Computational Neuroscience

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Week – 02

Lecture - 07

Lecture 07 : Patch Clamp Measurements

Hello, welcome to our seventh lecture in our computational neuroscience course. So, today we will be going over the patch clamp recordings that are crucial in our understanding of how excitable membranes work and how the properties of the membrane finally, give rise to activity which is action potentials in neurons. And so, we had briefly earlier I mean discussed that spiking is the major currency of computation in the sense that spikes are the basis of computation and spikes are the events that allow carry forward or communication between neurons. And, but underlying the spiking is the changes in membrane potential which are also extremely important in terms of understanding how spiking happens. So, what what what we mean by underlying action potentials is that things that are sub threshold in terms of the membrane potential that it when the membrane potential does not cross threshold which causes a spike when once the membrane potential crosses the threshold. So, things in the membrane potential that go on are also equally important in terms of understanding the basic mechanisms behind it.

And so, in order to access information about that membrane potential we have to take help of a specialized techniques which is what we will say as patch clamp. So, let us briefly review our previous understanding about how we measure activity from the brain. So, as you may recollect from one of the earlier lectures fMRI and EEG are used noninvasively and are useful for recording activity in humans, but their temporal and spatial resolution are not very close to what we want to achieve in terms of when we try to understand computation by single neurons. So, first of all we cannot gain access to single neurons here and so, we need to I mean, but still there are many many advantages and a lot of information is available from fMRI which is a example shown on the left hand side which is an indirect measure which provides an indirect measure of activity as you may recollect.

Similarly we have single unit recordings which is spiking when we put in a metal

electrode very close to a neuron, but extracellular and that picks up the signatures of action potentials happening in the neuron, but the this electrode being outside the membrane it does not it cannot pick up the small fluctuations that is the V_in minus V_out which is the difference in the in the potential inside and outside the neuron, but when there is a large excursion of this membrane potential then that is picked up because of a change in the current flow outside the neuron. And similarly we also talked about multi electrode arrays where we have essentially a parallelization of the single unit recordings and we also discussed how we gain access to multiple neurons simultaneously using two photon calcium imaging and here as you can see which we mentioned that here we have a whole cell recording that is a patch clamp recording where you had this is a neuron outline I mean filled with a calcium dye and you can see the outline of the pipette which also has the dye. So, inside the pipette there is the dye. So, here we are clamped on to the neuron that is what we are trying we will be trying to understand how this works. So, we are clamped on to the neuron and we are measuring the electrical, I mean we are measuring the voltage changes occurring in the ($V_{in} - V_{out}$) which is the voltage trace that is shown on the right hand side and simultaneously we are imaging here which is showing the calcium trace that is the change in calcium fluorescence.

And so as you can see with every action potential there is a jump in calcium, but this this change in membrane potential does not is not necessarily reflected in this calcium chain. So, we have to take recourse to more finer measurements in this case maybe with voltage sensitive dyes and can do these kind of recordings now. But in order to go forward and understand how the models of neurons have developed for for our in the entire field of computational neuroscience we have to need to gain information of the membrane potential of single neurons through patch clamp recordings. And as you may recollect here is an example where we have a glass pipette that is attached that is somehow attached on to the neuron and finally, we access the membrane potential in response to different current injections. So, this is an important process that we need to understand when we go into developing models of neurons the for spiking like in the Hodgkin Huxley equations.

So, what exactly is happening here? So, this is a more detailed version of the recordings of the patch clamp technique that we have talked about. So, in the top here as you can see that there is a recording pipette that is attached to the surface of a neuron. Essentially these patch clamp recordings are done with a pipette that is pulled to form this kind of an electrode a sharp electrode actually it is not too sharp micron or so and very smooth. So, the pipette is gradually lowered into the tissue if this is done on brain slice then we can actually observe the pipette going into the tissue and we can also observe in differential image contrast methods we can actually observe the neuron and

see the pipette going down and touching on top of the neuron. So, when we have a brain slice live brain slice we can do these experiments in this manner where we can actually see the neuron on which we are patching that is we guide our electrode accordingly.

Similar, but these techniques are also used in vivo that is in an intact animal when we are doing experiments where we have to go in blind and there we have to take recourse to current injections and what we are seeing in response to understand whether we are on a neuron or not and it is again sort of a fishing kind of experiment, but those are extremely valuable data that is generated with those kind of experiments. So, ok so once we lower the electrode into the tissue and we are on top of a neurons surface as we can see then we lower the electrode tip it is held usually at a particular angle that is preferred in that location and there is an indentation made on top of the on the surface of the neuron on top I mean you can actually see it in the DIC in the microscope. And then with mild suction as shown here the membrane is pulled up to coalesce to the sides of the electrode here. So, they coalesce and attach on to the sides of the electrode and this becomes a tight contact point that is there is hardly any leakage I mean ideally there should be no leakage of ions through here. So, in this condition it is actually what we call "cell attached".

Cell attached in the sense that we are tightly on to connected on to the membrane. And so this is a this is another form of recording where which is called cell attached recording or sometimes if the connection is not too hard it is also connection when the attachment here is not too tight it is loose patch recordings and so on. So, what we would want is to go whole cell and that is we break in that is with a strong pulse of suction now the membrane is broken. So, now the inside of the electrode or the patch pipette and the inside of the neuron become connected entirely and this condition is the condition where we have whole cell condition. And so if we want to do cell attached recordings where we can record from the neuron the action potentials perfectly from one neuron as opposed to when we are extracellular we do not know whether we are which neuron we are recording from.

In this case we are recording from exactly one neuron that cannot be any other signal picked up on there because the signal will be so large. So, now on this to do that recording in inside this we would need the solution to be what is present in the external medium. So, before going in we have to make sure whether we are going to do this cell attached recording or we will go to the whole cell recording. And accordingly fill up the pipette with the external solution in this case or the internal solution in this case. What we mean by internal solution and external solution is essentially the the the aqueous medium inside the cell has different ion concentrations and different molecules present different chemicals present and so ah we need when we are breaking in we want the

inside of the electrode to be exactly as the inside of the cell.

And here obviously, if we are not breaking in we want the outside region to be just like the external medium and so we would fill up the electrode with the external solution. So, as you can remember that the ion concentrations particularly for sodium potassium that we have discussed have to be ah exactly opposite in nature whether when it is when the solution is inside versus when the when we are talking of the external solution. Similarly, there are few other configurations of recording as you can see here this is what we call inside out recording ah where the inside of the neuron ah inside of the membrane is on the outside of the pipette as you can see. Ah here it is an outside out recording where you break it in and actually coalesce and make the outside inside of the membrane attached towards the inside of the pipette. And so accordingly it is the the internal solution has to be adjusted.

So, these are other there are few other variations also for and it depends on the question that we are asking ah and we accordingly apply the techniques. So, we will mainly be focusing on this whole cell recording because this is what we will be using ah in our ah in our discussions later when we are trying to model neurons and their excitability based on their sodium and potassium channels. So, as you might remember ah we ah we ah can ah model the membrane of a neuron if we think of just the membrane it is essentially mainly a capacitance and there are elements in the membrane like ion channels which we will discuss in detail later on that allows flow of ions through the membrane that provide a conductance. So, the equivalent circuit ah this is a very detailed circuit ah, but it shows all the elements when we are ah doing a patch clamp recording. So, this circle here represents the neuron cell body and this is the electrode.

And here we have a resistance that is our leak ah which is essentially ah we want that this to be of the order of giga ohms only then we will get good quality ah patch recordings. So, that is when the leakage through this corners that we talked about leakage through this region ah or this region this leakage is minimal. So, 10 to 100 giga ohms is probably ideal in order to ah do these recordings ah in the best possible way. There are ah there is a capacitance of the electrode also and here is the membrane capacitance these are the conductances of the channels that allow or the paths or ion channels specifically that allow current to flow through the membrane and this is the external ground. On top ah of on the side is what is basically ah denoting the patch clamp amplifier.

So, in in this patch clamp recordings there are essentially two modes of recordings that is what we will call the voltage clamp or another is the current clamp. So, for what we mean by the voltage clamp is that we have we set a voltage at which the membrane

potential will be held or clamped. So, that we will call the V_{clamp} let us say and that command voltage is provided here through ah a generator and essentially ah with a measurement of the current or rather we inject currents until we match the voltage. So, that is the command voltage. So, we are constantly with the amplifier able to measure the voltage across the membrane and we are injecting a current still it reaches the applied command voltage.

So, that in this in this output these two are essentially matching that is the voltage is matching the command voltage. So, that is the membrane potential is being set to whatever voltage we want it to be set the clamping voltage or V clamp and the current that we are injecting at that point to maintain the voltage or the current profile to maintain a voltage over time is essentially the current that is actually flowing across the membrane because the total current has to remain 0. So, the current that we are supplying if we can measure that that is equivalent to the current that has to flow across the membrane to maintain that voltage. So, in a voltage clamp method more we can measure the membrane current I is measured. So, ah and accordingly if we think of the other way around we can do the exact same thing where we set a current that is there is a fixed current flowing across the membrane and we measure the voltage required to maintain that current and or the voltage profile required to maintain that current or maintain that current profile level.

So, and so basically we are able to measure the voltage across the membrane in response to a current that is being supplied across the membrane in the neuron. So, these are the two modes that we need in order to go forward when we talk of how neurons behave when there is a current injection through inputs on to the neuron. So, to make it a little more clear. So, here are examples where this is a voltage clamp experiment. So, essentially this is from the squid giant action which we will talk about in more detail in later lectures.

So, this is the membrane potential at rest. So, this is V_{rest} at around minus 80 millivolt. It is being it is set there. So, the resting membrane potential as you have as you recollect in the in the beginning of our lectures where we said that that is the voltage that allows no net current across the membrane and the neuron stays the or the membrane potential stays there if the neuron is left undisturbed. So, that is the resting membrane potential and this is where the clamp is being set.

So, that is in this case let us say it is around minus 20 millivolts and it is held there for 15 milliseconds and then we come back to the the clamp back to the V_{rest} which is minus minus 80 millivolts. So, during that time with the patch pipette if we are recording the

current here are 7 successive repetitions of the experiment. So, in time if we go forward there is again another clamp like so which is again 15 milliseconds and the current corresponding to that is shown in the second trace here. Similarly the third pulse here the current corresponding to that is shown in the third trace here and so on. Now, the average of all these 7 turn out to be like this and one additional thing here is that in this particular case there was Cesium ion that was there in the in the bath.

So, what Cesium ion does it blocks any voltage gated potassium channels and there is no potassium currents. So, if we think that the sodium ion currents and potassium ion currents are the major currents that flow across the membrane then with the Cesium ion in there what we are seeing is the current through sodium ion channels and the average of 7 repetitions is like this. So, here are 2 things that are important to learn. So, why this is sodium and all that we will discuss in a in a later lecture, but let us focus on the points to be noted here that is when we apply a voltage clamp the nature of the current flow across the membrane is not deterministic in single trials. That is in every trial you get a different behavior or that is there is inherent randomness in the flow of the current.

But if you think of an average a large if you have a large number of sodium channels so to speak if these are from a one or very few given the size it is only actually one sodium channel then based on the law of large numbers we can think that on average across the neuron there will be a sodium current which takes this kind of a shape. And so stochasticity or the randomness that is there and the other thing is if you note the sodium current if you assume that it is the sodium current for now we will be assuming that then the current actually drops and then goes back up and goes back to 0 that is to the no current no sodium current situation. So, although we are not changing the voltage that is the voltage is fixed throughout the 15 milliseconds at a minus 20 which is 60 millivolts above rest the sodium current actually I mean the magnitude increases and then goes back to 0. So, this will show us an interesting phenomena about sodium channels later on. So, idealistically I mean in in a in a macroscopic macroscopic way when we average and incorporate all the possible I mean incorporate an estimated actual number of sodium channels then we can estimate the sodium current to be something that is shown to the right on top and it is a smooth curve again as you can see this is going down and then going back up to 0.

This is basically this is from the clamp being reset to the voltage there is capacitive current that is being generated from from because of the membrane. So, another important point here is that we have the current going negative this is a matter of convention the current going negative means that it is current into the neuron. So, when the this will be our convention throughout in this course that and current injected into the neuron that is positive ions going into the neuron will be depicted with a negative

current or with a negative sign and . So, why is I mean as you can think the sodium ions should be flowing inward if we change the resting membrane potential from minus 80 to minus 20 because we are I mean if there are parts available for sodium channel sodium then the when it is not at rest or it is away from the reversal potential of sodium the current or ion will flow in a manner such that the membrane potential is pulled towards the reversal potential of the neuron. So, now with different membrane potential sizes or with different weak clamp sizes we can calculate sort of the amount of current magnitude of the current which is equivalent to how likely the sodium channels are open.

So, based on this we come up with the idea that there is voltage gating that is a voltage dependent sodium current or so voltage dependent the voltage dependent activation of sodium channels. Similarly if we block sodium in this model that is using Tetrodotoxin which blocks sodium channels then we are left with potassium currents and the same exact experiment is being done here. And what we see here is in here is again the stochasticity in the nature of the current and it is varying throughout the time period that in this case 40 milliseconds and the average is such that it grows up and saturates and stays there. So, unlike the sodium current if we assume that this is the potassium current based on other ideas then the the potassium current is distinctly different in the sense that the there is no drop in the potassium current magnitude drop in the potassium current during the voltage clamp unlike what we saw in the sodium case where the sodium current dropped and then rather like this and then went to 0. This on the other hand stays high until the clamp is brought back down to V_{rest}.

So, what we see here that one difference and the other obviously has drawn here that the potassium current is positive which is the current is going out of the neuron . So, outward current is positive current and so the idealized curve in the macroscopic scale here based on the estimated number of potassium channels can be seen here and again by varying the clamp voltage we can get the the probability of the current I mean probability of the potassium channel allowing potassium ions to flow through at different voltages. We will discuss the probability part in much more detail when we talk about the Hodgkin Huxley equations. So, with this we will now move on to a more detailed discussion of ion channels. So, that later on when we come to the Hodgkin Huxley we can put all these things together. Thank you.