
$\alpha_m, \beta_m, \alpha_h$ and $\beta_h$	are functions of $E_m$ .

- **Explanation:**

Sodium and potassium in particular have unique voltage sensitive gates in membrane that will open and close in response to membrane voltage, and gives us an additional sets of differential equations to model (M,N,H) to represent the represent the probability that those gate open or close. Using a series of voltage clamp experiments and by varying extracellular sodium and potassium concentrations, Hodgkin and Huxley developed a model in which the properties of an excitable cell are described by a set of four ordinary differential equations.[1] Together with the equation for the total current mentioned above, these are. Moreover, Hodgkin & Huxley described this behavior by supposing that  $g_{Na}$  is determined by two variables,  $m$  and  $h$ , which vary with the membrane potential in opposite directions and with different time constants. The probability of gates being opened is determined by forward and backward rate constant of  $\alpha$  and  $\beta$ .

### 3. $\alpha$ and $\beta$ against $m$

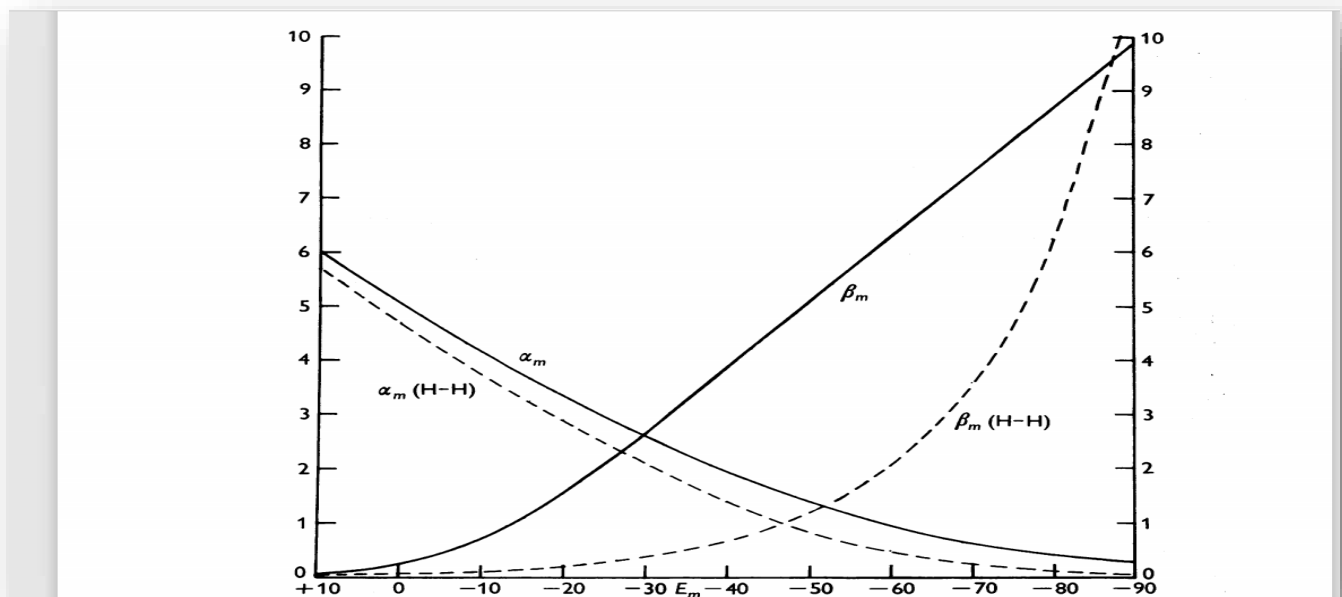


Figure 4:  $\alpha$  and  $\beta$  against  $m$

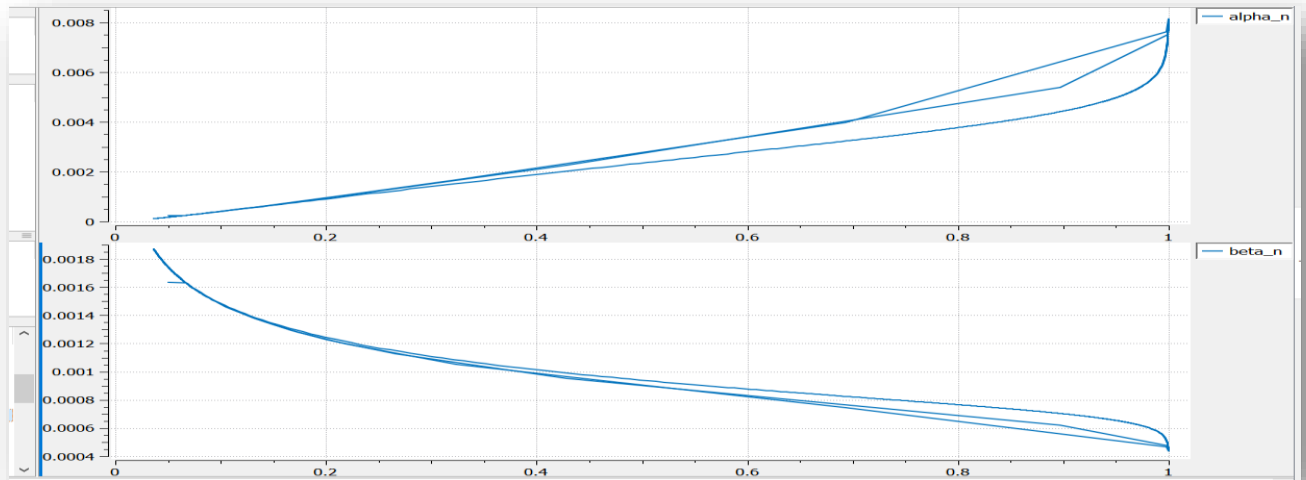


Figure 5: Alpha in response to m & Beta in response to m

- Equation:

Equation 4

$$\alpha_m = \frac{0.1(V + 25)}{e^{\left(\frac{V+25}{10}\right)} - 1}$$

$$\beta_m = 4 e^{\left(\frac{V}{18}\right)}$$

v	Potential different.
M	sodium gate
$\alpha_m, \beta_m,$	are functions of Em. The probability of gates being opened.

- Explanation:

Comparison of modified functions for am and Pm with those of Hodgkin & Huxley. Continuous curves: modified functions given by equation4. Interrupted curves: Hodgkin & Huxley's m functions after adjustment along voltage axis by same amount as for h



equations. These curves are given by equation4. Note that  $P_m$  changes less rapidly with  $E_m$  in modified function than in original Hodgkin-Huxley function. Alpha and beta related to  $(m)$  are rate constants for the  $i$ -th ion channel, which depend on voltage but not time. The probability of gates being opened is determined by forward and backward rate constant of alpha and beta.

#### 4. Alpha and beta related to $h$ :

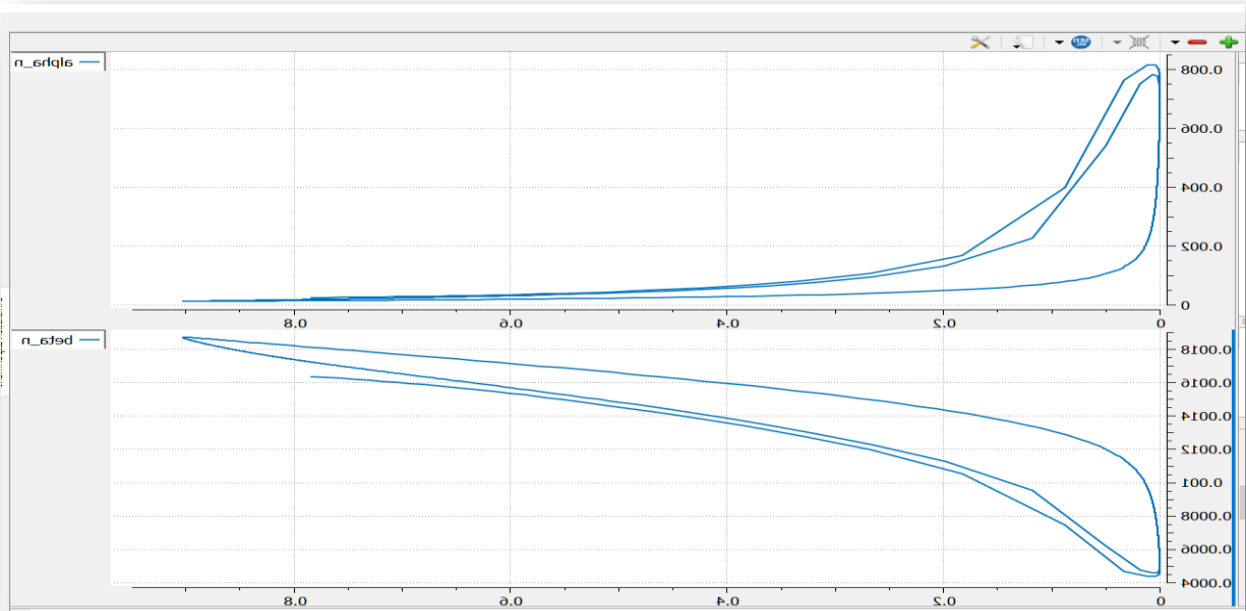


Figure 6: Alpha related to  $H$  & Beta related to  $H$

#### • Equation:

Equation 5

$$\alpha_h = 0.17 \exp [(-E_m - 90)/20],$$

$$\beta_h = \left[ \exp \left( \frac{-E_m - 42}{10} \right) + 1 \right]^{-1}.$$

- **Explanation:**

The Hodgkin-Huxley equations which describe the opening and closing of ion channels with deterministic equations for the variables  $m$ ,  $h$ , and  $n$ , correspond to the current density through a hypothetical, extremely large patch of membrane containing an infinite number of channels and the dependence of  $h$  on  $E_m$  describes the relation between the initial membrane potential and the maximum sodium current which may be produced by depolarization of the membrane. Using a modification of the voltage-clamp technique and using the maximum rate of depolarization as a measure of the sodium current Weidman (1955).

1-equation:

$$V_{eq} = V_{in} - V_{out} = -\frac{RT}{ZF} \ln \frac{[C]_{in}}{[C]_{out}}$$

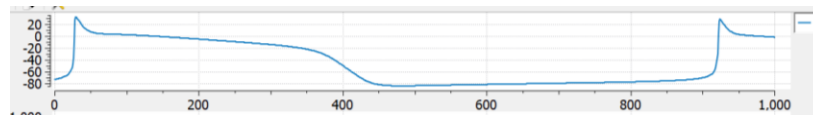


Figure 7: v in response to time

Explanation:

the graph represents the action potential from the resting point -80mv till it reach +20mv and it start to sloping again to depolarization by increase the time.

$$I = C_m \frac{dV_m}{dt} + g_K(V_m - V_K) + g_{Na}(V_m - V_{Na}) + g_l(V_m - V_l)$$

6.

*the change in ions release N, M, H, so n for potassium & m & h for calcium this change occurred due to the ions that enter and ions that get out from the cell*

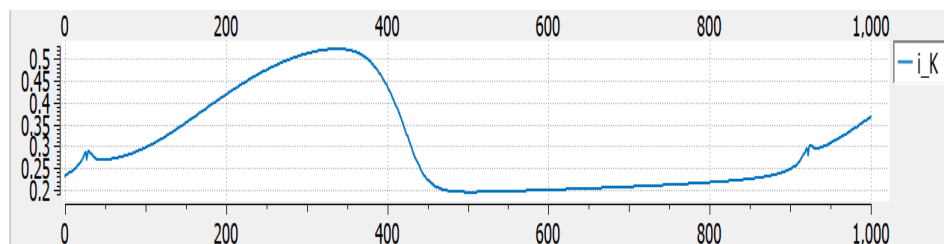


Figure 8: Potassium current in response to time

explanation:

in this graph (i\_k) it starts repolarization from 0.2 and starts increase 0.28 by increase the time bit by bit the graph sloping so i\_k is direct porportional by time(t) until it reach 0.5 then it starts to sloping down until it reach 0.5 the bottom and starts increase again.

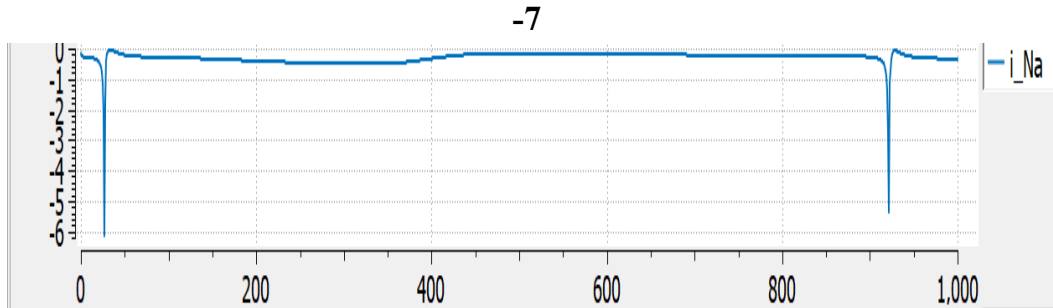


Figure 9: Sodium current in response to time

explanation:

this graph( $i_{Na}$ ) channels open & depolarized. Shows that it starts from 0 and starts sloping down under 1 until it reaches -ve numbers, so it reaches -6 then starts sloping up again to 0 and it still rest

**8.  $n$  for potassium the  $n$  against time in this equation  $k$  channel close in hyperpolarization so the Na& Ca channels open in this step**

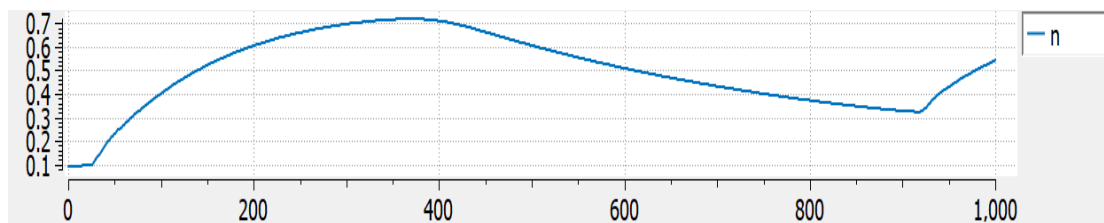


Figure 10: N (potassium) in response to time

Explanation:

this graph shows that it starts from resting point and start sloping up by time until it depolarize then starts to hyperpolarization

**9- equation:**

$$\alpha_n(V_m) = \frac{0.01(10 + V_m)}{\exp\left(\frac{10 + V_m}{10}\right) - 1}$$

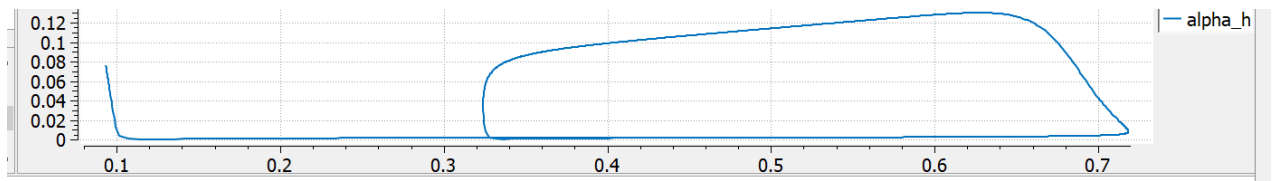


Figure 11: Alpha in response to n (potassium)

Explanation:

In this graph alpha n by time it starts sloping down until it reach a resting point 0, then by increasing the time it starts sloping up and turns back again and sloping down till it reach the resting point again.

**10- equation:**

$$\beta_n(V_m) = 0.125 \exp\left(\frac{V_m}{80}\right)$$

Explanation:

this graph show us how beta\_n helps in sloping down slowly untill the ventricles fills then it starts sloping up again and still rest till it reach 0.34 then starts in sloping down again till it reach the resting point.

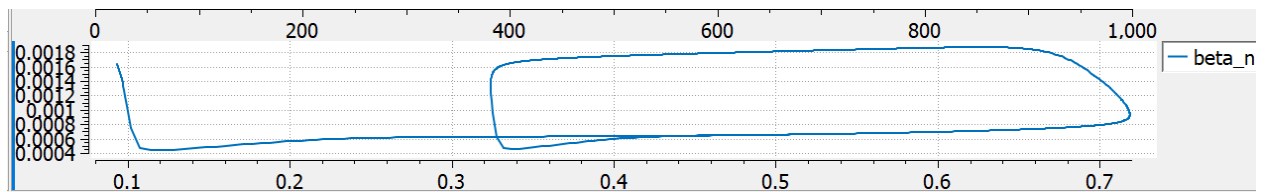


Figure 12: Beta in response to n (potassium)

## 11-Equation:

$$\alpha_h(V_m) = 0.07 \exp\left(\frac{V_m}{20}\right)$$

Explanation:

channel is open: the K<sup>+</sup> channel has four independent components, all of which are identical. The probability that the sodium activation gate is open is m<sup>3</sup> and the probability that the sodium inactivation gate is open is h

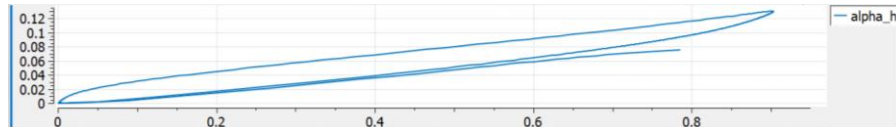


Figure 13: Alpha in response to h

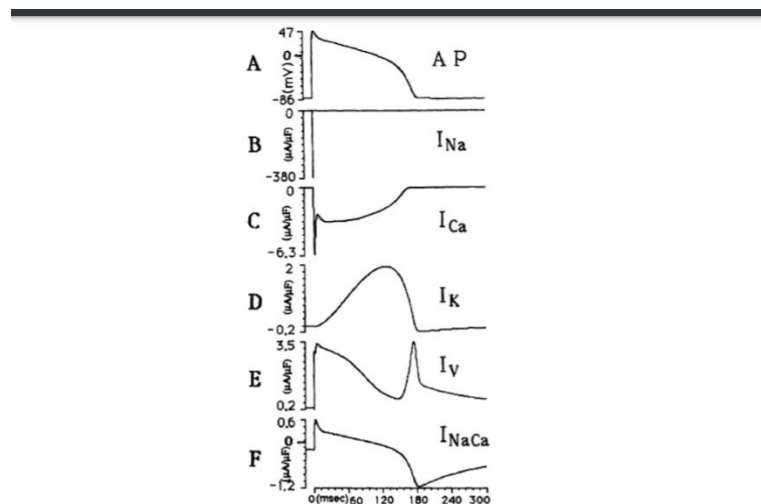


Figure 14: Shape of the h, o4Em relation

## Explanation:

This procedure leaves the shape of the h, o4Em relation unaltered. In fact, Weidmann's curve for Purkinje fibers is slightly steeper than that for squid nerve, but, as he has pointed out, it is very likely that this is only due to differences in experimental technique. In the voltage-clamp technique used by Weidmann, when the sodium conductance is greatly increased on depolarization of the membrane it is not possible to retain control of the membrane potential owing to the limitation on

the amount of current which may be passed through a micro-electrode.

## 5. Discussion

The purpose of this study was to discover the contraction of heart using the action potential by Hodgkin and Huxley. Firstly, aggregation information about the voltage clamp and analyzing potassium current using it. Secondly, analyzing the sodium current. It was observed the potassium and sodium currents and we conducted results for them by our selves. This section includes the main comparison between the methods we discussed and results we did.

- **Potassium current using voltage clamp:**

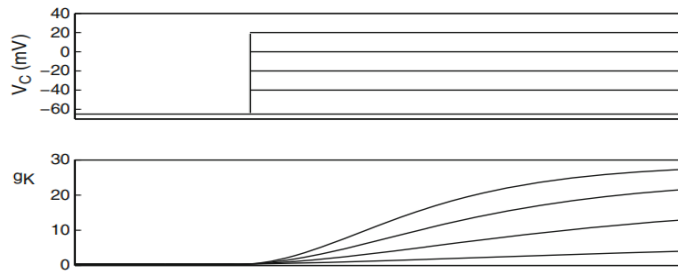


Figure 15: Potassium currents in response to four different voltages

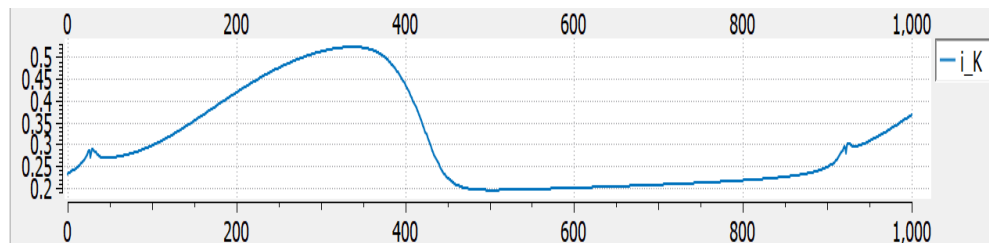


Figure 16: potassium currents in response to time

The potassium current ( $i_K$ ) in figure one from (The Hodgkin–Huxley Equations) vary over time as the membrane voltage is stepped to different potentials from the resting membrane ( $-60$ ) to  $-40$  mV,  $-20$  mV,  $0$  mV, and to  $20$  mV. The potassium current that results by software Open COR which appear in figure 2 starts repolarization from  $0.2$  and starts increase  $0.28$  by increase the time bit by bit. The graph sloping so  $i_K$  is directly proportional to time ( $t$ ) until it reaches  $0.5$  then it starts to slope down until it reaches  $0.5$  the bottom and starts increase again.

- **Sodium current:**

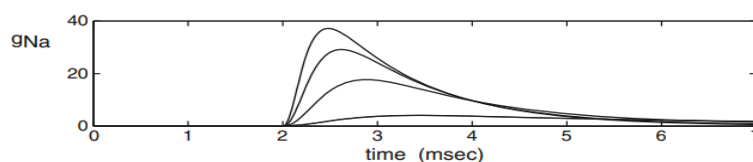


Figure 17: Sodium currents in response to depolarizing voltage steps



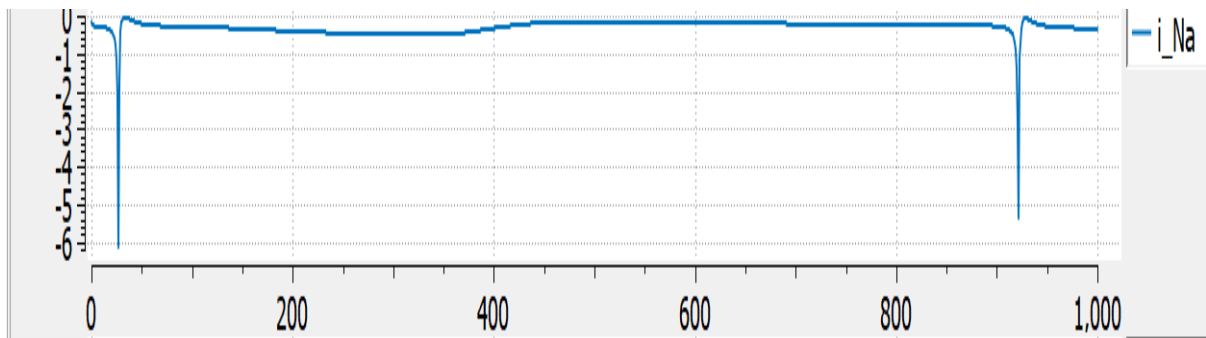


Figure 18: Sodium currents in response to time

In sodium figure 3 from (The Hodgkin–Huxley Equations), Negative values are used to indicate the bottom voltage step from resting potential (approximately -61 mV) to 0 mV, which causes a significant downward deflection inward current measured under voltage clamp. As a result, the downward deflection indicates a depolarizing negative inward current. The voltage-dependent sodium channels will open and enable sodium ions to flow down their concentration gradient into the cell, delivering positive charges into the cell and depolarizing the neuron if the membrane is stepped to a depolarizing potential. Unlike the potassium current, the sodium current eventually collapses to zero. This means that the channel is inactive. The sodium current that results by software Open COR which appear ( $i_{Na}$ ) starts from 0 and starts sloping down under 1 until it reaches (-ve) numbers, so it reaches -6 then starts sloping up again to 0 and it still rest.

## **6. Conclusion**

To sum up, this paper replicates the work of Hodgkin and Huxley model to characterize the long-lasting action and pace-maker potentials of the Purkinje fibers of the heart, with some adjustments. It describes the computations of the electrical Conduction in the Heart coordinates contraction. Also, it talks about the cardiac conduction system, and how the electrical signal travels from the SA node (the pacemaker of the heart) through internodal pathways to the AV node. Also, how Purkinje fibers play a major role in electrical conduction and propagation of impulse to the ventricular muscle, and that it allows the heart's conduction system to create synchronized contractions of its ventricles and are essential for maintaining a consistent heart rhythm. In addition, the action potential and the four phases, from phase 4 (resting phase) to phase 3 (repolarization). Moreover, the potassium and sodium current in the Hodgkin and Huxley model along with the results of the mathematical model.

## 7. References

<https://www.youtube.com/watch?v=70gXgnssdgE>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1396078/>

[file:///C:/Users/Lenovo/AppData/Local/Temp/Rar\\$EXa7752.38361/486-74dd66754e25/hSAN\\_FFWS\\_Documentation.html](file:///C:/Users/Lenovo/AppData/Local/Temp/Rar$EXa7752.38361/486-74dd66754e25/hSAN_FFWS_Documentation.html)

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1359535/pdf/jphysiol01248-0130.pdf>

[Analysis of sodium current in the Hodgkin Huxley model - YouTube](#)

<https://www.youtube.com/watch?v=0uCCKcoh1kQ>

<https://neurondynamics.epfl.ch/online/Ch2.S2.html#Ch2.E4>