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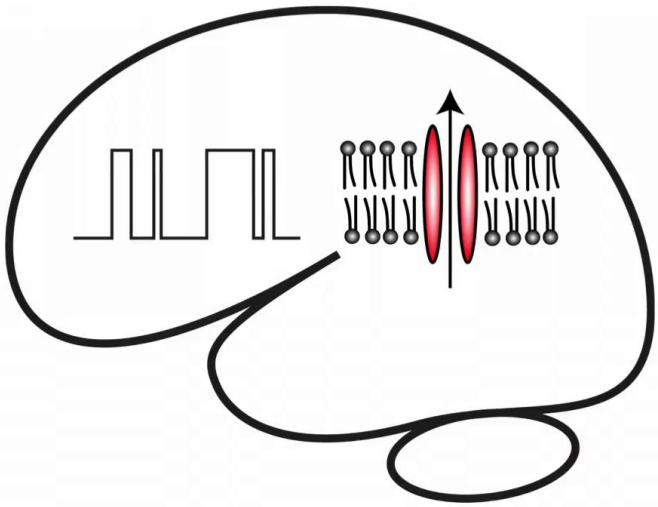
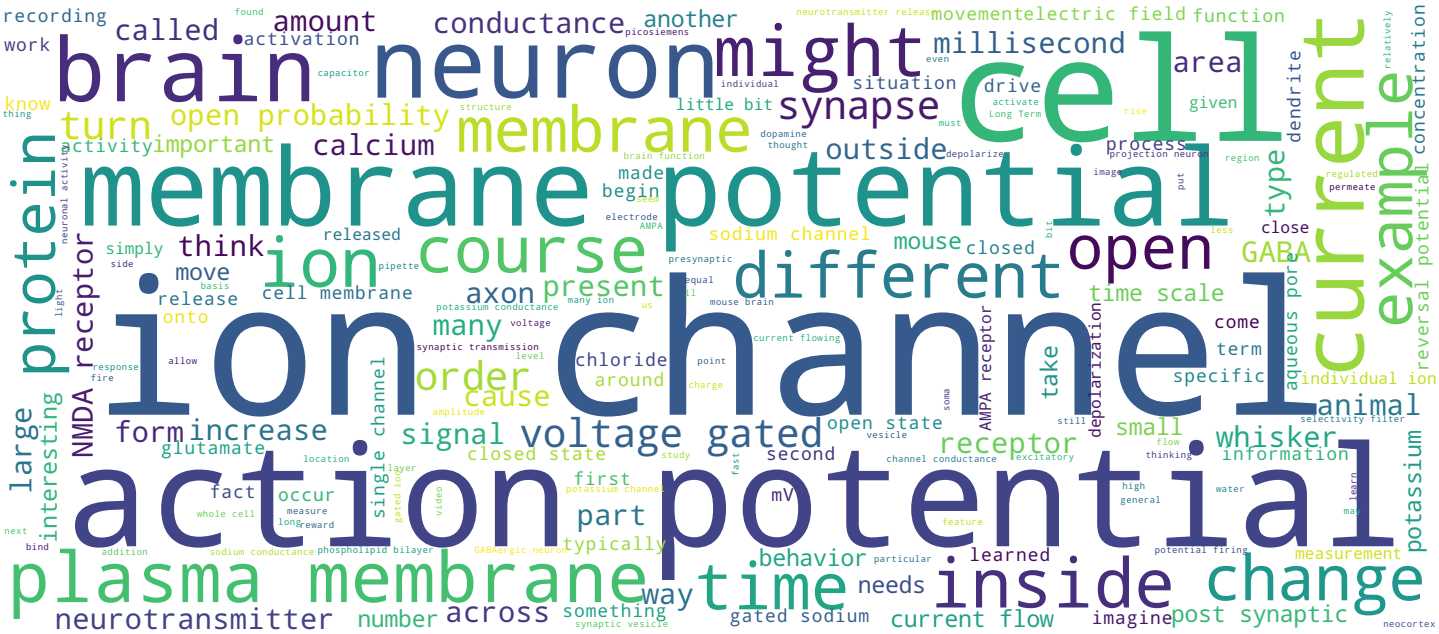


# 1.3 Ion channels

## Cellular Mechanisms of Brain Function

Prof. Carl Petersen

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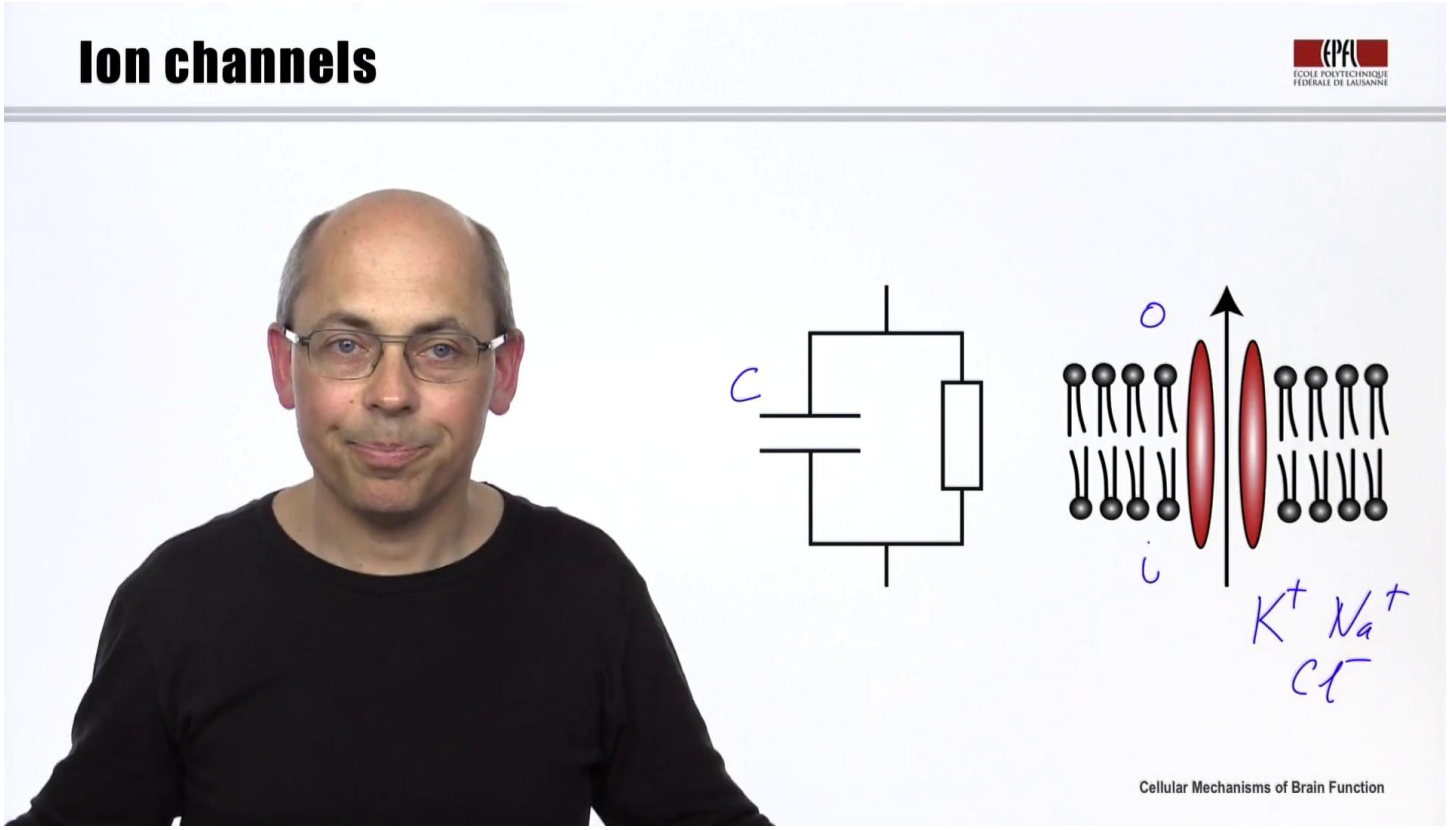
# Ion channels

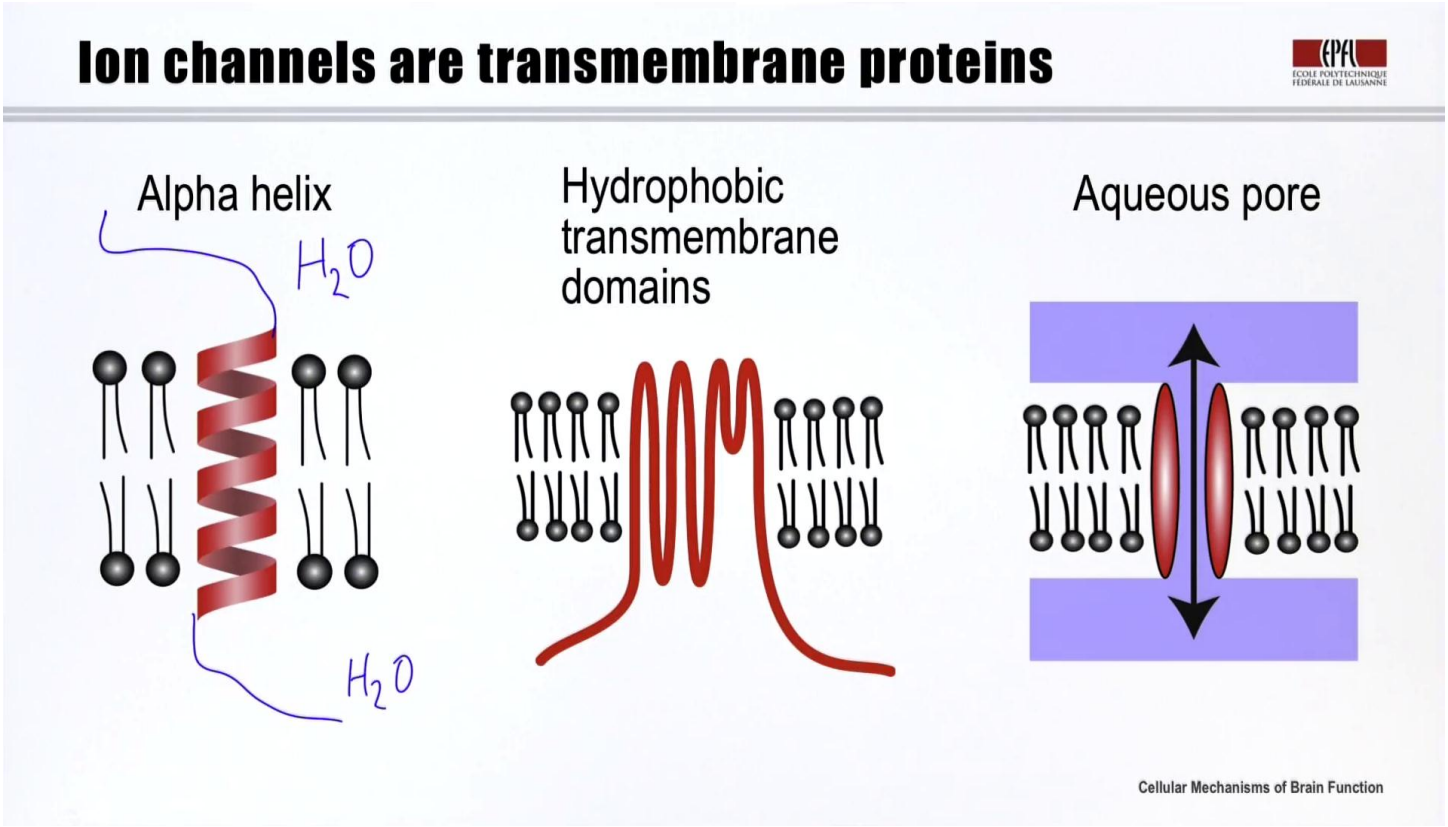



In the last lesson we learned that the cell membrane was made of a phospholipid bilayer that was impermeable to ions. The movement of ions, of charges, from the outside to the inside of the plasma membrane would make large changes in the electrical field but make small changes to ionic concentrations. Ion movement across the cell membrane can therefore give rise to large membrane potential changes, but, as we learned, the phospholipid bilayer isn't by itself permeable to ions. So, how do ions move across the cell membrane? How does membrane potential change? Inserted into the phospholipid bilayer that we learned, acts as a capacitor, are other elements: proteins, transmembrane proteins -- like this red ion channel-- that are inserted inside the plasma membrane, traverse the plasma membrane reaching from the outside to the inside and they have an aqueous pore in the middle through which ions like Potasium, Sodium or Chloride can traverse through these ion channels and move ions from one side of the plasma membrane to another.

Notes

Summary

0m 03s

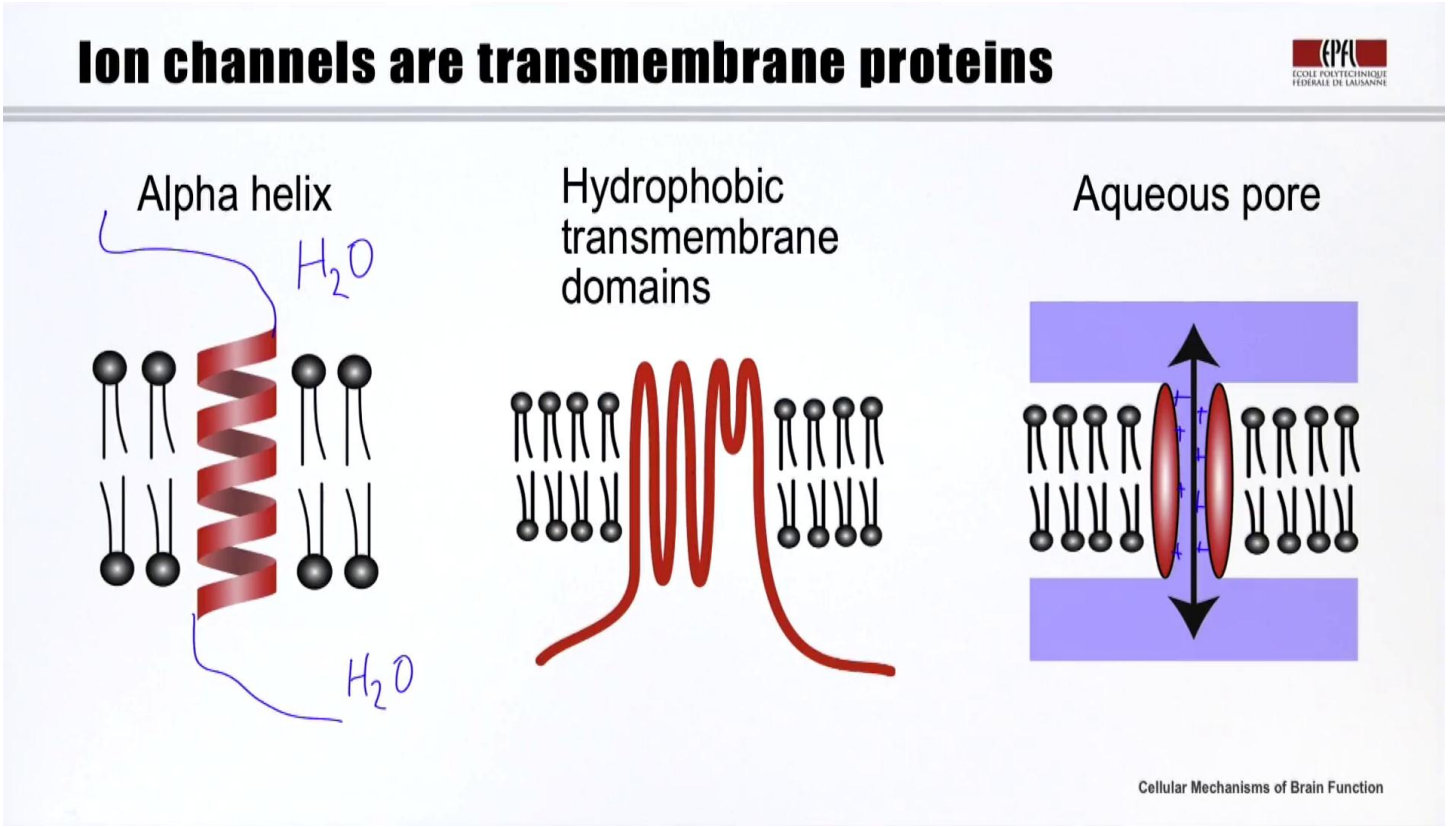



Ion channels are transmembrane proteins That is, they're made of a string of amino acids in a simple sequence. In order for the protein to cross the cell membrane it needs to have some specific features: It needs to have a part outside that likes water, because the inside and outside solutions are made of water. It needs to have an area here that is able to cross a lipophilic area because this is where we have the hydrocarbon chains of the phospholipids, and this area here is very hydrophobic, and of course on the other side it again needs to be able to interact with the aqueous environment, so it has to have hydrophilic aspects and a hydrophobic part here where actually crosses the membrane. And it turns out that for all relevant transmembrane proteins, the protein forms a so-called alpha helical structure where it can traverse the plasma membrane. In the alpha helix, the more hydrophilic parts of the amino acids, the charged parts, can hide on the inside of the alpha helix whereas the lipophilic parts of the protein can stick out and be happily interacting with the hydrocarbon chains. So, alpha helices are the ones that actually cross the plasma membrane.

Notes

Summary

1m 34s



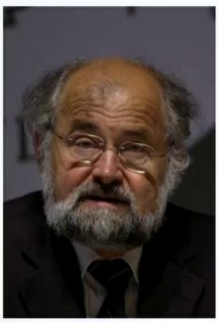

A typical ion channel doesn't just have one alpha helix but it might, for example, have six as it's drawn in this example here where we have one, two, three, four, five, six transmembrane regions and another part here that just dips into the lipophilic part of the plasma membrane. Typically, ion channels don't just have one of these but are associated with multiple sub-units that come together and form the ion channel itself, and one specific part of the ion channel, for example here, might then have some charged areas down the middle which allow it to interact with the aqueous pore of the ion channel. So, the ion channel is a complicated structure. It needs to have a part that likes water, that is present both on the outside and the inside. It also needs to have a part here that interacts nicely with water and so it's also a relatively polar environment here and that then allows the ions and water to flow freely across the plasma membrane through these ionic protein channels.

Notes

Summary

3m 06s

# Patch-clamp recordings of single channels



Erwin Neher and Bert Sakmann developed the patch-clamp recording technique allowing measurement of single channel currents (Nobel prize 1991).

Cellular Mechanisms of Brain Function

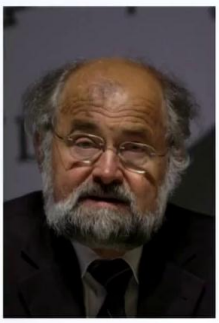
In the 80's two remarkable scientists, Erwin Neher and Bert Sakmann, developed the so called patch-clamp recording technique which allowed them from the very first time to measure the function of single ion channels, in fact, the first time that the function of an individual protein was studied in real time at a millisecond and microsecond tempo resolution. Erwin Neher and Bert Sakmann used glass recording electrodes, tips of about one micrometer in diameter filled with an ionic solution and attached to an electrical amplifier where they could differentially meausure currents. They could fix the potencial inside the patch electrode relative to the bath solution and measure the current flow inside the electrode. They moved this electrode up against a cell, they sucked gently here, and they found that they could suck a little bit of plasma membrane inside the electrodes -- This is still the glass pipette here -- and the remarkable thing that they discovered was that the electrical seal between the inside of the pipette and the outside solution was extremely tight and that the current flow, the leak around the seal here was on the order of 1 Giga-Ohm and going up to something like 10 Giga-Ohm.

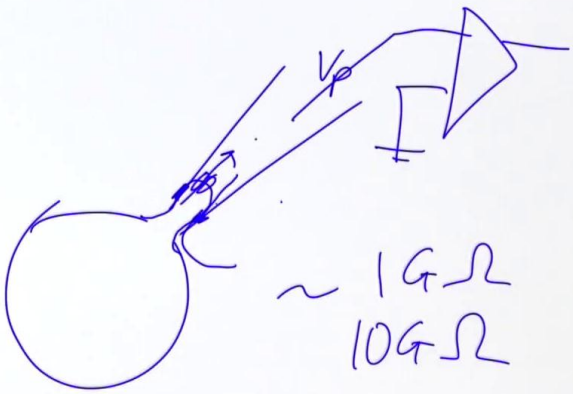
Notes

Summary

4m 14s

# Patch-clamp recordings of single channels






Erwin Neher and Bert Sakmann developed the patch-clamp recording technique allowing measurement of single channel currents (Nobel prize 1991).

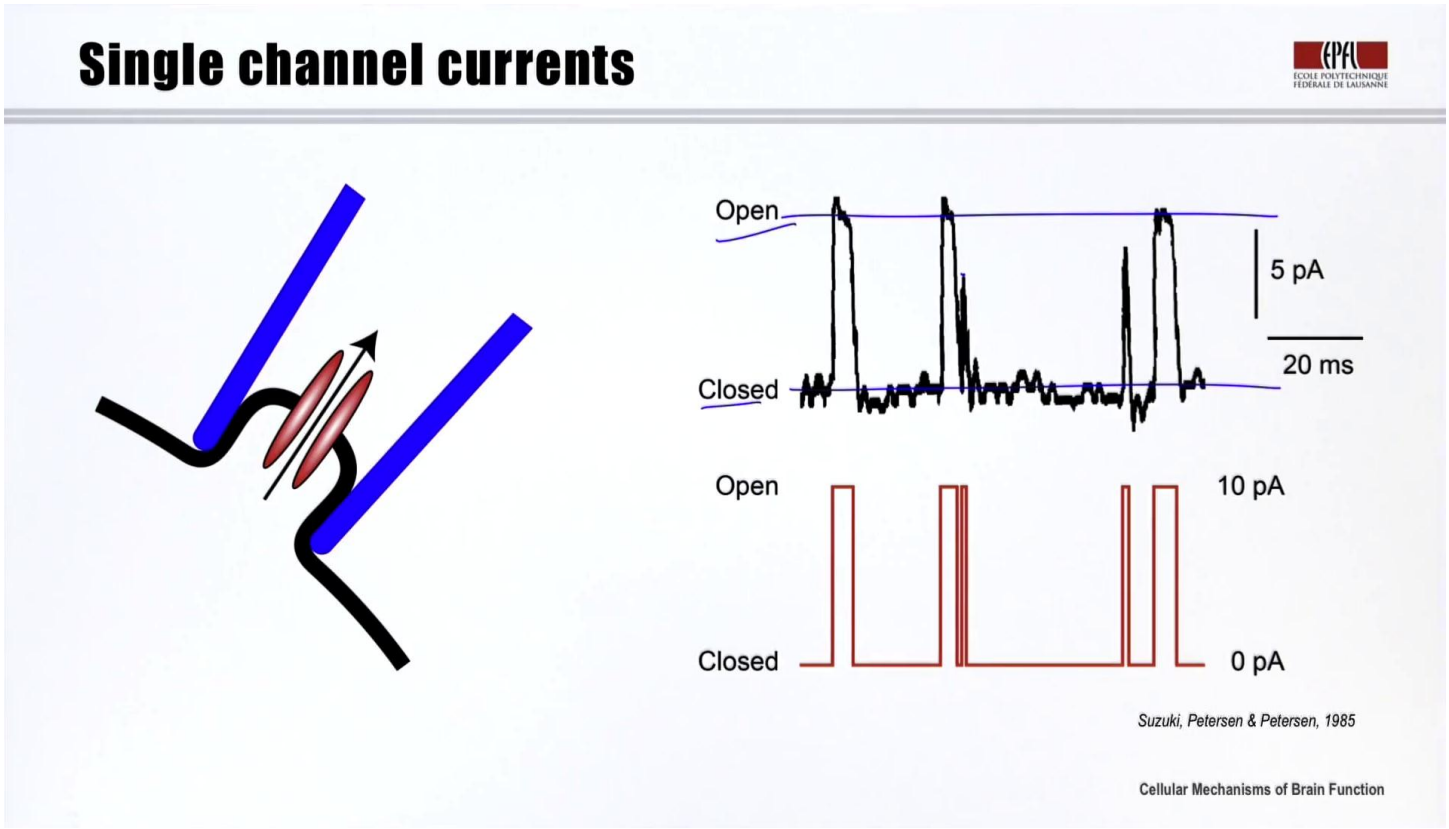


So, an enormously tight electrical seal was built here by the glass and the cell membrane for reasons that it's still poorly understood, and that very very tight electrical seal here allowed them to study the flow of ions across individual ion channels sitting inside this bit of patch of membrane that they sucked into the pipette and of course, they connected this to amplifiers, so they could measure the current flow through the pipette.

Notes

Summary

5m 50s




When they made these measurements they saw several remarkable features. The most remarkable one was the unitary opening and closing of ion channels. Ion channels don't open part way, a little bit, but they flip between two states: They can be in a closed state or they can be in an open state and it seems that the protein rapidly changes from a closed state to an open state, stays open for a while, closes, stays closed, opens, opens here so breafly that we don't even fully resolve it in this recording, it's closed again, brief opening, slightly longer opening. And these are two well defined states: a closed state and an open state, and here it's analyzed in a binary digital form down below.

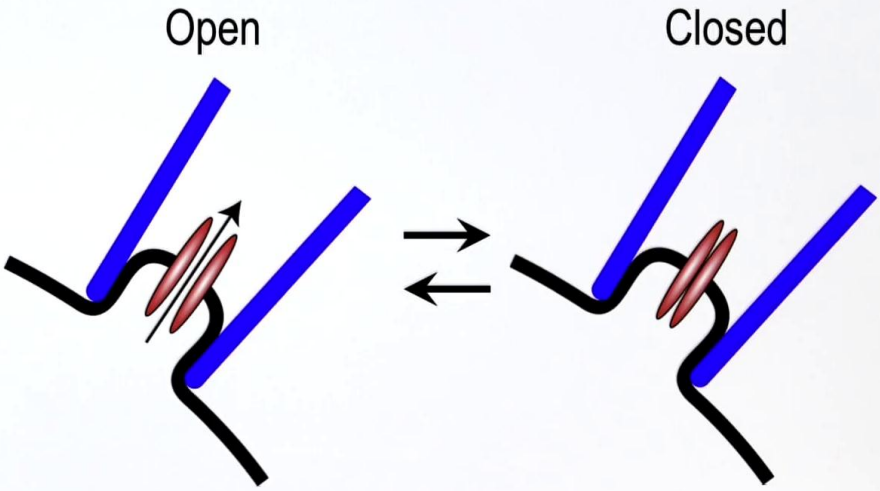
Notes

Summary

6m 22s

# Open and closed states

Single ion channels rapidly change protein conformation between open and closed states.



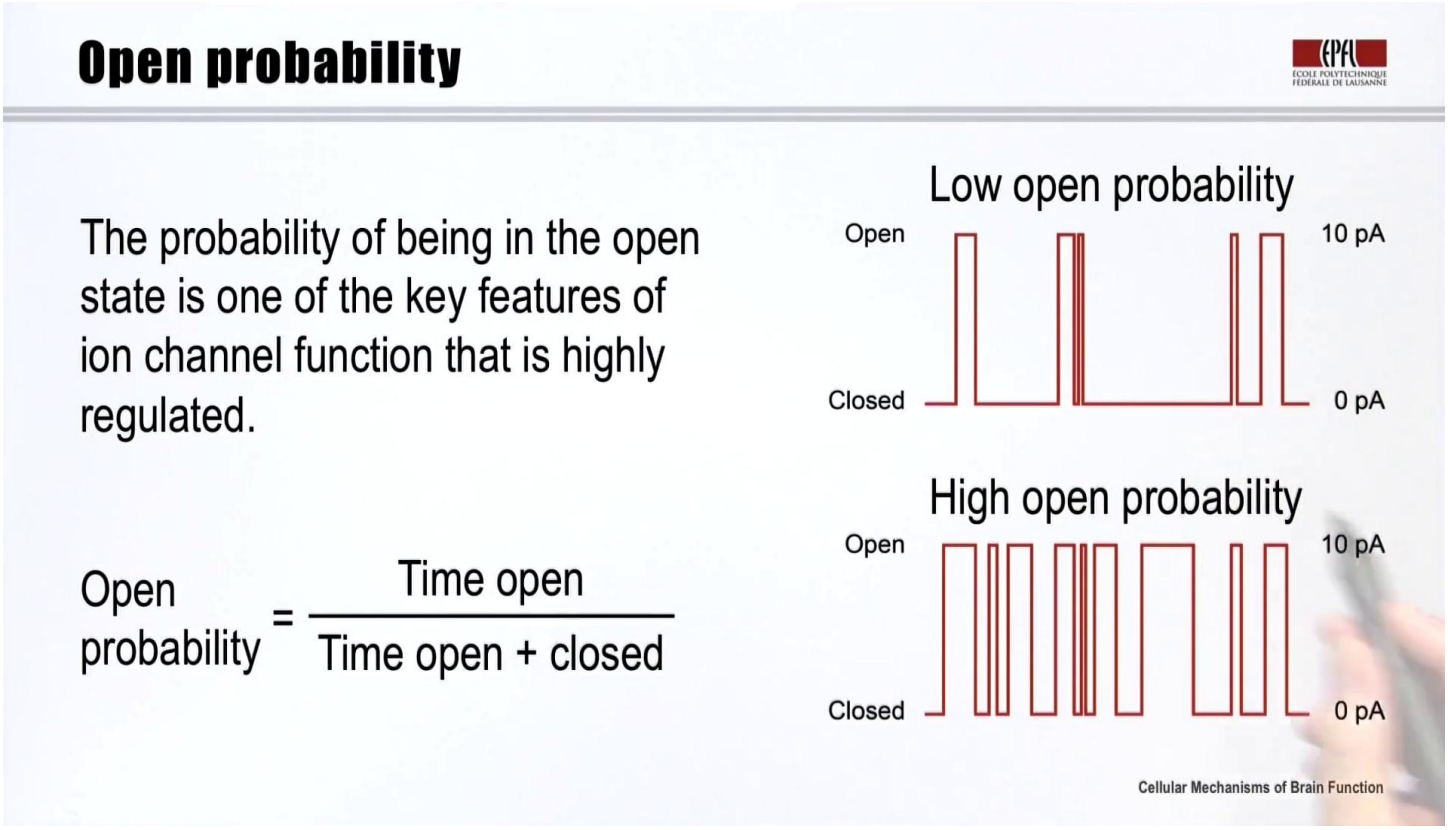

Cellular Mechanisms of Brain Function

So, ion channels transition between open states and closed states, and they can do so with different rate constants that then determine how long they spend in the open state, how long they spend in the closed state and the transition itself from open to closed and from closed to open takes place on a microsecond time scale. Extremely rapid changes in the protein structure open and close that ion channel and allow it to permeate ions or to have no conductance whatsoever.

Notes

Summary

7m 11s




The probability that an ion channel is the open state is one of the things that is most cranky regulated in relationship to ion channel function. Phosphorylation of the ion channel, changes in the electric field across the plasma membrane, changes in the concentration of different ions, and many other features can change the open probability of an ion channel and can change it on a variety of time scales from hours to minutes, to seconds, to milliseconds and microseconds. Open probability is highly regulated and forms the most interesting and convenient way to regulate the function of individual ion channels. We can calculate the open probability of an ion channel simply by summing up the amount of time it spends here in the open state and dividing it by the total amount of time that the measurement is taking place. So that's the open probability, it's the amount of time spent in the open state divided by the time in the open and the closed state. In this example, the probability that the ion channel's open is relatively low, maybe it's about 10%. In this lower example you see that the ion channel spends much longer periods of time in the open state than it did up here so the open probability in this recording might be about 50%.

Notes

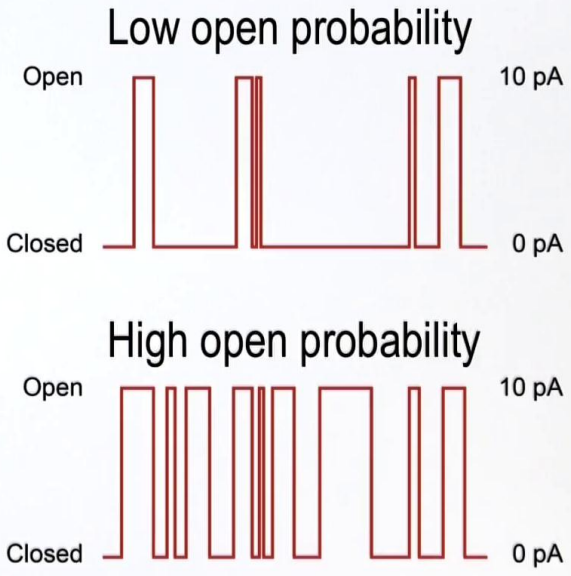
Summary

7m 47s

# Open probability

The probability of being in the open state is one of the key features of ion channel function that is highly regulated.

$$\text{Open probability} = \frac{\text{Time open}}{\text{Time open + closed}}$$



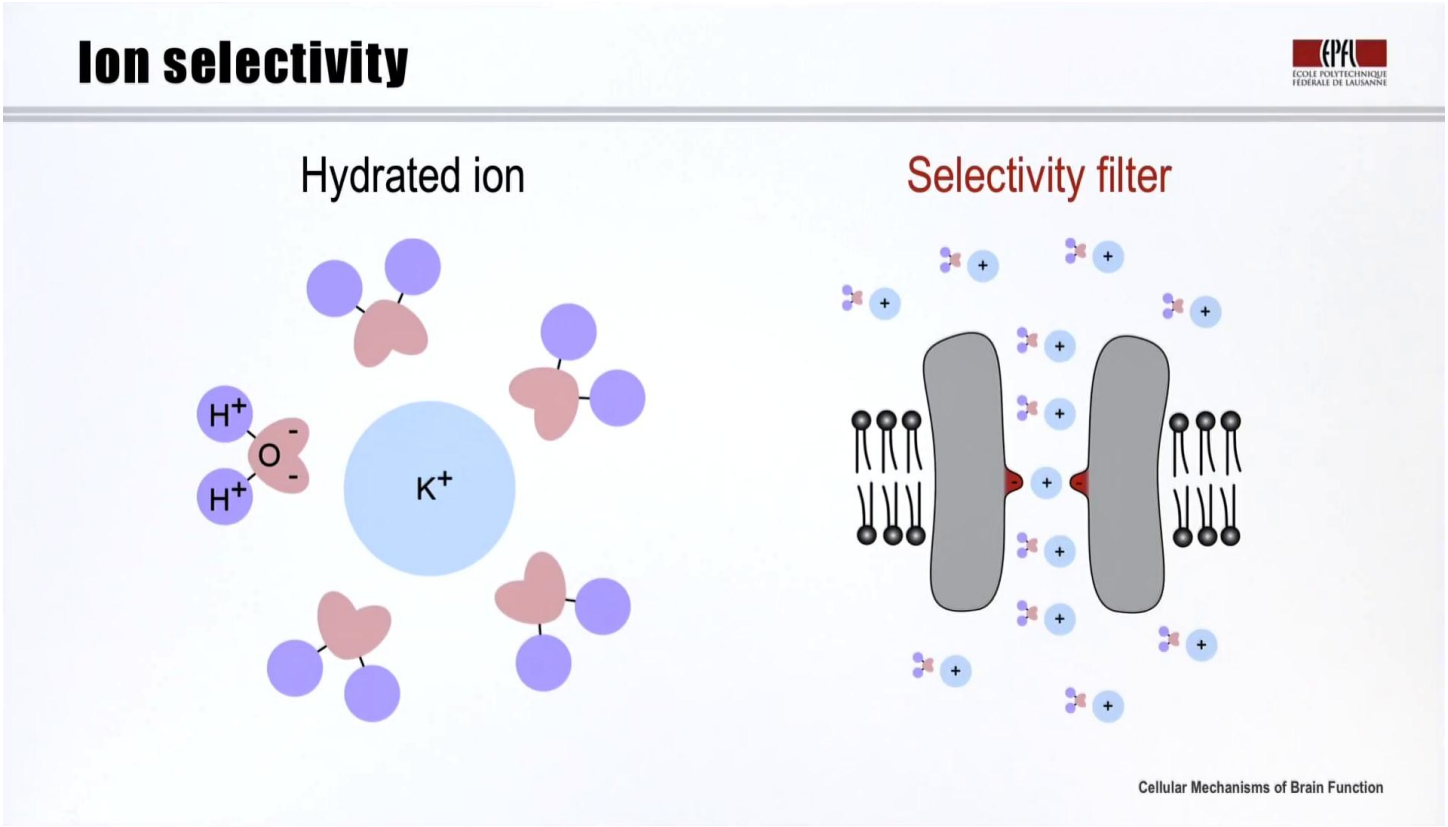

Cellular Mechanisms of Brain Function

And, as I mentioned, ion channels can rapidly transition from high open probability to low open probability states through, for example, different signaling mechanisms inside cells.

Notes

Summary

9m 12s

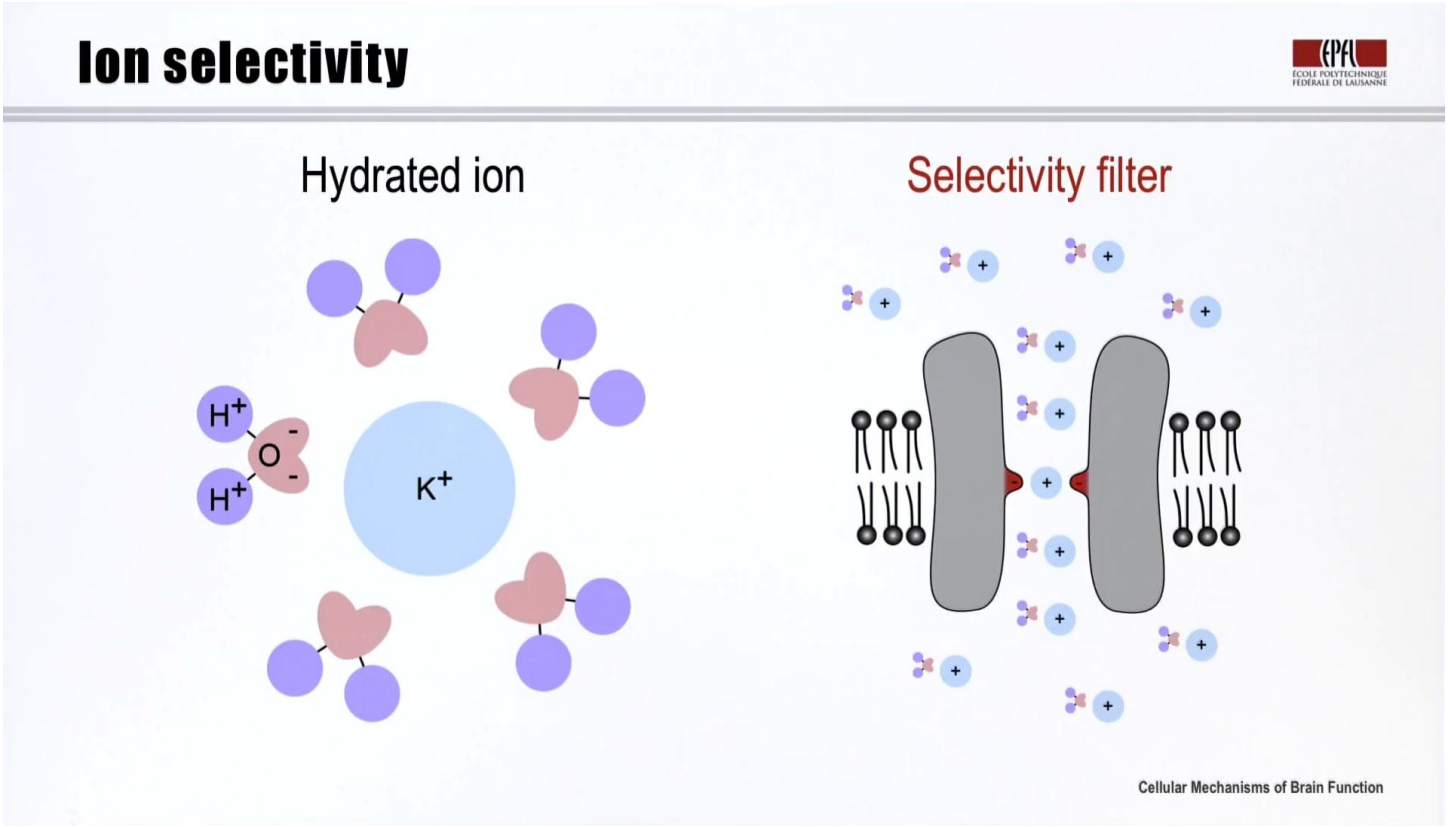



Another extremely important feature of ion channels is that they don't just let any ion through but very often they're highly selective for one specific species of ions. So there are, for example, ion channels that specifically allow sodium to permeate Different ion channels that specifically allow potassium to permeate, others that allow chloride to permeate, and so on. In order for that ion selectivity to occur there must be some specific interactions inside the ion channel. So the ion channel isn't completely an aqueous pore as I previously mentioned In fact, there must be a selectivity filter. And in general is thought to be that at that selectivity filter, in order to get a very specific selection for a given ion, there must be a close interaction. So, although ions are hydrated, of course, in the solution of the extracellular and intracellular solution, and they're also hydrated as they enter inside the ion channel pore at different sides, somewhere in the middle of it, for an ion channel selective for one type of ion, there must be a selective high affinity binding site which specifically says: This ion is the right one, it can permeate and other ions can't.

Notes

Summary

9m 25s

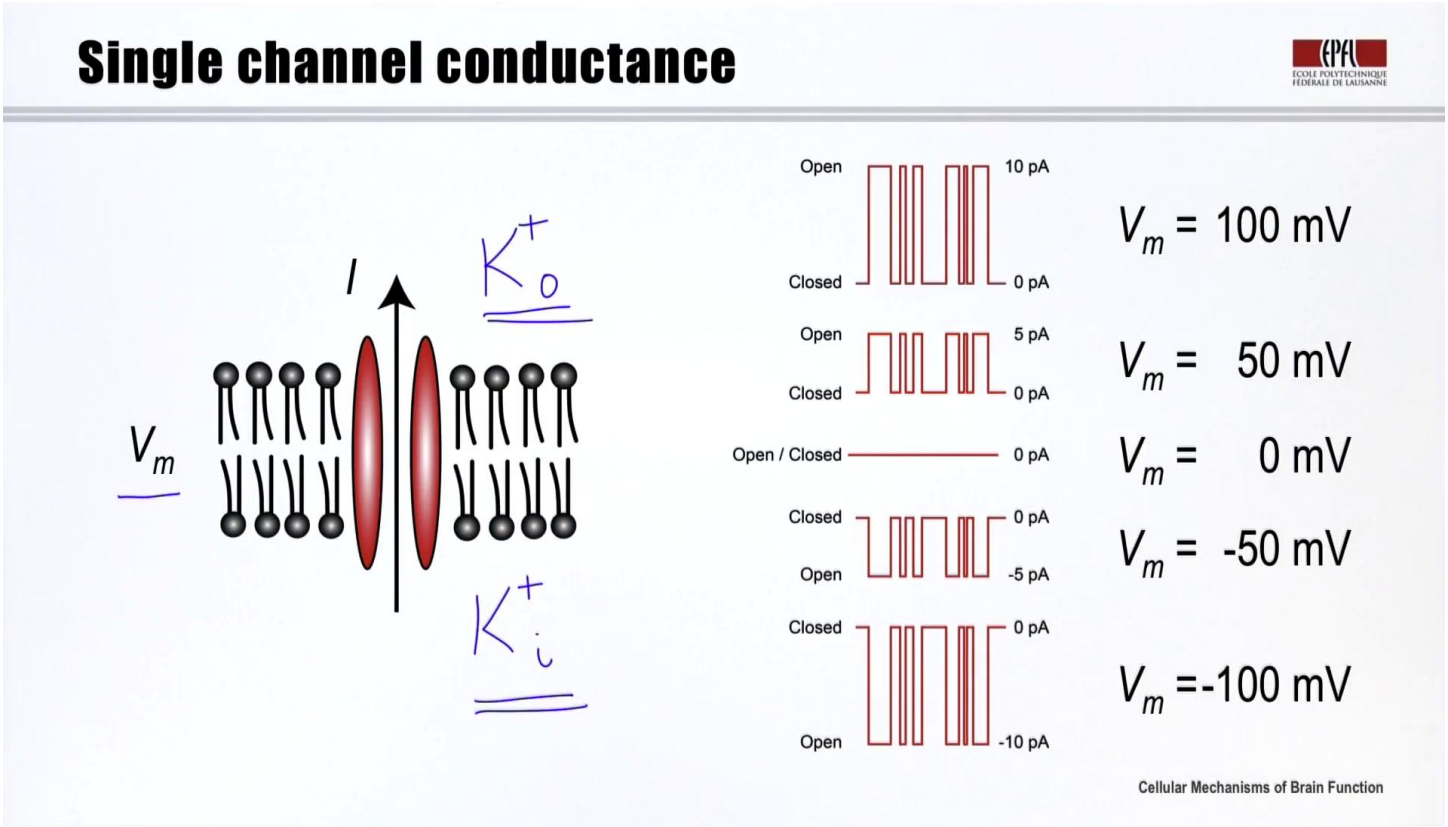



And so, for example, if you want to look at a cation channel -- cations are positively-charged ions -- that selectivity filter would have negative charges in it and would have a specific protein conformation that binds to that ion and doesn't allow other ions to permeate through that specific selectivity filter.

Notes

Summary

10m 48s



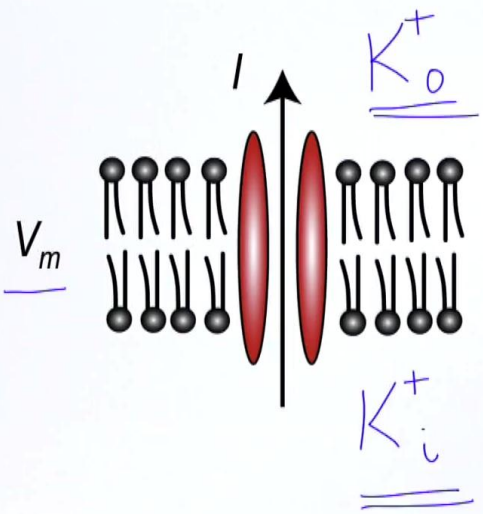

The current flowing through an ion channel is regulated by a variety of different features. We've talked about the open probability, and that's something that's regulated on a millisecond time scale. There are other features that also regulate how much current is flowing through an ion channel. The electric field is one of the key driving forces for how much current flow there is. So, if we imagine that there's a given concentration, say, of potassium, and there's the same concentration on the outside and the inside of the cell, then, the major driving force that'll move potassium through this ion channel will be the electric field across the plasma membrane, the membrane potencial. Equally important in terms of determining how much ionic flux it'll be at a given membrane potential is the concentration of the ions. If there's a much higher concentration of ions on either the inside or the outside, then the probability that that ion will enter in the ion channel increases. And again, we're dealing with the diffusion situation where these ions, in terms of moving through the ion channel, we think of them, basically, as being free hydrated ions and the ability to difuse will, of course, depend upon the concentration.

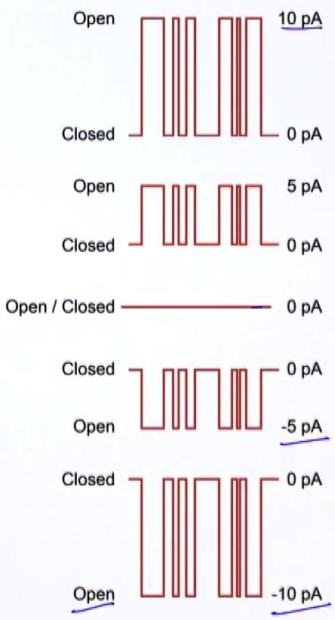
Notes

Summary

11m 11s

# Single channel conductance




$V_m$ = 100 mV

$V_m$ = 50 mV

$V_m$ = 0 mV

$V_m$ = -50 mV

$V_m$ =-100 mV

Cellular Mechanisms of Brain Function

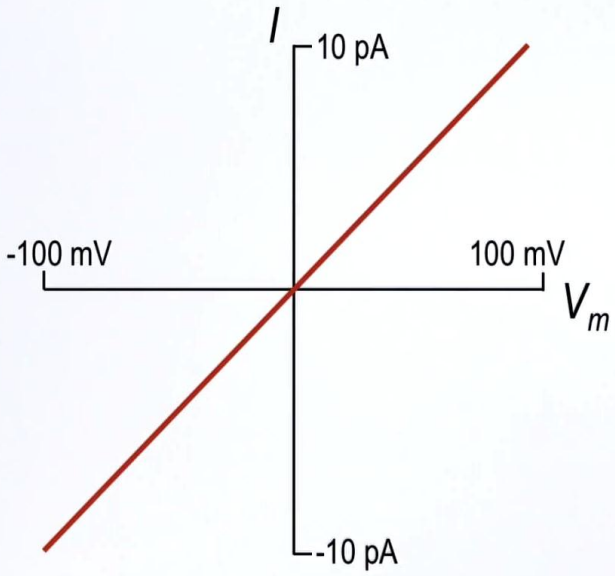
Here we think about the membrane potential dependence in such a situation where we have an equal concentration of the conducting ion on the outside and the inside of the plasma membrane. If there's no electric field, if the membrane potential is zero then there's also no driving force that tries to bring the potassium in or out across this patch of membrane. So, even if the ion channel is opening and closing there will then be no flow of current and we'll get a flat line in our measurement. If we go to very high positive potentials, we get a certain amount of current, in this example 10 picoampere. We go to negative potentials so we invert the electric field and now when the ion channel opens current flows in the opposite direction to where it went before. So we might have -10 picoampere of current. If we now halve the membrane potential, we halve the current. In this example, and it's not true of all ion channels, in this example we have a linear current-voltage relationship.

Notes

Summary

12m 39s

# Single channel conductance




Ohm's law

$V = I \times R$

$I = V \times G$

V, potential; I, current;
R, resistance; G, conductance
$G = 1 / R$

$G = 10\ pA / 100\ mV = 100\ pS$

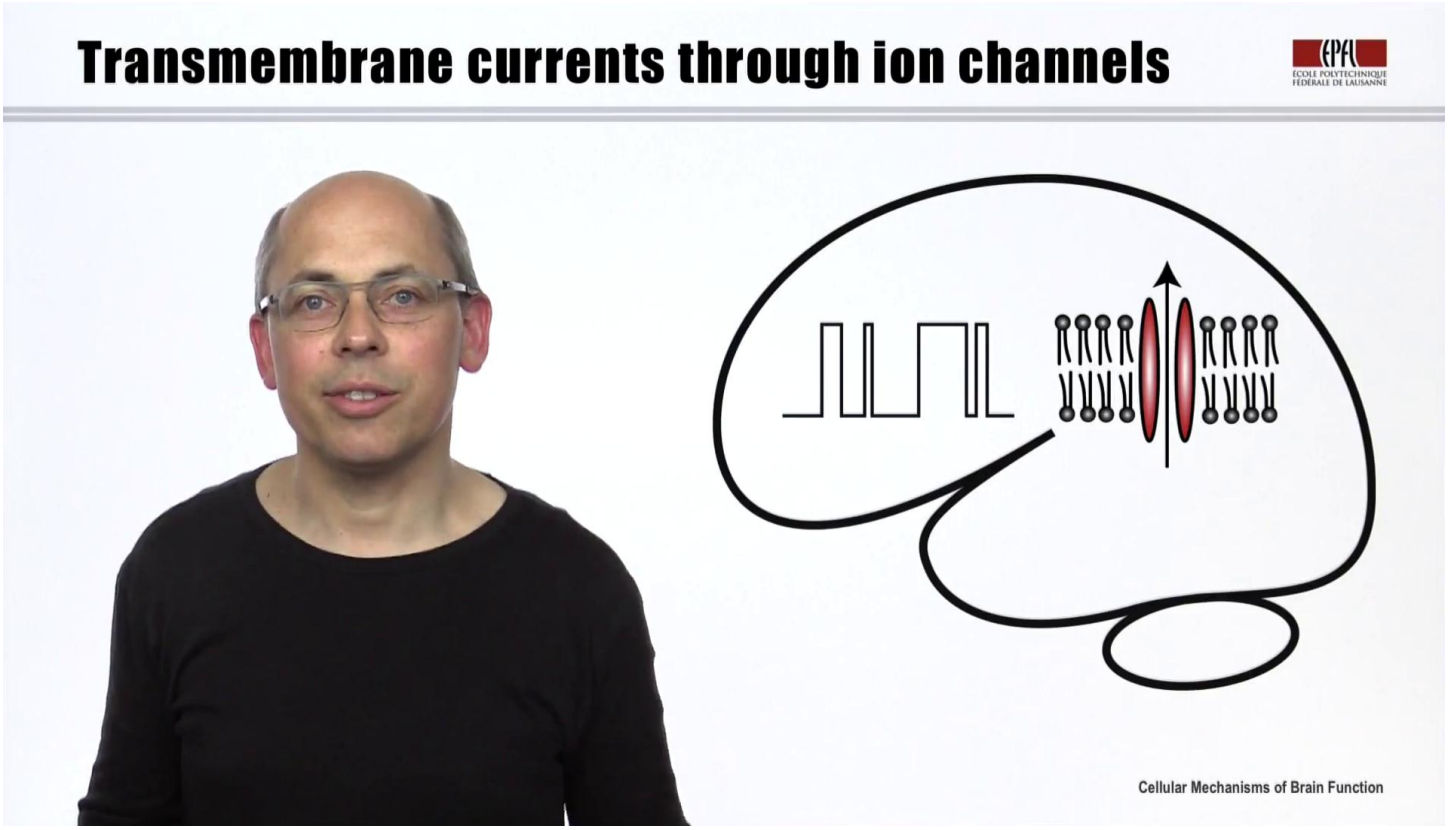
Cellular Mechanisms of Brain Function

At 100 millivolts we have 10 picoampere, 100 millivolts, 10 picoampere, we have a data point, we have 5, 50; we have 00, -5, -50; and -10, -100. This then forms a lineal conductance and we can, of course, calculate the single channel conductance from the basis of these measurements. We can do this using Ohm's Law: V = I xR We can invert that and say that the current flow is equal to the Voltage times the Conductance So, in order to work out the conductance of this ion channel we can say that it's equal to the current flowing -- 10 picoampere -- divided by the potential, 100 millivolts, and that gives us a single channel conductance of 100 picosiemens. It turns out that this is quite a large number. Most ion channels have much smaller single channel conductances. Something like 1 picosiemens or 10 picosiemens perhaps. 100 picosiemens is only for the very largest ion channel conductances.

Notes

Summary

13m 51s




So, we've now learned a number of interesting things about the plasma membrane. In addition to the phospholipid bilayer that forms a capacitor and sets up a strong electric field that can be charged by relatively few ions moving from one side of the membrane to another, we've now also found out that there are specific transport mechanisms that move ions from one side of the plasma membrane to another. And these are largely aqueous pores that allow high speeds of ions to traverse the membrane. They are highly selective, they can be selective for cations or anions, positive or negatively charged ions, and they can even furthermore be selective for very specific types of ions so there are sodium channels and potasium channels that specifically allow the permeation of one species of ions. These ionic movements through ion channels are the basis of how membrane potential can change nerve cells and those are the electrical signal that underline the neuronal computation. In the next few slides, I'd like to go through just a few examples, so that once again we learn a little bit about the ballpark in which we're operating and what the size of currents are, how much ions are moving second by second and how this works as we move from a single channel to thinking about a whole cell with many ion channels.

Notes

Summary

15m 06s

# Some numbers – single channel conductance

A typical ion channel has a conductance between 1 pS and 100 pS.

How many ions are transported per second?

10 pS

$I = V \times G$

$= 100\,mV \times 10\,pS$

$= 1\,pA = 10^{-12}\,A$

$e^- = 1.6 \times 10^{-19}\,C$

$\frac{10^{-12}}{10^{-19}} \sim 10^7\ ion/s$

Cellular Mechanisms of Brain Function

As we've already mentioned, a typical ion channel has a conductance somewhere between 1 picosiemens and 100 picosiemens. And it seems interesting and relevant to find out how many ions are actually transported per second through a single ion channel protein. So let's take the example of a 10 picosiemens channel, already quite a large ion channel, very respectable. The amount of current through that ion channel will depend upon the voltage, and, of course, the conductance. The voltage, the membrane potential, is typically around 100 millivolts or less and so we multiply this by our conductanceof 10 picosiemens and this then works out to be 1 picoampere. That is 10 to the -12 ampere. If we want to know how many ions this corresponds to, we need to know the unitary ionic charge: "e" which is equal to 1.6 times 10 to -19 coulomb So the number of ions flowing is 10 to the -12 over 10 to the -19, approximately 10 to the power of 7 ions per second. So, over a million ions flow through an ion channel that's open at any given time. A remarkable flux of ions.

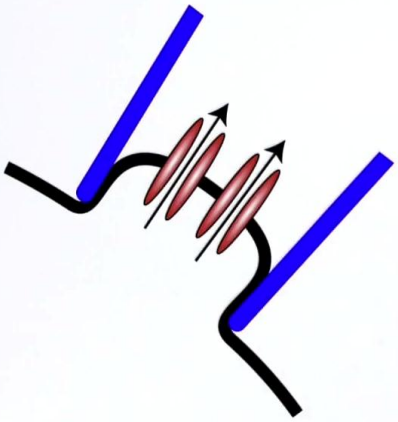
Notes

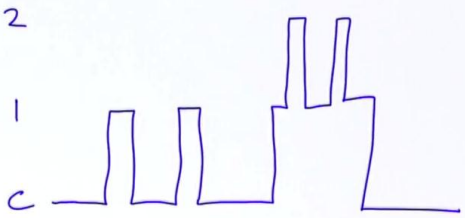
Summary

16m 39s

# Some numbers – many channels

A typical patch of membrane contains multiple ion channels.






Cellular Mechanisms of Brain Function

A given patch of membrane often doesn't just contain one ion channel. Typically, there might be two or more ion channels in the same patch of membrane that we have in our recording, in our patch-clamp recording set-up. So, typically the traces are more complicated than the ones we've already looked at. It might remain closed for a while, open, one ion channel might open, might close again, stay closed for a while, an ion channel might open and then a second ion channel might open, closed and open, closed, and both of them closed here. So you can see that there's now multiple states There's a state where both ion channels are closed, a state where one ion channel is open, and then another state where two ion channels are open. And in general, of course, we have to consider the situation where there's not just one type of ion channel, but there might be multiple different sorts of ion channels that might each individually have their own single channel conductance and their own specific ion permeability.

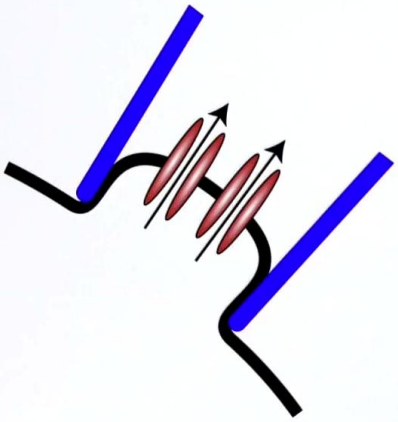
Notes

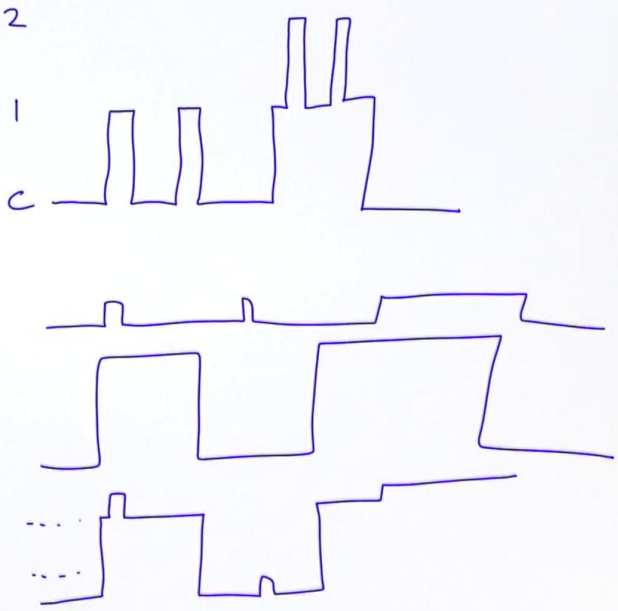
Summary

18m 18s

# Some numbers – many channels

A typical patch of membrane contains multiple ion channels.





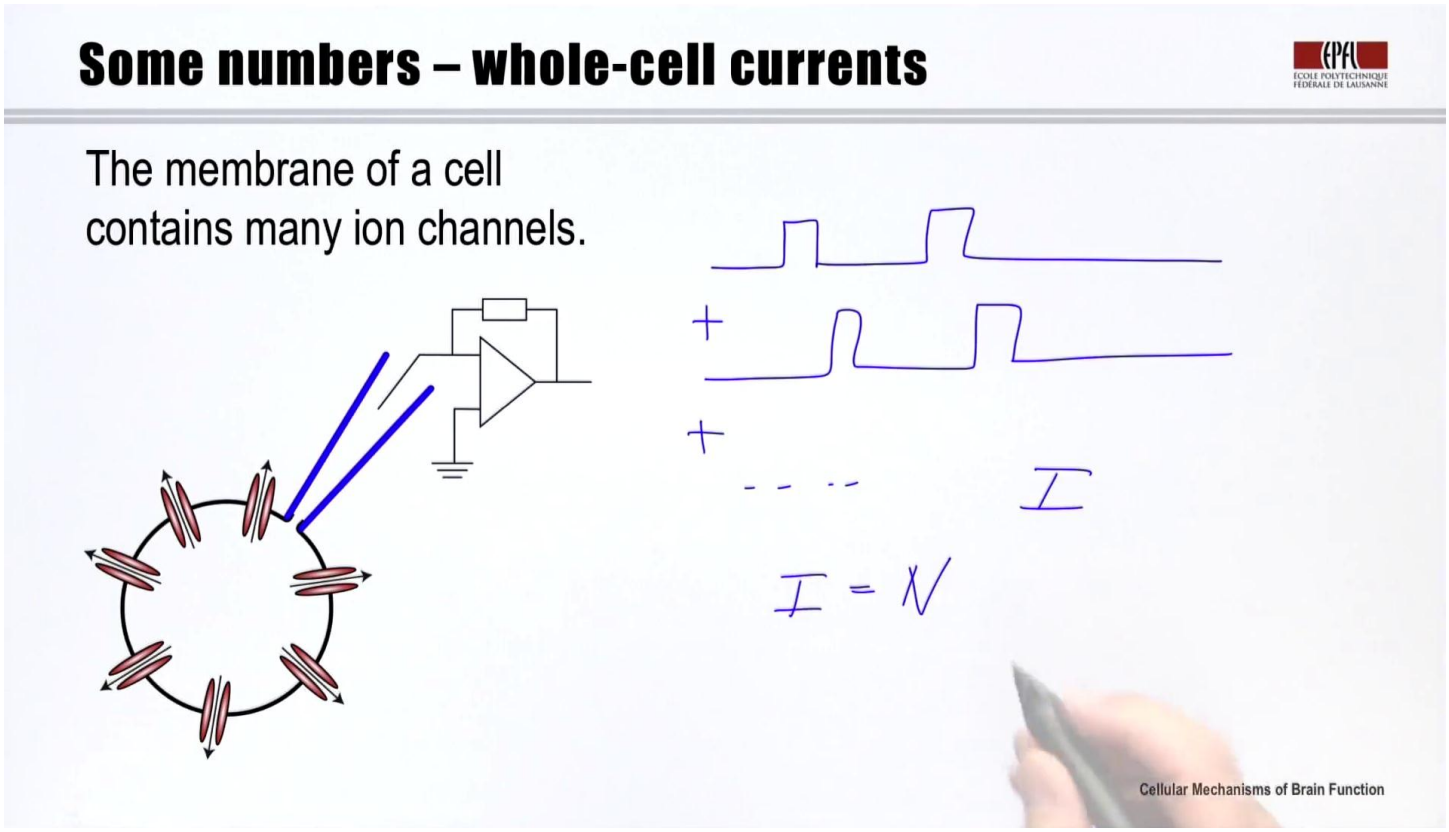

Cellular Mechanisms of Brain Function

So we might have a small ion channel mixed in with a large one, if we were recording them at the same time in the same patch of membrane we would have traces that looked a bit like this where you would see the individual channels but it wouldn't necessarily look exactly how we had it before with just open and closed states but we would have these multiple apparent sub-states here, but would actually be created by the mixture of ion channels that were inside an individual patch of membrane that we're recording from.

Notes

Summary

19m 32s




As we begin to think about cells, we need to begin to be able to put together the information from individual patches of membrane and see how that works across the entire plasma membrane. Clearly, we expect to have many ion channels present across the entire surface area of an individual cell, so we need to see how individual ion channels would summate to form so-called whole cell currents. So individual ion channels will be opening, closing, opening and closing, they'll do so independently at different random times and in order to understand the whole current, we need to add up all the currents from many different ion channels and that will then give us a total whole cell current flowing at any given time. The current across the plasma membrane will depend upon a number of things. One is the number of ion channels that are present. One, two, three, four, five, six, seven in this particular case but typically hundreds for real cells. The number of ion channels, the current flow for each individual ion channel -- so the single channel current -- and the open probability -- the amount of time that spends in the open time, relative to the total time -- And together, this then tells us on average how much current will be flowing across the plasma membrane.

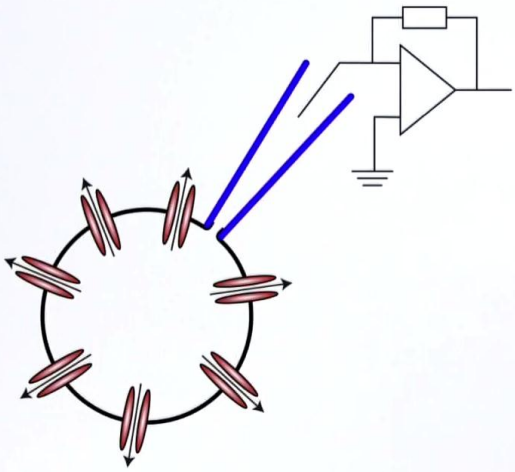
Notes

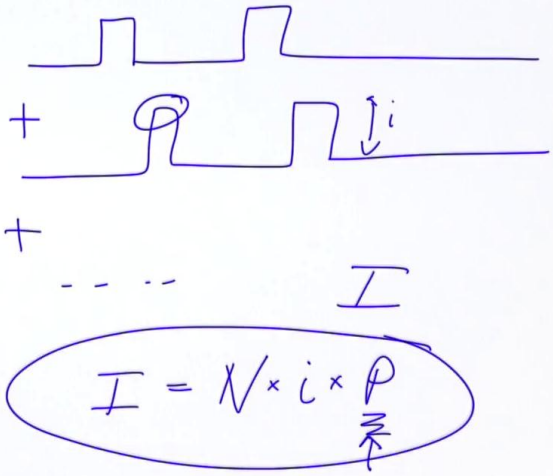
Summary

20m 11s

# Some numbers – whole-cell currents

The membrane of a cell contains many ion channels.





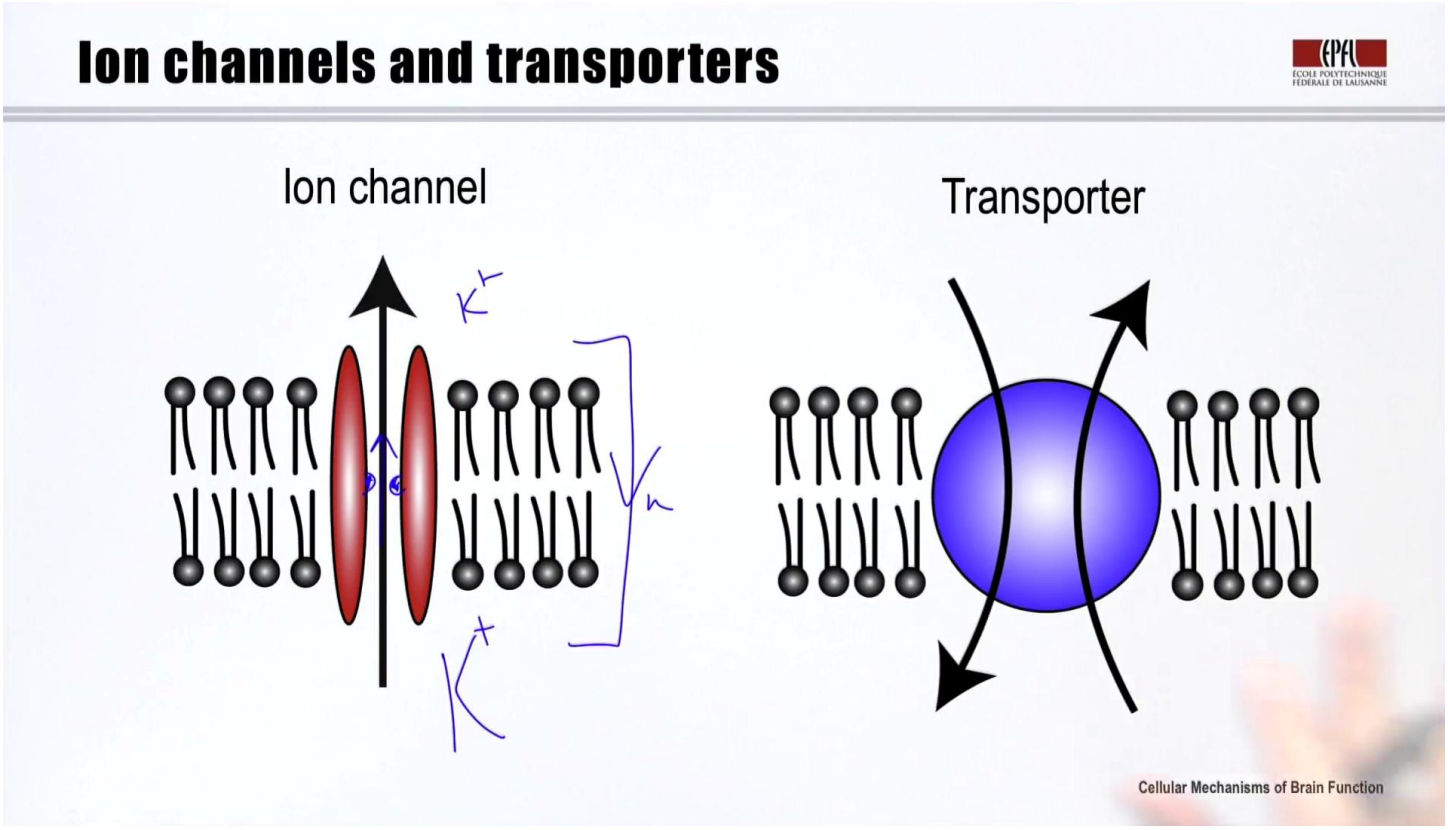

Cellular Mechanisms of Brain Function

It's also important to recollect that the open probability is the feature that's regulated most dramatically on the millisecond time scale. So, if you wanted to change the currents flowing across the plasma membrane then it would turn out to be full regulation of this open probability that decides the millisecond by millisecond current flowing across the plasma membrane.

Notes

Summary

21m 46s

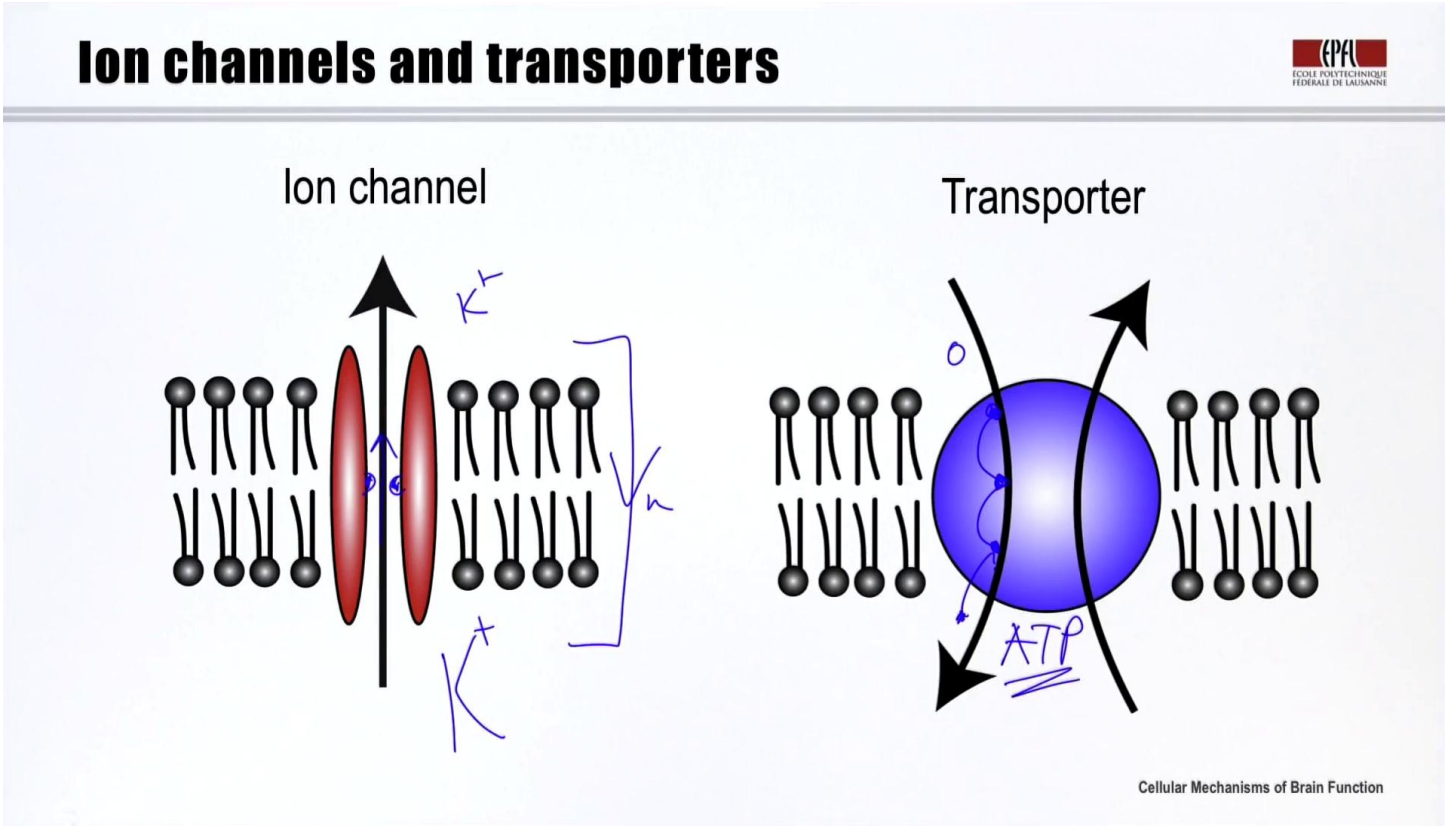



Finally, I'd like to point out that ion channels aren't the only type of protein that spans the membrane and moves ions from one side of the membrane to another. The ion channels are remarkable in that they have these very high transport rates where millions of ions are flowing through single ion channels across the plasma membrane. These high fluxes of ions are possible because the ion channel has an aqueous pore So the individual ions remain hydrated, fully diffusable, freely diffusable except, of course, for the selectivity filter as we discussed where the hydrated ion needs to come off, specifically bind with selectivity filter before it can continue traversing the ion channel. There are other types of proteins, transporters, as they're typically called, which operate on much slower time scales, so the transport rates are many orders of magnitude slower but they are able to transport ions against the electrochemical gradients. The ion channel, because of its aqueous pore, basically has to follow the electric field and the concentration gradients that are presented for the different ions. A transporter, on the other hand, can transport ions against gradients.

Notes

Summary

22m 14s

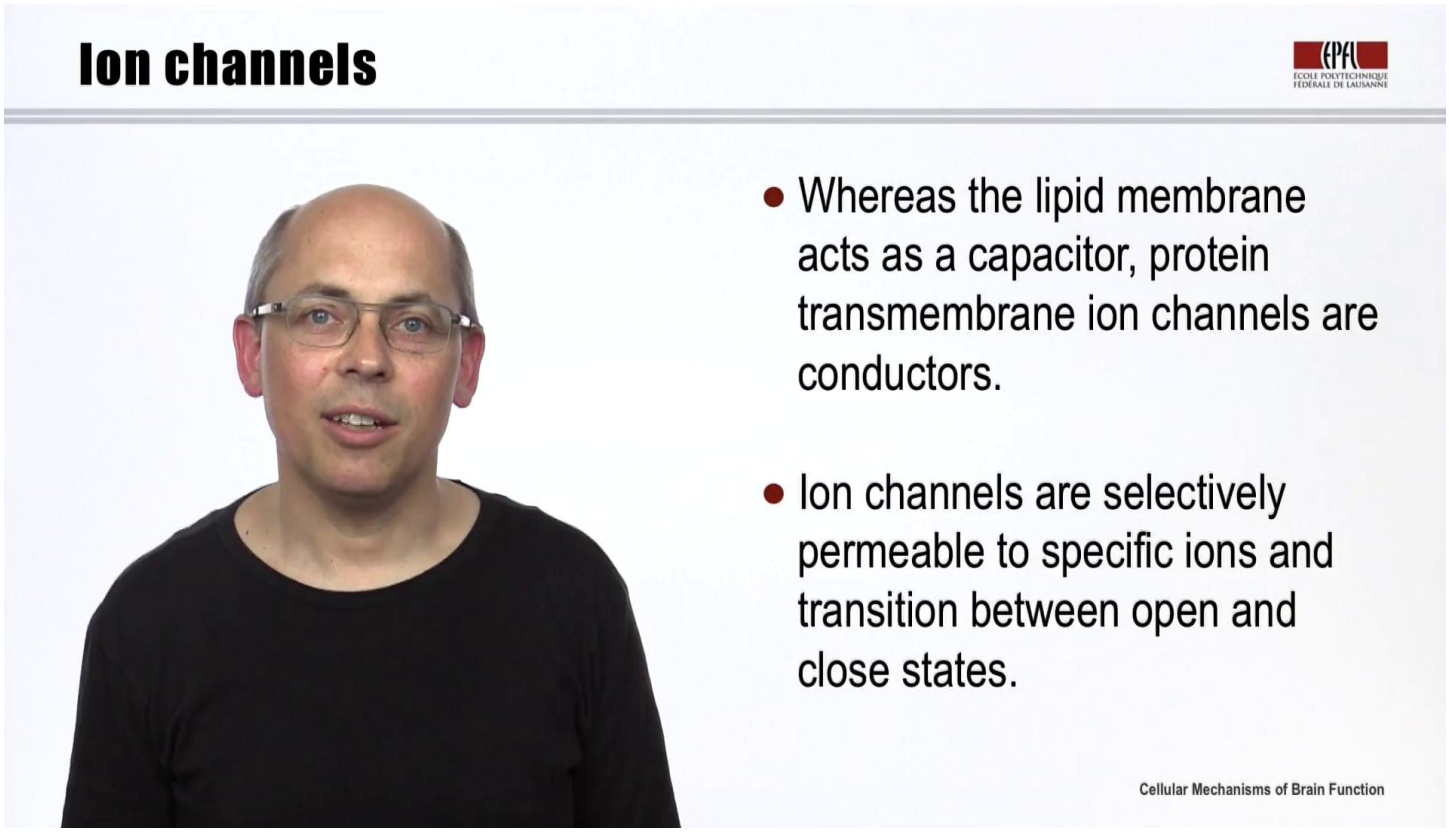



It can couple different types of ions and different sorts of ionic gradients and transport against electrochemical gradients perhaps even by using energy. So some proteins use ATP to get energy and will suck ions against the normal electrochemical gradients. In transporters, the ions are tightly bound to the protein as it changes conformational state, gradually moving these ions in tightly bound stages from the outside to the inside of the cell. Because of all these different conformational changes in the protein that are required to move the ion, this is a very slow transport phenomenon, but it has this very nice flexibility that it can move the ions against the electrochemical gradients through the use of energy or through coupling it to a favorable gradient.

Notes

Summary

23m 44s




So, we've now learned quite a lot about membrane biophysics. We've learned that there are phospholipid bilayers that act as capacitors and are impermeable to ions, and embedded inside these cell membranes are proteins, ion channel proteins that have an aqueous pore that transition between open and closed states allowing very rapid fluxes of ions, millions of ions per second to move in and out of cells. The ion channels can be highly specific moving only a single type of ionic species, for example, sodium or potassium, and the flux of millions of ions per second can then be used to change the membrane potential, and that then forms the basis of neuronal computation and electrical signaling. In the next lesson we're going to learn more about membrane potential and electrochemical diffusion and how these gradients are set up and regulated and control membrane potential in cells.

Notes

Summary

24m 41s