MICHALE FEE: Today we're going to continue developing our equivalent circuit model, the Hodgkin-Huxley model of a neuron. And we're still focusing on the mechanism that generates spikes. As you recall, there are two conductances, iron conductances, that lead to action potential generation. There is sodium conductance that is connected to a sodium battery that has a high equilibrium potential. There is a potassium conductance that is connected to a potassium battery that has a negative equilibrium potential, and those two conductances together have voltage and time dependence that lead to the generation of a positive going, followed by a negative going, fluctuation in the voltage that is the action potential.

And as you recall, the way that happens, there is a time dependence to these conductances so that when the sodium conductance turns on, this resistor gets really small, and basically connects the inside of the cell to that positive battery. When the sodium conductance turns off and the potassium conductance turns on, we're disconnecting the sodium battery and connecting the potassium battery, which has a negative voltage. And the voltage of the cell, then, is driven toward the negative potassium equilibrium potential.

So last time we worked out the voltage and time dependence of the potassium conductance. Today, we're going to focus on the, sorry, focus here on the sodium conductance and explain various aspects of the voltage and time dependence of the sodium conductance. And then once we do that, we're going to turn in the second half of the lecture to a really beautiful, simple model of a disease related to a defect in the sodium channel. And it's an example of how we can use modeling to test and elaborate on hypotheses about how defects in a circuit, or in an ion channel, can lead to very complex phenotypes in a whole animal.

So as you recall, our Hodgkin-Huxley model has three conductances and a capacitance that represents a capacitor that represents the capacitance of the membrane. The total membrane ionic current is just a sum of the sodium current, the potassium current, and this voltage independent, time independent, fixed leak current. So the equation for the membrane potential, the differential equation for the membrane potential in the Hodgkin-Huxley model, is just a simple first order

linear differential equation that relates the membrane current and the membrane potential.

So last time we described a set of experiments that were done by Hodgkin and Huxley to study the voltage and time dependence of these conductances in the squid giant axon. And as you remember, this axon is very large. It's 1 millimeter in diameter, which makes it very easy to put wires into it, and change the voltage, and measure the currents, and so on. So the experiment they did was a voltage clamp experiment, where you can hyperpolarize and depolarize the cell. There's a very fast feedback system that allows you to set a command voltage, and this operational amplifier injects however much current is needed to hold the cell at whatever membrane potential you command.

And the typical experiment that they would do would be to hyperpolarize or depolarize the cell to fixed membrane potentials and measure how much current passes through the membrane during and after that transient change in the command voltage. So if you take a squid giant axon, you start at minus 65 millivolts, and you hyperpolarize the cell, not much happens. And that's because all of those currents are already off when the cell is hyperpolarize at minus 60 or at low voltages. On the other hand, if you start at minus 65 millivolts and depolarize the cell up to 0 millivolts, all of a sudden you see a very large transient current that first goes negative, which corresponds to positive charges going into the cell followed by a positive current that's associated with positive charges leaving the cell.

And last time we talked about how we can dissect these two phases of the current, this negative phase and this positive phase, into two different ionic conductances. That they did that experiment by replacing the sodium in the extracellular solution that the axon was sitting in with a solution that has no sodium in it. They replaced that with choline chloride. So choline is a positive ionic-- has a positive charge and chloride, of course, has a negative charge. And so you can replace the sodium chloride with choline chloride.

And now, when you depolarize your cell, you can see that that negative part is gone. And the only current you see is this positive-- this kind of slowly ramping up positive current. And they identified that as being due to potassium ions.

And if you subtract the current curve without sodium from the current curve with sodium, the difference is obviously due to sodium. And so if you plot the difference between those two curves, you can see that the sodium current turns on very rapidly and then decays very rapidly, that that transient sodium current happens very quickly, almost before the potassium current even gets started. And we talked about how that fast sodium current, followed by a slower potassium current, is exactly the profile, that we showed here, that generates depolarizing change in the voltage followed by a hyperpolarize change in the voltage that looks like an action potential.

So now, let's just review quickly how we took these current curves, and from those, extracted the conductance of the sodium and potassium ion channels as a function of voltage and time. So what we did was we looked at the case where we do our voltage clamp experiment to different voltages. We start hyperpolarized. We step up to minus 40 and measure this potassium current.

We step up to 0, and you see this larger potassium current. If you step from minus 40 to 40, you see an even larger potassium current. And you can plot this peak current, or the steady state current, as a function of voltage. That gives you an I-V curve, and we'll look at that in a second.

If you do the same thing for the sodium currents, you see something different that's initially very confusing. If you step from minus 80 to minus 40, you see a small sodium current. If you make a larger voltage step up to 0, you see this bigger sodium current. But then if you step up from minus 80 millivolts to 40 millivolts, now you see you just have a tiny little sodium current.

Anybody remember why that would be? Why is it that you would see only a very tiny sodium current, if you step up to 40 millivolts? What is the equilibrium potential for sodium?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Good, good. So what would the sodium current be if I had stepped this voltage up exactly to 50 millivolts?

AUDIENCE: 0.

MICHALE FEE: It'd be 0. So this is pretty close to 50 millivolts, which is why the sodium current is actually pretty small. So now, let's plot the peak current as a function of voltage for potassium and the peak current here as a function of voltage for sodium.

That's what that looks like. So you can see that the potassium current is 0 for these voltages down here and grows. It's actually stay 0 for even more negative voltages.

The sodium current, on the other hand, has this very kind of funny shape. It's linear up here around high voltages, around the sodium equilibrium or reversal potential, and then at drops to 0. The sodium current stays at 0 for negative voltages.

And you recall that we use this to think about what the conductance must be. So let me just walk you through that logic again. So remember that the current is just a conductance times the driving potential.

The driving potential is positive when you're above the equilibrium potential, and it's negative when you're below. So this term here is a straight line. It's linear in voltage, and it goes through 0 when V is equal to EK. So there is the driving potential for potassium as a function of voltage.

Now, you can see clearly that the conductance as a function of voltage has some voltage dependence, because this doesn't look like this. So the difference between this and this is captured by this voltage-dependent conductance. And does anyone remember what that conductance, that GK as a function of V, looks like?

AUDIENCE: Sigmoidal.

MICHALE FEE: Yeah, sigmoidal. And what is it down here? It's 0.

So the way that you can get a 0 current, even with a very negative driving potential, is if the conductance is 0. You can see that the current is linear up here, and the driving potential is linear up here. So the conductance has to be constant.

And so we have a conductance that has to be 0 down here and a constant non-zero up here. Yes?

AUDIENCE: So why is the potassium curve 0 when it's more negative than GK? Why doesn't it go

in the other direction?

MICHALE FEE: Why doesn't this curve do something else? So what is it that you're--

AUDIENCE: Like why doesn't it-- why doesn't it--

MICHALE FEE: Why doesn't it keep going?

AUDIENCE: Yeah. Why is there like a [INAUDIBLE]?

MICHALE FEE: Ah. Because-- OK. That's a great question. So maybe you can answer it. How would I change the conductance curve to make this look more like this?

I could do something very simple to the voltage dependence of the potassium conductance to actually make it look like that. What would I do? The reason this goes to 0 and stays at 0 is because the voltage dependence of the conductance turns it off before the driving potential can go negative. So what would I do to the conductance to make this current dip below 0 before it comes back, any suggestions?

AUDIENCE: Translate it.

MICHALE FEE: Yeah, which way?

AUDIENCE: This way.

MICHALE FEE: Good, exactly. So if I took this curve and I shifted it that way, if I made the potassium conductance turn off at a more negative potential, then this would go down before it got turned off by the conductance. Does that make sense? Great question. Any other questions?

So the answer is, the reason this doesn't go negative is because the voltage dependence of the potassium conductance turns off the conductance before or on the positive side of the equilibrium potential of potassium. Yes?

AUDIENCE: Can you explain again why the [INAUDIBLE]?

MICHALE FEE: So if G were constant, if G had no voltage dependence and it was just a constant, what would this current look like? What would it look like? If this G were just a constant, no dependent on voltage?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Good. It would look just like this, right? So the reason this curve shuts off and goes to 0 is that the conductance goes to 0 down here, and it's constant up here. Does that make sense? And that curve just looks like that. It's 0 down here and constant up here.

Good question. Any other? There was another hand up here. Yeah?

AUDIENCE: I was wondering about notation. So it's GK of V. It's not like GK times V, right?

MICHALE FEE: No. It's GK as a function of V. Yeah, that's-- the notation is sometimes a little bit confusing. You kind of have to read it out from the context. Any other questions?

So now you can see why this curve looks the way it does. So now, let's plot the driving potential, V minus Ena. That's this curve right here.

It's Ohm's law, but it has a battery that makes it centered. It makes it give 0 current when V is equal to Ena, which is positive. So that's why that curve looks like that.

And what is it that makes the sodium current go to 0 down here? It must be that the what? What about the conductance?

AUDIENCE: Turns off.

MICHALE FEE: Good. The conductance, the sodium conductance, has to turn off down here. And what about up here? This is linear. This is linear, so the sodium conductance has to be what up here?

Constant, good. So you can see that the sodium conductance has exactly the same shape as the potassium conductance. It's not exactly at the same voltage, but it's close. Good.

So now you can see where this kind of weird shape of these sodium and potassium currents comes from. It's actually very simple. It's just a resistor in series with a battery that gives you this driving potential offset from 0, and that's multiplied by this voltage-dependent conductance.

Now, the time dependence of the conductance is entirely due-- sorry. The time

dependence of the current, that ramping up current that turns on and then stays constant for the potassium, is entirely due to the time dependence of the potassium conductance. So the potassium conductance just turns on. That process of the conductance turning on is called activation.

Same for the sodium-- the sodium conductance turns on quickly. That's called activation. The sodium conductance turns on very fast, and the potassium conductance turns on slowly.

Now, we talked about how the voltage gates work in our voltage-dependent ion channel. And the idea is that you have some gating charges that are literally charged residues, charged amino acids, in the protein. When the membrane potential is very negative, when the cell is at rest, you can see that there's a large electric field pointing that way inside the membrane, and that pushes the charges, pushes those gating charges, toward the inside of the cell, and that closes the gate.

When you depolarize the cell, this membrane potential goes closer to 0, the electric field drops, and those gating charges are no longer being pushed into the cell. And they relax back, and the gate opens. So that is the basic, sort of a cartoon, picture of the mechanism by which voltage-dependent ion channels acquire that voltage dependence.

So remember, we talked about how we can model that time dependence. We can model that opened and closed state of the ion channel as two states, an open state and a closed state, where the probability, n, of being in the open state, a probability of 1 minus n being in the closed state. Remember, this was for one subunit. For the potassium channel, there are four subunits, and all of them have to be open.

And we wrote down a differential equation for that gating variable, n. There is an n infinity, a steady state, that's a function of voltage. And remember, for the potassium, n infinity is negative down here and increases as a function of voltage to get close to 1 at voltages above minus 50, or somewhere between minus 50 and 0 millivolts, that gating variable. And the n infinity of that gating variable, n, goes from being very small to being large.

Now, so that's potassium. We went through that last time. And now let's talk about

sodium. Sodium looks exactly the same.

The sodium conductance can be modeled as having two states, an open state and a closed state. Remember, we did a patch recording on a single sodium channel. You could see that it flickers back and forth between open and closed. So we can model that process in exactly the same way that we did for the potassium conductance.

We have an open state, a closed state, a probability, m, of being in the open state. So m is our gating variable for-- our activation gating variable for the sodium conductance. Probability of being in a closed state is 1 minus m. There is that same kind of differential equation for the m gating variable, and a m infinity that has a voltage dependence that looks very much like the voltage dependence of n infinity.

So so far, the sodium and potassium conductances look very similar. They both have the same kind of activation gating variable, the same simple model for how to turn on and turn off, same differential equation, same gating variable that has this sigmoidal dependence on voltage. Any questions about that?

So you remember the way we thought about the time dependence of these is we simply integrate this differential equation over time. It's a first order linear differential equation, and you can think about the n, the gating variable, as always relaxing exponentially toward whatever n infinity is at that moment. And n infinity is a function of voltage, and any time dependence it gets comes from changes in the voltage.

So we're going to simplify things and just consider piecewise constant changes in the voltage. So let's do a simple experiment. We're going to hyperpolarize the voltage to minus 80. What is n infinity going to be, big or small? Remember what n infinity looks like is a function of voltage?

AUDIENCE: Small.

MICHALE FEE: Good. So at hyperpolarized voltages, n infinity is going to be small, and so is m infinity. Those ion channels are closed at hyperpolarize voltages.

So the gating variables that represent what the probability is of being open, those gating variables are small when the voltage is negative, very negative. So then we're going to step the voltage up. And what is n infinity going to do?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Anybody want to just draw for me what it's going to do in the air? It starts out small. So is it going to ramp up slowly?

Is it going to jump up? Is it going to wiggle around? What's it going to do? So why is it-- so I have several different answers.

I have some people saying that it's going to ramp up. I'm asking about M infinity now, not n. So how many people say it's going to jump up suddenly? OK, good.

That's what it's going to do. It's going to start out at a small value and jump up to a larger value when you depolarize the cell. And then what is n going to do?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Good. n is going to start at some initial condition and relax exponentially toward n infinity. And then when you turn the voltage back down, N infinity is going to go from this large value back down to a small value, and n is going to relax exponentially to that smaller value of n infinity. Any questions about that? We saw that last time.

Now, what is the conductance going to do? Where does the conductance depend on n, anybody remember, for potassium? How many subunits are there in a potassium?

AUDIENCE: Four.

MICHALE FEE: Four. And so if the probability that each one is open is n, and there are four independent, what's the probability that they're all going to be open?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Good. And so the conductance is going to turn on as this relaxing exponential to the fourth. And it's going to have that kind of gradual ramping up. Good.

It looks exactly the same for sodium. So if you start hyperpolarized, you depolarized the cell, that m infinity is going to start small, it's going to jump up to a high value. M is going to start small, and it's going to relax exponentially toward that higher value of m infinity.

Now, anybody want to guess at what the sodium conductance will look like? It's going to be some function of m, right? It turns out that it m cubed.

And the reason is that even though there are four things that have to all be open, they're not independent of each other. And so the exponent is not m to the fourth, it's m cubed. And Hodgkin and Huxley figured that out simply by plotting these relaxing exponentials to different powers. I imagine them saying, oh, the potassium is 4. Let's take m to the 4.

But it didn't fit. So they tried some other, and they found that m cubed fits. So that's it.

Now, the problem with this model is what? What is the problem with this model? Is that when you depolarize the cell, the potassium current turns on.

The potassium conductance turns on, but then what happens? What is-- sorry. The sodium turns on. What happens?

It doesn't do this. It doesn't turn on and stay on, right? The potassium, when you depolarize, turns on and stays on, just like that model. But the sodium does something else. What does it do?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: What's that?

AUDIENCE: It's a voltage clamp.

MICHALE FEE: This is voltage clamp, so it's we're controlling the voltage. m is already a maximum here, so it can't shoot up anymore, right? Anybody remember what sodium does that's really weird?

AUDIENCE: Deactivation.

MICHALE FEE: It inactivates. So the current turns on, a conductance turns on, but it doesn't stay on. It turns off, and that's what we're going to talk about next. And once we have that, we've got the whole Hodgkin-Huxley model. And that'll set us up for this really interesting sodium channel defect that we're going to talk about.

So that process there of shutting off is called inactivation. This process of n turning on is called activation. n turning off is called deactivation.

m turning on is called activation. m turning off is called deactivation. But this other thing has a different name. It's called inactivation. It's kind of a little tricky terminology.

So the potassium-- the probability of the sodium current being-- the sodium channel being open actually goes like m cubed times some other gating variable that describes how this turns off. And so there's another gating variable, called h. It's called the inactivation gating variable for sodium. And so now we're going to figure out how to think about h and how to describe it mathematically. You probably wouldn't be surprised to hear that it's just another first order linear differential equation-- activation gating variable, m, inactivation gating variable, h.

So how do we think about inactivation? Inactivation is literally just a little loop of goo or snot on the inside of the sodium channel, and it's charged. And when the sodium channel opens, it just falls in and plugs up that the pore. That's it.

So when the membrane potential is very negative, the inside of the cell is negative, there's an electric field pointing this way, and the inactivation particle is slightly positively charged. And that pushes it, keeps it out of the way. It turns out that that's a real thing. It turns out it's just a loop of amino acids on the inside of the ion channel. Hodgkin and Huxley, of course, they didn't have the structure of the sodium channel, but they actually predicted the existence of this thing that they called the inactivation particle.

When you depolarize the cell, when the membrane potential inside the cell goes more positive, that positive charge is no longer actively kept out of the pore. And so it falls in and blocks the pore. And that prevents ions from flowing through the ion channel.

So how would you model this? There's an open state and a closed state with energy levels. How would you want to do that?

AUDIENCE: Use the Boltzmann distribution.

MICHALE FEE: Yeah, you could use the Boltzmann distribution to compute the voltage dependence.

I haven't done that, but I'm sure it would work pretty well. How would you model the time dependence?

So let me ask you this. If there is a gating variable-- let's start with this. If there is a gating variable, h, that we're going to use to describe this thing getting open and closed, what is the voltage dependence of h infinity going to look like? When the voltage is very negative, what is h doing? You think it's big or small?

Here's the equation-- m cubed h. So when the-- yeah, right. h has to start out high and go small in order to explain this thing turning off. Does that make sense?

So what we're going to do is we're going to have-- we're going to model this again with two states, an open state and a closed state. h is the probability that this inactivation particle is in the open state. It turns out that there's only one of these particles, and so that explains why it's just times h, not times h to some power.

And we have a differential equation that describes how h changes as a function of time in a way that depends on h infinity. And Aditu, why don't you draw what h infinity probably looks like as a function of voltage.

AUDIENCE: High.

MICHALE FEE: Yeah. It just starts high and goes down. How do we actually measure that? Let me show you an experiment how you'd measure that.

So first, let me just show you this. So when you depolarize the cell, h starts out high, because h infinity is high. And then when you depolarize the cell, h infinity gets small, and h just relaxes exponentially toward the new smaller h infinity.

And what's really cool is that the tail, this inactivation, the way that conductance or the current turns off, is just a single exponential. It just falls like E to the minus some time constant. It's just given by this first order linear differential equation. Good.

This h getting smaller is called inactivation. Anybody want to take a guess at what this is called?

AUDIENCE: Deinactivation.

MICHALE FEE: Deinactivation, good. So there's activation and deactivation. There's inactivation

and deinactivation. Those are different things.

Just remember activation, which is easy, right? It's just things turning on. And then there's the same process that undoes the turning on. That's deactivation.

And there's inactivation, which is a separate particle. And it has a process of blocking and unblocking. So it's inactivation, deinactivation. Any questions about that? Yes?

AUDIENCE: If there is any activation, does that mean it's already charged up? So what does deactivation mean?

MICHALE FEE: Yeah. So when-- here. Let's just go back to this picture here. When the cell is hyperpolarized, the thing is hanging out outside not getting in the way. When you depolarize the cell, that electric field is not pushing it out anymore, and it falls in.

But when you hyperpolarize the cell again, that electric field turns back on. And what is it going to do? It pushes the particle back out to the other state, to the open state.

Any other questions? Pretty simple, right? Kind of very machine-like.

And then what we're going to talk about soon is how this thing sometimes doesn't work, this thing. There are genetic mutations that turn out to be fairly common actually, where this doesn't reliably block the pore. And we're going to see what happens.

First order linear differential equation. Exponential relaxation toward new h infinity. We can actually measure this h infinity as a function of voltage by doing the following experiment.

What we do is we hold the cell hyperpolarize. We can then step the cell up to different membrane potentials-- very low or very high. And then what we do is we jump the membrane potential up to turn on the activation gating variable.

And now we can see-- what you see is, that depending on where you held the voltage before you did this big voltage step, you get sodium currents of different size. And you can guess that if you hold the voltage very negative and then turn it

on, that activation gating variable for all those ion channels is [AUDIO OUT]. And when you turn on the sodium-- turn on the gating variable, you're going to get a big current, right?

If you hold the cell for a while here at a higher voltage, most of those sodium channels are going to have that inactivation gate already closed. And so now when you step the voltage up, turn on m, you're going to get a much smaller current. And so if you just plot the current size as a function of this holding potential, you can see that h is big for low voltages and goes to 0 for higher voltages.

And what this means is that when a cell spikes, that voltage goes up, and h starts falling, and the sodium channels-- many of the sodium channels in the cell becomes inactivated-- become inactivated. Yes?

AUDIENCE: The membrane potential on the x-axis, is that the difference in the-- is that [AUDIO OUT] or is that the [INAUDIBLE]?

MICHALE FEE: That's the absolute voltage during this holding. That's right. You can actually see at rest most cells actually have a substantial fraction of the sodium channels already inactivated.

So here's the plan. We now have a full description of the potassium and the sodium conductances as a function of voltage and time. So we're to put it all together and make a full quantitative description of the Hodgkin-Huxley model.

Our probability of the sodium current, sodium channel, being open is m cubed h. I just want to mention that this m cubed h assumes one thing about the gating variable and the inactivation variable. The mechanism for activation and the mechanism for inactivation assumes what about them?

AUDIENCE: They're independent.

MICHALE FEE: They're independent. And it turns out that that's not quite true. It's one of the very few things that Hodgkin and Huxley didn't get spot-on. So it's not exactly independent, but it's really not bad either. So it's a pretty-- it's still a pretty good model.

We can write down the sodium conductance as just the conductance of the sodium

channel when it's all the way open times m cubed h. Yes?

AUDIENCE: So do we know what the inactivation particle is?

MICHALE FEE: Yeah. We're going to see in a second. I'll show you exactly what it looks like and where these mutations are that have this effect on inactivation.

So we can write down the conductance, and we can write down the current. The current is just the open conductance times m cubed h times the driving potential. And that's our sodium current. Yes?

AUDIENCE: For the [INAUDIBLE], I'm not showing there is [INAUDIBLE] like a number, like sodium channel or something. It doesn't have it.

MICHALE FEE: Yeah, that's right. It's one, one single protein, but it has these transmembrane alpha-helices that act-- are multiple voltage sensors. And they act somewhat independently, but still a little bit cooperatively, and that's where this m cubed comes from. But you're right.

The potassium channel actually has four separate subunits that form a tetramer. The sodium channel [AUDIO OUT] that it's all one big protein. That's right.

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Yeah. You should really think of this-- I mean the n and the m were both-- it was empirically discovered that one goes as n to the fourth, and the other one goes as m cubed. And it turns out for potassium it has a really beautiful relation to the structure. For sodium, it's a little bit messier.

And I'm sure there are people who actually understand more about why it's exactly m cubed, but I'm not one of those people. So I'm going to refer you to the literature. And I'm happy-- maybe I can find a good reference for that.

So now that we have the sodium conductance and the sodium current, let's put this all together. So here's how we're going to now-- here's the algorithm for generating an action potential. And we introduced this last time, but let's just flesh it out for the full story.

So given an initial voltage, compute n infinity, tau n, m infinity, tau m, and h infinity

and tau h, as a function of that voltage. Those are just those algebraic expressions that give you the alpha n and beta n for each of those things-- one for potassium, one for sodium, the m, and one for the h for sodium.

So we're going to calculate all of those. Steady state gating variables as a function of voltage, we're going to start from our initial condition of n, m, and h, and integrate that differential equation one time step using-- it's going to relax exponentially toward n infinity. We're going to plug that n, m, and h into our equations for the potassium current, sodium current, and leak, which doesn't have those gating variables.

So the potassium current is Gn to the 4 times the driving potential. Sodium current is Gm cubed h [AUDIO OUT] driving potential. We're going to add all of those currents together to give the total membrane current.

That membrane current is going to give us a V infinity for our cell. Remember, the V infinity is just the current times the effective resistance. So we can use that to also calculate the membrane time constant.

And then we integrate the voltage one time step. Go back and recompute those n, m, and h infinities. And then we just keep cycling through this. When you do that, and you plot the voltage, you get an action potential.

Now, you can do that in a hundredth of a second in MATLAB. Hodgkin and Huxley we're doing this on their slide rules, and they got 2/3 of the way through an action potential and said, let's just publish.

[LAUGHTER]

So here's what that looks like. Here's V as a function of time for when you implement that loop in MATLAB. So you can see what you did.

So this is the injected current through the electrode, and can see it starts to depolarize the cell a little bit. And at some point, what happens is-- this is just a copy over here so you can line things up-- when you inject current, the cell starts to depolarize. And you can see that m starts to grow. The sodium current is starting to turn on.

And at some point, m gets big enough that it's turning on a substantial amount of sodium current into the cell. And what does that do? It depolarizes to cell more, which causes m to grow faster, which causes more current, which depolarizes the cell faster.

And it just runs away-- bam-- until you reach essentially the equilibrium potential of sodium. And then what does the sodium current do? The sodium current actually stops even though the channel's open.

Then what happens is, during that whole time, n has, in this hyperpolarized voltage-- the potassium channel is starting to open and grows, potassium current conductance turns on, and that starts hyperpolerizing the cell. During that whole time, the inactivation gate-- this cell is very depolarized, very positive. That little bit of goo falls in, h drops. That shuts off the sodium conductance. Potassium conductance finishes bringing the cell back.

Beautiful, right? Yes?

AUDIENCE: Is h just the voltage-dependent or it's also time-dependent?

MICHALE FEE: Time-dependent in exactly the same way that n and m are time-dependent. There is a-- h infinity changes as a sum-- as a function of voltage. And then h relaxes exponentially toward h infinity. Any questions about that?

So for the problem set, you'll have code for this, and you can play around with this and try different things. And then there's a particular problem that Daniel and I cooked up for you for this. I'll basically show you what that looks like. Here's the crux of it.

If you inject a little bit of current into the Hodgkin-Huxley neuron, you get a spike. And then if you wait a few milliseconds and inject another current pulse, what happens? You don't get a spike. Can anybody guess why that is?

AUDIENCE: h is still inactivated.

MICHALE FEE: Yeah. That thing is still stuck in there and hasn't had time to fall out yet. And if you plot h, you can see that it hasn't recovered back to the state it was at the

beginning. So that's called a "refractory period." So cells don't like to spike two times in a row to close. Yes?

AUDIENCE: So what things like [INAUDIBLE] h at which it's a spike?

MICHALE FEE: Yeah. So you want to just like-- what would be the intuitive answer? So there's not a hard cutoff, right? If h is right here, it will be much harder. You'd have to inject a lot more current to make it spike.

If h is recovered to here, then it would take a little bit less current to make it spike. So basically, there's a gradual decrease in the amount of current it would take to make the neuron spike again. So there's no one answer.

So let's take a look at what happens when sodium channels go bad.

[VIDEO PLAYBACK]

[MUSIC PLAYING]

- Most of the animals on this petting farm, on Maui, Hawaii, are sweet, but nothing too unusual. And then there are the goats-- Myotonic goats, to be specific-- more commonly known as stiff-legged goats, wooden-leg goats, nervous goats, fainting goats. Fainting goats are indigenous to North America. But that name is a bit of a misnomer, because they never lose consciousness when they keel over.

If they're startled, a genetic condition causes their muscles to lock up. But it only lasts a few moments, and then they're back on their feet. Now, until the next time they're spooked.

[END PLAYBACK]

MICHALE FEE: So these fainting goats have a particular mutation in their sodium channel. Now, it turns out that the sodium channels that are in your brain that control action potentials are a different gene than the sodium channels that are in your muscles that produce muscle contractions. So you can have a mutation in the skeletal isoform of the sodium channel that produces these muscular effects without having any effect on brain function. But that same mutation in the brain, isoform of the sodium channel, is lethal.

So this is actually a condition that exists in humans. It's called-- there are actually a whole set of these, what are called "sodium channel myotonias." One of them is called hyperkalemic periodic paralysis. And this just shows a different-- this is a different phenotype of one of these sodium channel defects.

So the goats became very stiff and fell over. It turns out there's a different phenotype that looks like this. So basically, it causes extreme weakness. The muscles are completely paralyzed. They can't contract anymore, and it seems like that would be a completely different effect-- what would cause muscles to just go rigid and a very similar thing would cause paralysis-- and it turns out that actually those two things have very similar cause.

That hyperkalemic-- kalemic refers to potassium. And so this condition is very sensitive to potassium levels. At high potassium levels, it's much worse than at low potassium levels. So there can be an attack of weakness or paralysis, and then just a few minutes later somebody's all better, and that paralysis goes away.

So to understand what's going on in this condition, we need to take a look at how muscle fibers actually work. So let's take a little detour in that. So basically, let's start here with the action potential that drives muscle twitches.

So the way this works is that it an action potential will propagate down an axon toward the neuromuscular junction. That action potential will cause the release of neurotransmitter that then causes current to flow into the muscle fiber. That current flowing into the muscle fiber depolarizes it, turns on sodium channels, and that causes an action potential in the muscle fiber that looks very much like the action potential that we just saw for a neuron for the squid giant axon.

Now, there is this famous problem, called the "excitation contraction coupling problem," which is, how does an action potential here on the surface of a muscle fiber get down into the myofibril and cause a contraction of the muscle? So we'll get to that question, but let me just describe what these things are. So the myofibrils-- the myofibril is this little element inside of the muscle fiber itself. And these are bundles of thick fibers and thin fibers that essentially-- here, I think it's on the next slide.

So let me just finish the story about how the action potential gets inside. So the

action potential propagates through these little structures called transverse tubules. These are little tubes that go from the surface of the muscle fiber down into the muscle cell.

They're like axons. But instead of going out from the cell body, they go into the cell body. That's pretty cool. This thing is huge. This muscle fiber is about 100 microns across. So in order for that signal to get into the myofibril to cause contraction, it actually has to propagate down an axon that goes into the muscle fiber.

So that action potential propagates down into the t-tubules that's a voltage pulse that opens up voltage-dependent calcium channels that activate calcium release in something called the sarcoplasmic reticulum. So you may remember that in neurons the endoplasmic reticulum sequesters calcium. In a muscle fiber, the sarcoplasmic reticulum does the same thing. It's sequesters calcium.

But when this voltage pulse comes down the t-tubule, its voltage-dependent calcium channels, which cause the release of calcium, which then activates calcium-dependent calcium release through another set of channels, and it basically floods the myofibrils with calcium. And that triggers the contraction. And here's how that works.

Within these myofibrils are bundles of thick filaments, which are myosin and thin filaments, which are actin. The thick filaments are these structures right here. The actin are filaments, thin filaments, that intercalate between the myosin thick filaments.

The myosin thick filaments are covered with these myosin molecules that stick out. The myosin heads that are like little feet reach out. And if they bind to the actin, then these things basically grab the actin and start walking along. And they pull the actin.

They pull this actin filament this way. The ones over here walk this direction and pull this actin filament that way, and that causes these two end plates to pull, sorry, these two, what are called "z disks," to pull together. And the thing shortens. Does that make sense?

And then when the contraction stops, these little feet stop walking. They relax, and

those actin filaments now can relax and retract. Pretty cool, right?

So how does the calcium connect to that? So the calcium goes in, floods this myofibril. The calcium goes in and binds to these little molecules, called troponin, that are sitting in grooves of the actin filaments. And when the calcium binds to troponin, it moves out of the way and opens up the binding site for these myosin heads to grab onto the actin filament. They grab on and they pull.

And as soon as they pull, an ATP comes off. These things open up, ATP binds, boom. They pull again. So they just walk along with one ATP per cycle.

Then when the calcium-- what happens is that the calcium starts being sequestered back into the sarcoplasmic reticulum that unbinds from the troponin. The troponin falls back into the groove, and the myosin heads can no longer connect to the actin. And that's the end of the muscle twitch. Pretty amazing, right?

So what goes wrong when sodium channels are inactivated? And that's what we're going to talk about next-- when sodium channels fail to inactivate. So here's what the sodium channel looks like.

There are these clusters of transmembrane alpha-helices. These things together, these four things together, form the pore. And there's a loop between them here that produces the inactivation.

And you can see, if you look at the sights of different mutations of the sodium channel that produce defective inactivation, they tend to be clustered in these cytoplasmic loops of the sodium channel. So myotonia and the periodic paralysis that we just saw in those movies are caused by these different sets of mutations on those loops. And again, for these myotonias, these mutations are in the skeletal isoform of the sodium channel.

So now, what do those mutations actually do to this? So now, let's take a look at-- let's do a patch clamp experiment, where we take muscle fiber from a wild-type. So you can just take a muscle biopsy-- extract a little pinch of muscle.

You can culture it in a dish. And you can do that for wild-type, normal human muscle fibers. And you can do it for muscle fibers from a person with this particular mutation of this sodium channel.

And you can see that just like for the neurons, just like for the sodium channels in neurons, you can see that depolarizing this ion channel produces brief openings that are aligned at the time when you do the depolarization step. And then there are no more openings. The sodium channels turn on, and then that gating variable, that inactivation gate, shuts off the pores, and there are no more openings.

But in the muscle fiber that has this mutation, you can see that you get this burst of openings right at the time of depolarization, but you keep getting openings at later times. And if you plot the average current over many trials, you can see in normal fibers there's this very brief pulse of opening, and in these fibers, muscle fibers, with a mutation, there is a constant extended high probability of that sodium channel turning on, opening up. And that's what causes all the problems right there. In these conditions, that only represents about a 2%, a 0.02 probability, of turning on at a time when a normal muscle fiber would be inactivated.

So you can actually study these things in more detail. So this shows a set of experiments that were done in rat fast twitch muscle. This shows a control, and this shows a muscle fiber that's been treated with a toxin that comes from the sea anemone that produces a toxin that uses this toxin to actually help catch prey. And it turns out, what that toxin does is it mimics the effect of this blockage of the inactivation of the sodium channel. So you can see that applying this toxin also produces these extended openings or failures to inactivate.

If you take that toxin and you [AUDIO OUT] to a muscle fiber, you see something really interesting. You take a muscle fiber. You can hook it up to-- tie a string to one end, and tie a string to the other end, and kind pull it tight a little bit, and measure the force that that muscle fiber is exerting. So you can measure force as a function of time.

If you stimulate that muscle fiber with a little electrical shock, you can elicit what's called a muscle twitch. And in the presence of this ATXII toxin, you can see that that twitch is very extended in time. Is there a question? Did I see a hand? No.

So what's going on? So you can now record from this muscle fiber when it's been treated with this toxin that produces what's called a myotonic run. And you can see

that [AUDIO OUT] muscle fiber produces a single or maybe two action potentials when depolarize it. That's what a muscle fiber normally does.

But when you treat it with this ATXII, it generates many action potentials. Now, why would that be? Does that make sense? We're going to explore why that is. We're going to look at a particular model for how the sodium-- the failure to inactivate of the sodium channel produces these myotonic runs.

What's really crazy is that after you turn off that current injection that activates the muscle fiber, the neuron keeps spiking. The muscle fiber keeps spiking. That continued spiking corresponds to continued contraction of the muscle.

So you can trigger the muscle to generate some action potentials in a normal muscle that produces a very brief twitch. But in these muscles with this mutated sodium channel-- in this case it's with the toxin, but the same thing happens in the muscle fibers with the mutated sodium channel-- it produces continued contraction of the muscle. And that's what was happening to the goats. Their muscles contracted, and then they didn't relax. And so they were stiff like this, and then they fall over.

Now, that's called a myotonic run. It's really interesting and was a big clue to what the mechanism is that produces this. If you take these muscle fibers and you put them into a solution that doesn't have the right osmolarity-- so too much, two too many ions, too high an osmolarity, or too low an osmolarity, just like pure water, for example-- produces what's called an osmotic shock.

And what it does is it breaks all the t-tubules from the membrane. So it doesn't break the membrane, but it disconnects all the t-tubules from the membrane. Now, what happens is you see the myotonic run goes away. So something about the t-tubules is causing this myotonic run.

So there's a really beautiful set of papers from David Corey and a person named Cannon, who proposed a hypothesis for why this actually happens, and I'll walk you through the hypothesis right now. So here's the idea. So when you have an input from a motor neuron onto the muscle fiber you get synaptic input, [AUDIO OUT] muscle fibers.

So this is the motor neuron synapse. That's the muscle fiber. So you should think about this as being a very long cell here, and here's a t-tubule that's represented by a channel coming in from the surface. So this is a cross-section of the muscle fiber.

So the idea is that that current injection causes an action potential, which causes sodium to flow into the cell. And on the hyperpolarize phase of the action potential, potassium goes out of the cell to bring the cell back down to a negative voltage. Now, that actual potential propagates into the t-tubule, which means you're going to have sodium flowing into the cell and potassium flowing out of the cell. But out of the cell means into the t-tubule, right?

So what normally happens is, after an action potential, you're left with an excess of potassium in the t-tubule. So what happens-- think is going to happen, anybody? Think back to the first lecture. Yeah?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Yeah, there's going to be some pumping going on here. But actually, most of the potassium gets out of the t-tubule by a different mechanism. It gets out by diffusion. So these extra potassium ions diffuse out through that t-tubule back into the extracellular space.

Now, can we estimate how long it takes that to happen? Any idea how we would do that? Anybody want to take a guess? Does anyone remember how long it takes an ion to diffuse across, let's say, a cell body, 10 microns? Kind of a few tens of milliseconds, right, 50 milliseconds?

This thing is about 25 microns long. And so it will be maybe four times that. So that timescale we can calculate by just using our equation for the relation between time and distance for a diffusion, and you find that that's about 300 to 400 milliseconds. So that's how long it takes those potassium ions to diffuse out of the t-tubule.

Now, what happens when we have a sodium ion that isn't inactivating? What happens is you're going to get a lot more spikes. You're going to get a lot more spikes generated, because this sodium current turns on, but now it's not properly inactivating.

And so you're going to get extra spikes. And those failure to enact [AUDIO OUT]

extra spikes, and extra spikes means you're going to have a lot more potassium going into the t-tubule. So what is all that-- and remember, we now have 300 or 400 milliseconds before that potassium can get out of the t-tubule by diffusion.

So what's going to happen when you have all that extra potassium in the t-tubule? What's it going to do? Yeah, [INAUDIBLE]?

AUDIENCE: It corrects the muscle fiber [INAUDIBLE].

MICHALE FEE: Yeah. So remember, the equilibrium potential, the negative equilibrium potential of the muscle fiber, which is normally, like any cell, is down around minus 80, that negative potential is caused because there's so much more potassium inside the cell than outside the cell. And so the potassium ions are normally kind of leaking out of a cell, and that keeps the membrane potential low.

But now, if you-- remember, this is outside the cell. So you have now, suddenly, a very high concentration of potassium ions outside the cell. And what do they do? They push their way back in. They start diffusing back in, which does what to the cell?

You now have potassium ions going the wrong way, which does what? I think you already gave the answer. Say it again.

AUDIENCE: Depolarizes it.

MICHALE FEE: Depolarizes the cell. Puts potassium back in, and it depolarizes the cell. And what is that going to do?

AUDIENCE: Cause more spikes.

MICHALE FEE: Cause more spikes, which is going to do what? Push more potassium into the t-tubule. It's runaway instability.

So that's kind of a cool hypothesis, right? You could imagine all sorts of experiments to test this. Like you could put a little thing in there to measure potassium concentration in the t-tubule. Well, that's only a few microns across.

How do you test this hypothesis? How would you-- it's a great idea. But how do you know if it even makes any sense when you put it all together, any suggestions?

Yeah?

AUDIENCE: [INAUDIBLE] the potassium.

MICHALE FEE: Yep. So it's already known that at low potassium this problem is less severe. The disease is even named after that observation-- hyperkalemic periodic paralysis.

Any other suggestions? What are we here for? What is this class? Introduction to neural computation, right? So what can we do?

This is a word model, right? When you actually put it all together, you could do all this, and when you model it, it makes no sense whatsoever. There's something wrong with this word model.

Neuroscience is full of word models. The only way to know if a word model makes any sense is to actually write down some equations and see if it works the way you think it is going to work. See if your word model translates into math.

And so that's what David Corey and Cannon did. They took this picture, and they developed a model for what that looks like it. And it started with just the Hodgkin-Huxley model.

Here's Hodgkin-Huxley. That's what we've been using all along. They added another little compartment that represents the conductances and the batteries associated with the membrane in the t-tubule.

And notice, there's a EK here. What does EK depend on?

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Say it again. EK depends on--

AUDIENCE: [INAUDIBLE]

MICHALE FEE: Of--

AUDIENCE: Potassium.

AUDIENCE: Potassium.

MICHALE FEE: Of potassium ions, and potassium ions are changing. So let's actually-- so this part you already know. That's just Hodgkin and Huxley with a few extra resistors attached to the side of it.

What about the potassium part? Let's just flesh out that model a little bit more to see how spiking activity would lead to changes in potassium, how that change in potassium would change the battery, and how that would feedback and change the spiking activity. So let's do that.

So we're going to imagine that we are going to model our potassium conductance in here. So we're going to write down a variable that's the potassium concentration inside the t-tubule. And what is going to affect that potassium concentration? What are the sources of potassium? What are the sinks of potassium, anybody?

Well, one is just diffusion. So we can model that, and that looks an awful lot, actually, like Fick's first law. So the change in potassium concentration as a function of time has a contribution from the difference between the potassium concentration inside and outside. That rate of change through diffusion is proportional to the difference in concentration inside and outside divided by that time constant that we've just calculated.

Now, what-- so that's how potassium leaves. That's one way that potassium leaves. So the potassium gets into the t-tubule at a rate that's just proportional to the potassium current. The rate of change of the potassium concentration is proportional to the potassium current.

And the potassium current-- so let's just flesh this out a little bit more. This, we already calculated. This is the conductance times the driving potential. But that current, we have to do a little bit of changes of units to get current into the right units for a change in potassium concentration as a function of time.

So current is coulombs per second, and here we have moles per liter per second. So we need to divide by two things. We need the volume of the t-tubule, and we need Faraday's constant, which is just coulombs per mole. That's a well-known number that you can just look up. Multiply those two things together, you get the contribution of the potassium current to the rate of change of potassium concentration.

The potassium current is just conductance times driving potential. Notice the EK is a function of potassium concentration. I haven't written it in here, but that's just the Nernst potential. And so we have a differential equation for the potassium concentration as a function of time. It's a function of the potassium concentration voltage and equilibrium potential.

And now, we just take that and add it to the code that we already have for Hodgkin and Huxley. And here's what you get. So here's a normal muscle fiber. You get a single action potential.

What they did was they modeled-- they made some fraction of those ion channels fail to inactivate. And here's what happens to the model when you make 2% sodium channels fail to inactivate. You see that you get this large number of action potentials, because the sodium channels are not inactivating properly. And when you turn the current off, you get this high potassium concentration in the t-tubule that's now causing additional spikes.

That is continued contraction of the muscle. That is this myotonia. The model is exhibiting myotonia.

How do you explain periodic paralysis? That's totally different, right? Now the muscle just goes completely limp. How do you do that? Any thoughts about this?

What do you think would happen if we made a slightly larger fraction of the sodium channels fail to inactivate? Here's what happens. You get more and more action potentials. And at some point, what happens is the voltage just locks up.

The sodium channels go into a different state where the system is no longer oscillating. It's just fixed at a high voltage. It's called depolarization block, and it's what happens when there's no longer enough-- there aren't enough sodium channels active to give you spiking, but there are enough non-inactivated sodium channels to just hold the voltage high.

And this muscle fiber is no longer able to contract, and it's completely flaccid. It's completely loose. And so this is the hyperkalemic periodic paralysis.

So you get both of these really interesting phenotypes in this disease just

depending on one little parameter, which is what fraction of these sodium channels are failing to inactivate. And so you can see, you get this very complex phenotype from a simple mutation of an ion channel. And in order to understand really how it's behaving, you have to do modeling like this.

It's the way you understand a system and how it works. Until you do this, you don't really understand it. So I'll leave it there. Thank you.