PROFESSOR: So let's get started.

[CHATTER]

Oh, it's interesting that some of your questions had to do with things that I didn't quite cover in lecture, but that's fine. I'm going to cover them at the beginning of today's lecture.

This is a really important question. How do you know what channel is at-- how do you know what ion a particular channel is conducting? It's actually hard to determine. There are various ways to do it, where you can specifically label the ion, or follow a particular ion, and address whether or not it's getting into a cell or across a membrane, when there's a particular ion channel present. But it's not completely trivial.

One of the things I didn't have time to go through with you, but which is one of the PowerPoints though, is the fact that ion channels are really selective. And they don't conduct ions on the basis of size, OK? So you're not going to get a large ion channel that could accommodate a large ion, also accommodating a small ion. It's not a matter about of just opening up a space.

There are also charge considerations where the ions actually interact with the molecules in the channel, in the actual pore through the membrane. And it's that interaction which selects for a particular ion. But this notion of exactly what ions channels are conducting has been many, many decades of work. And what I've written up here is correct. But if you want to explore it more with me, come and talk during office hours.

And then a number of you started asking me about modulation of neurotransmitters, which I'll talk about in today's lecture. And these are clearly of interest, because many recreational drugs and medications modulate neurotransmitter amounts. And that is how they work.

So for example, some of you might be taking things called SSRIs, specific serotonin re-uptake inhibitors like Prozac, which make you feel less anxious and better about things. And these act by prolonging serotonin activity, by preventing it from being retaken up into cells. And I'll touch on this at the beginning of the lecture. And it takes a while-- is what I've written here-- for these medications to start working, because you're really asking for a rearrangement of the whole

synaptic process and the synaptic structure, in order that they can work.

And here is another one. What about amphetamines? Are they neurotransmitters? No. They increase the release of dopamine, which is a neurotransmitter, and they also seem to inhibit re-uptake of dopamine and serotonin is all. I'll talk about re-uptake very briefly in a moment.

For many medications, both-- actually, for many medications, no matter whether or not they affect your brain or other parts of your body, the precise mechanism of action is really not known. There are guesses. There's data. But the precise mechanism is often not known.

So let's use that as a segue into our lecture. I won't have office hours today, due to my schedule. I will have them next Wednesday due to the vacation schedule. And you're welcome to email me in the meantime. All right.

We've been walking through the cells involved in nervous system formation-- the connections between the cells. And now today we're going to finish talking about the connections between the cells and segue into the incredibly complex topic of circuits in the nervous system.

So today, the first thing I want to talk about is regulating synapses. And you remember that we're talking about chemical synapses. And the second thing I want to talk about are circuits.

Is there a problem? Can you hear me OK at the back? Thumbs up. Great. Good.

When I introduced circuits-- when I introduced synapses to you, I told you that one of the reasons that there was this chemical synapse in the midst with a slow chemicals synapse, in the midst of this rapid electrical transmission, was because you could regulate synapses. And that is really what fine tunes us.

It allows us to respond in a graded way to stimuli both from within the body and outside. It allows the body to adapt in ways that are not all or none, where action potentials are all or none. The overall response of the body clearly isn't. It's very nuanced. And all of this has to do with regulating chemical synapses. And that's what we'll talk about for a few moments.

You can regulate chemical synapses by changing the amount of neurotransmitter.

And if you think about this for a moment, if you think about the synapse, there's the neurotransmitter released into the space between the two cells, diffuses across the synaptic cleft, and then does something to the post synaptic cell, potentially to lead to an action

potential.

Now if that neurotransmitter stuck around in the space between the cells, it would keep stimulating the postsynaptic cell over and over. And as more neurotransmitter was released, so the postsynaptic cell would be further stimulated. And you would get to a point where the post synaptic neuron was completely over stimulated. And that's clearly not a way to regulate responsiveness to any stimuli. So neurotransmitter does not stay in the synaptic cleft for very long.

So it's changing the amount of neurotransmitter via degradation. Once neurotransmitter is released, in some cases, it's degraded by specific enzymes. For example, in the case of acetylcholine, there's a particular enzyme that breaks down acetylcholine. And if that enzyme is inhibited, you go into respiratory shock. You cannot breathe anymore, because you have to activate and inactivate the muscles via the nerves, as you breathe.

You can also regulate the amount of neurotransmitter by something called re-uptake, sometimes called reabsorption, where the neurotransmitter-- absorb-- tion-- where the neurotransmitter is released. And then it's taken up by the presynaptic cell, which is a frugal way of doing things. It doesn't have to keep synthesizing the neurotransmitter, and that-- so reabsorb-- re-uptake by the presynaptic cell.

And that is the case for serotonin and dopamine.

And then in some cases, you can regulate the synthesis, the amount of neurotransmitter that's being made. And the big class of neurotransmitters regulated in this way are the endorphins, which are a group of peptide neurotransmitters that are the natural opiates of the body, the natural pain regulators of the body. And all of these processes are regulatable both for modulating normal synapses, and also in all cases, for medication targets, for drug targets-- so normal modulation and drug targets.

Let's look at a couple of slides.

Acetylcholine is a neurotransmitter that binds its receptor on the postsynaptic membrane. And after it's done so, acetylcholinesterase, the E here, comes and breaks it down and stops it restimulating the postsynaptic cell.

Many nerve gases-- sarin was one of the famous ones that was used in the Japanese underground some years ago-- inhibit acetylcholinesterase. And in that case, you get a

buildup of acetylcholine in the post-- in the synaptic cleft. You get repeated stimulation of the postsynaptic cell. And that leads to, as I said, respiratory paralysis and death.

Our troops overseas have with them vials of atropine. Atropine is a competitive inhibitor of acetylcholine-- binds to the receptor and prevents acetylcholine from binding. And in the case of a nerve gas attack, if you inject yourself with atropine, you'll stop the acetylcholine from working. And you'll be OK. You get a little kind of floppy, but you can survive, because they're ultimate mechanisms of stimulating those nerves. OK.

Here's serotonin, the re-uptake pathway. Serotonin is released as all neurotransmitters, and then it's reabsorbed by the presynaptic cell. And SSRIs, whose mechanism of action is really not understood, do something to block the re-uptake of serotonin. All right.

The other way, clearly, that one could modulate how often synapses are active, or how often the postsynaptic cell is stimulated, is by modulating the receptors. Intuitively, if you have more receptors, the neurotransmitter has more places to bind. It can send a greater signal by changing membrane potential, if you decrease the number of receptors, and so on. And it turns out, if you modify the receptors, if you put phosphate groups on them, sometimes you can also change how well they act.

So the other thing to do to modulate synaptic activity is by changing the receptors. And there are really three ways to do this-- change in number or change in the type-- the subtype of receptor-- where there might be a subtle change in amino acid. Because you are using now a different gene to make the receptor.

You can increase or change the affinity for neurotransmitter. And you can change receptor responsiveness.

All of these three things have got something to do with learning and memory. They have to do with addiction. And all of these changes are slow. They occur over minutes, days, weeks, even. And for some, we understand how these changes occur, but for many, we don't.

The outcome of changing the receptors-- and I see we have a board issue here. So I'm going to put this on-- actually, let me put this board down-- and this board up.

One of the outcomes of changing all of these parameters about the receptors is that over a long period of time, you really change how a synapse works. And you can change how a

synapse works through these parameters, by repeatedly stimulating that synapse, OK?

This is what practice does. When you practice your musical instrument, or you practice your biochemistry problems, and you do it over and over, you're changing the synapses that allow you to engage these problems.

And these processes have got names. So let me just complete this. So by changing receptors so that repeated synaptic stimulation-- changes the responsiveness of the synapse-- generally, as I say, through the receptors in this case. OK.

And there are two outputs. One, you can increase the synaptic response. You can make it more likely that there'll be an action potential. And that would take place at excitatory synapses.

And this process is known as a long term potentiation. It's the stuff you want when you're trying to learn something. It's believed to be the way memory works.

You can also decrease the response of a particular synapse, and that would work if it was an inhibitory synapse. And in that case, the process is called long term-- let's just write it out-- long term depression.

Both of these processes have been shown to work in the lab, in culture-- hard to do those experiments on real animals. But it's believed that that's how memory works.

So in your first handout, I've drawn out for you the idea. Here is a normal axon. The presynaptic and the postsynaptic cell-- neurotransmitters released. It engages receptors. And at some frequency, there's an action potential elicited.

If you change-- if you repeatedly stimulate that axon over time-- days, minutes-- you change the receptor spectrum on the postsynaptic cell. And in long term, potentiation responsiveness increases. Whereas in long term depression, you decrease receptor number, or some other parameter, and you lead to decreased responsiveness.

OK. Let us move on to our second topic, which is that of circuits, which refers to multiple connectivities-- multiple synapses forming in a way that is stable, and that can lead to particular outcomes of particular stimuli.

Let's look at your next handout to try to get a sense of where we are. So I've diagrammed this

out for you. And I've started here with some kind of sensory neuron, an interneuron, and a motor neuron-- three connected neurons. You could put a lot of these other things called interneurons in the way.

Sensory neurons receive signals. Motor neurons tell things to happen, like muscles to work. And interneurons, of which there can be many, are the connectors between sensory and motor neurons.

There's an input, and there's an output. And what happens in between-- this is a synthesis of what we've talked about over the last couple of lectures. The sensory neuron makes multiple synapses onto the interneuron. They can be excitatory or inhibitory. They're summated.

The interneuron then decides whether to make an action potential or not-- action potential-- yes or no in each case. If it makes an action potential, it sends its signal to the next neuron, the motor neuron. That motor neuron is getting a bunch of inputs-- excitatory and inhibitory. And it, again, has to decide whether or not there's an action potential, OK? So that's the context that puts circuits, where we have been discussing the process of setting up the connections in the nervous system.

The thing about circuits-- and here's a term you should know-- is that they have to do with axon pathfinding. And so let's, for a moment, forget this diagram, which is a diagram of what there is in the adult. And let's think about how these circuits are set up.

The circuits that we have in our brains and then our bodies are of the close to uncountable. I'm not sure we ever will count them in an animal as complex as ourselves. It's been done in caenorhabditis, which has got 1,000 cells-- the little worm we talked about-- it's got 1,000 cells.

And all of the connections in caenorhabditis have been mapped. And they are extremely complex. But that's just a handful of neurons. When you're talking about 10 to the 10th, 10 to the 11th neurons, those connections are enormous.

But we can ask some basic questions, OK? And let's just state it. Neurons are connected to form circuits.

We can say that they're functional circuits, if you like. That is implicit. And the big question is, how do these circuits know where to form?

Or if you like the restatement, how do the neurons know where to go-- know to go and where

to connect?

And there are two kind of intuitive answers, both of which turn out to be correct. Either the neurons just go every which way. And if it works, that's retained, and the rest of the connections just are dissolved and go away. And that's true.

But the bigger truth is that the neurons are told where to go. So you can phrase these that the neurons undergo some kind of random process where neurons survive if connections are made.

Or there's some kind of guided process where the neurons are told where to go.

And both of these turn out, after many, many years of work, to be correct.

Let's look at some slides here. This is work from Professor Fee, over in Brain and Cognitive Science. He studies these little birds and their song circuit, which forms and reforms during the life of the bird.

The circuit is complicated. These are not single neurons. They are bundles of neurons. But it's kind of mappable.

And this is one of the circuits. It's got something to do with song within the bird brain. It's not the entire circuit. That's one of the things that's going on here on campus.

But when you start thinking about things like language-- I really like this slide. Because these are activity plots of the brain that can be done in a number of ways by monitoring oxygen uptake, or glucose use. But you can look and see different parts of the brain are active.

And when you think about language-- here are these four parts of the brain. Actually, here is a huge part of the brain used to generate words. Each of these parts of the brain are millions and millions of neurons, which are connected to one another within these regions.

And then each of these regions is connected to one another. And each of those regions is connected to the output, which would be all of your vocal apparatus. And all of this is connected to your auditory apparatus, to your visual system, so that you can read words. They can be processed, and so on.

The connections that give language probably take a very large part of your brain. I would throw out 15% to 20% of your brain is involved in some aspect of language-- receiving,

generating, or output. And the circuits there, as I said, are enormous. OK.

So do neurons know where to go? Let me go with you. This isn't on your handout. This is to look on the screen. Do neurons know where to connect?

There was a very famous experiment done by Sperry, some many years ago-- Roger Sperry-- who got a Nobel Prize for his work-- that involved a frog. And it involved figuring out where the neurons in the retina went.

So it turns out, if you look at your eyes, OK, there are neurons that go from your eye back into your brain. The neurons on the side of your nose are called the nasal neurons. And the neurons on the side of you, or the outside of your face, are called temporal neurons.

And what Sperry did was to take an adult frog and rotate the eye 180 degrees so that the nasal neurons would now be on the outside of the head. The temporal neurons would be on the inside of the head, OK? And for a while after the operation, the frog was really confused. But after a while, it actually recovered.

Now let's lay out this-- and the recovery, as I'm going to tell you, told Sperry and the world that neurons were told where to go. So here is the retina depicted as a circle. And here's a part of the brain called the optic tectum, that the neurons connect to. It's the first part of their circuit.

Here are the temporal neurons connecting to the C region of the brain, and the nasal neurons connecting to the R region.

So after this rotation, there were two possibilities. One was that the axons growing out.

So let's just be clear. In this rotation, these axons were severed. They were cut. They had to regrow and find their targets, if they could.

So one possibility would be that the axons would grow out of this retina, and they'd grow everywhere. And they wouldn't make really wrong connections. And things would be a mess.

In actual fact, what really happened was that the nasal neurons, even though they were on the wrong side of the face, found their way to the R region. And the tactile neurons found their way to the C region, just like they had connected to before. And this was a really profound experiment that told investigators that axons knew where to go. And we know the molecular basis of this now, but I'm not going to tell you about it.

In order to understand more about this guidance process, you need to understand a part of the axon that we haven't discussed, and a time in the neurons life that we haven't discussed either. And that is a time when neurons are growing.

Most neuronal growth takes place during embryonic development, but it continues throughout life. And so this is a developmental process, but also in that experiment, that was an adult frog. OK.

So guidance of neurons-- and as we'll discuss, particularly axons-- occurs during development and repair-- and learning as well. We can put that up as well-- during development, repair, and learning. Although I'd say for learning there's a bit of a question mark whether that's true. But I think it's probably true. OK.

And the cell that we need to consider is a committed neuron, which is called a neuroblast. It doesn't matter. But here's your committed neuron.

You have to think back to past lectures-- a neuron that knows that it's going to be a neuron but hasn't decided to-- hasn't become one yet-- hasn't differentiated. It's called a neuroblast. And here it is. It's just a cell body with the nucleus.

And over time, this neuroblast sends out processes that are called neurites. They initially look the same. Soon some of them become dendrites. And one of them becomes an axon-- some neurite, axon, plus dendrite outgrowth.

So you have a cell now with some processes. And one of these is going to be the axon. And that axon grows. And it's during that growth process that it figures out where to go.

The cell body doesn't move. The dendrites don't move. It's the axon that is doing the pathfinding.

So the axon extends and eventually finds its target.

And this extension is a growth process that we'll talk about. OK.

For every-- a nerve-- when we talk about nerves-- there are actually many-- many neurons, many axons. There are bundles of axons. And there is something about the first axon to find a target, that's very important-- the pioneer axon. The first one finds the target. And then others follow-- the same path that lays down a path.

And they form bundles, also called fascicles. And these fascicles together make the nerve.

The part of the axon that's really important for this process is the very tip of the axon, and it has a special name called the growth cone. So the axon tip, or the growth cone, is crucial.

And we can draw it on the board. If we now draw an axon-- and we've blown it up now. So usually we blow up and we lose bits, but now we're not going to lose any bit of it. Here's the axon.

And down the length of the axon are parallel microtubules-- many of them. And these microtubules both stabilize the axon and also transport substances to and from the cell body. So they stabilize the shape of very long axons, and they also transport, like little railway tracks, substances to and from the cell body.

That's not the tip of the axon. At the very tip, the microtubules end, and they interdigitate with these finger-like protrusions, which are very dynamic. That means they change all the time.

And these protrusions protrude because of polymerized actin. And you might be saying, we heard about that already. Yes. You've heard about that in morphogenesis. We've talked about this.

Cell biology is cell biology, whether it's about neurons, or cells in your stomach, or cells in your-- that give rise to the hairs on your head. All of these cells are just cells.

So there are these protrusions at the end. So this whole thing is the axon. But the very end is the growth cone. This very end is the growth cone.

And these protrusions go by the name of filopodia, filamentous, and lamellipodia, more flattened feet. And they protrude because of the polymerization of actin within them. So there's F-actin leads to protrusions.

Well, that's one important thing. And I'll show you a movie in a moment to show you that as an axon grows, it's sending out zillions of these things all the time that are feeling their way around the place. Actually, I'll-- no. I won't show you now, because there's an intervening slide.

But the other thing you should know is that like all cells, there are receptors on the surface of the growth cone that are also sampling what ligands are in the environment. And if the ligands are favorable, they will either stabilize these protrusions and make more of them to take place.

So here are receptors all over the place. This is a receptor. And if receptors contact ligands, then again, you will get F-actin formed. And protrusions will be formed. And they will be stabilized. OK.

The growth cone is paramount to axon outgrowth. There are signals which both attract axons towards them, and signals which repel axons. We can call those, not surprisingly, attractive signals. We'll talk about some in a moment.

And in that case, the growth cone extends. And it extends-- not to beat a dead horse here-- but because F-actin increases or is stabilized. And the flip is that the growth cone can be destabilized and literally collapse under repulsive signals.

The growth cone collapses. And it collapses because F-actin now becomes G globula, or unpolymerized actin, using exactly the same processes that we talked about during the morphogenesis lecture.

Let's look at a couple of slides. You don't have these. So just look on the screen.

This is a cell drawn in Professor Lodish's book. Here's the leading edge of the cell. The direction of the cell is where there is lots of polymerized actin.

This I've just drawn on the board.

And here is an axonal growth code. And I really like this movie. It's a time lapse taken over 10 minutes. And you can see all these things at the end and on the sides-- these protrusions from the cell-- very active.

They're forming. They're disaggregating, forming again and disaggregating as the cell-- those of the lamellipodia and the filopodia-- as the cell is feeling its way through the environment-- trying to find somewhere to go, OK? So this is a real exploratory process by the cell. Good.

What are these guidance signals? Well, this is not a mystery either. Ligands-- we know about receptors. You know about the guidance signals are ligands.

And these ligands, when they're bound to a receptor, lead to signal transduction and axon outgrowth. So ligands-- and we'll put in parentheses, plus receptors-- to signal transduction-- and a change in the growth cone.

There are two kinds of guidance signals that are reasonably separate from one another.

They're called short range and long range guidance signals.

Short range signals require contact between the axon and the extracellular matrix, or the axon and another cell. So there's some localized accumulation of a signal, and that has to be directly contacted by the growth cone-- so require axon ECM, or cell contact.

These signals are not diffusible. Therefore-- and they include things like laminin, which is a part of the extracellular matrix, but also an axon guidance signal.

And then there are long range signals, which would be the flip of the short range. These are diffusible. And they can be concentration dependent in their effect.

And we talked about things like this previously when we talked about morphogens-- other ligands that can act at different concentrations in different ways. OK. And an example that I'll explore more with you is the netrin protein.

All right. Let me see what I have here. OK, this is nice. This is on your-- this is your next handout. And this is an assay for short range guidance signals. It's called a stripe assay. And this was how it was found.

What those nasal and temporal retinal neurons grew on-- the idea is you take a plastic dish, and you put stripes of different molecules on the dish. And then you put neurons all the way along one side of the dish. And you look at them. You ask where they grow.

And they'll choose where to grow. And if they like one of the molecules, if they can interact with one of the molecules on the dish, they will grow in particular stripes, and not in other stripes. And that gives you your experiment and control in one dish.

This is what it looks like. So here are the neurons from the nasal side of the retina. And those nasal neurons, which project to the R side of the tectum, grow on our membranes, but not on C membranes, which is where they don't go. You may not have this, but you can go back and look at this later on. All right.

But let's move on now to an example that I want to spend a bit of time on. And the example of a long range signal is that the netrin in the spinal cord.

And what we're going to talk about are two types of neurons, one of which are growing down the spinal cord from the back, more towards the belly, and another type of neuron that's

growing more from the belly side of the spinal cord, back towards the back, OK? And those neurons always know where to go. And it turns out they are told where to go by the same signal.

So there are two types of neurons. There are these things called commissural neurons. And they grow ventrally, or down, towards something called the floor plate. I'll show you in the diagram. And then there are these other ones called trochlear neurons that grow dorsally, or up. And they grow away from this thing called the floor plate.

And it turns out, using an explant assay, that I'll go through with you in your slides-- but you should understand-- it was found that a single molecule called netrin, which is a secreted ligand, a secreted protein, that's expressed in the floor plate-- which we'll talk about in a moment-- is attractive for the commissural neurons and repulsive for the trochlear neurons.

And it also turns out, as we'll go through in a moment, that this has to do with different receptors and different receptor dimers, which bind the same ligand. And so here there is something called, for the commissural neurons, there is something called a DCC-- DCC receptor dimer.

And for the trochlear neurons, there is a DCC UNC5 receptor pair. And this will not mean much to you, but now you can write it down. And then you can go through your slides. And you'll have a reference point on right with you. OK.

So here is the diagram. The cell bodies of the commissural neurons are up in this region of the spinal cord called the roof plate. It's the top of the spinal cord.

And on the bottom of the spinal cord, near your belly, there is a cone-shaped group of cells that forms this thing called the floor plate. The floor plate doesn't actually make neurons. It turns out to be a really important source of signals. It's an organizer, if you like.

And it not only organizes these axons in the spinal cord, it actually also organizes your midline. And it's one of the reasons, if you're missing the midline of the body, things go wrong. It's because the floor plates are not there.

So the floor plate is an organizer. They are the commissural neurons growing towards it. And here are the trochlear neurons growing away from the floor plate.

This is what it looks like if you do an immunostain. The cell bodies are in red. And here are the

commissural neurons coming down in the spinal cord.

And this is a section through the spinal cord, OK? You've cut through-- cut through at the waist and then turned the section on its side. So you're looking into the spinal cord, which would be coming out in its length from the board-- from the screen.

All right. Here's an explant assay to figure out whether or not that floor plate has got something to do with the direction that those commissural neurons grow. So on your handout, the idea was to take a piece of dorsal spinal cord that hadn't started to send neurons out yet, and to culture it together in the laboratory, in a plastic dish, with some fluid nutrients, and so on, and ask what happened. And if you did that, that dorsal spinal cord sent out neurons towards the floor plate.

On the other hand, you can see that that experiment was specific, because if you put the dorsal spinal cord together with some roof plate, nothing happened. There was no outgrowth. So there was something special about this floor plate that elicited that commissural acts on outgrowth. And the idea is that the floor plate was attracting the commissural axons.

This is what it really looks like. Here's a chunk of dorsal spinal cord and floor plate. And here are the growing axons. And here's the control experiment. All right.

So what is the protein involved? Well, the idea was that it was something in the floor plate. And it was really hard to find this, because there's not much of it.

Professor Tessier-Lavigne, who is now president of Rockefeller University, but at the time he was running a research laboratory, and he and many undergraduates, and graduate students, and post-docs, and so on, went ahead and dissected many, many little floor plates. And they also found that the brain of the chicken contained the same kind of activity.

So they dissected out many thousands-- I believe it was 35,000-- chick brains and spinal cords. And they did biochemistry on this material. And they used the explant assay that I just showed you, where instead of the floor plate, you'd have a little pellet of material in which you had soaked the material that you've purified by biochemistry, from your smushed up, dissected brains.

And from this-- it was really successful-- from this, they got a single protein. Here's the RNA for the protein. That's right expressed in the floor plate. That's what the white is. That's the RNA. This is an in situ hybridisation. And they called it netrin.

Netrin protein diffuses away from the floor plate, but it's still mostly on this ventral side of the spinal cord. And you could show that netrin was important, because if you meet a mouse that lacked netrin-- here's the mouse that lacks netrin-- the commissural neurons go all over the place. They really don't know where to go. All right.

The netrin receptors, as I drew on the board-- you can look on your last handout-- are two-fold. One of them are called DCC, the tyrosine kinases. They also activate GTPases and do some other signal transduction.

And when netrin binds to a dimer of DCC, you get F-actin that's made. Microtubules grow. And the growth cone extends.

On the other hand, when you get this hetero dimer DCC and UNC5, or a different homodimer, you get cytoskeletal remodeling, G-actin made, and the growth cone collapses. OK. Close enough. We'll stop there.