MICHALE FEE: Today we're going to continue building our equivalent circuit model of a neuron. Again, this is the Hodgkin-Huxley model, and the model was really developed around explaining how neurons generate action potentials. There are two key ion channels that are associated with making spikes. There's a sodium channel that we model as a conductance in series with a battery, and there's a potassium conductance that we model the same way. And again, those two conductances cooperate to produce an action potential.

And we saw, essentially, how those two conductances, together with their batteries-- the sodium battery is up at plus 50 or so millivolts. The potassium battery is down at minus 75 or so millivolts. And those two conductances then work to essentially connect the inside of the neuron to the plus battery and then to the minus battery to give you an action potential. And you may remember that we saw what that looks like.

So here is plotting membrane potential in blue. Here, we turn on this sodium conductance. The voltage of the cell races up to about plus 55. Then we turn off the sodium. We turn on the potassium. The voltage goes down to minus 75 or so. And then we turn off both conductances, and the cell recovers. So you can see that that basically produces what looks like an action potential. That's the basis of action potential production.

But in order to understand how these things turn on and off in the time course that they do, we need to understand a little bit more about how these sodium and potassium conductances work. And that's what we're going to focus on today. So let's just start building our model. So each one of those conductances with a battery is associated with a current. There's a current that goes through each of those conductances.

The total current through the membrane, the ionic current through the membrane in the Hodgkin-Huxley model is a sum of three components, actually-- a sodium current, a potassium current-- those two we just talked about-- and a leak current that is just a fixed current. So the sodium and potassium currents are functions of time and voltage. The leak current, the leak conductance, is just fixed. And it has a

battery of about minus 50 millivolts. And it just tends to keep the cell sort of hyperpolarized. And these two currents, these two conductances, do the job of making an action potential.

So the total membrane current is just a sum of those three parts. And now we can just take that membrane current and plug it into this equation for the voltage as a function of current now and solve that differential equation to get the voltage to calculate how the voltage evolves in time in the presence of these membrane currents.

All right, so you recall from the last lecture that you watched on video that these currents can be written down as a conductance. So let's just start here. This is the most similar one to the one that you saw in the previous lecture. The current is just a conductance times a driving potential. And we described how that equation can be summarized in electrical circuit components as a resistor, which is one over the conductance, times a driving potential, which is basically just the voltage drop across the conductance.

Now, each of these conductances, each of these currents, is going to be written down by a very similar equation. So the potassium current is just the potassium conductance times the driving potential for potassium, which is just the membrane potential minus the Ek. And the sodium current is just the sodium conductance times the driving potential for sodium, which is the membrane potential minus the sodium battery. Any questions? Yes?

AUDIENCE: How did the [INAUDIBLE].

MICHALE FEE: Yeah. So unless I've made a mistake, I've tried to put these-- remember that the potassium battery is minus, is negative, right? And the sodium battery is positive. So I've tried to show that by putting the batteries in the opposite direction, right? So the battery symbol has one side that's supposed to indicate positive voltage, and the other side is negative. So because they have the opposite sign, I've put them in backwards, in the opposite direction. Does that make sense?

Don't worry about that too much. If I ask you to draw this, I don't really care that much which way these things go. I just want you to know that this one is negative on the inside and that the sodium is positive on the inside. This is the inside of the cell

here, right? The sodium battery drives the inside of the cell toward positive voltage, because you have positive ions flowing into the cell.

So you can see now that the membrane potential here depends on the membrane currents through this differential equation. But the membrane currents-- the sodium, potassium, and leak currents-- all depend on these conductances, right? And these conductances for the sodium and potassium are voltage dependent and time dependent. So you can see that the conductances depend on the voltage, right?

So the membrane potential depends on current. Current depends on conductances, but the conductances depend on the membrane potential. So it goes around and around and around, right? So those things all depend on each other. And so what we are setting out to do is to write down the way those things depend on each other, the way you can think about that system evolving in time.

So let me just show you what the plan is. So the plan is to write down an algorithm-- basically, a for loop-- that describes how the neuron generates an action potential. Let me just walk you through the steps of that, and then I'll get to your question. So we're going to start with some membrane potential V, and we're going to calculate that the voltage-dependent parameters of the sodium and potassium conductance using that membrane potential.

And once we have those parameters, we can actually calculate the conductance for each of those, for the sodium and potassium. Once you know the conductance, you can get the currents. Once you have the currents, you can compute the total membrane current, just by adding them all together. Then, you can compute-- once you have all those currents, you just compute V infinity, which is just the current times the resistance, the effective resistance.

Then we're going to integrate our first order linear differential equation to get a new voltage as a function of time and V infinity. And we're just going to go back and start again. The so that's the algorithm that a neuron uses to generate a spike. And that's what we're going to work out.

Now, we've talked about these things over the last few lectures, how you can relate

total current to V infinity and then integrate a first order linear differential equation, which is just relaxing exponentially toward V infinity. But now we're going to put these things in, figure out the voltage and time dependence of the sodium and potassium conductances. Yes?

AUDIENCE: [INAUDIBLE].

MICHALE FEE: Yes. It's primarily potassium. It has a negative potential. But it's just constant, so we're not really going to pay much attention to it. So if the sodium and potassium currents are off, then the leak current still keeps the cell hyperpolarized. All right, any questions? That's the big picture.

So here are our learning objectives. I'd like you to be able to draw that circuit diagram, not worrying about the long and short sides of the battery. We're going to talk about how we measure the properties of ion channels. That's called a voltage clamp. So I want you to be able to describe what that is. I'd like you to be able to plot the voltage and time dependence of the potassium current for today.

The next lecture, we'll talk about the sodium current, so we'll add that to our list of things that we need to know. But for today, I'd like you to able to plot the voltage and time dependence of the potassium current and conductance. And be able to explain, biophysically, where the time and voltage dependence of that potassium conductance comes from and be able to write it down in terms of quantities that are called the Hodgkin-Huxley gating variables. So that's the plan.

All right, so let's come back to our circuit. Again, we have a sodium current that's sodium conductance times the sodium driving potential. The conductance is voltage and time dependent. The equilibrium potential for sodium, again, is plus 55 millivolts. The potassium current is just potassium conductance times the potassium driving potential. The driving potential is reference to a battery in equilibrium potential at minus 75. And the leak has a battery at minus 50 millivolts.

So those are the parts we're going to use. We're going to describe now the experiments that Hodgkin and Huxley did to extract the parameters of the sodium and potassium conductances. Today, we're going to focus on the potassium conductances. And then on next Tuesday, I guess, we're going to do the sodium.

All right, so the reason Hodgkin and Huxley studied-- so they studied these channels, the potassium and sodium channels, in the giant squid axon. Now, most of our axons are about a-- most of the axons in our brain are about a micron across. This axon is about a millimeter across. Action potentials propagate much faster in large axons, and this axon is involved in transmitting an action potential from the brain to the tail that drives an escape reflex.

The squid squirts water out of sort of a chamber that has water in it. If the squid senses danger, it contracts muscles that squeeze water out of that, and it makes a jet. And it squirts the squid forward away from danger. So that action potential has to propagate very quickly from the brain to the tail, and it does that through this enormous axon. That axon is so big you can now put multiple wires inside of it. You don't even need to pull these glass electrodes. You can just take little wires and stick them in-- chop out a little piece of the axon and stick wires inside of it and study it. Yes?

AUDIENCE: So if the body of this squid is like a giant [INAUDIBLE] it has arms coming out of its head?

MICHALE FEE: Yes. You eat that part, and you eat that part. Not that. You throw away the most interesting part. OK, any other questions?

All right, so now we're setting out to measure these sodium and potassium conductances, OK? So how do we do that? So what we really want to do is to set-- we want to measure conductance, which is the relation between voltage and current. So we'd like to do is to be able to set the voltage at a certain level and measure the current that flows through these channels. So you really want to plot the IV curve, right? You want to set the voltage, measure the current, and do that at a bunch of different voltages.

And you recall that the conductance is basically just the slope of that curve, right, that line. So the job is set voltage, measure current, extract conductance. Now, the problem with that is that as soon as you set the voltage of the axon somewhere up here in an interesting range, the thing begins to spike. And then the voltage is no longer constant, right? So it becomes really hard to make measurements like this if you depolarize the cell a little bit to set try to set the voltage and all of a sudden, it's

[MIMICS BUZZING]. It's generating spikes.

So what do you do? So the trick is to develop a device called a voltage clamp. This thing basically holds the-- so, look, if the action potential were really, really slow, then you could actually set the voltage. You could change the current being injected into the cell by hand. You say, OK, I'm trying to set the voltage at zero. Oh, it got a little bit too high, so I turn the current down. Now the voltage has gone too low, so now I turn the current up.

You could do it by hand if the action potential were super slow, if it took a minute to generate, right? But the action potential takes a millisecond. So that's just too fast for you to follow. So you just make a little electrical circuit that does that job for you. It uses feedback to set the voltage where you want. So you put a-- here's your cell. Here's your membrane resistance or conductance that you're trying to measure.

You put a electrode in the cell. You put it into a little amplifier called an operational amplifier. And then on the other side of that amplifier, that differential amplifier, you put the command voltage that you're trying to set. So here's the way it works. Basically, this thing tries to set the membrane potential. It tries to make this value, this voltage, equal to that voltage. And it does that by feeding current back into the cell. Does that make sense?

OK, so you use an operational amplifier. An op amp has two inputs-- a plus input, a minus input. The output is just a gain times the plus input minus-- the positive input minus the negative input. And the gain is really big. It's about a million. So if this input is a little bit above that input, this output is big and positive. If this input is less than that input, you can see this is negative. And so the output is big and negative. Any questions?

So don't get confused here. That G is gain, not conductance, just for the next few slides. So how does this work? You can see that if the membrane potential is less than the command voltage, then the output voltage is positive and big. That drives current into the cell, which increases the membrane potential and makes it approach the command voltage. If the membrane potential is larger than the command voltage, then this thing-- this is bigger than this. So this is negative.

And that pulls current out of the neuron and decreases the membrane potential.

And in both cases, the membrane potential is being pulled toward the command voltage. And you can show, if you just plug in these variables into a couple of equations, that as long as the gain is big enough, the membrane potential is forced to be very close to the command voltage.

All right, so that's the voltage clamp. It drives whatever current is necessary to clamp the voltage of the cell at the command voltage. And then what we do is during an experiment, we step the command voltage around. The cell tries to spike. Those currents turn on. The cell tries to spike, but this thing keeps the voltage locked at whatever it is that you want the voltage to be.

And then you measure the amount of current required. You just measure the amount of current flowing through this resistor here that's required to hold the cell at any voltage. All right, any questions? Voltage clamp-- very cool. Yes?

AUDIENCE: Can you explain gain again?

MICHALE FEE: Gain is just the multiplier here in this equation. So if there's a tiny difference between the two inputs, the output is bigger. That's what gain means, right? If the gain is a million, if there's a microvolt difference between the two inputs, the output will be about a volt. And it would be a plus or minus, depending on which of those two was more positive.

OK, so now let's get to the actual voltage clamp experiment that Hodgkin and Huxley did. Here, we have two wires in our cell-- one to measure voltage and the other one to inject current. That's exactly what they did. There's one wire here. That's a little piece of axon. You can literally cut the squid open, find that axon. It's a big, white-looking tube about a millimeter across. Cut two pieces of it, take two wires, stick one in each end.

I drew it like this, but if you did it that way, they'd probably short together. So then one of those measures the voltage. You set a command, and the other wire allows you to inject current inside the axon. And then you seal the ends with a little bit of Vaseline. Yes?

AUDIENCE: Sorry, what is the command VC?

MICHALE FEE: VC is the command voltage that you're trying to set the inside of the cell to. Remember, here, we're setting the-- VC is what you're controlling as the experimenter. You're setting the voltage with that command. So the voltage clamp then holds the inside of the cell at that command voltage, and then you're measuring the current with this device. There's a readout that tells you how much current it's putting into the cell, or it goes onto an oscilloscope, because it's time dependent.

So let's do an experiment. Here's an example of an experiment. They hold the command at minus 65. And suddenly, they drop the command voltage to minus 130. What does the cell do? What is the current? Nothing. There's a little transient here, which is the amount of current. It took to charge that capacitor up to minus 130 millivolts, and then nothing happens.

All right, let's do another experiment. Now we're going to start our cell at minus 65 and suddenly jump the voltage up to zero. So we're going to depolarize our cell. And now something happens. We get a big pulse of current that's negative. What does negative mean? Anybody remember what negative current means by our definition?

Negative means that there are positive ions going into this cell. So it's charging the cell up. This is membrane current now. So you just have to remember that definition. Negative membrane current means that positive charges are going into the cell, and that's depolarizing the cell. And then that negative current lasts for a few milliseconds, and then the current reverses sign and becomes positive and stays on.

So what is that? So the first thing that Hodgkin and Huxley did was they tried to figure out what causes that pattern of currents. So here's what they did. They had this idea that part of this might be due to sodium. And so what they did was they did an experiment where they replaced sodium outside the cell, outside the axon, with an ion. They kept the chloride, but they replaced the sodium with choline. So they used choline chloride, so it's a salt solution, but it has no sodium in it.

And then they redid that experiment. And here's what they found. What they found was-- so here it is-- with sodium, and if they replace the sodium, they find that they

get almost the same thing, except that initial negative pulse goes away. And so they hypothesized that that part is due to sodium. And so now you just subtract this from this to get the difference. And that is the sodium current. Does that make sense?

And then one other thing. Through another set of experiments, they were able to show that this part that's left after you block or remove sodium is actually due to potassium. So this is the potassium current. That is a sodium current. So by doing different kinds of experiments-- so one of the things that you're able to do that they didn't do initially, but later, they were able to do things like take that little piece of axon, take out a little miniature paint roller, squish the axon, roll the roller over the axon, squish its guts out, and then fill it up again with solutions that they control that have different ions in them.

And so they're able to study-- just do multiple different kinds of experiments to be sure that this slow thing that turns on, this slow positive current is potassium, and this fast negative current is sodium. All right? So now what you can do is you can do this experiment at different voltages here, right? We want to measure how these currents depend on voltage, right?

We can see here how they depend on time. We can also see how they depend on voltage, by doing this experiment at different voltages. You start at some negative potential. You step the voltage up to minus 40, or you step it up to zero, or you step it up to 40. And now you can measure that potassium current as a function of time, or the sodium current as a function of time. All right? At different voltages.

All right, so those look kind of weird, especially that one. That looks kind of scary, like what the heck is going on there? But it turns out that both of these things are actually pretty simple. Once we dig a little bit more into how these currents are produced, you're going to see that there's a very simple way to understand what's happening there. All right, any questions? Bear with me.

So now what we want to do is we want to measure the voltage dependence of these things kind of separately from the time dependence. So what we're going to do is we're going to measure the peak potassium current, kind of this steady state potassium current as a function of voltage. So here's our IV curve that I promised that we were going to plot. Peak current as a function of voltage.

You can see that it's approximately linear above minus 50 or so millivolts. The sodium current looks kind of weird. We're going to plot the peak sodium current as a function of voltage. And we see that the peak sodium current has this weird shape. It's sort of linear up here at positive voltages, and then it crashes down to zero at negative voltages. All right? Still kind of weird and scary.

So let's see if we can understand where this comes from. I just replotted them here. So now, remember that we use this voltage clamp to measure current as a [AUDIO OUT] voltage. But what is it that we're really trying to understand? We're really trying to understand the conductances, those resistors, right? We're under trying to understand the voltage and time dependence of those conductances.

So we're trying to extract conductance as a function of voltage. And remember that [AUDIO OUT] is just conductance times a driving potential for potassium. Sodium current is just sodium conductance times the sodium driving potential. So we you could imagine extracting the conductance as current divided by the driving potential. This is what we're really trying to find.

Rather than doing this division, because this one over-- this thing goes to zero at the places where the voltage is equal to the equilibrium potential. So we don't want to do that. We're going to solve this problem graphically. So here's the driving potential, right, V minus Ek. It goes to zero at Ek, right?

And we want to find a conductance that makes this look like this. So if this is kind of a straight line, if the current as a function of voltage is kind of a straight line and this is a straight line, what does that tell us about the conductance in this region up here? It's constant. Excellent.

Now, if the driving potential is very negative here but the current is zero, then what's the conductance? Driving potential is big and negative. Current is zero. What does that tell us about the conductance? It's zero. OK, so not so hard, right? We have [AUDIO OUT] zero here and constant here. So can anyone just show me what that might look like?

Good. It could be like a jump. It could be kind of smooth. And that's exactly what it looks like. So the conductance is zero here, which it has to be, because the current is zero, even though the the potential is negative. And the conductance here is

constant, because the driving potential is constant here. The driving potential is linear, and the current is linear, so this needs to be constant, all right?

So the conductance is very simple. It turns off at negative voltages and turns on and then stays on at higher voltages. Let's do that for sodium. This thing looks crazy, weird, right? But let's go through the same operation. Here's our driving potential for sodium. Remember, it's got a reversal at plus 55. But it's a straight line, right? That's a battery and a resistor. So there's our driving potential.

Now, this is linear. This is linear. So what does the conductance look like up here? Good. This is zero, but this is big and negative, so what does the conductance look like down here? Good . Starting to look pretty familiar, right? Boom. It's exactly the same. Both of these conductances are off at negative potentials, and they turn on at positive voltages and remain constant. Yes?

AUDIENCE: [INAUDIBLE].

MICHALE FEE: Great. Great question. Because the sodium conductance turns on, and then it shuts off right away. And the shutting off is a different mechanism. So we're trying to figure out how to ignore that and just understand the voltage depends of how it turns on. Does that make sense? And we'll get into that next Tuesday.

But I'm showing both of these at the same time because they look similar at the level of the voltage dependence. In fact, the way they turn on is very similar. It's just that the sodium has some other weird thing that shuts it off after a few milliseconds, and we'll talk about that next time. Yes?

AUDIENCE: [INAUDIBLE].

MICHALE FEE: No, it's not. But any non-linearity here, we're going to account for by changes by voltage dependence of the conductance. So great point. It's a subtlety that we have kind of imposed by this way of writing down the voltage dependents of the current. Any other questions? No? OK, pushing on.

So this kind of gradual turning on, this sort of zero conductance down here and a [INAUDIBLE] up here, that's called a sigmoidal voltage dependence. And it's the voltage dependence of activation. It's how these channels turn on. And as I said

before, we're going to deal with the other properties of the sodium channel that turn it off later. So both have a sigmoidal voltage dependence of activation.

And if you plot this, you can see that-- if you plot this on a log scale here-- log scale on conductance, linear here on potential. You can see that both of these curves, both the potassium and the sodium, have this very characteristic exponential turn-on followed by his saturation and constant conductance at higher voltages. All right, any questions?

That's voltage dependence. Now we're going to turn to time dependence. So you can see that the time dependence-- so this driving potential, that's just constant. That just depends on the voltage and the reversal potential. And so we can separate out-- this thing is just any time dependence this has is dependent on the time dependence of the voltage. But in our voltage clamp experiments, the voltage is constant. So this thing is constant.

So any time dependence [AUDIO OUT] current has to be due to time dependence of the conductance, right? And what that means is we can just look at the shape of this potassium current-- remember, this is the potassium current here. That time dependence is just due to time dependence of the conductance. Does that make sense?

So what's happening is the potassium conductance is starting at zero. The moment you step the voltage up, that thing begins to grow gradually and then runs up to a constant potassium conductance in time. It starts off, ramps up, and then becomes constant, all right? So that's the time dependence. Sort of gracefully turns on.

That process of turning on is called activation. The sodium conductance-- or current, the same thing. The sodium current is just the sodium conductance. The sodium current is a function [AUDIO OUT] is the sodium conductance as a function of time, times a constant. In our voltage clamp experiment, again, this voltage is constant. So the sodium conductance turns on. That's activation. But then it turns off, and that's called inactivation.

So the sodium current has [AUDIO OUT] sodium conductance has two things going on-- one, activation, and the second is inactivation. And it turns out these are two separate biophysical mechanisms. And we're going to spend more time on this next

week.

So, notice something interesting. The sodium conductance turns on. You depolarize the cell. Sodium conductance turns on right away and then shuts off. The potassium conductance has a delay, and then it turns on. Does that look familiar? It looks an awful lot like this, right? Here's the sodium conductance. Turns on and then shuts off. And then the potassium conductance turns on with a delay. And that gives us an action potential.

So you can see that when you use voltage clamp and dissected out the time difference of the sodium and potassium conductance, it looks just like the thing we concocted earlier, just sort of our toy example for how to make an action potential. Pretty cool, right? OK, it's starting to come together piece by piece.

So we're now going to dig in a little bit deeper into the biophysics of how you get these voltage and time dependencies. So we're going to derive the equation for the voltage dependence. Anybody want to take a crazy guess on how we're going to do that? Just a wild guess, how you might derive the voltage dependence of something? No? OK. We're going to use the Boltzmann equation.

And we're going to derive different equations that describe the way those channels turn on, how those conductances turn on. All right? And once we do that, we're going to have a simple set of equations-- and not just equations. We're going to have a set of processes that we can think of as happening a loop, in a for loop. That's our algorithm for an action potential.

All right, so let's dive into single channels and see how they work. So, of course, currents result from ionic flow through ion channels. It's actually possible to record currents from single ion channels. We can actually make a version of our voltage clamp that we can attach to a single ion channel. And the way you do that is-- so when you take this piece of glass and you pull it, instead of poking it through the cell, instead of making it really sharp and poking it through the cell, what you do is you make it a little bit blunter, so it's got kind of a rough end.

And then you can fire a polish-- you can hold the end of that electrode into a flame. Not quite. It's usually a filament that heats up hot. You hold the end of the electrode near this hot filament and it melts the tip into a nice, round-- it's still a tube, but the

edges of the tube are nice and smooth. And now when you take that tube and you press it up against the cell-- actually, you attach a little plastic tube to the end of the glass, and you press that that electrode up against the cell.

And you actually literally suck on it with your mouth onto that tube. And it sucks the membrane up against that smooth end of the electrode. And it sticks. The lipids of the membrane actually seal themselves onto the end of the glass. So now no currents can flow out through these edges here, all right?

And then you hook it up to a very sensitive current amplifier. And now you can control the voltage. You can actually just rip that off of the cell, so now there's no more cell. You just have an ion channel sitting there on a piece [AUDIO OUT] on the end of your glass. Now you can do a voltage clamp experiment and study the current-- the voltage dependence of the current through that ion channel.

So here's what this looks like. Here's one experiment. We're going to start at minus 100. This is a potassium channel. You depolarize the potassium channel up to 50 millivolts, and you see that that current, through that single channel, starts flickering on and off. Here's another trial. Turns on, turns off, turns on, turns off. You can do that a bunch of times.

You can see something interesting. The current is either off-- doesn't turn on gradually, doesn't change smoothly. It just flickers between on and off. That's a very important aspect of ion channels. But if you average all those trials together, you see that you get an average current that looks just like the current that Hodgkin-Huxley measured in the whole axon. How is that possible?

AUDIENCE: [INAUDIBLE].

MICHALE FEE: Yeah. Good. So, basically, what we're doing is we're measuring one ion channel many times. But on a cell, you're measuring a bunch of ion channels, each of which is doing something like this. But they're happening all at the same time, and the current is being averaged. So here, we're averaging the current one at a time. And on a whole cell, we're just averaging a bunch of them at once. It's called ergodicity in physics. It's called the ensemble average.

OK, you can do the same thing for sodium. You take your patch, a new patch

electrode. Fire polish it. Push it up to a cell. Apply some suction. Glues on. This time, we had a sodium channel. And now you can see that the thing, again, flickers on, flickers off, flickers on, flickers off. But now, they all flicker on right at the beginning, and then they flicker off and stay off.

And if you average all those different trials, you see an ensemble average sodium current that looks just like when you measure the sodium current on a whole axon, OK? But the key thing is that these channels have two states-- on and off-- and they flicker back and forth between those two states, conducting and non-conducting. So we can now write down-- we could start working with this idea that our ion channels are either open or closed.

And we can think of a probability that the channel is being open, that the channel is open. And we can have a total number of channels. The number of open channels is just the number of channels you have times the probability that any one of them is open. If g is the inductance of one open channel, then we can write down the total potassium conductance as the probability that any given ion channel is open times the number of channels times the inductance of one open channel. Does that make sense?

And now the claim here [AUDIO OUT] all of the interesting voltage and time dependence of these channels happens here. Obviously, the number of them isn't changing very rapidly. The conductance per channel is constant, per open channel. So the interesting stuff is in the probability that the channel [AUDIO OUT]. And if we want to get the current, we're just going to plug this conductance into here, OK?

All right, so let's start with a potassium channel. Let's dig in a little bit deeper into what the potassium channel looks like. Potassium channel is formed by four identical subunits. They're produced separately by ribosomes. They form a heteromer, a tetramer. And that tetramer has a hole that runs down the middle of it, which is where the ions flow.

Each of these subunits has a voltage sensor that allows it to turn on and off. In order for the channel to be on, all four of those subunits has to be open. So each subunit has an open state and a closed state. And for the channel to be open, all four of them have to be in the open state.

So if n is the probability that any one subunit is open-- I meant to make you guys answer this question before I showed the answer. But is it clear how if any one subunit has a probability of being open of n, then the probability that the whole channel is open is n to the four? This n is called a gating variable. I would like you to know that the probability that a sodium potassium channel is open is n to the four. That's an important thing for you to remember.

That assumes that those four subunits are independent. And in potassium channels, that's a very good approximation. So we can now write down the conductance of our potassium channel-- something times n to the four, where that something is that inductance of one ion channel.

So we can now write down the current as n. Open conductance times n to the four times a driving potential. And that n is called the gating variable for the potassium conductance. All right, any questions? No? Yes?

AUDIENCE: [INAUDIBLE].

MICHALE FEE: n absolutely does depend on voltage. Very good. That's where we're going next. But before we go on to that, I wanted to add one other thing, which I think is really cool. We're going to do the voltage dependence of a potassium channel using the Boltzmann equation.

So here's the way you think about a potassium channel working. Here's a potassium channel. We're showing a cross-section. Here it is. Here's the membrane, the lipid bilayer. Here's our potassium channel, sitting in the membrane. And we're taking a cross-section through that tetramer that shows two subunits. And I'm showing the voltage sent-- I'm showing the mechanism that that opens and closes one of those subunits.

This subunit we'll also have a voltage sensor and a gate that looks the same. So look, the voltage sensor-- how do you sense voltage? You sense voltage with charge, right? Voltage differences, I should say, you sense with a charge. Because voltage gradients are electric fields, and electric fields push on charges. So if we want to detect the voltage difference across this membrane, we put a charge in the membrane.

When the voltage difference is zero, there's little force on those charges. Now, if we suddenly hyperpolarize the [AUDIO OUT] cell so it's very negative, now there's an electric field inside the membrane that points toward the inside of the cell, which pushes those charges toward the inside of the cell. And now you can just have a little mechanical linkage. That's not really what it looks like, but there's some way that the amino acids and the protein are configured so that when those charges get pushed on, it closes a gate. And now the current can no longer flow through the ion channel.

OK, so now we're going to derive how this-- we're going to see how to derive this voltage dependence from the Boltzmann equation. All right, so, everybody, this is just for fun. I don't expect you to know how to do this. I just want you to see it, because I personally get chills when I see this. It's really cool. But I'm not expecting you to be able to reproduce it, OK? So just watch.

So, again, the Boltzmann equation says that the probability of being in two states, open or closed, depends on the energy difference between them. So we have an open state and a closed state. And when the voltage inside the cell is zero-- when the voltage difference between the inside and the outside of the cell is zero, [AUDIO OUT] know that the sodium channel likes to be open.

So what that means is that the open state has a lower energy than the closed state, right? Sodium channel likes to open when the cell is depolarized. That means the voltage inside and outside are close to each other, right? Open state has a lower energy than the closed state. Let's call that energy difference delta u. And it's close to kt, because when it's open, the channel kind of flickers back and forth between open and closed. Does that make sense?

Now let's put on-- let's hyperpolarize the inside of our cell. So now the voltage inside is low. There's a voltage gradient, an electric field that is trying to push those charges in. Now, you can see that those charges here are sitting at a lower voltage. So in the closed state, those charges are down here at a lower potential. What does that mean for the energy of the closed state when the cell is hyperpolarized? Its lower.

The energy of the closed state is low because those charges are toward the inside

of the cell, and the voltage is low. Now, what happens if the cell is hyperpolarized, but it happens to be in the open state? You can see those charges are closer this way. So you can see that the energy of-- these charges are still sitting in a voltage that's lower than outside. So that open state has a slightly lower energy. But you can see that the closed state still has a much lower energy than the open state, OK?

And we can write down that voltage difference as a gating charge times this voltage difference. So now let's just take-- here is an open state. It has an energy difference of a little amount, w. Open state is lower than closed by an amount w. When the voltage inside the cell is low, we've decreased the energy of the closed state by this amount-- gating charge times membrane potential.

And now we have an energy difference in the open state and the closed, the energy difference between the open state and closed state as a function of voltage. We have a simple equation that describes the energy difference between the open and closed state as a function of the membrane potential. And now we can just plug that into the Boltzmann equation and derive the probability of being open and closed.

So we just plug that delta u into here, w minus gating charge times voltage. Now let's calculate the probability of being open. This gives us the ratio of open to closed. How do we calculate the probability of being open? Well, n is the probability of being open. That's just probability of being open divided by open plus closed. What's the probability of being open plus the probability of being closed?

Well, if it's in one or the other, then the sum of those has to be one, OK? And now divide both top and bottom by p0. The probability of being open it's just 1 over 1 plus p closed over p open, which is just the inverse of this. And that's equal to that.

All right, that may have gone by a little bit too fast. And I wasn't very smooth on that. But you can see the idea, right? It's estimating how the energy difference between the open and closed state depends on the voltage of the cell, and it's just an energy difference. So it has to be a charge times a voltage, yeah? And that's right [AUDIO OUT] charge times a voltage.

And now we're just doing a little bit of algebra to extract the probability of open from open divided by closed. And now if we just plug that into there, we get this. All right? So now let's see how that compares to the actual answer. Probability of open

is just 1 over 1 plus this exponential. Here's what that data looked like. Remember, that was the data for the conductance as a function of voltage.

Here's a fit to a functional form that looks like that. And here is the prediction from Boltzmann. You can see that it almost exactly fits. And you can actually extract, biophysically, what the gating charge is inside this tiny, little protein simply by fitting this to the data. Pretty cool, right? Yes?

AUDIENCE: What is w?

MICHALE FEE: It's the energy difference between the open and closed state when the voltage is zero. So you kind of have to fit that, too. If the voltage is zero, it's the energy difference between the open and closed state when the voltage is zero. And then you subtract from that the energy of the gating charge as a function of voltage inside the cell, OK? Yes

AUDIENCE: So is that the [INAUDIBLE].

MICHALE FEE: Yes, each has the sensor, and they all have to be open for the ion channel to be open. Yes?

AUDIENCE: Do you not need to put it to the power of four?

MICHALE FEE: No, because this is the probability that one subunit is open. But that's a good point. If you want to compare that to the-- so you're right. If you want to compare that to the conductance of the whole channel, then it has to be raised to the power of four. And that's been accounted for here. Good question. Any other questions? Boltzmann men equation is pretty cool.

If you know the mass of a nitrogen molecule and the acceleration due to gravity, what can you calculate with the Boltzmann equation? Any idea? The mass of a nitrogen molecule and the acceleration due to gravity.

AUDIENCE: Pressure of nitrogen? The partial pressure of nitrogen?

MICHALE FEE: Close. You can calculate the height of the atmosphere. You can do all kinds of really cool stuff with the Boltzmann equation. OK, there was another question here. No?

So you can extract, actually, these quantities-- the gating charge and this energy

difference in the zero voltage state. And the fit is very good. OK, so that's voltage dependence. I highlighted these slides that I don't expect you to be able to reproduce in blue, just to make it more clear for your review what you have to focus on.

OK, let's look at the time dependence. The time dependence is pretty simple. It's going to just involve a linear first order differential equation. You guys are all super experts on that now, right? So we have an ion channel-- sorry, a subunit that's either open or closed, right? We have an open state, closed state. What we're going to do is-- so the way to think about this is the ion channel, the subunit, if the cell is polarized, is sitting in the closed state, right?

When you depolarize the neuron, that changes the energy levels. [AUDIO OUT] Which way was it? I forget. The closed state has a lower energy. Now, when you depolarize the cell, the closed state suddenly has a much higher energy, and it's close to the open state. And so, at some point, that subunit will jump over to the open state, right? But that takes time. You change the energy levels, but it takes time for the system to jump into the open state.

Why is that? Because that transition is caused by thermal fluctuations. And so you have to wait for one of those fluctuations to kick you over into the open state. So we're going to model those transitions between open and closed states with a simple rate equation that's voltage dependent. We have an open state, and we imagine that n is the probability of being in the open state.

And we can equivalently think of it as if we have a population of subunits. And let's think of it more as the fraction. It's also equivalent to-- just whichever way you want to think about it, either works well. But you can also think of it as the probability of being in the open state, or the number of subunits that are in the open state in a population.

You also have a closed state. So if n is the probability of being in the open state, [AUDIO OUT] of a closed state with probability 1 minus n, right? If you're in the closed state, you have some transition rate, probability per unit time, of going from the closed state to the open state. And if you're in the open state, you have some probability per unit time beta of going into the closed state.

So those things have units of per second, probability per second. Yes? Rebecca, right?

AUDIENCE: Yeah. What's the cause for the fluctuations? Just regular [INAUDIBLE]?

MICHALE FEE: Just warmth. And these things are voltage dependent, remember? Those depend on the energy difference between that open and closed state. All right, let's develop our first order linear equation. It's going be very simple. We have a closed state, an open state. The change in the number of open states is just going to be the number of closed states, the number of closed subunits that open, minus the number of open subunits that close. That makes sense?

All right, that's simple enough. The change in the number open subunits per unit time is going to be the number of closed subunits that there are times the probability that a closed subunit opens per unit time-- that's alpha-- minus-- remember, the number of closed subunits that open is the number of closed subunits times the probability per unit time that a closed subunit opens, all right?

And the number of open subunits that close is just the number of open subunits times the probability that any one of them closes per unit time. Does that make sense? A lot of words, but the equation ends up being very simple. The change per unit time of n is just the number of close subunits, one [AUDIO OUT], times the probability that those open per unit time alpha minus beta times n. Alpha times 1 minus n minus beta times n.

Any questions about that? Alpha, beta are voltage dependent. So I've rewritten that equation. n is the probability that a subunit is open. Let's just rewrite this. Let's expand this. Alpha minus alpha times n minus beta times n. Factor out the n. So you have dn dt equals alpha minus alpha plus beta times n. Divide both sides by 1 over alpha plus beta.

What's the steady state of this-- the steady state solution of this equation? That is the steady state solution, right? If you set dn dt equal to zero, then n is equal to that. [AUDIO OUT] just n infinity. And what's that? Alpha and beta have units of per unit time. So what is one-- what units do 1 over alpha plus beta have? Time. So what might that be?

AUDIENCE: Tau.

MICHALE FEE: Tau. It's a time constant. So, after all of this, what we end up with is an equation that looks exactly like what we had for-- we have a first order linear differential equation exactly the same form as the equation we used to understand the way the voltage changes in a cell in response to current injection.

So if we change n infinity, what is this thing going to do? What is n going to do? It's going to relax [AUDIO OUT] n infinity with a time constant tau. In all of these things, the tau is tau sub n, because it's for the n gating variable. So that's why this has an n here. So n infinity and tau are voltage dependent, because they come from alpha and beta, which are voltage dependent.

But we actually just derived the steady state voltage dependence of the potassium conductance, right, from the Boltzmann equation? What is n infinity for very negative voltages? Do you remember it, just approximately? Big, small, [AUDIO OUT]? What is the steady state? What's the probability that a potassium channel is open, that a subunit is open, at very negative voltages? Do you remember? Zero. It's off.

For big voltage, n infinity has to be-- if we think of it as a probability, it's close to one. So n infinity goes from zero at negative voltages, sigmoidal activation up to one at high voltages. OK, so now let's look at how n changes as a function of time. So here's our [AUDIO OUT] potential. We're going do a voltage clamp experiment. We're going to start at minus 80 millivolts and step up to zero.

So what is n infinity going to do? n infinity is just a function of voltage, right? It's like those energy levels. They change immediately. So what is n infinity going to do? Good. It's going to start at close to zero, jump up to one, and then jump back to close to zero immediately following the voltage.

But what is n going to do? Now let's plot n. n is going to start at zero. When you step the voltage up, n infinity will jump up, and n will relax exponentially to a high n infinity close to one, right? And then when we turn back, n infinity jumps back down to zero, and n relaxes exponentially back. Yes?

AUDIENCE: On the n [INAUDIBLE], that's not relaxing [INAUDIBLE] to one. It's to n infinity, right,

at the top?

MICHALE FEE: Yes, but for a voltage around zero, n infinity is going to be close to one . Any questions? That is called activation. That's the activation. Now, this looks a little funny, right? This thing is turning on immediately. It doesn't have that nice sigmoidal shape that the potassium current had, or the potassium conductance. Why is that?

What are we looking at here? We're plotting n. What is the potassium conductance or the current? How does that relate to n? n to the fourth. So what do we do to plot the potassium current or the potassium conductance? We just take this [AUDIO OUT], right? So what does that look like?

This process of turning off-- the gating variable getting bigger is called activation. The gating variable getting smaller, n getting smaller, is called disactivation. So now let's plot this to the fourth. So this conductance turns on gradually, but the conductance is proportional to n to the fourth. So let's plot that.

So if we plot n to the fourth, you can see that that function now turns on smoothly in time. This is time now, right? So that gating variable n relaxes exponentially, but the conductance goes as the gating variable to the fourth. And so it has this nice, graceful turn-on, right? Because it's the gating [AUDIO OUT] exponential to the fourth looks exactly like this.

In fact, that's how Hodgkin and Huxley figured out that it's n to the four, because they knew that if they assume that it's an exponentially decaying gating variable, that the only way they could fit the turn on of the conductance was by raising it to the fourth power. If they raised it to the second power, it was still too sudden. If they raised it to the third power, it was still not quite right. But if they raised it to the fifth power, oops, it's too delayed. If they raised it to the fourth power, it exactly fits the shape of the conductance turning on.

And so they inferred-- they didn't know about subunits. They just had a piece of axon, a piece of squid lying on a table in front of them. And they were able to figure out that there were four independent processes that turn on the potassium conductance. Pretty cool, right? That's what you get by doing things quantitatively.

So they could [AUDIO OUT] the shape of that potassium conductance turning on by

this exponential gating variable raised to the fourth power. And from that, they were able to infer that it's four independent first order processes that combine to produce that activation.

OK, the offset also fits if you raise it to the fourth power. They were able to measure the size of the potassium conductance to measure n infinity directly. So we derived it using the Boltzmann equation, but they measured it directly just by the size of the conductance. And you can also measure the time course. You don't need to worry about this. I'm not expecting-- you should know this. I'm just showing you that just for fun. You don't need to know that.

And you can extract these tau's just by measuring this exponential decay at different voltages, or measuring the inferred first order process. You can infer the time constant of the first order process on the onset and the offset to extract these tau's as a function of voltage. This is tau as a function of voltage.

From these two quantities, you can actually extract alpha and beta. And so you can write down a simple algebraic expression for alpha and beta. And that's the way they actually wrote those things down. They wrote them down as alpha and beta, rather than n infinity and tau. Those are simple expressions for alpha and beta in units of per millisecond as a function of voltage in units of millivolts. I think-- yeah, it's millivolts. There it is right there.

So you can actually just take those parameters and calculate n infinity and tau n and calculate the gating variable from that using that differential equation. So we have these nice expressions for what the steady state, n infinity, and tau n are. Now, why did we-- yes?

AUDIENCE: [INAUDIBLE]?

MICHALE FEE: Yes, they're per unit time. Yeah, so they have units of per millisecond. OK, so now let's come back to our picture. We have n infinity and tau n as a function of voltage. Now we just can plug those into this differential equation and solve for n. Well, we already know what that does. n relaxes exponentially toward n infinity with a time constant tau. But you can integrate that numerically.

You get the potassium conductance as n to the four, g times n to the four. You get

the potassium current as g n to the four times the driving potential, or V minus Ek. And now let's come back to our algorithm for making an action potential. So we have the parts related to the potassium current. We still have to add the parts related to sodium, but it's going to look very similar.

So here's the idea. We start with the membrane potential at time step t. We compute n infinity and tau n. We integrate dn dt one time step to get the next n. Plug n into our equation to get the potassium current. We then add that to all the other currents to get the total membrane current. We compute V infinity of the cell. We integrate dv dt one time step to get the next voltage. And you plug that in and calculate the next n infinity, all right?

So we still have to add the sodium parts, but you can see we've gone through all of these steps for the potassium. And so we're just this shy of having a full-blown algorithm for [AUDIO OUT] an action potential in a neuron. And not only do you understand all the little steps, but you understand the fundamental biophysics that leads to that voltage and time dependence.

All right, so, again, what I'd like you to be able to do is to draw that circuit, the Hodgkin-Huxley model. I'd like you to be able to explain, at a basic level, what a voltage clamp is and how it works. I'd like you to be able to plot the voltage and time dependence of the potassium current-- remember, this sigmoidal activation of the potassium current-- and the conductance, voltage and time dependence. And be able to explain the time and voltage dependence of the potassium conductance in terms of the Hodgkin-Huxley gating variables. OK?