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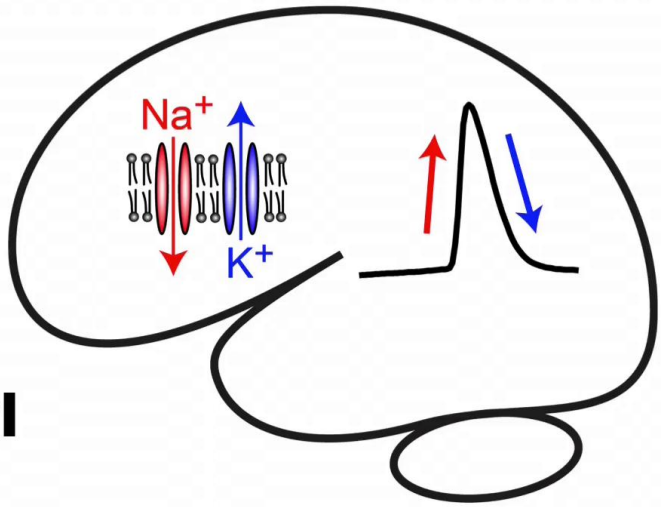
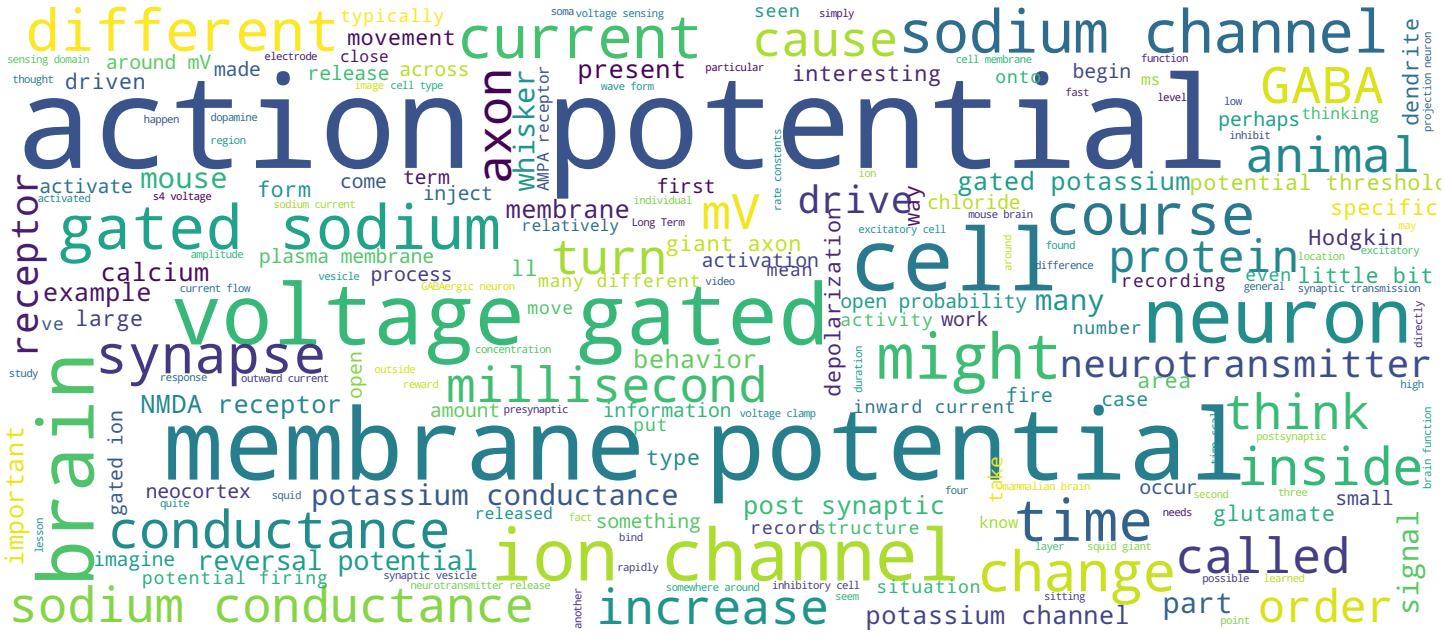


2.3 The action potential

Cellular Mechanisms of Brain Function

Prof. Carl Petersen



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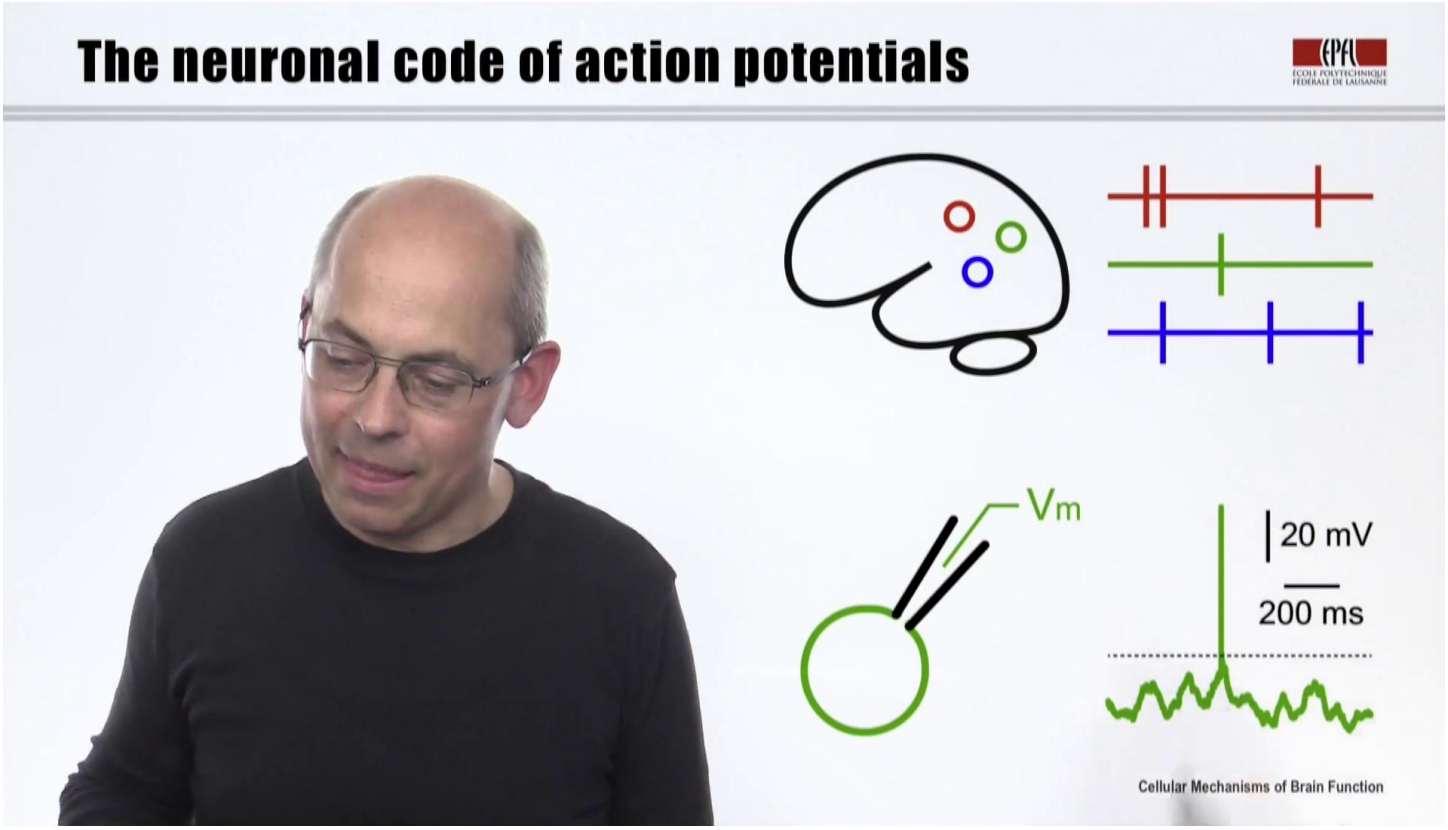
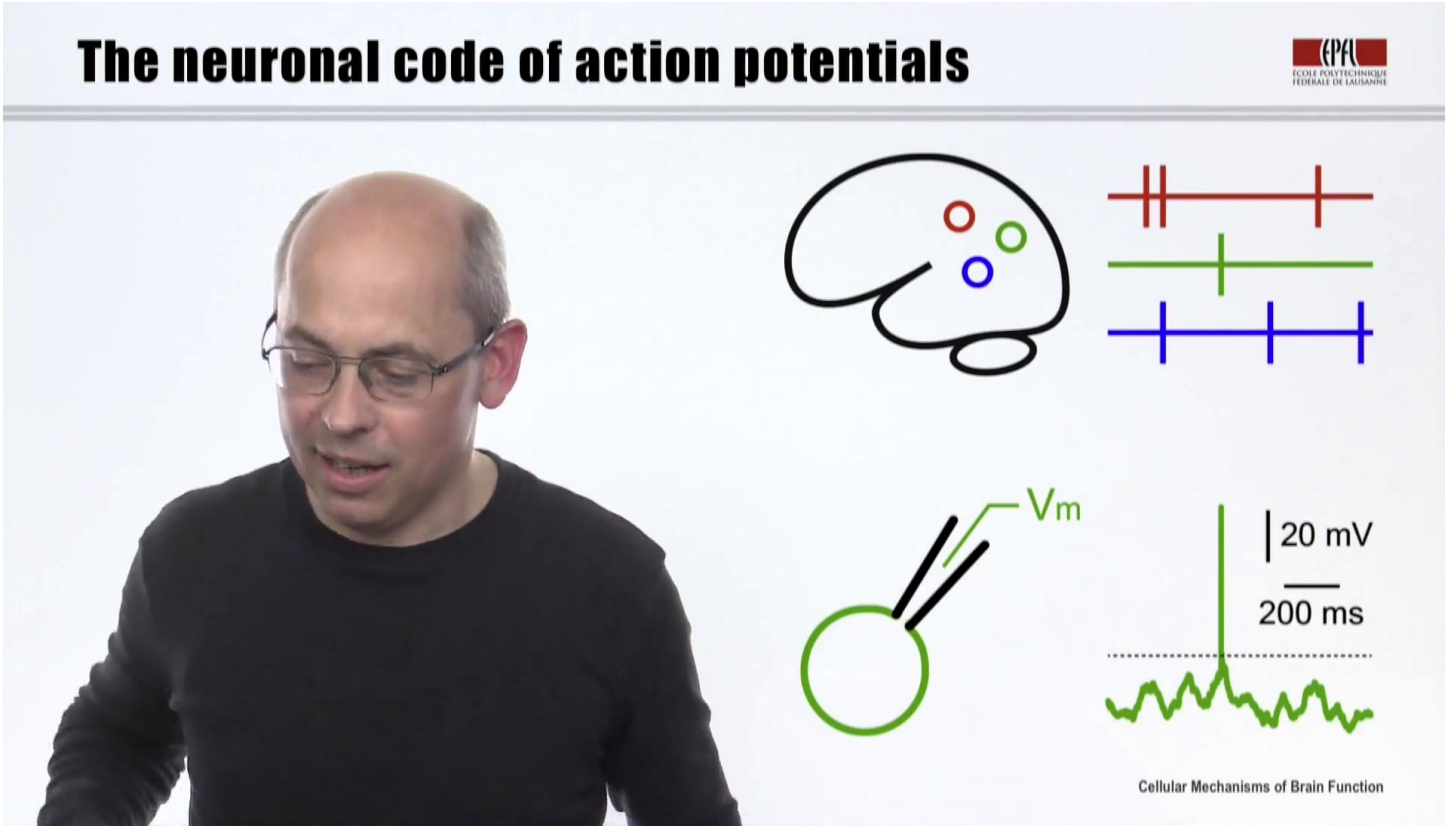


We've now studied the biophysics of the cell membrane in some detail. We have learned that the phospholipid bilayer electrically acts as a capacitor. And we've learned that the protein ion channels act as conductors for specific ionic species. We've also begun to think about the complex, nonlinear membrane potential dynamics that could result from the voltage gated activation of ion channels. In this lesson, we're going to see how these basic biophysical mechanisms can explain the ionic basis of the action potential, probably the most important signal that takes place in the brain. The action potential is a unitary, all-or-none event that can easily be recorded in the brain of living animals. If you put a small microelectrode inside the brain of a mammal and you record the electrical potentials at the tip of that electrode, you'll easily see the all-or-none action potential wave form that is recorded near the neurons that are firing. Here in this example, we record from a red, a green, and a blue cell. And looking at the potentials of the electrodes across time, we see that there are unitary, all-or-none events, spikes or action potentials, each of which looks identical, has a duration of about 1 ms, and the different nerve cells can fire these action potentials at different times.

Notes

Summary

0m 03s

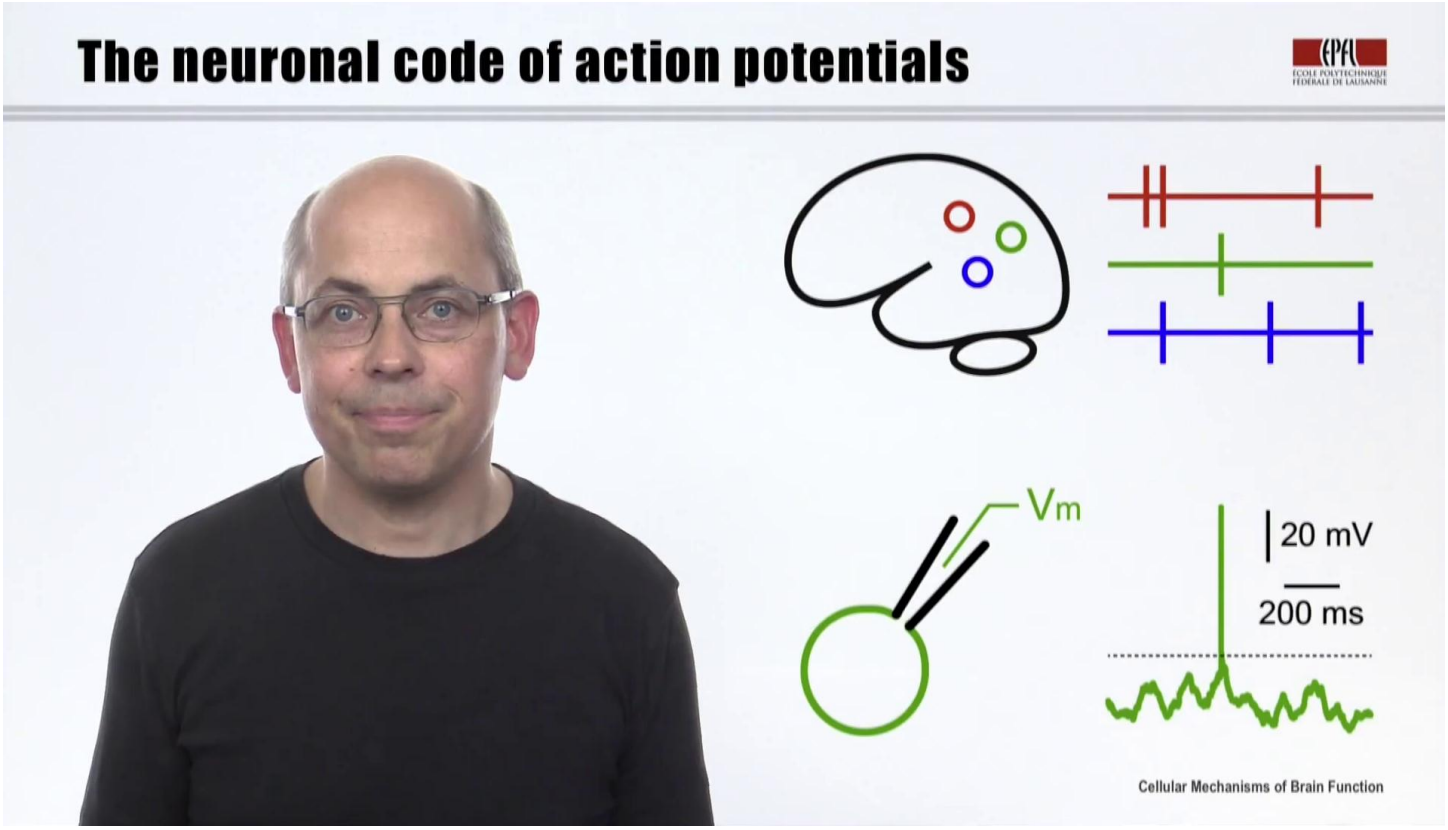


If you record these action potentials in the visual part of the mammalian brain and you present different visual stimuli, you'll see that these action potentials correlate spectacularly well with different features of the visual scene presented to the animal. The action potentials therefore code for the visual stimulus. If you put these electrodes into the motor cortex of the mammalian brain and you record spikes from many different cells, you're able to predict the movements that an animal makes. These action potentials code for the movement that the animal will make. Action potential discharge, across time and across different neurons, therefore carries a lot of information about what the animal is seeing, doing, and feeling, and thinking. It's thought that the pattern of neuronal action potentials forms the most important coding of information in the brain. In order to understand how these action potentials are evoked, we need to look at the membrane potential of individual neurons. Here, below is schematically indicated what the membrane potential of this green cell might look like as it fires it's action potential.

Notes

Summary

1m 54s

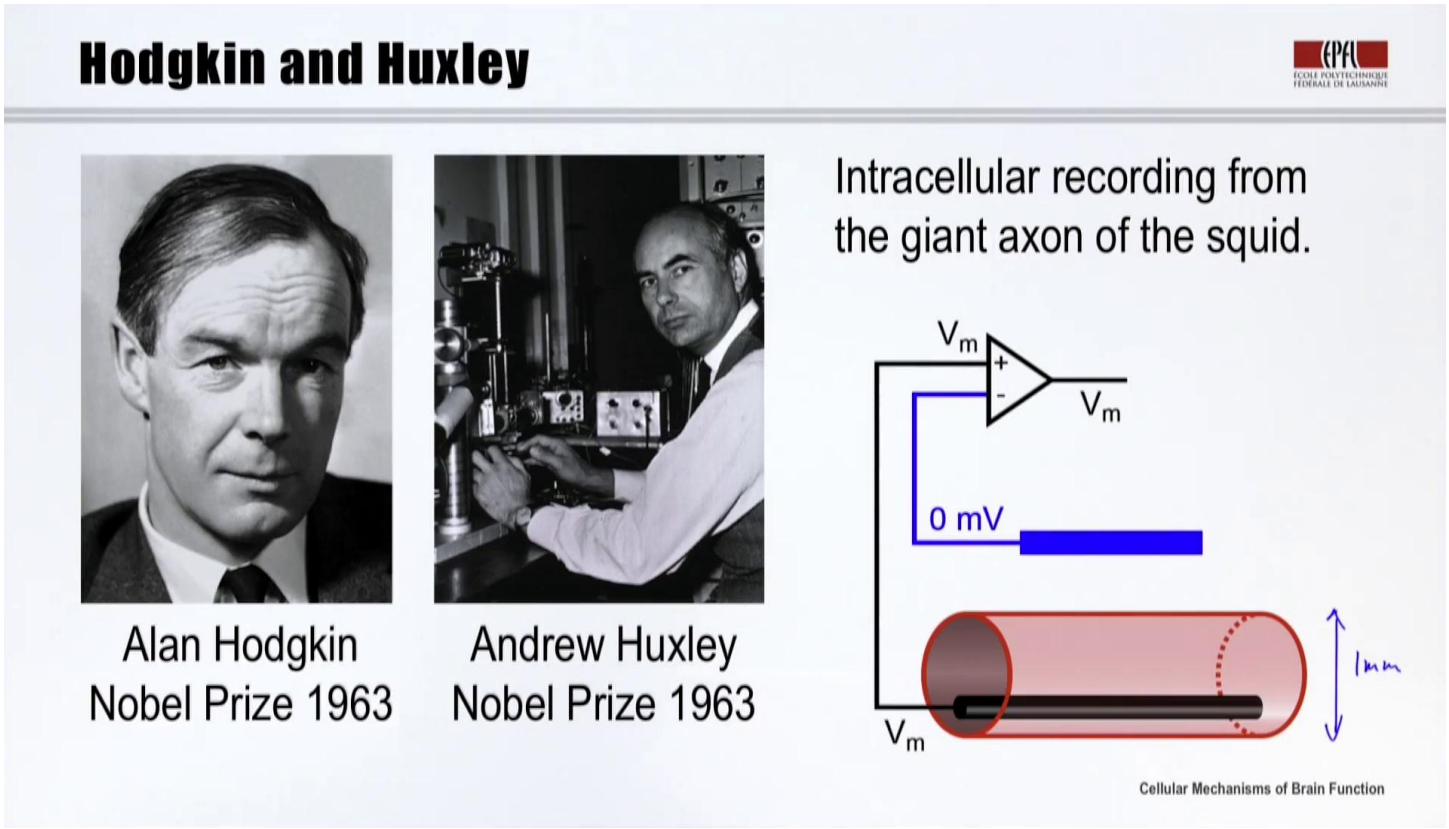


The membrane potential fluctuates in a so-called subthreshold regime and at one given point, it rises steeply, becomes positive, and then repolarizes, and all of that spike takes about 1 ms and that event that stands out from these background fluctuations is the action potential, the all-or-none digital signal that encodes information in the brain.

Notes

Summary

3m 18s

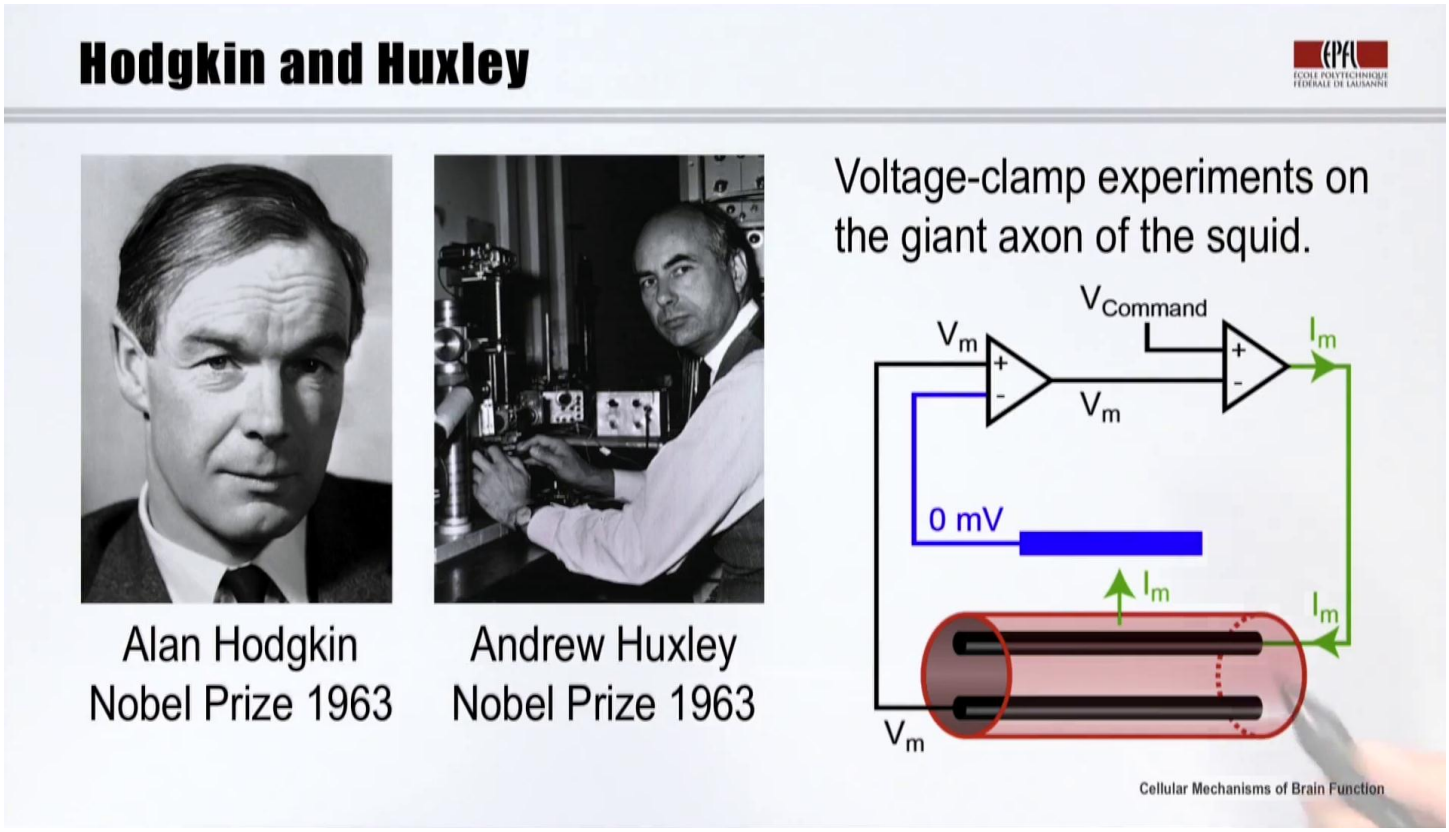


In this lesson, we'll take a historical perspective. And we have two heroes of the action potential, Alan Hodgkin and Andrew Huxley. They were the first, true record that action potential intracellularly, in terms of the membrane potential changes that underlie the action potential. Hodgkin and Huxley worked with the giant axon of the squid. The squid has a escape reflex that it needs to activate very rapidly in case of danger. A predator is about to eat the squid, the giant axon fires an action potential, and that in turn, causes contraction of muscles that drive the squid to swim away from danger. The axon of the squid, this giant axon, is more than a thousand times larger in diameter than most neuronal arborizations. A typical axon of a mammalian neuron has a diameter of around 100 nm. The squid giant axon has a diameter somewhere close to 1mm. This made it possible in 1939 for Hodgkin and Huxley to insert electrodes inside this large diameter axon, place another electrode outside in the bathing sea salt water in which this nerve was bathed, and through a differential amplifier comparing the absolution and the intracellular electrode, they could then record the membrane potential of this axon as it fired an action potential.

Notes

Summary

3m 51s



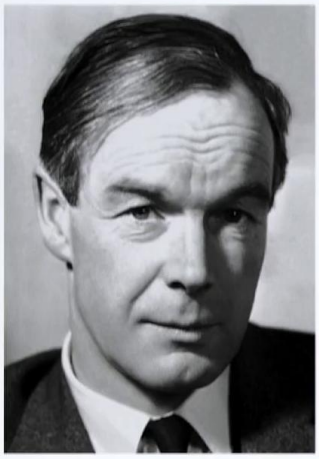
And what they saw looked something like this, a very steep, rising positive phase, a very steep, negative falling phase, and importantly, the action potential overshot 0 mV and went somewhere up to around +40mV. The duration was somewhere around 1 ms. Hodgkin and Huxley had thus, recorded the intracellular membrane potential correlate of the spike event that had previously been seen from the outside of cells as little extracellular blips. Before they could study the biophysical mechanisms that gave rise to this change in membrane potential, the second world war broke out. These first membrane potential recordings had been done in 1939 and there would then be an interruption of many years before Hodgkin and Huxley could return to their experimental work on the squid giant axon. When they got back together again, they applied one further advance in electronics that had been developed during the war years. They, again, applied the voltage measuring technique to the squid giant axon, but this time, the membrane potential output was fed into a further voltage clamp amplifier that compared the measured membrane potential with a command potential which experimenters imposed upon the system.

Notes

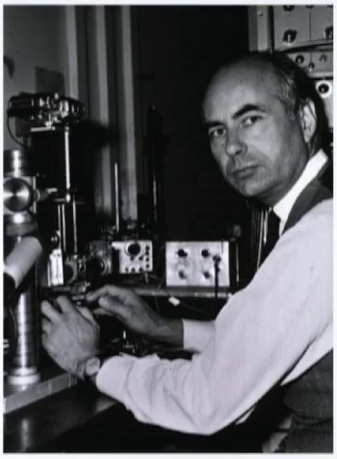
Summary

5m 40s

Hodgkin and Huxley

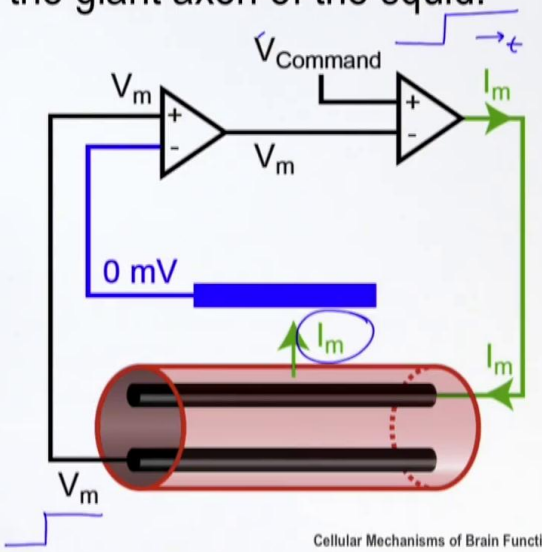


Alan Hodgkin
Nobel Prize 1963



Andrew Huxley
Nobel Prize 1963

Voltage-clamp experiments on the giant axon of the squid.



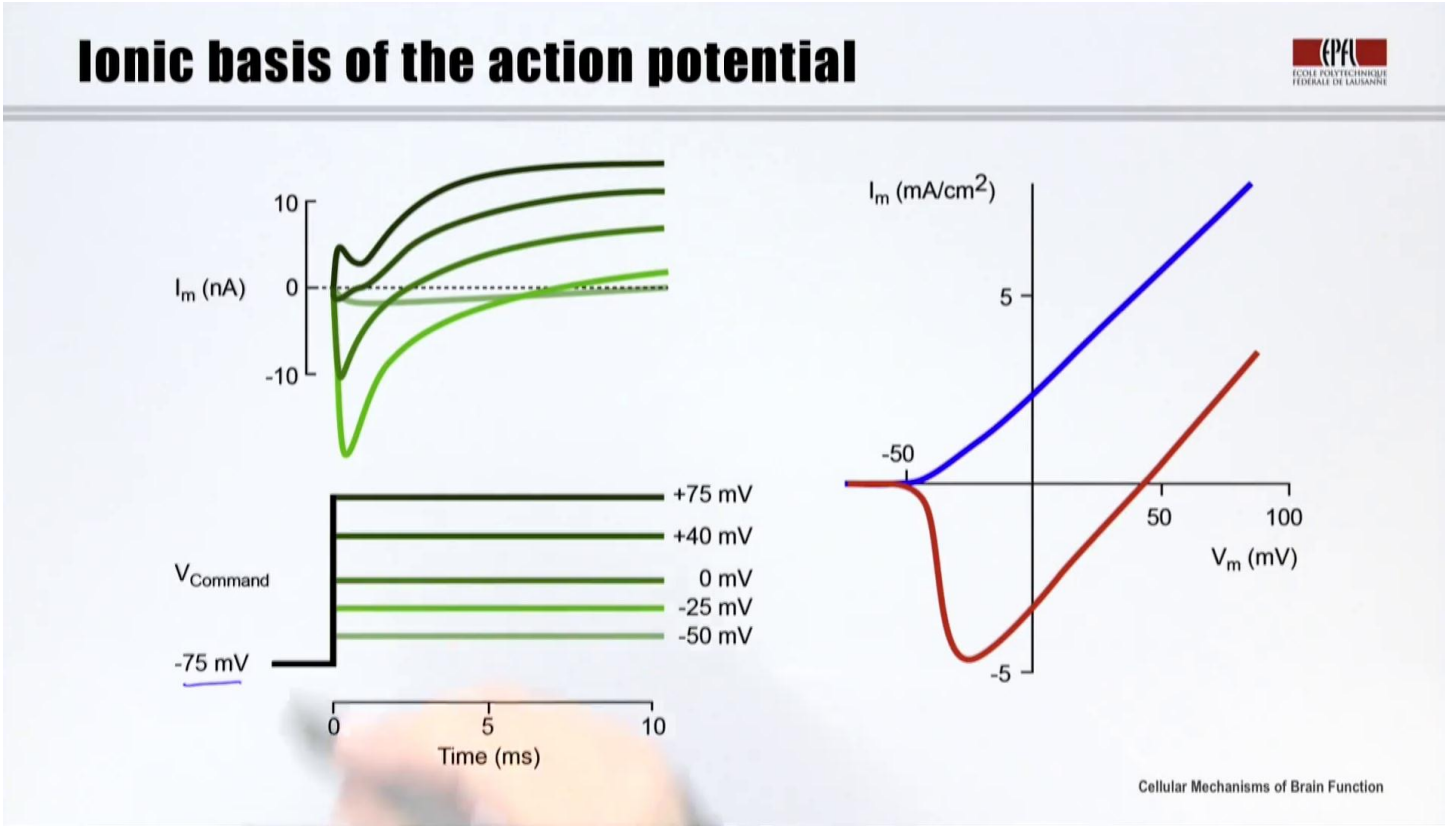
Cellular Mechanisms of Brain Function

The differential amplifier would compare the command requested voltage with the measured voltage and inject the right amount of current to bring the membrane potential to the command potential. Hodgkin and Huxley were thus, able to impose voltage clamp steps across time that would then result in voltage steps in the membrane potential and they would then measure the membrane current that would flow across the membrane.

Notes

Summary

7m 10s

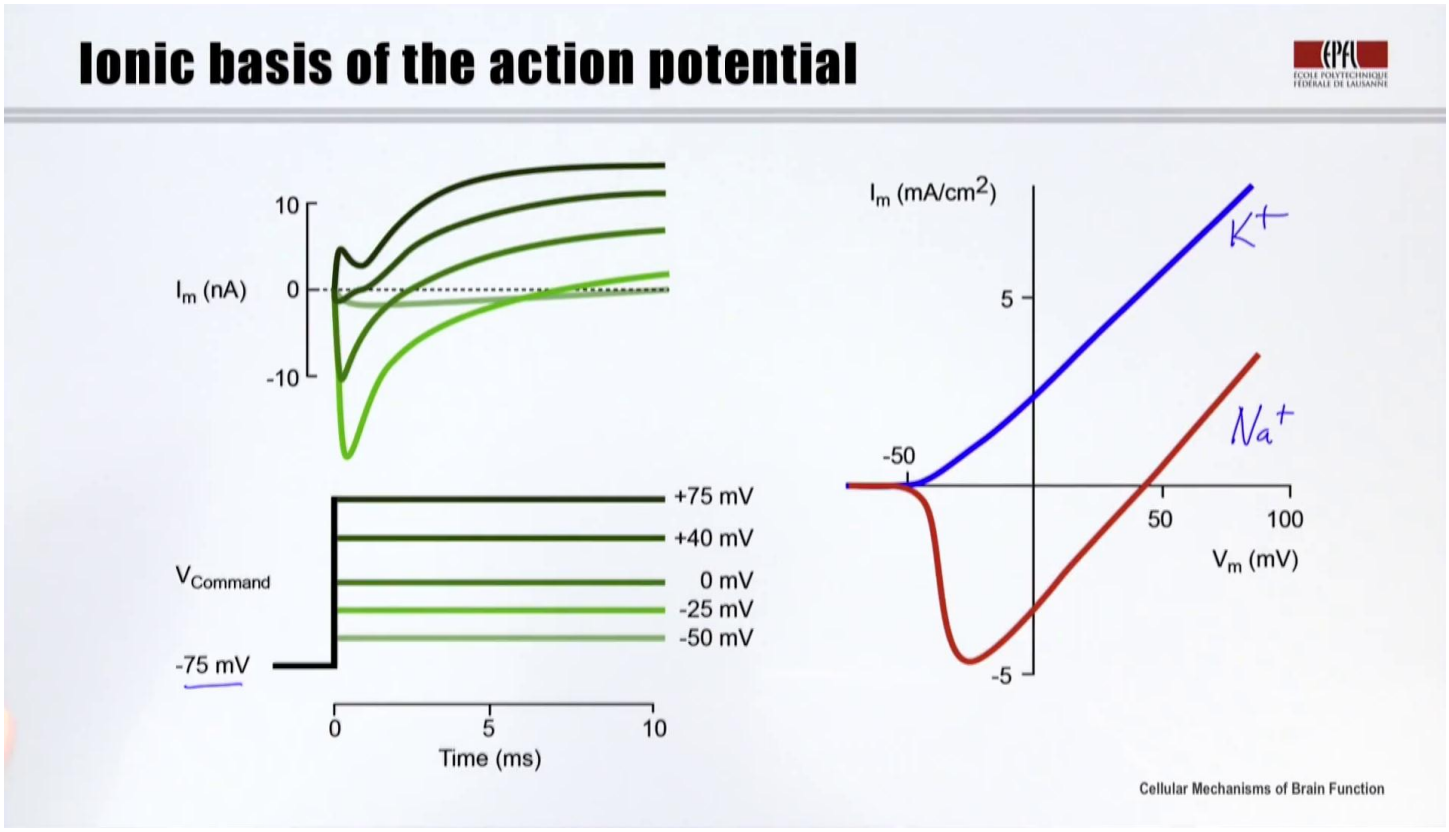


The type of data that they found is shown in these green traces. We begin with a membrane potential of around -75 mV and at time 0, the membrane potential is then stepped to different potentials, and they measured the transmembrane currents. As they stepped from -75 to -50 mV, very little happened. Very little current flow was activated. Going to -25 mV however, they saw a rapid inward current that then rapidly disappeared and gave way to a delayed outward current. At 0 mV, the situation was similar, fast, inward current, delayed, outward current. At +40 mV, the inward current was almost gone and the delayed outward current was even bigger, and at +75 mV, the early component was outward as was the delayed component. Hodgkin and Huxley realized that this wave form in the current flow could be accounted for by two different conductances, a fast, early conductance, and a delayed conductance that they could compute later on. By measuring at an early time point, they could plot a current voltage profile that looks like this red curve, somewhere positive to minus 50 mV, the inward current is steeply activated as we see here, at around +40 mV, it reverses, and at more positive potentials, it's an outward current.

Notes

Summary

7m 43s

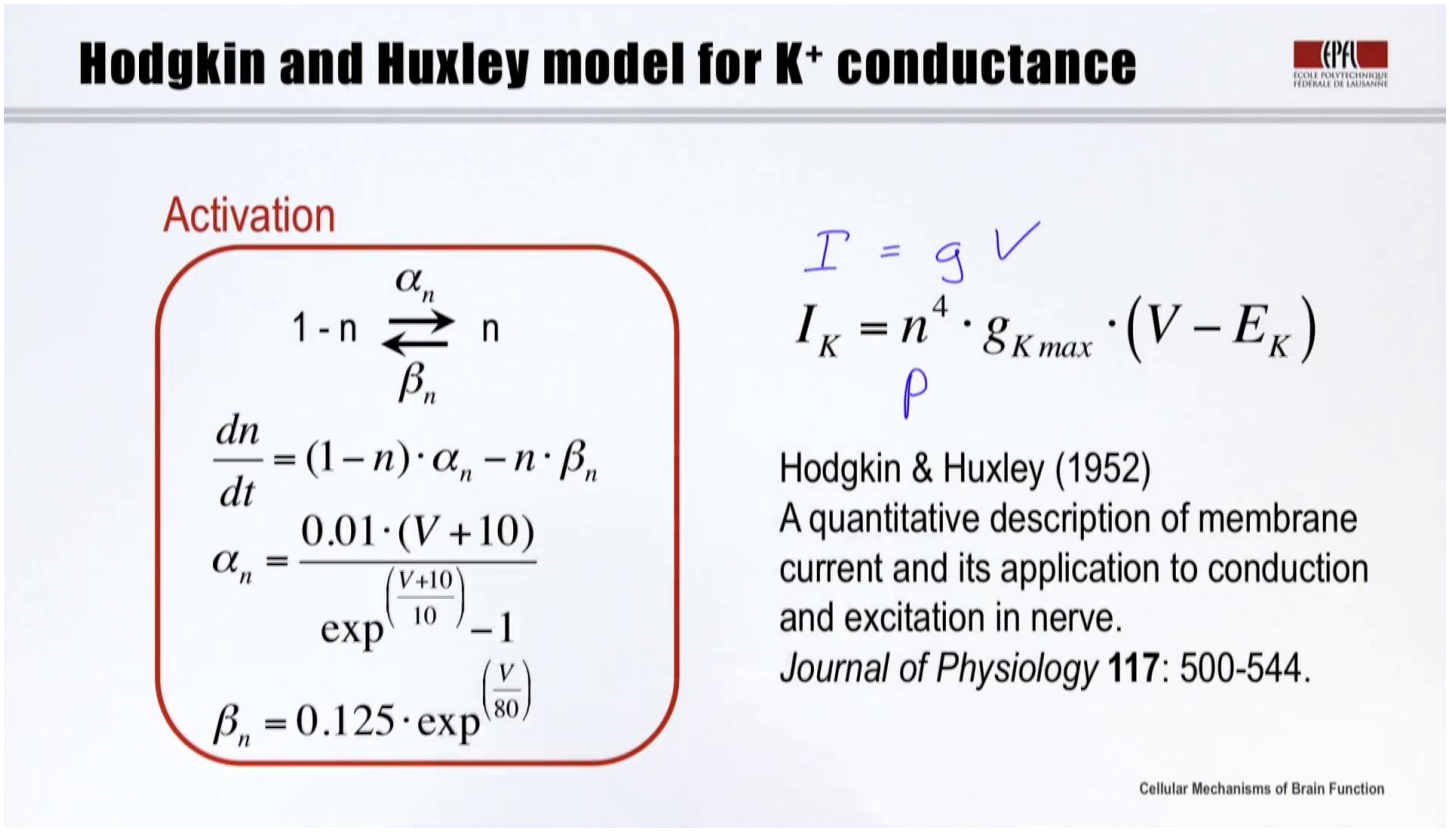


The delayed current is represented by this blue curve. Now, because we've been studying voltage gated ion channels, these curves look extremely familiar. This, indeed, is the voltage gated sodium conductance, and this is a voltage gated potassium conductance, as Hodgkin and Huxley correctly concluded from their early measurements. They repeated these measurements from many different starting potentials, many different potentials and time intervals between the different voltage clamp steps, and they were able to build up a quantitative description across many different conditions for how these voltage gated currents developed in the squid giant axon.

Notes

Summary

9m 25s

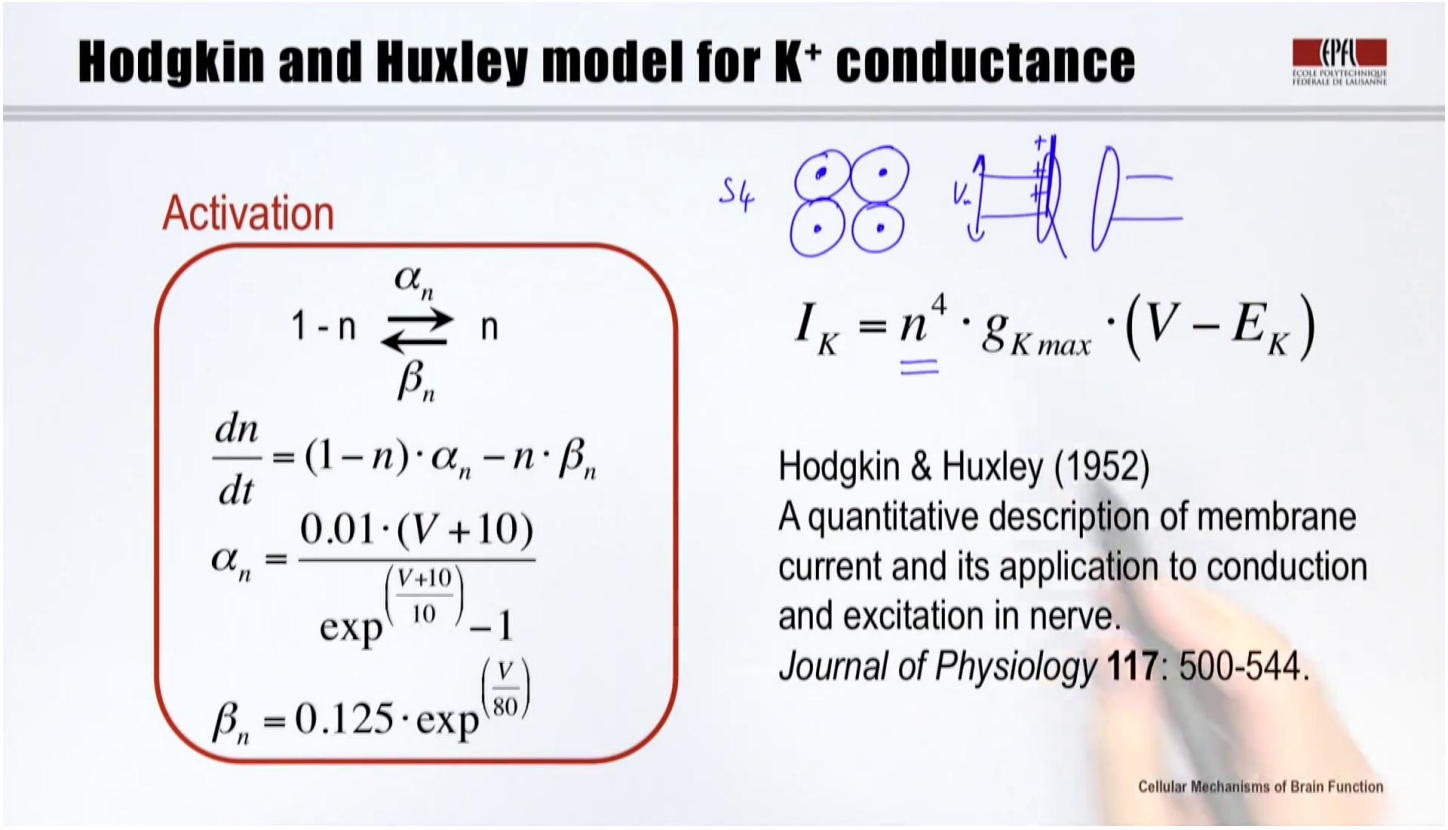


The measurements that Hodgkin and Huxley made were already remarkable, but even more remarkable was their quantitative description of these conductances that were activated in the squid giant axon. They used a simple model which could describe the currents that they saw. As we've been using ourselves, they say that the membrane current is equal to a conductance times a voltage and for the potassium conductance, we, of course, need to think about the offset with the nernst equilibrium potential for potassium. There's some sort of maximal conductance that, presumably, relates to the number of potassium channels that are present in the cell membrane, and then there's another term here, a voltage and time dependent term that basically, represents the open probability of the potassium channels in the membrane. In order to describe the open probability of these ion channels, Hodgkin and Huxley described a particle gating scheme where there would be a probability, n, of a gating particle being in an activated state and, 1-n, that it's in the other impermeable state and there would be rate constants, alpha and beta, that would then go between the permissive and the non-permissive state of this gating particle.

Notes

Summary

10m 12s

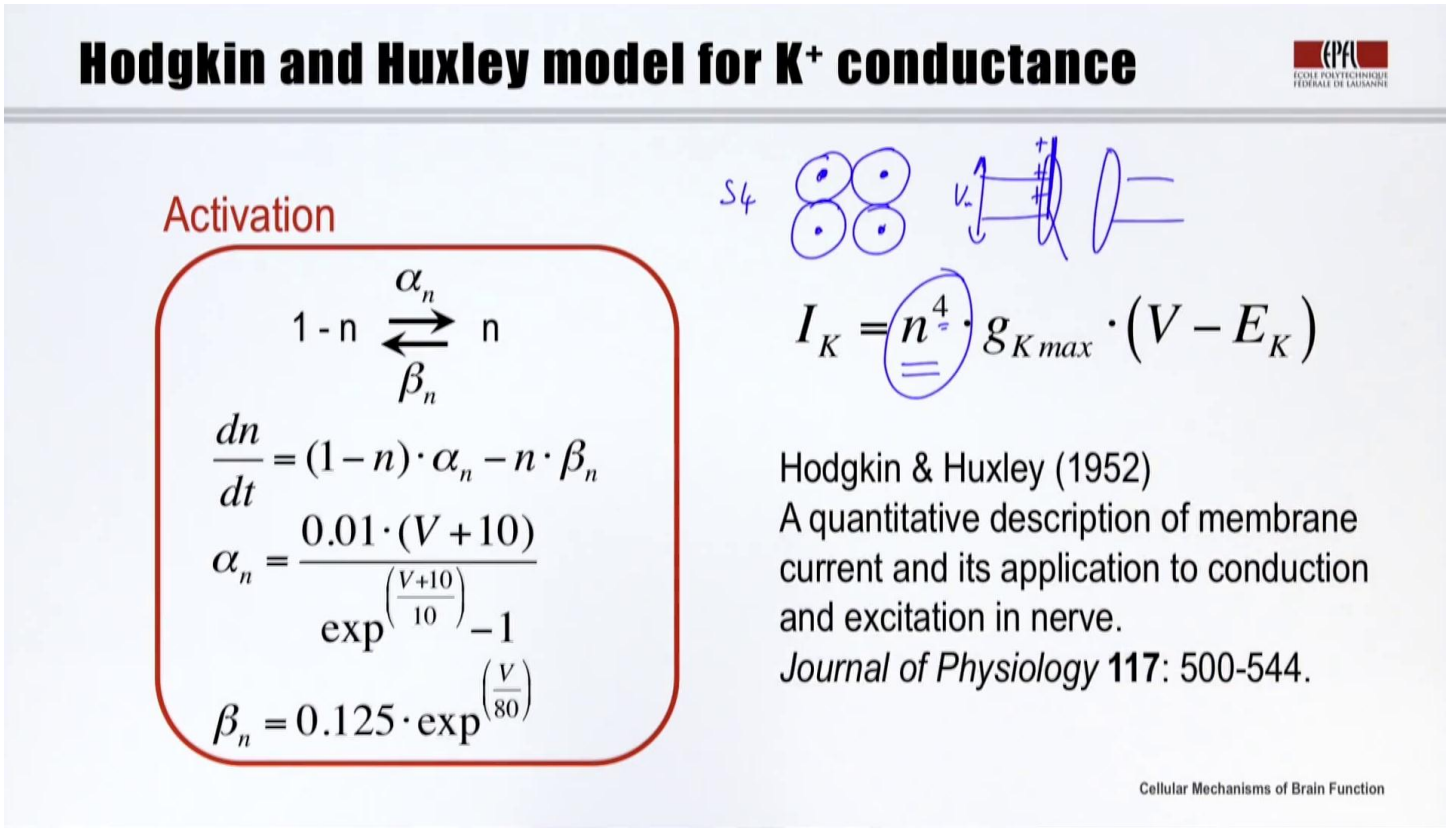


This then describes a first order differential equation for n across time with these rate constants and the rate constants, alpha and beta, are of course in themselves, voltage dependent. This is a voltage dependent ion channel and these empirical equations here then relate to expectations from boltzmann statistics. This is the description of the voltage gated potassium conductance that is voltage activated, but has no inactivation. The power of four for the activation gate is interesting because we can think of n now as being the probability that the s4 voltage sensing domain is in the open, permissive state. We remember that the voltage gated potassium channel has four subunits, each of which has an s4 voltage sensing domain, that's the domain that, inside the ion channel-- if this is now the voltage gated potassium channel, there's an s4 domain that you will remember as positively charged that s4 voltage sensing domain moves depending upon the membrane potential and it then causes an opening of the voltage gate. The fact that there are four of these s4 gating domains might then relate to this fourth power here and the movement, alpha and beta, these rate constants, then might relate to the movement of the s4 voltage sensing domain in four independent gating particles.

Notes

Summary

11m 50s

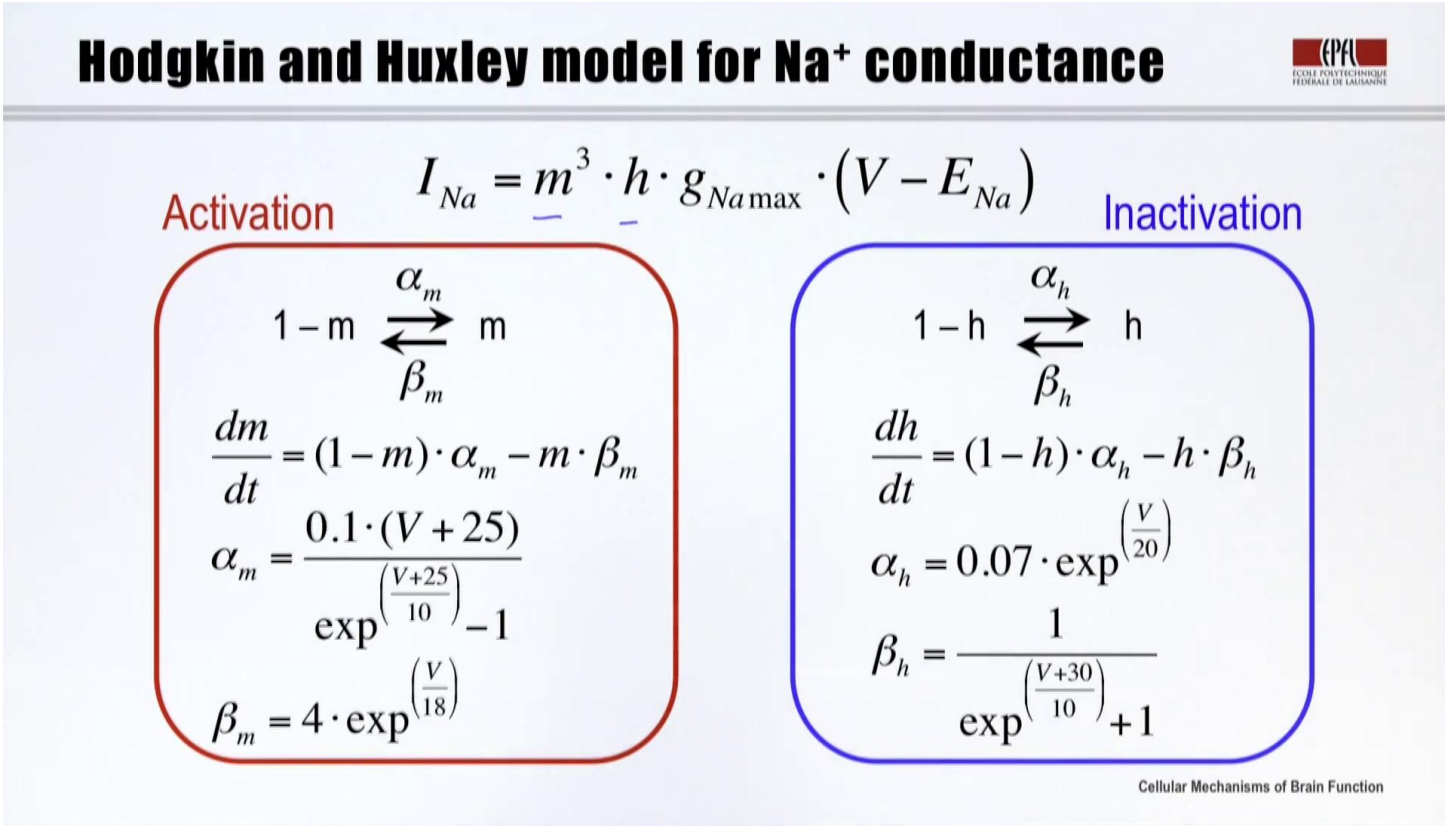


And so there could be a biophysical reality to this empirical equation that Hodgkin and Huxley empirically fitted to their experimental data. The 1952 description of the membrane currents, in the journal of physiology, is a historic paper which incorporates these equations that are quantitatively able to account for the voltage gated potassium conductance and also for the voltage gated sodium conductance with a small modification.

Notes

Summary

13m 36s

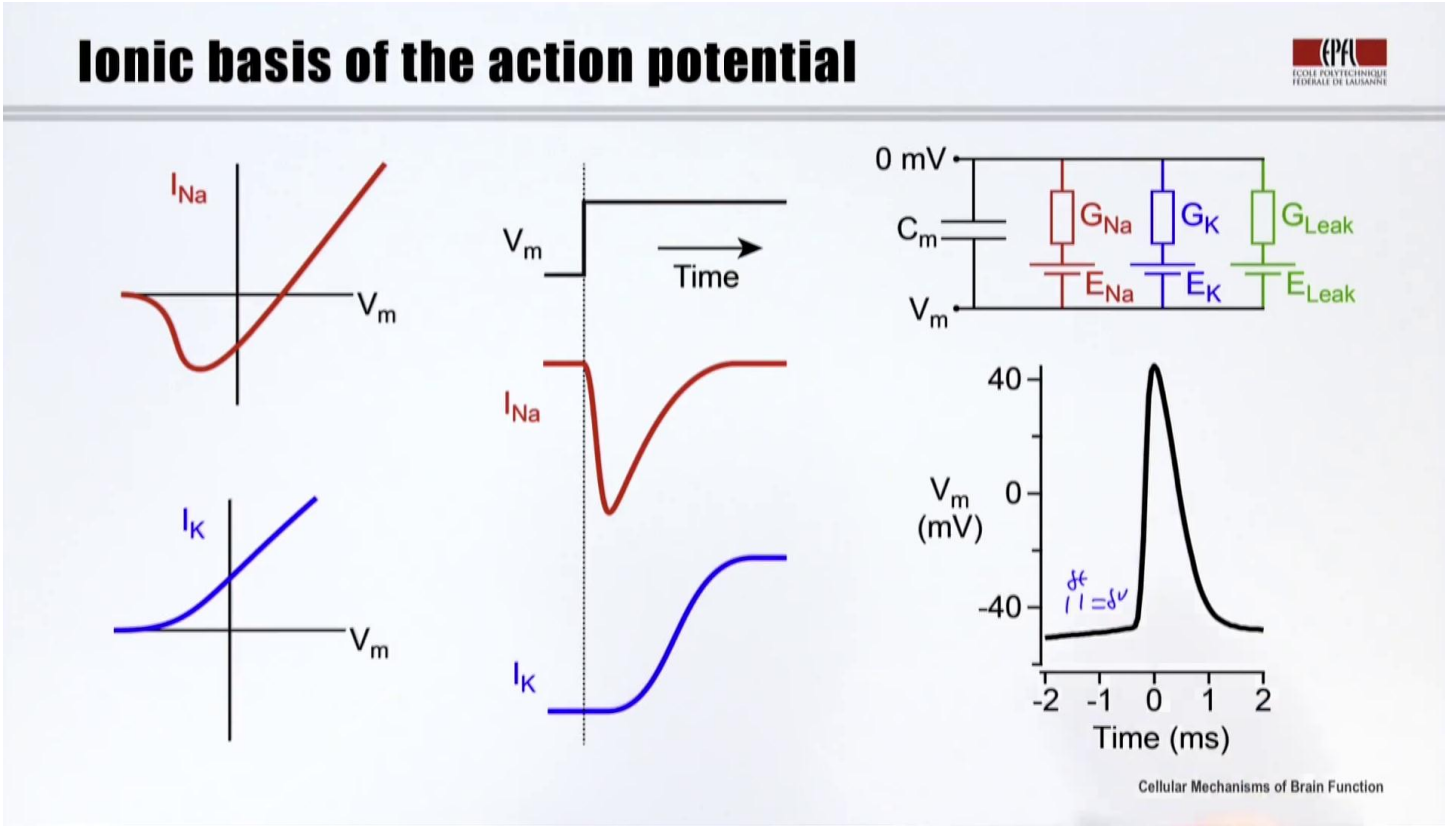


The voltage gated sodium conductances are more complicated because they have both a voltage gated activation and a voltage gated inactivation. And so the sodium current is, again, described relative to the reversal potential and some maximal number indicating the number of sodium channels present and there's then an activation and an inactivation term, n being the activation particle, again, governed by alpha and beta rate constants, a first order differential equation and again, boltzmann type statistics for the voltage dependence of these rate constants. The same is true for the inactivation term, now termed h, and not raised to any power. Hodgkin and Huxley fitted their experimental data and obtained values that they inserted into these equations and were able, quantitatively, to reconstruct the action potential on the base of their voltage clamp data.

Notes

Summary

14m 11s

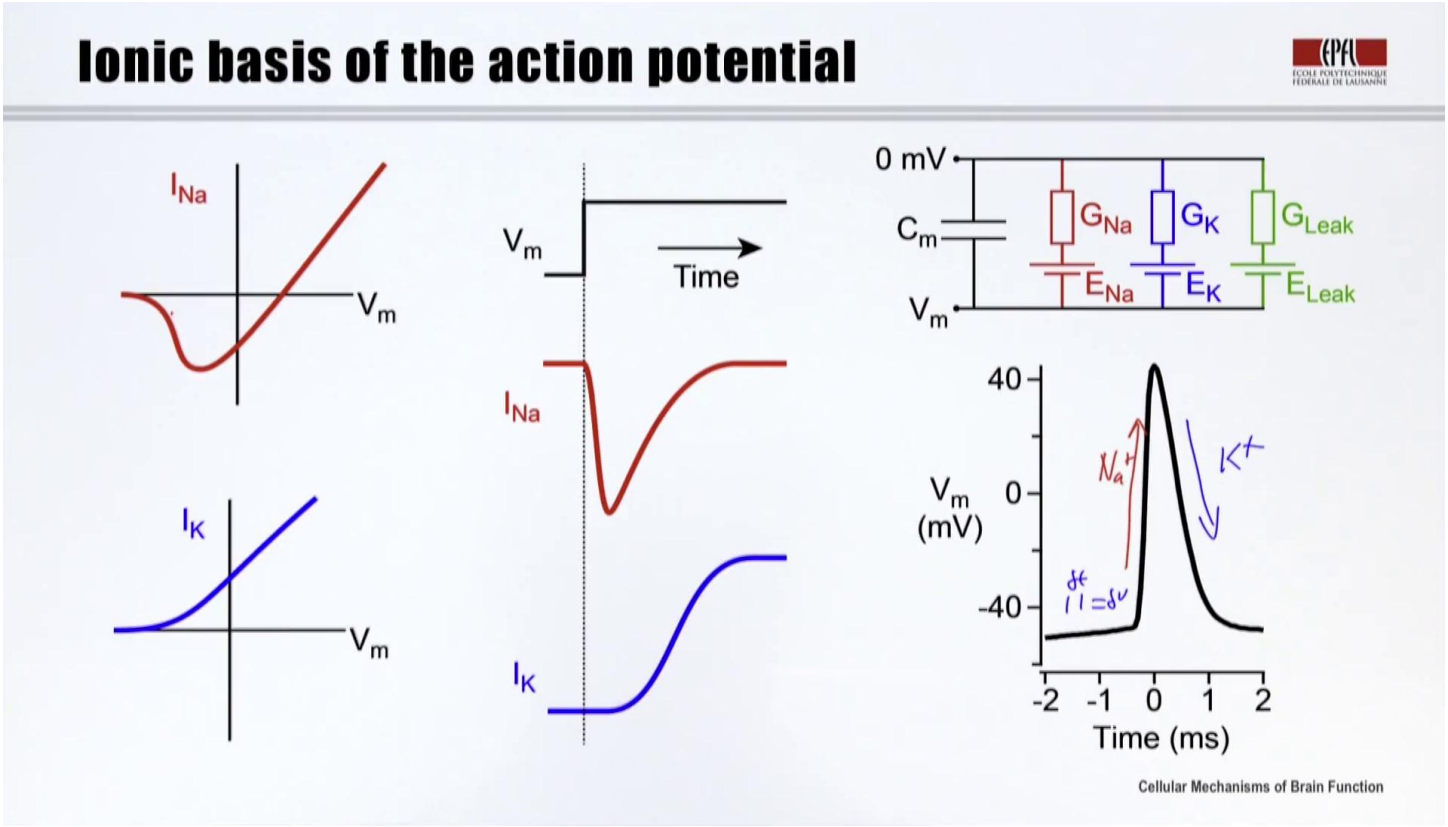


So they began with voltage clamp data looking at current-voltage relationships and also the current with respect to time across different voltage steps. They came up with equations, empirical equations, modeling these currents and conductances and they then went and placed those currents and conductances inside a simple model of the cell incorporating capacitance, sodium, potassium, and leak conductances and by going through and integrating the membrane potential across small steps in time, they were able to compute the step by step changes in membrane potential for different time steps and were then able to iteratively go through and compute the trajectory of the membrane potential during an action potential. The work is all the more heroic because in those days, the digital computer didn't exist. The description, however, was remarkable and quantitatively agreed with their experimental results. They found that this upstroke of the action potential was driven by the voltage gated sodium conductance.

Notes

Summary

15m 13s

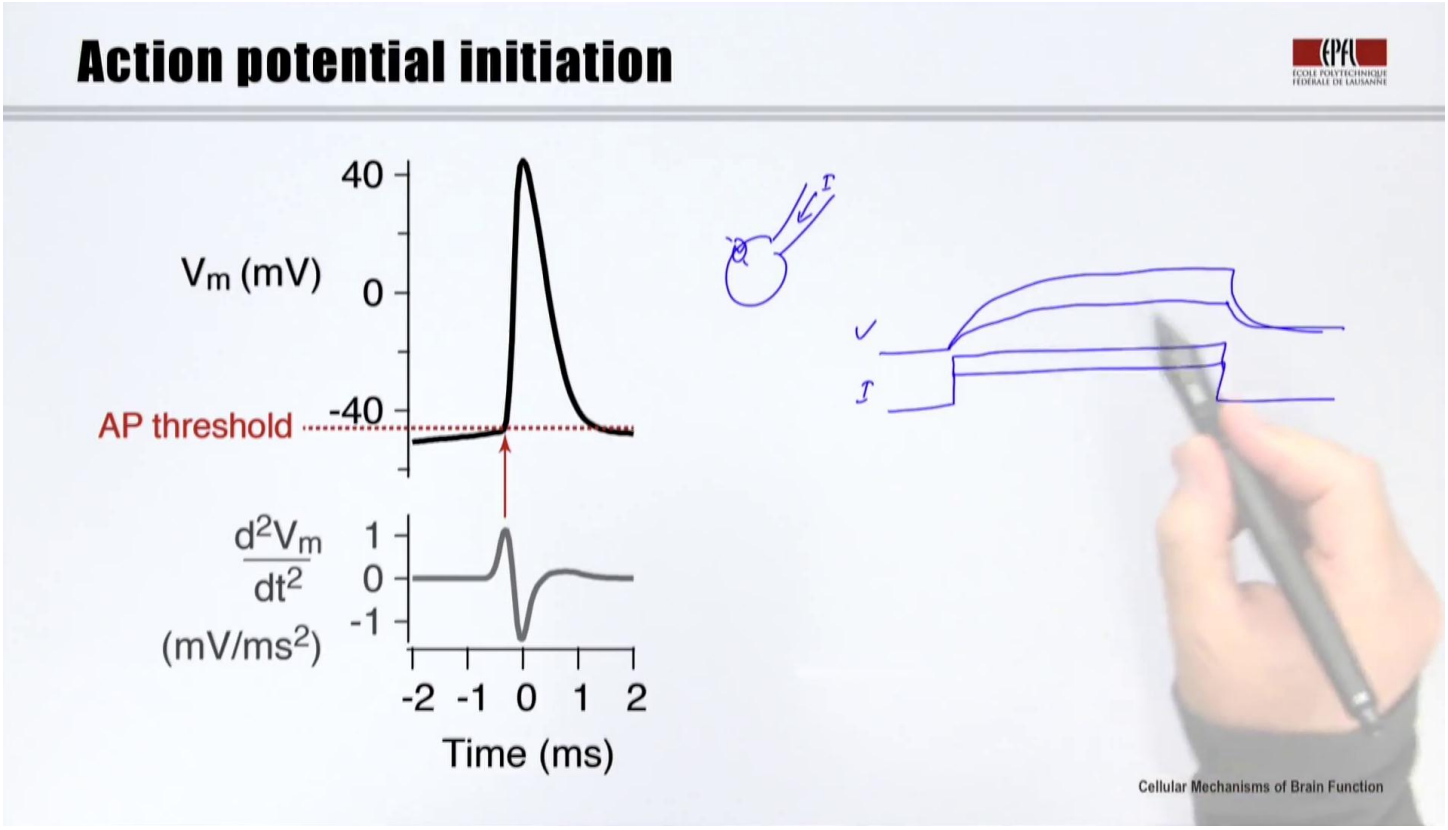


It's rapidly activated and has a very steep voltage activation and so above some given threshold potential, the voltage gated sodium channel becomes activated, drives an inward current, that inward current causes more depolarization and increases, once again, the sodium current driving an explosive depolarization going towards the sodium reversal potential. The sodium channels then rapidly inactivate, up here at some 200 µs and after which the voltage gated potassium channel kicks in and drives the repolarization of the membrane potential back down to its previous state. The description of the action potential, by Hodgkin and Huxley, was made on the squid giant axon, and the ionic conditions, and temperature, are very different from that seen in mammalian nervous system. Nonetheless, the Hodgkin-Huxley formulas with very minor adjustments, turns out to make a good description of action potentials that are being observed in every living species to date. The Hodgkin-Huxley model of the action potential is of enormous importance for neuroscience because it provides the first quantitative description of an obvious and important biological phenomenon in terms of biophysical mechanisms. And it changed neuroscience to a new, quantitative science that still continues today.

Notes

Summary

16m 29s

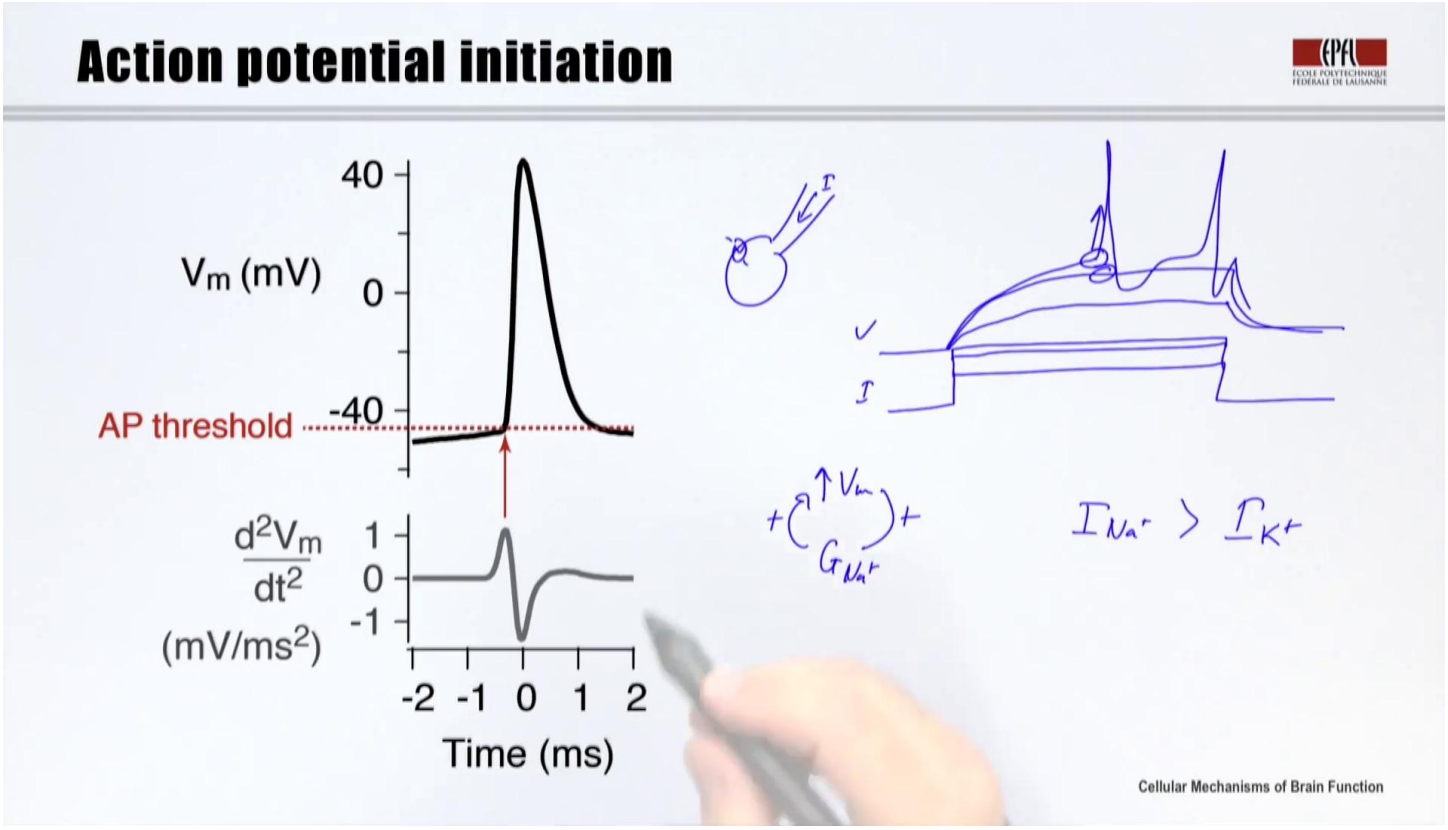


And thinking about the action potential, there's one particularly interesting point the initiation point of the action potential. What happens there at the initiation of the action potential? Here, in this case, we define a threshold potential at which the rate of change of the velocity of the membrane potential is maximal. the point of maximal curvature in this experiment is set to define the action potential threshold that's somewhere around -45 mV in this case. Let's look and see what happens at membrane potentials close to threshold. Let's imagine we're recording from a cell, we can inject some current into it, and we can of course, measure the potential difference across the cell. Inject a little bit of current, and when we inject current, the membrane potential depolarizes, we fill the capacitance of the cell and depending upon the leak conductance, we'll reach a larger or smaller membrane potential. And as we turn the current off, the capacitance discharges and will return to the resting value of membrane potential. If we now inject a larger current, we'll get a larger depolarization in the membrane. If this depolarization was just below action potential threshold, nothing happens.

Notes

Summary

18m 10s

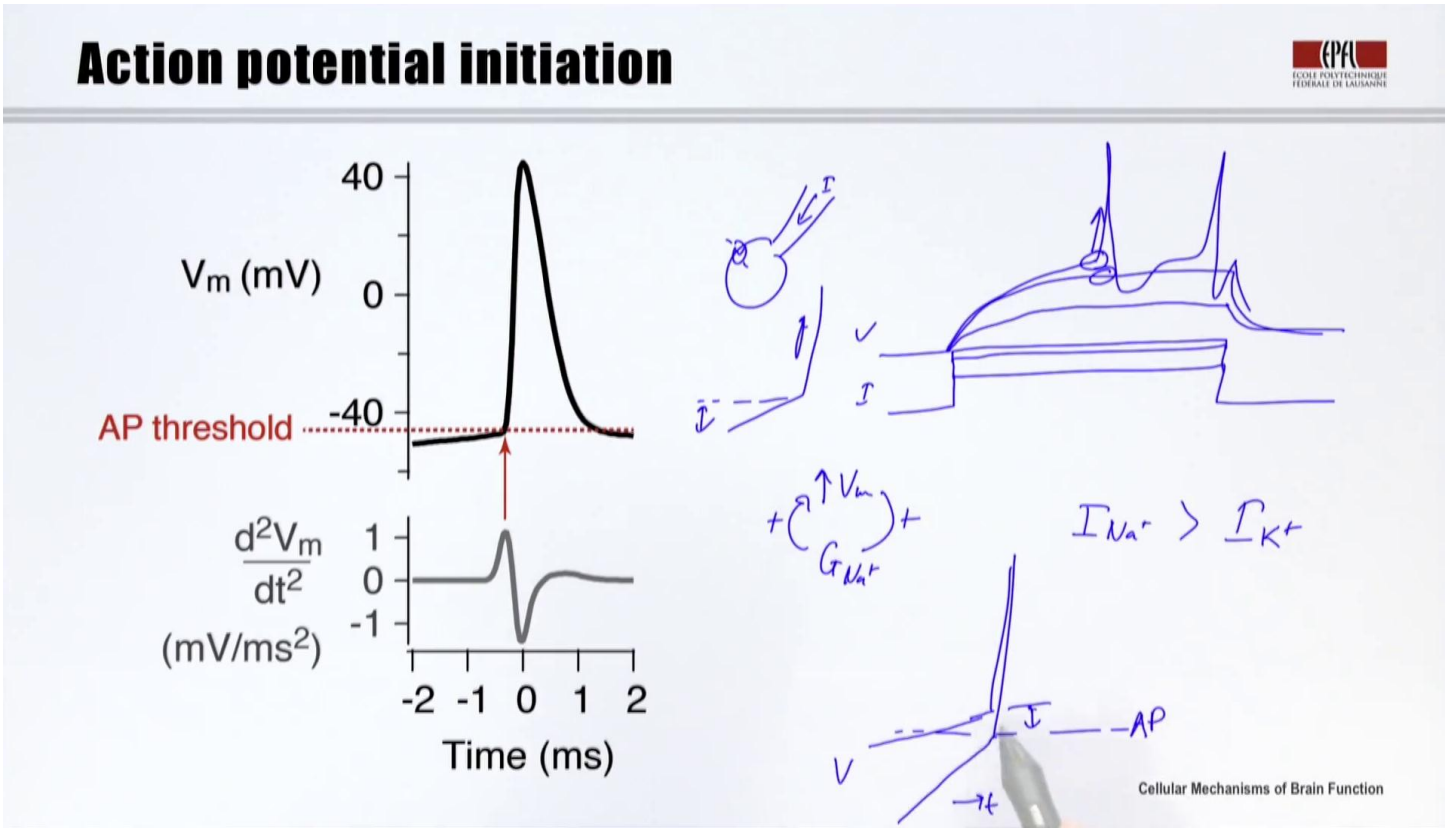


We then inject a tiny bit more current and now, we hit the action potential threshold and an action potential is initiated. What is the difference between here and here? Somehow here, we've reached the autocatalytic step where the voltage gated sodium conductance goes into that positive feedback loop where an increase in membrane potential causes an increase in the sodium conductance and then we get this positive feedback loop that drives the explosive rise of the action potential. And the key step is really that the sodium current must be bigger than other hyperpolarizing currents like the potassium conductance. And if that's the case, then we'll get into this explosive regime whether sodium conductance takes over. The sodium conductance that's available at any given time varies a little bit because we know that sodium channels inactivate, and so just after this depolarization on the rising phase of the action potential the sodium channels inactivate and they remain inactivated for a period of some milliseconds. And so there's an absolute refractory period where for some milliseconds, it's impossible to fire another action potential because the voltage gated sodium channels are inactivated.

Notes

Summary

19m 48s

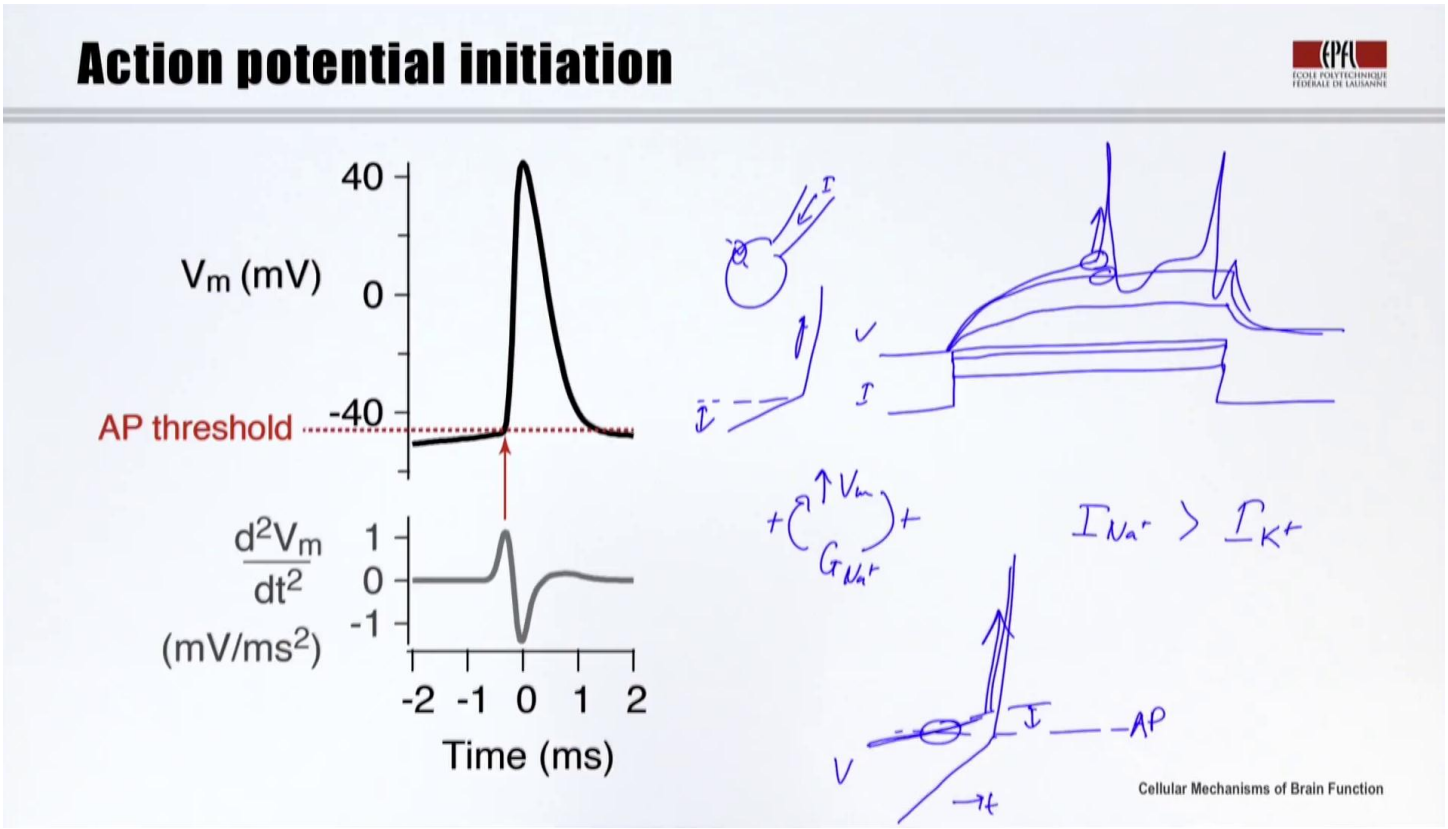


The voltage gated sodium channels then recover with a time constant of some milliseconds, and so after, say, 5 ms or so, the most of the voltage gated sodium current is again, available, but perhaps not quite all of it and this means that the threshold is now increased a little bit because the number of sodium channels, the total sodium current density that can be driven, is actually a little bit lower. And so, we need to be able to go a little bit more depolarized in order to activate more voltage gated sodium conductance in order to get to that steep, rising phase of the action potential. Action potential threshold can also vary in another way, depending upon the trajectory of the membrane potential towards threshold. If we come with a very steep membrane potential trajectory--this would be voltage, this would be time, and we hit action potential threshold. This action potential threshold is lower than if we came with a very flat membrane potential trajectory, in which case AP threshold will be, perhaps, a couple of millivolts higher. And again, this is because of voltage dependent sodium channels inactivating.

Notes

Summary

21m 15s



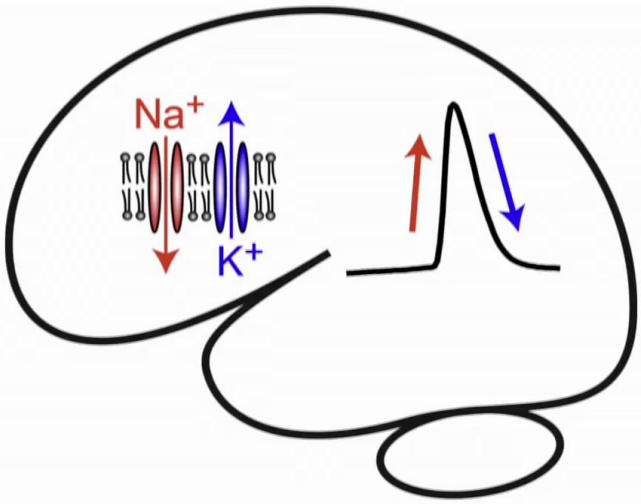
We come very slowly towards threshold, the voltage gated sodium channels have some time to inactivate and that then means that there's less sodium channel that's available to drive that explosive rise of the action potential. So action potential threshold isn't absolute. It varies, perhaps, by a few millivolts. And so action potential threshold of around -45 mV is typical, but we must remember that there are small variations in that threshold that are possible.

Notes

Summary

22m 33s

The action potential



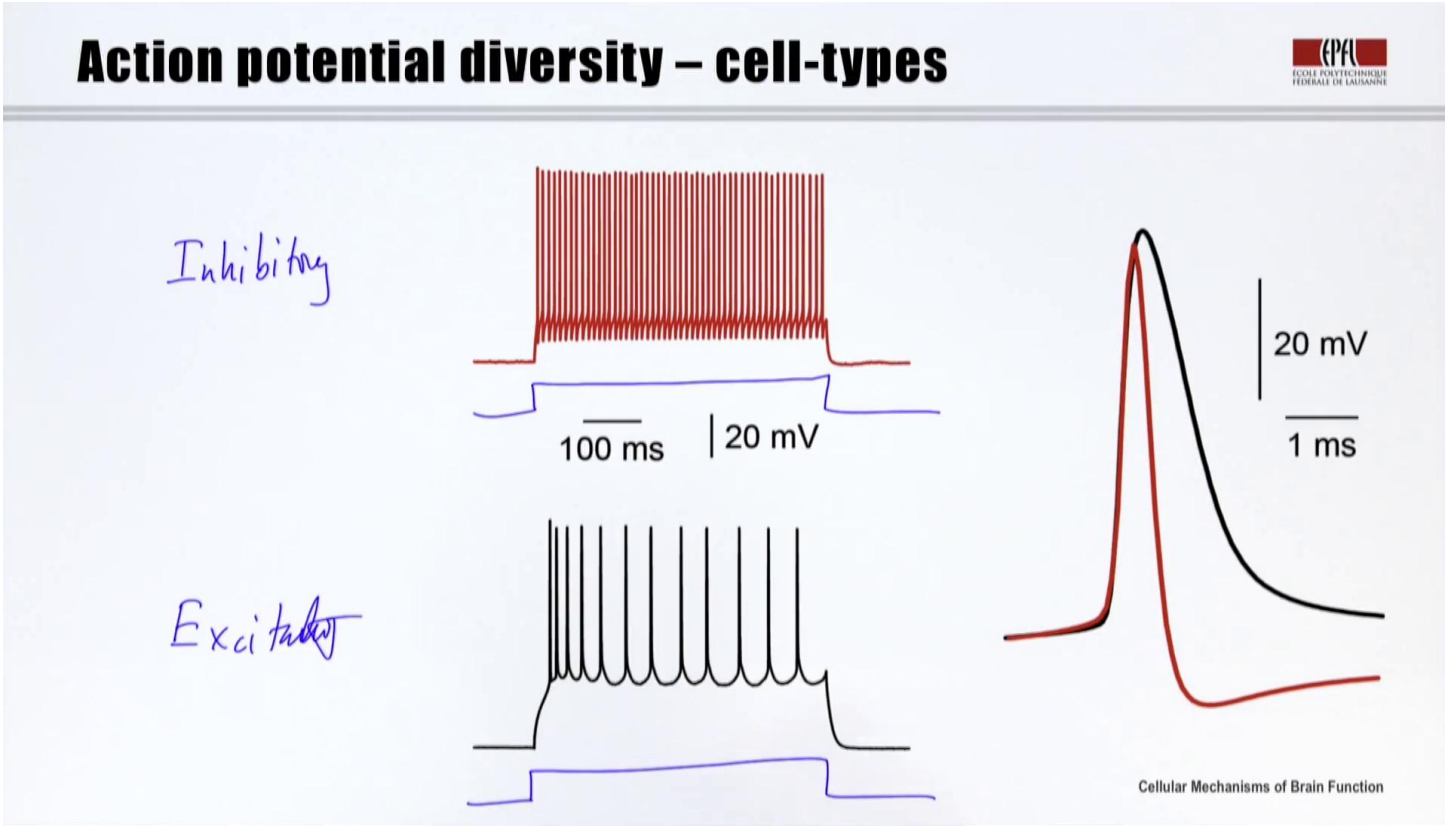
Cellular Mechanisms of Brain Function

So we've now learned about the ionic basis of the action potential, the most important electrical signal in the brain. The action potential is an all-or-none event initiated at a relatively well defined threshold, somewhere around -45 mV, the upstroke is driven by the steep voltage dependence and rapid activation of voltage gated sodium channels, they inactivate after 200 microseconds and then the voltage gated potassium conductance takes over and repolarizes the cell back to negative potentials.

Notes

Summary

23m 07s

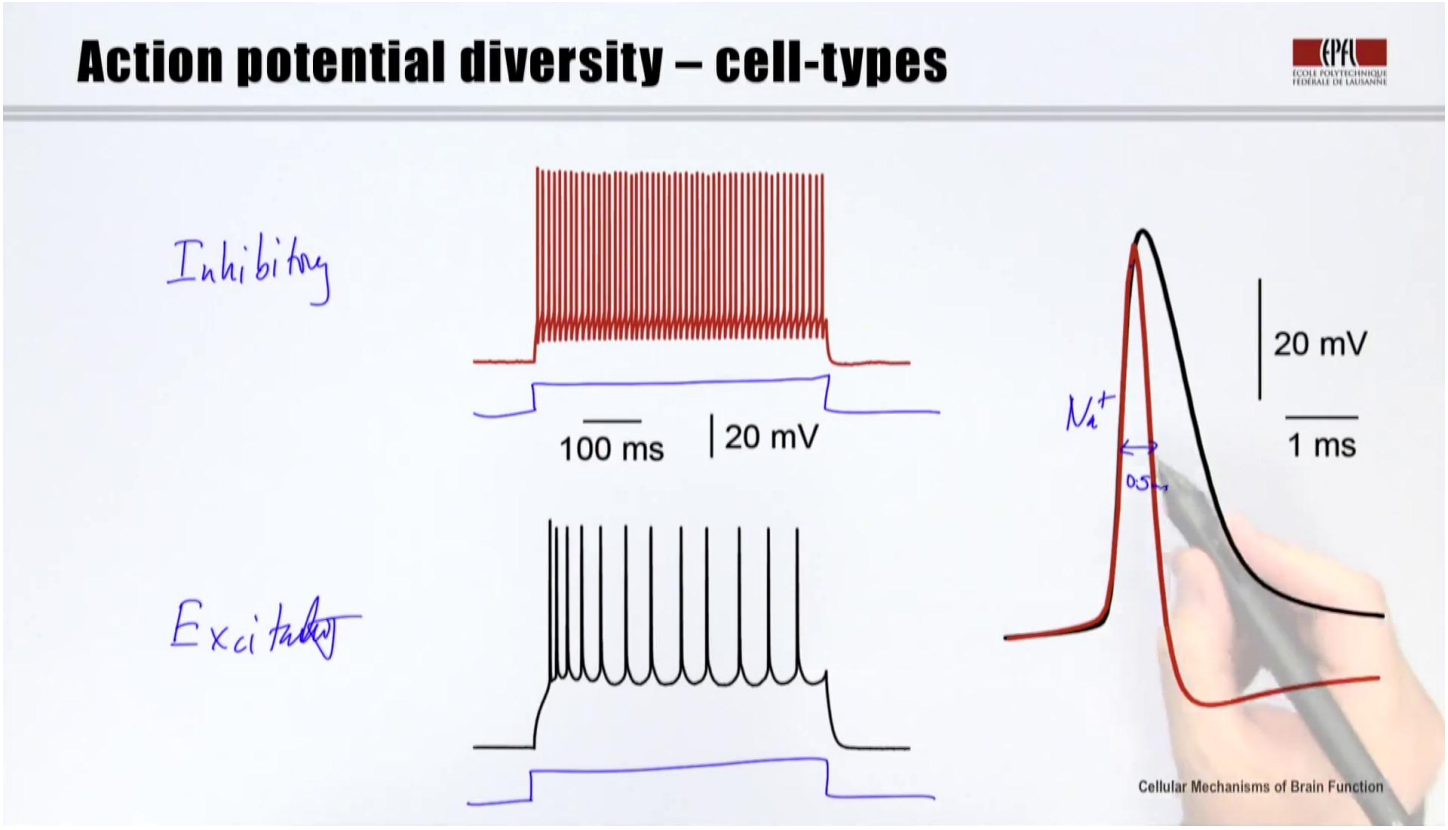


Action potentials show some diversity. So although an action potential is an all-or-none event in a given cell, there's some differences between action potentials across different cell types. And I'd like to illustrate that by an example of two neurons that are recorded next to each other in the neocortex of the mouse. The red cell here is an inhibitory cell that will tend to inhibit its neighbors in terms of the interactions of the neuronal networks. So this cell has an inhibitory influence on its surrounding cells whereas this black cell is an excitatory cell that tends to excite other neurons nearby, and this one will then try to drive action potentials in other nearby cells and this would try to prevent action potentials from being driven in other nearby cells. If a current is injected into the cell, action potentials are fired and they're fired at a very high frequency. The excitatory cell also fires action potentials, but you can see that the rate of action potentials is much lower. And as we increase current, we can certainly increase the frequency of action potential discharge here a little bit, but it's never going to get to the action potential firing rates of this inhibitory neuron that can fire several hundred hertz, more than ten times what's possible for this excitatory neuron.

Notes

Summary

23m 43s

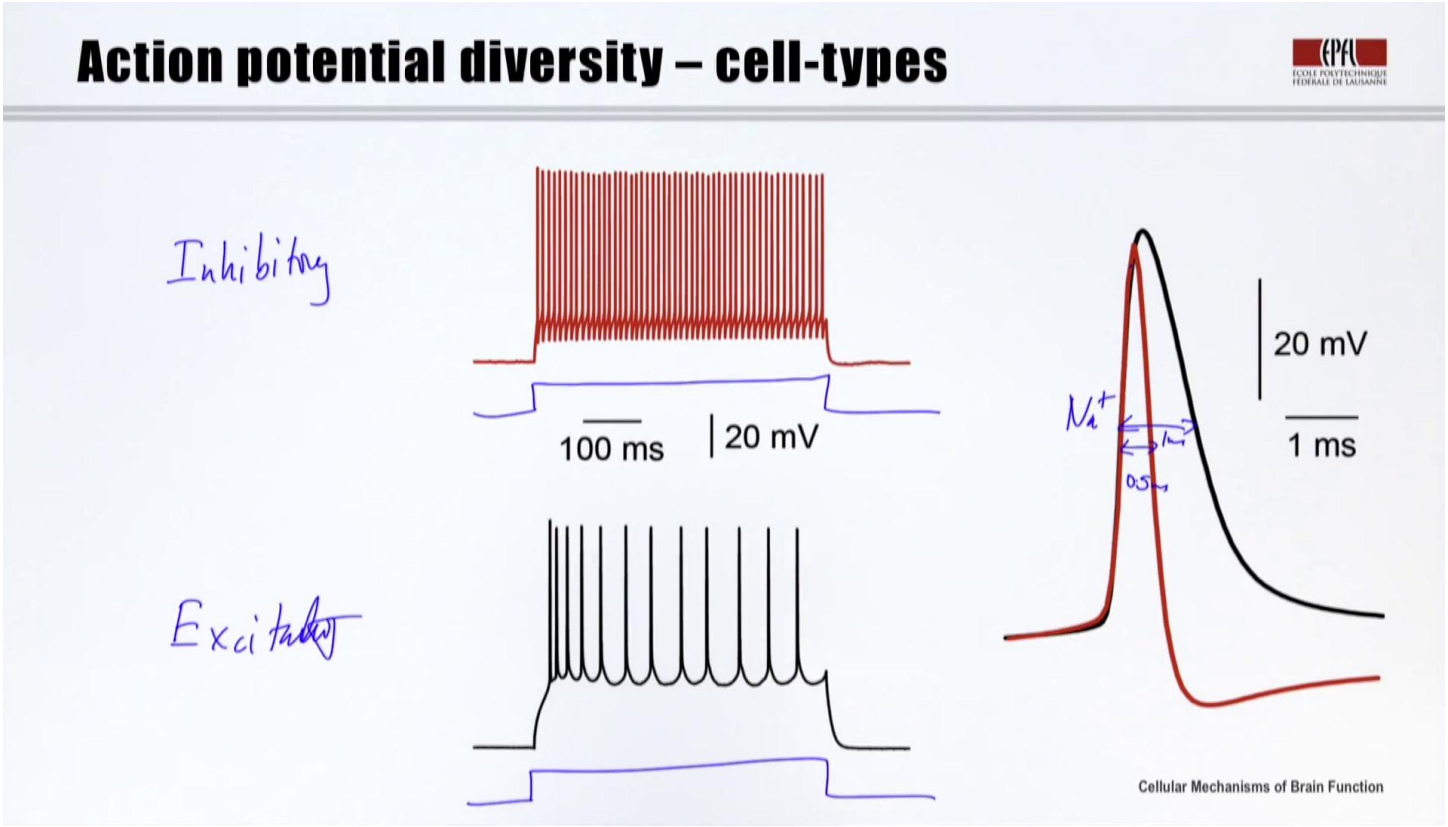


So there are differences in the action potential discharge patterns that different types of cells can have. If we zoom in on these individual action potentials in these trains, we'll see that the wave form of these action potentials is also rather different. The upstroke of the action potential appears to be relatively similar. It's very fast in both the excitatory and the inhibitory cell and of course is driven by the activation of the voltage gated sodium conductance. The repolarization, however, is rather different in these two cell types. In the inhibitory cell, we have a very rapid repolarization. The action potential wave form lasts some half millisecond or so and at a expression of a specific voltage gated potassium channel contributes to the rapid repolarization of this action potential wave form. Excitatory cells have a different potassium channel and here the wave form is almost twice as long, being about 1 ms in duration.

Notes

Summary

25m 17s



The rapid repolarization of the action potential in the inhibitory cell means that the voltage gated sodium channels can deinactivate more rapidly and that then allows this high rate of firing of the inhibitory cell which is simply not possible in the excitatory cell in part, because the availability of sodium channels simply takes longer and so it's more difficult to get the cell to fire again and we need longer delays to deinactivate the sodium channels in the excitatory cell. And so the expression of different voltage gated ion channels, and of course different ion channels in general, will contribute to different patterns of action potential discharge in different neurons.

Notes

Summary

26m 24s

The action potential

- The action potential is a brief (~1 ms) all-or-none signal in the membrane potential, initiated at a threshold voltage.
- The upstroke of the action potential is driven by positive feedback activation of voltage-gated sodium channels. The downstroke is driven by voltage-gated potassium channels.

Cellular Mechanisms of Brain Function

So in this lesson, we've learned about the most important electrical signal in the brain, the action potential. The action potential is an all-or-none signal that's initiated at a relatively well defined threshold voltage and once that threshold is crossed, then the action potential rapidly depolarizes to something close to +40 mV and then repolarizes again back down to something like -50 mV. All of that takes place in the space of about 1 ms. The upstroke of the action potential is driven by the positive feedback voltage gated activation of the voltage gated sodium channels, they then inactivate after a couple of hundred microseconds, the membrane potential is now reached close to the reversal potential of sodium and it's then repolarized by the delayed activation of the potassium conductance that brings membrane potential back down to negative values. The all-or-none digital signal of the action potential is a reliable signal. It's one that's useful for communication across long distances and the spread of that action potential across the neuronal processes is what we'll look at in the next lesson.

Notes

Summary

27m 08s