MAT323/APC323 Fall 2017 - Topics in Mathematical Modeling: Mathematical Neuroscience

Final Project: Hodgkin-Huxley model

Gabriel Toneatti Vercelli, Thiago Tarraf Varella

Princeton University, January 12th

Over the last decades, the Hodgkin-Huxley model (HH) not only introduced an insightful understanding of the form and propagation of action potentials, but also served as the basis to new experimental approaches and modelling techniques in Neuroscience. The authors of this model successfully designed a system to depict action potentials even though they did not know many of the currently basic molecular details of neuronal activity. This achievement opened doors to new studies on nerve cells and earned them the 1963 Nobel Prize [1].

Qualitatively speaking, Hodgkin and Huxley found out that the relevant quantities to the study of action potentials were the membrane conductance of specific ions, mainly Na^+ and K^+. Through their experiments, the authors showed that the change in these conductances is dependent on the membrane potential and that the conductance of each ion is independent of the others.

Quantitatively speaking, the authors realized that the dependence of the membrane conductance on membrane potential introduced a feedback element in the system. Thus, using their knowledge about electrical circuits, they derived differential equations to describe the observed phenomena [7]. All these conclusions and derivations will be explained and discussed in the following sections, focusing on the first four papers of the published series separately [4, 3, 5, 6] and using the fifth paper [2] to outline the information and build the final model.

1 Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*

The first paper is concerned with an analysis of the total ionic current (I_i) through the membrane of nerve cells and its dependence on membrane voltage. With this goal in mind, the authors prepared neurons in a normal ionic environment and used two different set-ups for their experiments: the first stimulated the membrane with a rectangular voltage pulse and measured the membrane potential and total current over time, the second controlled the potential difference across the membrane through a feedback loop and measured the evolution of current over time.

These two set-ups were inspired by the belief that the "membrane current may be divided into a capacity current which involves a change in ion density at the outer and inner surfaces of the membrane, and an ionic current which depends on the movement of charged particles through the membrane" [4]. This can be described mathematically as:

$$I = C_m \frac{\partial V}{\partial t} + I_i, \tag{1}$$

Where I is the total current, C_m is the membrane capacity per unit area and $\frac{\partial V}{\partial t}$ is the change in membrane potential over time

Calculating membrane capacity

Since the membrane capacity is a function of the shape of the membrane, this capacity is a constant that had to be measured experimentally. To do that, the authors used the set-up that fixed the voltage difference across the membrane and recorded the instantaneous capacity current that surged after the initial change on membrane potential. By integrating this capacity current over the charging period, they acquired the total charge of the membrane gained from the change in potential and calculated the membrane capacity as the ratio of these two quantities. On average, the value of membrane capacitance per unit area obtained was $C_m = 0.9\mu F/cm^2$ and the ionic environment, temperature, and shock strength had little effect on this value.

The capacity current was distinguished from the ionic current in this case by their time-scale. The time-scale of the charging period (around $60\mu s$) was shorter than the time-scale of the movement of ions (around $200\mu s$). Furthermore, after the end of the charging period, the membrane current returned to approximately zero, what corroborates the separation by time-scales (fig 1).

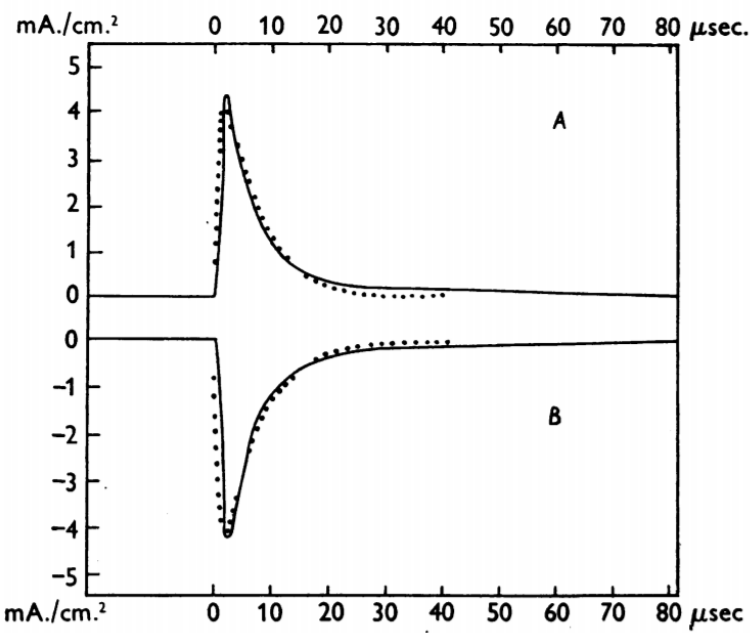

Figure 1: Current through capacitative element of membrane during a voltage clamp. Abscissa: time in μs. Ordinate: membrane current density (mA/cm^2) with inward current taken as positive. At t = 0 the potential difference between external and internal electrodes was displaced +40 mV. in curve A or -40 mV. in curve B. The continuous curves were traced from experimental records. The dotted curves were calculated according to the equation $I^* = 6.8[\exp(0.159t) - \exp(-t)]$ where I^* is the current in mA/cm^2 and t is time in μs. Figure and caption taken from fig. 16 on [4]

Stimulation with rectangular pulses

The first experiment was performed with rectangular stimuli and the membrane potential varied freely. The authors measured the progression of voltage and current over time and the threshold voltage to trigger an action potential. Consequently, using their experimental results,

Hodgkin and Huxley calculated I_i from the total current, I, the change in membrane potential, $\frac{\partial V}{\partial t}$, and the previously experimentally calculated membrane capacity, C_m.

From their results, the authors realized that at times greater than $200\mu s$ the total current was negligible. This means that I_i could be calculated through the formula $I_i = -C_m \frac{\partial V}{\partial t}$. Therefore, using the shape of $V(t)$ in a graph of membrane voltage over time, the authors estimated the relationship between membrane voltage and ionic current in the rising phase of the action potential (fig 2).

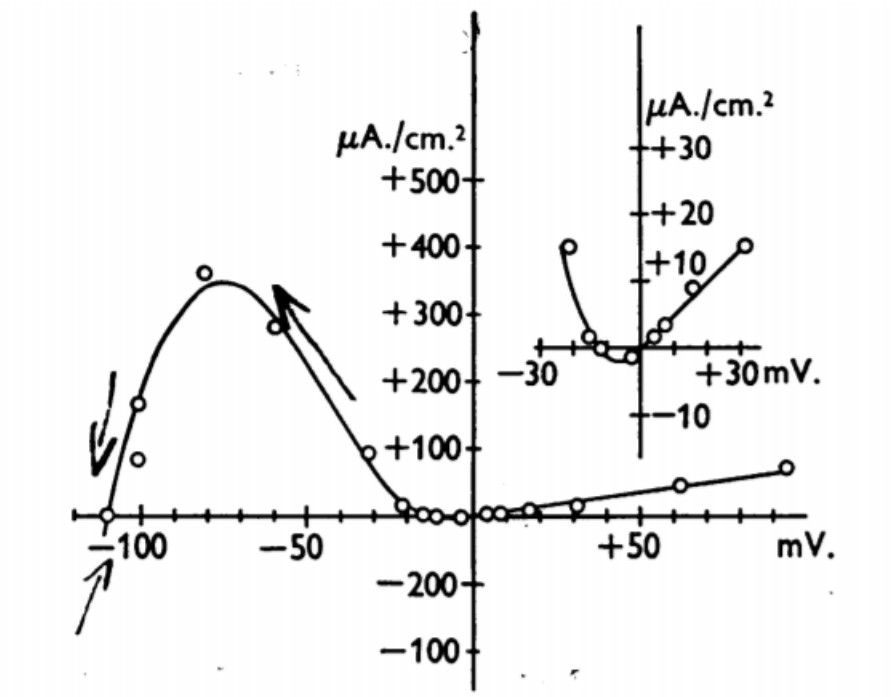

Figure 2: Relation between ionic current density (I_i) and displacement of membrane potential (V). Abscissa: displacement of membrane potential from its resting value. Ordinate: ionic current density. Inset: curve in region of origin drawn with tenfold increase in vertical scale. Measurements made $0.29ms$ after application of shock. Figure and caption taken from fig. 10 on [4].

The graph on figure 2 can be interpreted as an one-dimensional dynamical system with two stable equilibria in the edges and one unstable equilibrium in the middle representing the threshold voltage to trigger an action potential. This explains why the system slowly converged to the resting potential for stimuli below $12mV$, rapidly converged to $110mV$ for stimuli greater than $12mV$ and could remain at this threshold voltage for an indefinite amount of time.

Stimulation with controlled potential

The second experiment was performed with a feedback loop in the voltage clamp to maintain the potential difference across the membrane constant. Hodgkin and Huxley designed it by connecting the output of a cascade of amplifiers to its input in such a way to cause negative feed-back, that is, for any change in membrane potential, the system generated a current in order to restore the potential difference. In figure 3, the simplified system used by them is depicted next to a simple example of a modern amplifier connected in a negative feed-back fashion.

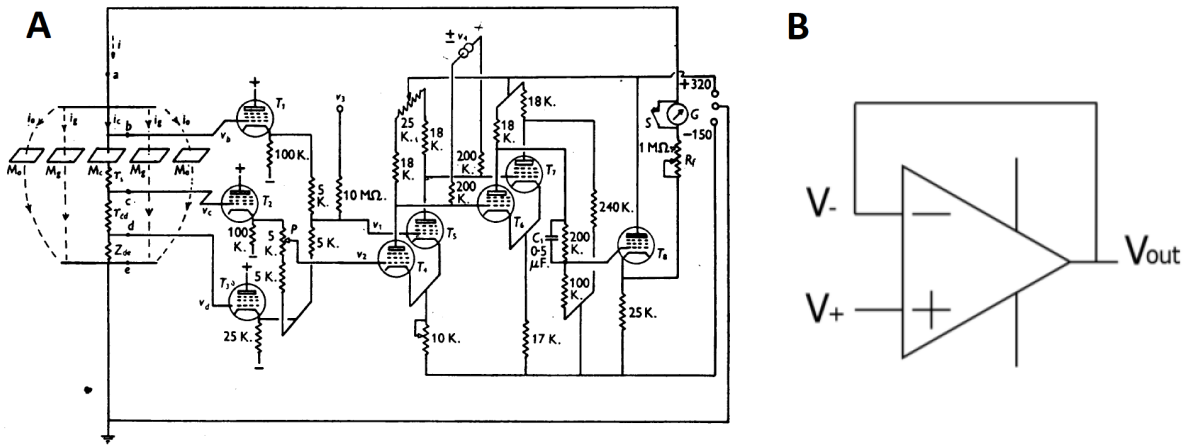

Figure 3: A: simplified electrical system used in HH experiments, T1, T2, T3 and T8 are cathode followers that amplify the current passing through them and T4, T5, T6 and T7 are d.c. amplifiers. All valves used were 6AK5 except T1 and T2 which were 1223. The M's represent the electrodes in the membrane of the neuron and the I's their respective currents. Figure and caption taken from fig. 6 on [4]. B: Sketch of amplifier in a negative feed-back wiring. Figure taken from a Handout of the Integrate Science Curriculum, a Princeton course.

The effects of negative feed-back can be seen in fig 3.B because if there is a non-null difference between the input potentials V_+ and V_-, V_{out} would be an amplification of this difference and would be fed back to V_-. Thus, the system would reach equilibrium when $V_{out} = V_-$, using that $V_{out} = \alpha(V_+ - V_-)$ we get the condition $V_- = \frac{\alpha}{\alpha+1}V_+$. For high gain α, this means that the amplifier would bring the voltage difference between V_+ and V_- to almost zero. Furthermore, the ratio between these two voltages can be controlled by adding resistances to the path from V_{out} to V_- and this is how Hodgkin and Huxley were able to set the membrane potential to virtually any value.

After designing this circuit, the authors set the input voltage (V_+) to a value so that the membrane potential of neurons were equal to their respective resting potential. The input voltage is represented by v_3 in fig 3.A, to the right of the cathode follower T_1. Moreover, they had a second input voltage, $\pm v_4$, that was used to introduce a rectangular stimulus to the system. Thus, the authors had total control over the membrane potential of neurons and over changes in this potential.

Using this machinery, then, Hodgkin and Huxley were able to measure the evolution of current over time at a fixed potential. In this experiment, they found that after a certain threshold of depolarization the qualitative behavior of the current changed. While small depolarizations caused only a small current as response, big depolarizations at short times triggered an inflow of current that would lead the system to depolarize even further if the potential weren't being held by the feed-back circuit. Big depolarizations at long times, however, generated an outflow of current indicating a tendency to repolarize the system.

Taking these data, the authors analyzed measurements taken a short time after the shift in potential and since $\frac{\partial U}{\partial t}$ was equal to 0 over the whole experiment, what they were measuring was purely the ionic current across the membrane. This allowed them to reconstruct the graph of ionic current versus membrane potential already used in figure 2. After doing so, they got a continuous relation whose shape was very similar to the one found previously. This

result supports the belief stated earlier that the membrane current depends on two elements, a capacity current and an ionic current that add to the total current. Further quantitative comparison, however, is not possible because the ionic current measured in this experiment is a function of both potential and time.

2 Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*

Sodium current

Initially, on [3], they recorded the current after depolarizing $65mV$ and maintaining it at this level in three different situations: first in sea water, then in a choline solution, and finally back to sea water. The choline solution was used in the concentration necessary to have the same resting potential, being similar to sea water but replacing just the sodium by choline, so that it was totally sodium free. Only in the middle medium, the choline solution, that the initial inward current was not present. Also, the steady state was lowered in all the situations but in the choline solution it was slightly less negative. They also measured that difference for different voltages on the voltage clamp, as seem in figure 4. As it was known, even in sea water there was a voltage below which the initial inward current disappeared.

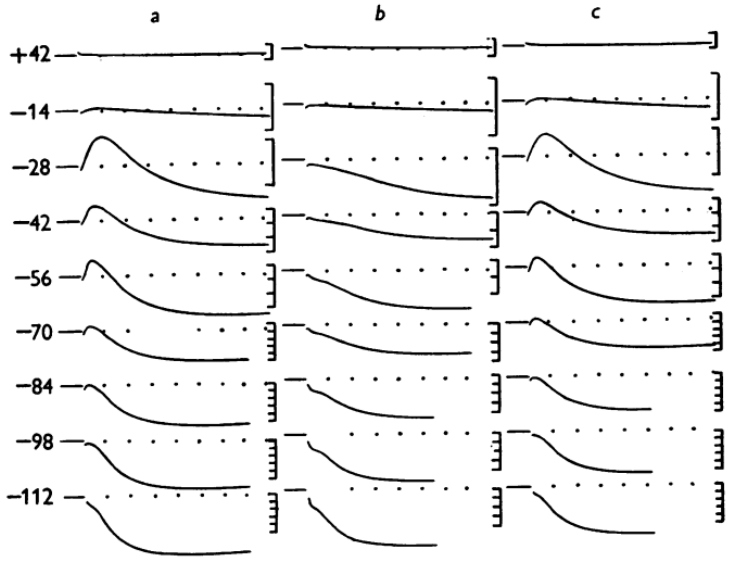

Figure 4: Records of membrane current during voltage clamps. a is on sea water, b is on choline sea water and c is back to regular sea water again. The potentials are in mV. In vertical scale, each division is 0.5 mA/cm^2 and on horizontal scale the interval between dots is 1 msec. Figure and captions taken from fig. 2 on [3]

These results can be used to support the hypothesis that the inward current is carried by sodium ions, since when they are not present the current disappears. Furthermore, this current also disappears when the voltage decreases more than a certain level. This phenomenon can be explained by a Nerst potential

$$E_{Na} = \frac{RT}{F} \log \frac{[Na]_i}{[Na]_o}. \tag{2}$$

Subtracting two of these potentials with different external sodium concentrations ($[Na]_o$), we get a formula composed only by known quantities. From now on, we will call this difference

the sodium potential shift. If E_{Na} is the Nerst sodium potential in sea water with a sodium concentration of $[Na]_o$ and E'_{Na} the Nerst sodium potential in a reduced sodium solution with sodium concentration $[Na]'_o$, the sodium potential shift is $E'_{Na} - E_{Na}$.

Now, consider E_r as the resting potential in sea water and E'_r as the resting potential for the reduced sodium solution. The experimental value for the sodium potential shift, thus, can be measured using that the membrane displacement V_{Na} is $E_{Na} - E_r$, so that the shift can be written as

$$(V'_{Na} - V_{Na}) + (E'_r - E_r).$$

On the other hand, using the theoretical prediction from the Nerst potential equation, we arrive at another expression for this shift:

$$E'_{Na} - E_{Na} = \frac{RT}{F} \log \frac{[Na]_o}{[Na]'_o}.$$

The experiment from figure 4 was adapted to measure the previous quantities and repeated for several different values of $[Na]_o$. These measurements, then, were used to calculate the sodium potential shift in the two ways above. If we compare the model predictions for the sodium potential shift with the experimental data varying the sodium concentrations, we get:

Axon#	Reduced V_{Na}	Model prediction	Experimental data
20	-78	+28.9	+30
20	-45	+55.3	+55
21	-48	+55.6	+56

Therefore, it was possible to conclude that the initial inward current is indeed driven by the sodium concentration.

Other application of that model is to calculate the internal sodium concentration using the sodium potential. Using equation 2 and considering the concentration of sodium in sea water to be about $460\, mmol/kg$, one would get an internal concentration of $60 - 70\, mmol/kg$. This is a reasonable result, since the concentration in freshly dissected axons is approximately $50\, mmol/kg$.

Current driven by other ions

Consider three assumptions:

1. The potassium current, I_K, will not be affected by the sodium concentration of the external medium
2. The sodium current in a high-sodium medium is proportional in amplitude to the one in a low-sodium medium even though the time scale might change.
3. There is a small time interval before the sodium current's reaching it's maximum amplitude where I_K is constant.

With theses assumptions, we can find the currents driven by specific ions in terms of the general current. Since $I_i = I_{Na} + I_K$ and $I'_i = I'_{Na} + I'_K$, where the prime currents are in a medium with a different sodium concentration, we have that $I_i - I'_i = I_{Na} - I'_{Na} = I_{Na}(1 - k)$, so

$$I_{Na} = \frac{(I_i - I'_i)}{(1 - k)},$$

$$I'_{Na} = \frac{k(I_i - I'_i)}{(1-k)} \text{ and}$$

$$I_K = \frac{(I'_i - kI_i)}{(1-k)}.$$

The value for k was determined by looking at the initial behavior of I_i and I'_i, where the influence of the I_K is not considered, because of assumption 3.

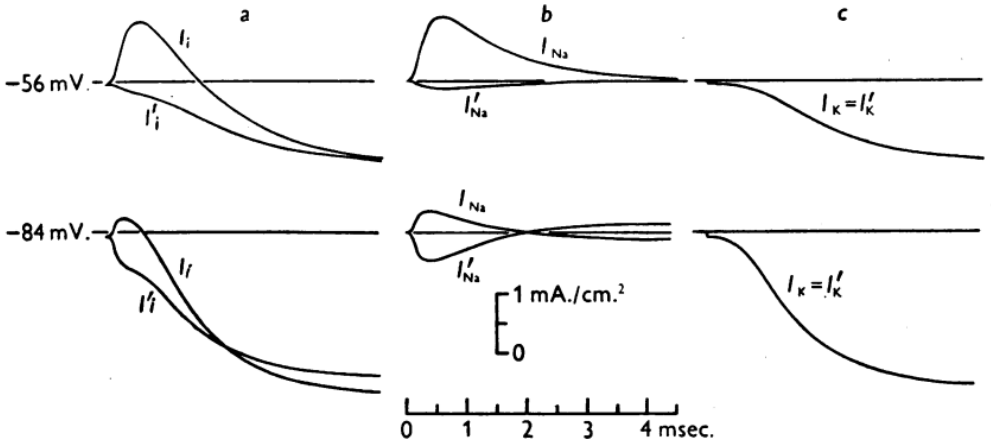

Figure 5: Time course of different currents separately in two different potentials. The currents $I'i$, I'_{Na} and I'_K are recorded in a solution with 10% sodium. Figure taken from [3].

Moreover, if you further assume the "Independence principle", which states that the ions act independently regarding their passage through the membrane or contribution to the total current, you can conclude that the ratio I'_{Na}/I_{Na} will be given by

$$\frac{I'_{Na}}{I_{Na}} = \frac{([Na]'_o/[Na]_o)\exp[E - E_{Na}]F - RT}{\exp[E - E_{Na}]F - RT}.$$

The formula allows us to predict a reduced sodium current I'_{Na} given the sodium current in sea water, I_{Na}. It turned out that the prediction was accurate, so the independence principle is reliable.

Conductance

The direction of the ionic current through the membrane can be given just by the concentration difference of the ions and the electrical potential difference across the membrane. To calculate the magnitude of this current, however, it is necessary to have also the permeability of the ions, which can be modeled using the formalism of a conductance. This way, the sodium permeability is the sodium conductance g_{Na} defined as

$$g_{Na} = \frac{I_{Na}}{E - E_{Na}}. \tag{3}$$

Notice that it makes sense to consider $E - E_{Na}$ as the driving force because when the potential is the sodium potential ($E - E_{Na} = 0$) we have an equilibrium for that ion.

Origin of the action potential

With the previous results, the authors could predict that the mechanism in which an action potential could happen is the following:

A current from a neighboring region would depolarize the membrane and with that allow the sodium to flow. The higher external sodium concentration would create a current inwards depolarizing even more until it reaches the sodium equilibrium. As a delayed result of the depolarization, other ions (that are thought to be potassium ions by other studies) will generate an outwards current and decrease the sodium permeability. When the other ions exceed the sodium current it repolarizes the membrane until the other ions reach equilibrium.

3 Components of membrane conductance in the giant axon of *Loligo*

Discontinuity of sodium current and continuity of sodium conductance

Differently from the previous papers, the authors now investigate what happens if you maintain the depolarization just for a short time, returning the membrane potential to $0mV$ after that. What we observe is shown in the figure 6.

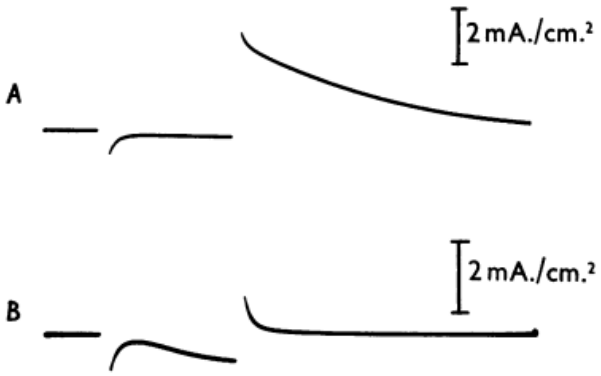

Figure 6: The figure A represents the membrane current after a depolarization of 110 mV during 0.28 ms in sea water. The figure B represents the same but in choline sea water, which is a sea water but replacing sodium with choline. Figure taken from fig. 3 on [5]

This experiment proves that the tail on the current after the repolarization is given by a sodium current. The fact that the period of inward sodium current is reduced when you cut the period of depolarization suggests that the sodium permeability is reversible.

If you calculate the conductance using equation 3 you find that the sodium conductance is continuous in the process, which explains the discontinuity on the current since the voltage is discontinuous.

Analogue of Ohm's Law

If you measure the current immediately after the repolarization on the membrane potential you can observe the Ohm's law. To measure that, they set an incomplete repolarization of different magnitudes. The current immediately after the repolarization is linearly proportional to the magnitude of it, suggesting that the mechanism of sodium's going through the membrane

might be similar to that of a resistor. This is another evidence to consider the conductance, which is the inverse of the resistance.

It is important to notice, however, that the Ohmic behavior does not happen in a sodium-free solution. That information does not affect greatly the results of our experiments, since their aim is to study the action potential and no action potential can be triggered in a sodium free solution.

Time course of the sodium and potassium conductance

It is possible to calculate how fast the sodium conductance is restored to its resting value by fitting the tail observed after the repolarization with the function $\exp(-bt)$, where b is the time rate. Also, it was observed that the dependence of the rate b on the voltage follows a sigmoid curve, increasing with the voltage applied.

To study the behavior associated with potassium it is necessary to study a longer depolarization. To do that the experiment described on the previous section was recorded in choline sea water. It was observed that by repolarizing the membrane to a value equal to +7 mV or below, the ionic current was outwards and returned to its resting value. When the membrane was repolarized to a value equal to +21 mV or higher the current was inwards. The conclusion is that other ions studied must have the Nerst potential near +12 mV.

By comparing the curve associated with potassium to the one associated with sodium, it was possible to observe that both had similar shapes. The time rate of the sodium b_{Na}, however, was about 30 times greater than the time rate of the potassium b_K. Other differences found were that the peak of conductance was greater for sodium than the one for potassium, and the sodium conductance fell after reaching the peak while the potassium conductance remained at its resting value.

Furthermore, there is a rise of both the sodium and potassium conductance when the membrane is depolarized. During the repolarization, both conductances fall and show a similar tail converging to their resting values. The dependence of each rate on the membrane potential were also similar and the analogue of the Ohm's law works for both ions, in which case the current equals 0 at the ion's Nerst potential.

Leakage of the ions

When you change the potassium concentration you do not observe the expected changes in the system that would occur if the only ions participating in the process were sodium and potassium. Therefore, it is important to include a leakage current, which includes leakage of sodium, potassium and other ions. This addition should enable us to find a leakage current I_l, an apparent equilibrium potential V_l at which I_l is 0, and a conductance g_l. By studying different situations where you would expect no sodium nor potassium current, for example in specific situations on a choline sea water solution at the potassium potential, when you measure the current, you find the leakage current. In these situations you find the constants necessary to describe this mechanism.

4 The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*

The forth paper deals with the ability of membranes of nerve cells to change their sodium conductance depending on their membrane potential. The approach Hodgkin and Huxley used to assess this feature of the membrane was applying two rectangular stimuli to it. The important variables studied in their experiments were the duration of the first stimulus (called conditioning step), the potential difference between the first and second stimuli, and the time window between them.

Time dependence

To study the effects of the duration of the conditioning step on sodium conductance, Hodgkin and Huxley performed a series of measurements varying this quantity for a small range of potentials and applied a second rectangular stimulus right after the conditioning time. The potential of this second stimulus was the same for all measurements ($-44mV$) enabling the authors to make more quantitative comparisons. Thus, the authors could use the size of the action potential potential triggered by the second stimulus as an indicator of sodium conductance. The results can be seen in figure 7.

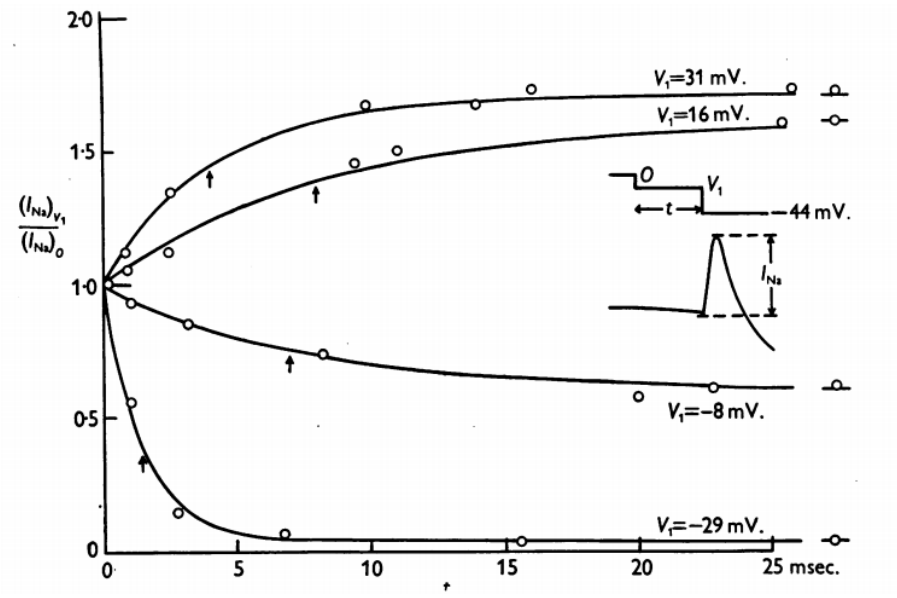

Figure 7: Time course of inactivation at four different membrane potentials. Abscissa: duration of conditioning step. Ordinate: circles, sodium current (measured as inset) relative to normal sodium current; smooth curve, $y = y_\infty - (y_\infty - 1)exp(-t/\tau_h)$, where y_∞, is the ordinate at $t = \infty$ and τ_h is the time constant (shown by arrows). Figure and caption taken from fig. 6 on [6]

It is interesting to notice that all curves in this figure resemble exponential functions that converge to a value at infinity. By fitting these curves to equations of the form

$$y = y_\infty - (y_\infty - 1)exp(-t/\tau_h),$$

the authors were able to estimate the time constants of this process for each value of potential. The results from this measurement were that the time constants varied greatly with potential, with values ranging from $1.5mV$ to $10mV$ and that they had a maximum near $V = 0$. Also,

these constants were useful in future experiments, when the authors needed to know how long they needed to wait to consider the system in a steady state.

Voltage dependence

To study the effects of membrane voltage in sodium conductance, the authors did a similar experiment. They measured, nevertheless, the sodium current only at the steady state for each potential and used a much greater range of potentials. This yielded a curve of current versus potential that had the form of a logistic curve with center near the resting potential of the membrane (fig. 8). This inspired the authors to define a variable that represents the percentage of the sodium-carrying system still active (h), which is 1 when the sodium current in the action potential is maximum and 0 when it is minimum. At the resting potential h was about 0.6 meaning that close to 60% of the sodium-carrying system was active.

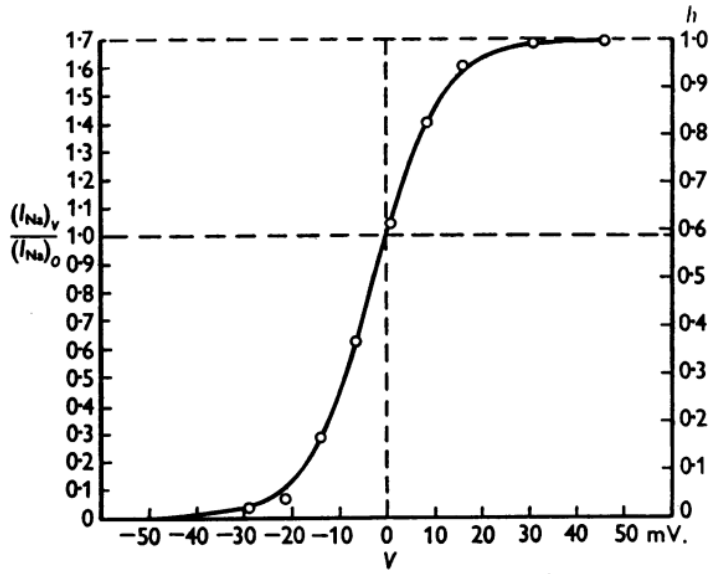

Figure 8: Influence of membrane potential on 'inactivation' in the steady state. Abscissa: displacement of membrane potential from its resting value during conditioning step. Ordinate: circles, sodium current during test step relative to sodium current in unconditioned test step (left-hand scale) or relative to maximum sodium current (right-hand scale). Figure taken from figure 1 of [6]

Refractory period

After having understood how the conditions before a stimulus affect the sodium conductance of the membrane, Hodgkin and Huxley wanted to measure how long they needed to wait to recover a complete action potential. Therefore, they fixed the duration and potential of both rectangular stimuli and varied the time interval between them.

The first result was that the time constant for this recovery process was close to the time constants measured in the first experiment for potential changes close to $-44mV$. This supports the hypothesis that the stimulus used to trigger the action potential also behaved as a conditioning stimulus and converted a part of the sodium-carrying system to an inactive state. The second result was that, by extrapolating the curve of inactivation over time to a time difference of $0s$ between the two rectangular pulses, one would get an inactivation of 63%, in

other words, right after the first stimulus 37% of the sodium-carrying system was active. This number agrees with the expected inactivation generated by a conditioning step similar to the first pulse used. Thus, this also supports that the inactivation of the membrane after the action potential was mainly due to the conditioning behavior of the first stimulus.

The sodium conductance of the membrane, hence, have two different controlling systems. The first has a short time scale and was discussed in paper [5]. This is related to the direct dependence of this conductance on the potential of the membrane and how the depolarization and repolarization of the membrane affect it. The second has a long tim e scale and can be understood as a conditioning of the membrane that can activate or inactivate the sodium-carrying system, leaving the membrane in a refractory state. This hypothesis is supported by the previous experiments and is what originated the idea of the neuron having a refractory period.

5 A quantitative description of membrane current and its application to conduction and excitation in nerve

With the previous articles, we could find these equations:

$$I_i = I_{Na} + I_K + I_l$$

$$I_{Na} = g_{Na}(V - V_{Na})$$

$$I_K = g_K(V - V_K)$$

$$I_l = g_l(V - V_l)$$

and we have calculated all the constants. Also, we have that

$$I = C_m \frac{\partial V}{\partial t} + I_i,$$

just like the circuit on the figure 9.

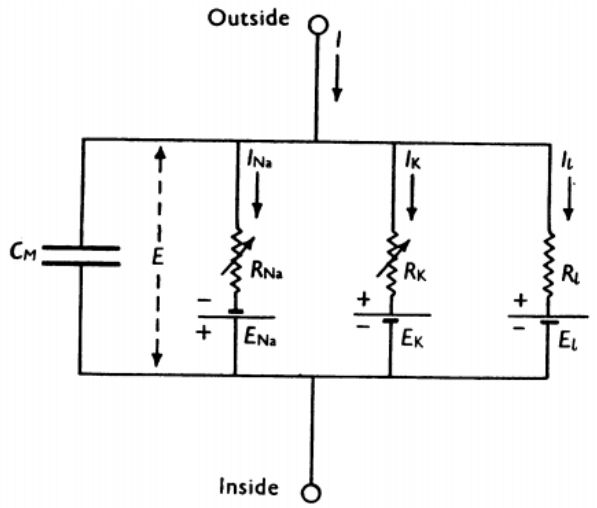

Figure 9: Electrical circuit representing membrane. R_x is the inverse of g_x. R_{Na} and R_k vary with time and membrane potential; the other components are constant. Figure and caption taken from fig. 1 on [2].

In order to have equations to describe the dynamics of this system, we are missing a way to describe the evolution of the conductances on time. If you consider that the permeability g

will depend on what is already inside or outside the membrane, you find that it is propotional to a quantity n raised to the forth power. This quantity is the proportion of particles inside the membrane and is given by

$$\frac{dn}{dt} = \alpha_n(1-n) + \beta_n n$$

where α_n is the rate of transfer from outside to inside and β_n is the rate from inside to outside. This can be written as:

$$g_K = \overline{g}_K n^4$$

For sodium, on the other hand, two variables like n are necessary, one for particles activating the process on the inside (m) and one for particles inactivating the process on the outside (h), so that

$$g_{Na} = \overline{g}_{Na} m^3 h$$

Finally, by fitting the parameters with the experimental data and solving the equations we can obtain very accurate approximations for the dynamics of the action potential, as can be seen in figure 10

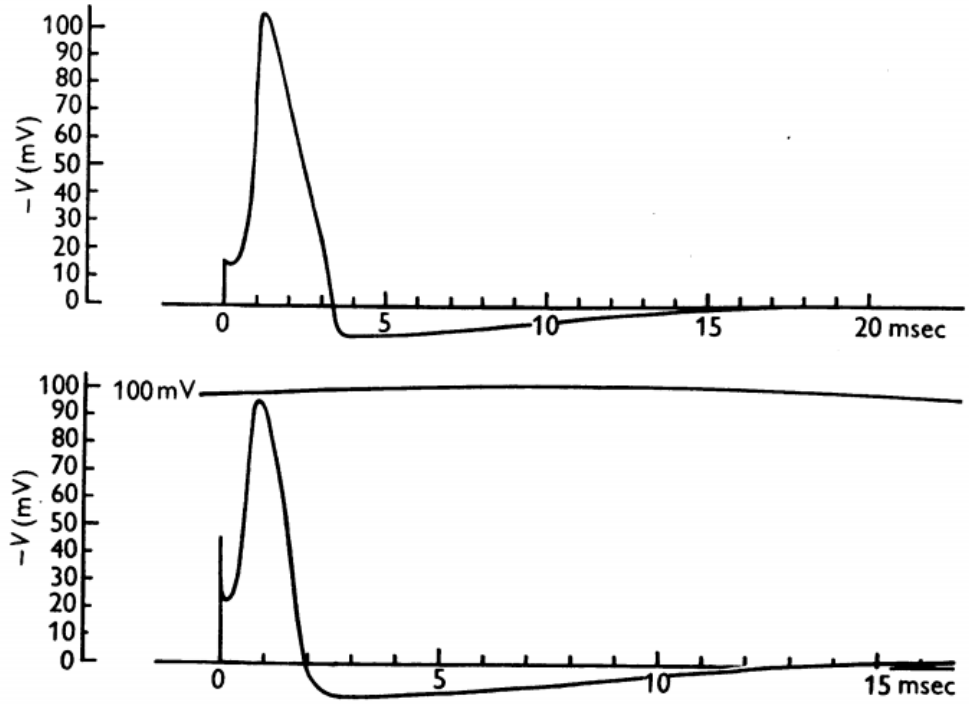

Figure 10: The upper curve is the solution of the equations for a initial depolarization of 15 mV, whereas the lower curve is the experimental data. The horizontal scale differs by a factor appropriate to the temperature difference. Figure taken from fig. 13 on [2].

References

[1] Nobel Media AB. *The Nobel Prize in Physiology or Medicine 1963*. 2014. URL: `http://www.nobelprize.org/nobel_prizes/medicine/laureates/1963/` (visited on 12/31/2017).

[2] Alan Hodgkin and Andrew Huxley. "A Quantitative Description of Membrane Current and Its Application to Conduction and Excitation in Nerve". In: *The Journal of Phisiology* 117.4 (1952), pp. 500–544.

[3] Alan Hodgkin and Andrew Huxley. "Currents Carried by Sodium and Potassium Ions Through the Membrane of the Giant Axon of *Loligo*". In: *The Journal of Phisiology* 116.4 (1952), pp. 449–472.

[4] Alan Hodgkin and Andrew Huxley. "Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*". In: *The Journal of Phisiology* 116.4 (1952), pp. 424–448.

[5] Alan Hodgkin and Andrew Huxley. "The Components of Membrane Conductance in the Giant Axon of *Loligo*". In: *The Journal of Phisiology* 116.4 (1952), pp. 473–496.

[6] Alan Hodgkin and Andrew Huxley. "The Dual Effect of Membrane Potential on Sodium Conductance in the Giant Axon of *Loligo*". In: *The Journal of Phisiology* 116.4 (1952), pp. 497–506.

[7] Arnon Levy. "What was Hodgkin and Huxley's Achievement?" In: *The British Journal for the Philosophy of Science* 65 (3 2014), pp. 469–492. DOI: `https://doi.org/10.1093/bjps/axs043`.