Chapter 2 – Action potential

Chapter 2 has 6 course videos:

- Observations and Hypotheses
- Sodium Channel and Unit Na+ Current
- Total Na+ Current
- Potassium Channel and Unit K+ Current
- Total K+ Current
- Conclusion

In general, for every chapter, we will follow the steps below:

- Observation of a phenomenon to explain the hypothesis (hypotheses) on the underlying ion mechanisms
- Study of the ion mechanisms starting with an explanation of the operation of a single ion channel
- Explanation of the operation of several such ion channels
- By doing this, we explain little by little the operation of the neuronal membrane at the time of the phenomenon being investigated in the chapter (glutamatergic synaptic transmission, for instance).

Here also, some questions (not provided) asked after some videos will enable you to check that you have grasped the key concepts.

CHAPTER 2 INTRO (2:43)

You are going to learn about the ion mechanisms behind the sodium action potential which is the signal emitted by a neuron's axon and propagated along the axon. What are the objectives of this chapter? At the end of the chapter, you will know about a ion current through a voltage-gated ion channel. You will know the various states of a voltage-gated ion channel: closed, open, and sometimes inactivated. You will know how to construct and understand the significance of a current-voltage curve and you will be able to apply Ohm's Law to biological systems. Can you give me a quick definition of action potential? The sodium action potential is the signal emitted by neurons' axons and propagated along the axons all the way to the axonal terminals. Their role is to enable the release of a neurotransmitter. The sodium action potential is a binary signal, that is to say, it either exists or it does not. This has several consequences. First, it means that the action potential of a given neuron will always have the same amplitude. And the second consequence is that the language of neurons, or action potentials emitted over time is written in binary notation. If we say that 1 is the action potential and 0 is the absence of an action potential, there are neurons that discharge in an irregular fashion and first, there is silence followed by an action potential, a somewhat shorter silence, followed by two action potentials, then a long silence, two action potentials, a short silence, three action potentials, etc. There are others that discharge in a regular way, that is to say, an action potential will always occur at regular intervals always separated by silences of the same length. And then, there are also neurons that discharge in bursts, i. e. you have 5 or 6 action potential at once followed by a long silence followed by 5 or 6 potentials at once, etc. Over time, as action potentials were recorded, there were sounds, that is, we heard things

like toc, toc-toc, toc-toc, toc-toc-toc or we heard a regular toc toc toc toc toc. And if it was in bursts, we heard: pschit, pschit. Obviously, the challenge is to understand how biological systems can create a binary signal. What learning tools did you provide with this Chapter? In addition to the course videos, the supplements, quiz, practical exercises, there are videos of experiments stages at the Mediterranean Neurobiology Institute in Marseilles which is the lab where I work.

CH. 2-1: OBSERVATIONS AND HYPOTHESES (08:01)

To study neuron action potentials, neuron activity must be recorded. To do this, we use the patch clamp electrophysiology technique described in the previous chapter and also in a supplement. Neuron activity is recorded using the patch clamp method in whole-cell mode. Here, a neuron is represented by a round cell. So, we are in the whole-cell configuration and in current clamp mode to record the changes in potential in a neuronal membrane. Here, we see a potential of -80 mV. We see that the neuron is silent at that potential. If we use the patch to send a bolt of positive current, we register a slight membrane depolarization. If we increase the intensity of the positive current now in a step-wise manner, we will see that the membrane continues to depolarize and the depolarization is proportionate to the magnitude of the current step: we call this an ohmic response. But starting with a certain depolarization threshold, we start to see a large-amplitude signal, sharp and short. We see here a time scale of approximately 1 millisecond coming back to normal. This is an action potential. Therefore, an action potential is abrupt depolarization. We see here, that starting with a certain threshold, there is a depolarization phase, followed by one of repolarization. The depolarization is very fast, it is quick. We have a time scale here. All or none, that is to say, either we have everything or nothing. And here, for example, we see that had we increased the value of the depolarizing current, we would still have had a response of the same amplitude. Thus, either there is everything or there is nothing. The action potential of a given neuron always has the same amplitude. Again, there is a threshold above which it appears, the depolarization threshold. Let us revisit the properties of the action potential. Starting with a threshold potential, here is the potential scale, there is a depolarization phase. What does it mean, exactly? It means that the interior surface of the membrane is increasingly less negative with respect to the exterior one, and even inverts, i. e. becomes more positive than the exterior surface. Then, the membrane repolarizes and returns to its initial values. What causes the two phases? To find out which ions are involved, because there are always ions involved in neuronal signals, we use ion channel blockers. We have here TEA which is a potassium channel blocker. And what we see is if the depolarization phase is the same, repolarization takes much longer. It would seem that potassium ions play a part in this repolarization phase but not in depolarization. If we also add TTX now, i. e. tetrodotoxin which is a sodium channel blocker, we will see that there isn't anything any more, no action potential just an ohmic response. We say there is no doubt that sodium and potassium ions are involved in action potential, with potassium ions in the repolarization phase while for sodium ions, we do not know whether its is only the depolarization phase or depolarization and repolarization. Let us test if others are involved in this action potential recorded here. If we add a calcium channel blocker, even several, we see nothing, no change. We still see the same action potential. If we add a chloride channel blocker, nothing happens either. It seems that these two ions are not involved. To verify, we could also suppress the extracellular calcium ions and

observe that the action potential is unchanged. And we could also replace the extracellular Cl- ions with equivalent non-permeant anions and again, nothing happens. Therefore, we are sure that calcium and chloride ions are not involved in the action potential referred to as sodium, and we will see why. Following these experiments, we hypothesize that potassium ions participate in the repolarization phase here while sodium ions participate in depolarization or in both depolarization and repolarization which we do not know yet. We will start by trying to understand the exact part played by sodium. Better to understand the order in which sodium and potassium currents participate in action potential, we will now record some current values and to do that, we will record in voltage clamp mode. We record using patch clamp in whole-cell mode here and in voltage clamp mode to capture the currents crossing the entire neuronal membrane. Thus, membrane potential is maintained at -80 mV, and we apply a depolarizing voltage. We see that for as long as the potential is maintained at -80 mV, the current is stable. This is the maintenance current. And as soon as there is a jump of sufficient amplitude in depolarizing potential, we record a drop in negative current followed by positive current. Here, we are dealing with an inward current by definition (negative currents are inward currents for + charges) followed by an outward current of + charges. This is also explained in the supplements. What if we now re-injected the blockers that we saw recently; so, we are going to put tetrodotoxin blocking the sodium channels? Still the same experiment, maintenance potential of -80 mV, depolarizing potential excursion. We see right away that the inward current has disappeared; while the outward current remains. Thus, it would seem that this inward current here is a current of sodium ions and that the outward current is that of potassium. We are going to verify it using tetra ethyl ammonium, a potassium channel action potential blocker. Thus, we now see that the inward current is there. that it is greater than here, and that the outward current has disappeared. It can, therefore, be deduced that the outward current is sensitive to TEA and that it is an outward current of potassium ions. We now know that the sodium current is responsible for action potential depolarization. This is about entering + charged sodium ions, which naturally depolarize because there is going to be a + charge surplus inside the membrane. This sodium current is followed by a potassium current which enables repolarization. Why? Because there is and outward transport of + charged potassium ions, and the interior repolarizes losing these + charges. Looking at this recording, we could ask a number of questions. Why is it that during this phase, the inward sodium current is the first current to appear? And is it incoming? And why is it so short? We could also ask why the outward potassium current is delayed with respect to the sodium current and why it is of longer duration? So, we are now going to study these two currents, sodium and potassium, one after the other.

CH. 2-2: SODIUM CHANNEL AND UNIT NA+ CURRENT (7:28)

The sodium channel is voltage gated. This is a protein channel; and therefore, it is made up of a sequence of amino acids which we see here: some 2,000 amino acids. And we can distinguish four identical domains that we label I through IV with Roman numerals. These four domains contain six membrane-spanning segments each (1, 2, 3, 4, 5, 6) and a P-loop which we see here and which makes up the interior of the channel that transports the ions. We also point out Segment 4 which is seen there. This Segment 4 which we injected with small + charges is in fact made up of a regular positively charged amino acid structure, and it is thanks to this structure that the protein is sensitive to changes in potential, or "voltage-gated" for

short. Now, likely, this protein that we see here folds, or the four domains fold into a pseudo-tetramer which delimits the aqueous pore through which sodium ions will cross. On another illustration below, we see I, II, III, IV representing the four domains that we saw here in the principal subunit on the left. And if re represent sodium ions, this potential exists in a state that is closed as we see here: the aqueous pore that lets the ions through is closed. And a potential can also exist after a depolarization, a delta V, a change in the membrane potential , and we see that the aqueous pore opens, and sodium ions pass through. We are now going to record sodium current through a single sodium channel. This current is known as unit current and is denoted with a small i, small i sodium. To record a unit current, we have to have a very small piece of membrane underneath the pipette and hope that there is only a single channel in that little piece of membrane, and that it is a sodium channel. Thus, we go to the cell-attached configuration and voltage-clamp mode because we want to record current. We decided to record the activity of a muscle fiber because there are many sodium channels, and we are in a cell-attached configuration and recording the small piece of membrane that is under the recording electrode. We maintain membrane potential at -90 mV. And we apply a bolt of depolarizing voltage to get -40 mV. So, we depolarize a small piece of membrane that is under the electrode here and record the current going through the electrode. We see that we record a negative current step from time to time. I will remind you that negative current is incoming current of + charges, or the absence of a response. Here, a negative current step, there a very short response, a negative current step but of a very short duration, no current, and another negative current step, and again no current. Now, if we display the appearance of these current and remove the noise. or rebuild the plot of the currents that we are recording, here they are. What do we see in this plot? We see that for a jump in potential of the same amplitude, the unit current always has the same intensity. Its duration is variable, and the time lag between the start of the depolarizing shock and the appearance of the current also changes. When there is no current, the sodium channel is in a closed state. Then, when there is current, it is in an open state. And then, once the current disappears, it is again in a closed state. But it is not really in a closed state because we observe the channel never opens. It is in what is known as an inactive state, in a state from which it cannot re-open directly. And finally, it is a state that protects the membrane from excess sodium current. We observe that this channel has three states: closed, open, and inactive. And one can describe the path from state to state: so, from closed to open to inactive. It is an equilibrium every time, and here, to re-open, it goes through the closed state. What causes it to open? It is a change in depolarizing potential. What happens when it deactivates? It is a matter of time. Once it has remained open a certain time, the channel deactivates. What happens when it closes? It is when the membrane repolarizes. Under what conditions does the sodium channel open? To find out, we will record a unit sodium channel, still in voltage clamp mode. If we increase the potential, we will start with the maintenance potential of -80 and apply increasingly depolarizing potentials ranging from -70 all the way to +60. We see that initially, we do not have a sodium current. At -70 mV, the channel does not seem to be opening. At -50, it opens, and the current is strong. And the current will become ever smaller, then stop and change direction: from inward it will change to outward. First, we have sodium ions entering and then exiting towards +60 mV. Let us build a current-potential, or current-voltage, curve. For a step up to -50 mV, we could say that the current is about -2 pA. So, here -2 pA. For -20, we have -1.5. Here, -20 mV, -1,5. For 0, 1 pA. For +20, about 0.5. It reverses direction

at +50; so, 0 pA here, and at +60 it is outward at several picoamps. And if we plot it now, we see that it is almost a straight line; therefore, the current-potential relationship is linear for an ion channel.

CH. 2-3: TOTAL NA+ CURRENT (4:39)

To record sodium current across the entire membrane, we record in the whole-cell configuration, as we see here when we record the activity in all the channels within the neuronal membrane, and in voltage-clamp mode to record the current. If we jump from 80 mV to 30 mV, i. e. apply a bolt of depolarizing potential, we will see that we are over the sodium channel opening threshold because the threshold is about 50. In and response to this bolt of potential, we record a current which is no longer rectangular but which shows a descending leg, a peak, and an ascending leg. This is a negative inward current. This is an inward + charge current. And if we want to make sure that this is a sodium current, we apply some TTX at which point the current disappears completely. In fact, this current represents a sum of unit currents that opened up one after another. The curve looks very smooth, but in fact, many currents occur one after another and make up the descending leg. And afterwards, we have a gradual inactivation of the channels that produces the ascending leg. If we repeat the same experiment at several test potentials, we will see that we get a family of sodium currents, always with this descending leg which represents the gradual but rapid opening of the sodium channels and the ascending leg which represents the gradual inactivation of the sodium channels. We can build an I/V curve that corresponds to the experiment here on the left. Let us first look at the reversal potential here which is not at all the calculated reversal potential for the extra- and intracellular sodium concentrations. Here, we are at +30 mV. Now, let us look at the curve and its bell shape. Why do we get this bell curve? Although, as you recall, for the unit current we had a linear graph. We get this bell curve because initially, the channels will open gradually. Whereas a single channel either opens or does not open. Here, the channels open gradually. Why? Because the first sodium channels open, and that depolarizes the membrane somewhat because there are + charges coming in. And this will cause other channels to open which opens still other channels, which opens more channels, which opens even more channels. It's like when you start a chain reaction. All at one, everything is aflame. It goes very fast but in spite of everything, it is gradual. Then, at some point, we get back to a plot that is more or less linear because we approach the reversal potential; hence, the current is less intense. It is increasingly weak. This is a little difficult to grasp; therefore, I will repeat this one more time. When we move from 80 up to about +60, we first see a tendency for the current to become smaller in amplitude because we are approaching the reversal current. Then, we see it increasing because what is happening is the action of two opposing forces. The first one is the gradual opening of sodium channels and the second one is the drop in the driving (electrochemical) force. But the gradual opening wins which makes the current stronger. And then, when all the membrane channels are open that can open, there is only the electrochemical force which slackens off and makes the current slacken off as well. This total sodium current underlies the action potential depolarization phase because this is an inward current of + charges. This makes the membrane depolarize and abruptly because the channels open up extremely quickly and abruptly. Then, suddenly, when they are all open, they will inactivate and we will have the repolarization phase begin. So, to go back to the beginning about an action potential threshold, what is an action potential

threshold? In fact, it is the opening threshold for sodium channels. Once the sodium is inside, this depolarizes the membrane greatly and very quickly. Then, there is a peak because the channels inactivate, and sodium stops entering.

CH. 2-4: POTASSIUM CHANNEL AND UNIT K+ CURRENT (5:01)

We just saw a sodium current that underlies the action potential depolarization phase. Let us now look at a potassium current that underlies the action potential repolarization phase. The action potential potassium channel is made up of 4 subunits. It is not the same as the sodium channel which had four domains within the same protein. There, there are for separate proteins. But we find other very similar things, such as transmembrane segments 1 through 6, i. e. six such segments. Here we also find the P-loop which is located in the channel pore and we find Segment 4 which is made up of certain positively charged amino acids which make the protein sensitive to potential, and we say that the channel is voltage gated. This channel is a tetramer made up of four similar subunits, and we see here that the aqueous pore is closed. A change in potential, a depolarization, a delta V, a change in depolarizing potential is required for it to open. And then, the aqueous pore opens, and potassium ions which we see here exit under the action of their force which pushes them out. Now, we are going to look at the function of the potassium channel which is to convert membrane depolarization into an outward current of potassium ions. To record a unit potassium current, we record in the outside-out configuration and voltage-clamp mode. Thus, the voltage clamp mode is to record current, and the outside-out configuration is about a very small piece of membrane that has been excised from the neuron and in which we are hoping to find a potassium channel. To record a potassium current only, we apply sodium and calcium blockers since these channels are also voltage gated. So, if we hold the membrane potential at -80 for now and apply an increasingly depolarizing potential in steps, we record, top to bottom, first a small outward current in steps, thus, rectangular steps like those of a unit current. Then, this current becomes stronger and stronger as we depolarize. There is also a peculiarity: we see here that initially, this current comes with a delay with respect to the moment when we depolarize the membrane. And then, the channel opens, closes again, re-opens, re-closes, then it re-opens and then it will re-close again, then it closes here which is a very very brief closure, then it re-opens and closes. The action potential potassium channel has very different properties than the action potential sodium channel. First off, it has a long opening delay. It may re-open several times in the course of a single increase in potential which is very different; hence, we say that it has two states: closed and open. It is the change in potential, or more specifically, a depolarization, which makes it transition from closed to open and it is repolarization that makes it close. This channel does not inactivate. If we wanted to build the current/voltage curve now, or the current/potential one, based on that potassium unit current. We are going to do it together for a potential of -50. So, 1, 2, 3, 4, 5. There, we have an outward current of about 0.5 picoamps. For a potential of -30 here, we have an outward current of a little more than 1 picoamp. For -10 millivolts, we have around 2, in fact. And for +20 here, we have about 2.5. So, we are going to build this current/voltage plot which is a straight line. And this is a unit current which follows Ohm's Law here. To see the reversal potential, we extend this plot and find it at about -80 millivolts. We cannot observe this reversal potential in our experiment and we have to have the I/V plot to illustrate in graphically because at -80 the channel is closed.

So, since it opens starting at -50, the I/V plot needs to be extended to see the reversal potential.

CH. 2-5: TOTAL K+ CURRENT (1:57)

A neuronal membrane contains a lot of potassium channels. What does the total potassium current look like? To find out, we record in the whole-cell configuration and voltage-clamp mode because we still want to record current. And there, we are going to record the activity of all the potassium channels in the cell that open in response to an increase in potential. So, we apply a potential of -80, and we make this potential increasingly depolarizing. And in response, we record a family of increasingly strong outward currents which always spring up following a certain delay. If we wanted the I/V curve corresponding to this experiment, it is right here. Let us analyze it. Initially, the current is zero because the channels are closed. The membrane is not sufficiently depolarized. Then, once the opening threshold of the potassium channels is reached, we see a potassium current that is growing ever more as a result of depolarization. Obvious question, why is it growing? Because we are going away from the potassium reversal potential which makes the current increasingly strong because the force that is pushing potassium out is increasingly large. It is this total current that underlies the action potential repolarization phase. Why does it underlie the repolarization phase? Because it make positive ions exit which makes the membrane interior less and less positive. So, the action potential repolarizes.

CH. 2-6: CONCLUSION (7:45)

Let us look at the state of the sodium and the potassium channels through the various action potential phases. Let us start with the sodium channels. Initially, they are closed. They are closed because the membrane is not sufficiently depolarized for them to open. Starting with the moment when the membrane is going to depolarize and all the way to the action potential opening threshold, they will open and they do, extremely quickly. But they also inactivate very quickly, that is to say that a certain period of time after they open, which is about 1 millisecond, they inactivate automatically. So, the first ones to open will inactivate. During this entire peak and repolarization phase, sodium channel currents inactivate. Then, they will close. When will they close? They will begin to close when the membrane crosses the potential threshold again. As soon as the membrane hyperpolarization exceeds the sodium channel threshold potential, these channels will close little by little. Now for the potassium channels. Initially, everything is as with the sodium channels. They are closed because the membrane is below their opening threshold potential. Once this threshold potential is exceeded, the channels open but we saw that they open late, with a lag. And this lag is extremely important to keep sodium ions from exiting at the same time potassium ions are entering, because if we had + charged ions exiting and entering at the same time, the membrane would not change potential. To have both a depolarization and a repolarization phase, it is extremely important that there should be a gap in the opening delay of sodium and potassium channels. When will potassium channels re-close? Same as sodium channels, it is when the membrane is more hyperpolarized than their opening threshold potential, and then they gradually close. Thus, we see here a phase that we have not mentioned so far which is a small transient hyperpolarization phase resulting from the fact that potassium channels close slowly. They open slowly and with a lag and they close slowly and with a lag. Because of this, a potassium current still exists for a long time after the membrane is over the threshold, and so, the

membrane has a tendency to go to the potassium current reversal potential. Afterwards, the membrane repolarizes as soon as more and more potassium channels close and there is no longer a potassium current. The action potential is initiated at the initial segment. How do we know that? We know because we have made a recording simultaneously in the soma and in the initial segment here. And every time we recorded different neurons we saw that an action potential first appeared in the initial segment before reaching the soma. Why is it initiated in the initial segment? The reason is that there is a very high concentration of sodium channels there and that these sodium channels have a somewhat lower opening threshold than those in the soma; hence, they will open at lower depolarization. Now, why is it that this membrane is depolarized? How is it possible? It must first depolarize to the threshold there to be an action potential. This depolarization results from synapse activity which we see here, synapses which are very numerous in dendrites. And when these are excitatory synapses, these depolarize the initial segment membrane all the way to the opening threshold potential of the sodium channels. Once initiated, the action potential will propagate towards the soma which is why it can be recorded in the soma and also to the axonal terminals. In the soma, it stops because most frequently neuronal dendrites do not have voltage-gated sodium channels. I say most frequently because in biology, there are always exceptions. So, the action potential is, in fact, looking to descend and to propagate along the axon. How does action potential propagate along an axon once it has been initiated within the initial segment. Frequently, axons have a sheath that is referred to as a myelin sheath which is a sheath made of glial cells. These sheaths are spaced out, and in between the sheaths, there is what is known as nodes of Ranvier. If we record at one of these nodes of Ranvier, we see that action potentials at each Ranvier node has exactly the same amplitude. We say that the action potential propagates in a non-decremental fashion. How is it possible? What are the mechanisms that ensure that the action potential does not lose amplitude while traveling down an axon? Let us look at the propagation mechanisms. Here, we record at the first node of Ranvier and we record an action potential. It is very stretched because of the way it is shown but it is really an action potential. Here, the sodium channels that are very numerous in the nodes of Ranvier are open because there is an action potential. The sodium channels in the initial segment, on the other hand, are in an inactive state because they opened before, and therefore, are still inactive. And the sodium channels at the second Ranvier node are closed because they have not undergone depolarization yet. This sequence is very important. We also see the importance of deactivation here: it is there so the action potential cannot go back because there, the channels cannot re-open. So, the action potential can only travel in one direction: from the soma to the axonal terminals. Let us look at the next stage. Now, we are recording at the second Ranvier node. And therefore, we have this action potential at the second Ranvier node. We are at time T + delta T. Those at the first Ranvier node are inactivated because they were just open while those at the initial segment are re-closed. The action potential cannot travel back to Ranvier Node 1 because the channels are inactivated and those at the initial segment are too far and the current lines are too weak. So, it will continue to propagate like this towards the axonal terminals without weakening because the action potential is recreated at every Ranvier node. At each Ranvier node, the sodium channels re-open and re-establish the action potential. The action potential is an all or none signal, all or none because either the sodium channels are open or they are not open. They open all at once one after another because every time they open they bring sodium ions in that depolarize the membrane, and

this depolarization makes even more sodium channels open because these channels are voltage gated. So, they open extremely rapidly one after another. The action potential propagates without decrementing because it is recreated at every Ranvier node. It is referred to as sodium action potential because its depolarization phase only depends on sodium current. It initiates at the initial segment because this is where there is a great concentration of sodium channels with an opening threshold that is lower than in other parts of the neuron, such as the soma. It propagates in the axon and not in the dendrites because dendrites mostly, in the greatest majority of neurons, have almost no sodium channels. So, once started in the initial segment, it will propagate in the soma and stop there. And in the other direction, it will travel down the axon and the axon collaterals all the way to the axonal terminals. What is its role? It is to achieve the fusion of vesicles containing neurotransmitters to the membrane in the axonal terminals and the release of the neurotransmitter. Therefore, the essential part played by the action potential is to bring about synaptic transmission. This is what we are going to see in the next chapter.