PROFESSOR: We're talking about axon growth, and I'd like to spend most of the time today talking about various kinds of neuroplasticity, how axons respond to damage in the brain, either by regeneration or various kinds of spotting.

So we were talking about chemicals, effects on growth, and this is where we ended last time. And I think this was on your quiz. Caitlin and I wrote very different courses, so we're giving Caitlin's today. We debated about which one's was easier. Well, it's sometimes hard for me to judge what will be easier. So we went by her judgment today.

So this was one of your questions, right? So what is the difference? It's pretty straightforward. Tropic always means affecting direction. So remember we talked about nerve growth factor axons can throw up a gradient of nerve growth factor. But trophic always means survival or growth.

So I divided into two types-- survival promoting effects, many cells that depend on nerve growth factor won't survive. Their axons don't take it up. But we know that these neurotrophic factors also increase the amount of growth. So you get very little growth without growth factor, and you get a lot of growth with it. And they effect just how robust the growth is too.

Now, this question has come up a number of times, or had come up a number of times in trying to decide how these growth factors work. So for example, there's a particular-- this will come up later in the class, but we can cover it right now. The base at the floor plate of the spinal cord, there are molecules called netrins that are secreted. And they attract the axons of the spinothalamic tract-- that is, axons of dorsal horn neurons.

So they grow down to the floor plate. But then they cross over, and they go up into the lateral columns on the other side. Well, if netrins had only one effect, they attract these axons, why do they grow past the floor plate at all?

So that raised the question, well, could the same molecule be critical for both attraction and repulsion? How could that be?

And it was Mu-Ming Poo at Stanford who found a specific mechanism involved in just such a change. We won't go over the words here, but this is from an experiment. And this-- you just look at these pictures. See the growth cone here? And here's where they put a little pipette that's oozing the growth factor. This is semaphorin-3 that they're working.

So it appears to be repelling the axon. It's turning away. Then, they add cyclic GMP to the solution. And see what happens? It changes and turns towards the source of semaphorin-3, And he did a number of experiments like that. And he tried both cyclic GMP and cyclic AMP, very similar in their actions. And both of them could have this effect on the way an axon responded to these semaphorins, which like netrin, they could affect the growth of axons-- at a distance, just by diffusing through the intercellular space.

AUDIENCE: [INAUDIBLE].

PROFESSOR: That's right. Yeah, he's a good experimenter. He did all the controls.

This just shows another way. The graphs can be a little hard to figure out, but basically it's always that same thing. He varies the substance in its concentration and looks for the directional effects on the axon. And he finds this dependence not just on the growth factor but also on the presence of these other molecules. That doesn't mean we completely understand what's happening in the floor plate region, OK? That story has never to my mind been finished. We know that netrins are important in the attraction effect. But whether these energy-supplying molecules, how they change when they reach the floor plate, that's not as clear.

You can imagine other things that could be going on. The axon, as it matures, could change in its metabolism. So it simply changes in the way it's responding to various chemicals as it grows. So a lot of that still remains to be figured out.

This is a summary of some of the work on this molecule, semaphorin-3. It's found in the ventral part, the ventral horns of the spinal cord. So where you see the darker brown here, you have some semaphorin-3.

And notice, as is well known from anatomical studies, these large axons from the muscle stretch receptors, grow right into the ventral horn. But the fibers that terminate in the dorsal horn just won't grow into that ventral horn region. So the proposal is that it could serve as a kind of molecular sieve. It's just sorting the axons. Only some of them can grow. See that here.

So this is what we talked about. Here I've already written answers because I posted these already, what's meant by exuberant axonal projections. This is a well-known phenomenon in axon development. Many times, axons form a lot of-- they distribute terminals in areas that they don't later on. So they disappear. So we say they have exuberant projections, but then they regress with maturation. And that's true for a number of different fiber bundles, both subcortically-- it was named initially for transcortical projections by Innocenti and [INAUDIBLE] who was working with transcortical projections in the cat.

But it's been discovered in other systems too. It's been discovered in thalamocortical axons that branch profusely in the cortex, and then they regress to sort of prune themselves back to their adult form. It happens for optic tract, where they're truly exuberant. They grow even into the ventral basal nucleus, it's somatosensory structure and into the medial geniculate body. They don't stay there normally. They do in the case of some early brain damage. Then you sometimes can get them to stay there.

OK. And then, I think you were asked about this two modes of growth. I'll just show you the pictures of that and how they differ. These are the pictures. This is one of the figures in the book also. This is from the work in my lab, where we were looking

at stages in the growth of the optic tract. We've used a number of different techniques to look at this.

And when you first see the axons-- these aren't Golgi, but Golgi-stained axons in this early period look very much like this. We were normally using other methods to look at them. And you can use various methods to do it.

But you need a macro that can show the entire axon and its little swellings and collaterals. So here, when they're elongating, they look like this. They don't really form collaterals. They do have these little filopodia. And notice, the filopodia aren't always at the growth column. You find filopodia along the length of the axon too.

And normally, if they do from a collateral, it starts at one of these swellings where filopodia appear and then a growth cone appears, and a collateral starts to form. But that's normally not what happens right away. So when they're elongating, they're growing quite rapidly. In the optic tract, we measured it. It's between 60 and 100 microns an hour.

So then here's the next stage. They start to form branches. And you'll notice the exuberant projections-- they're forming branches in many places. If this, from here to here, is all superior colliculus, that means they're terminating the whole length of the colliculus early on. And that's typical of many of these axons in the optic tract. And apparently in other places too, similar phenomena had been seen for the fibers that grow up into the visual cortex from the thalamus. That was done by another student that I worked with in the lab, Jan Naegele, who's been at Wesleyan University now for some time.

And then, we call this initial focalization-- when they lose most of these very rudimentary little branches, they lose most of them, and they retain one of them. Here's one that's retaining one here, but it's still got that trailing axon. And because we don't see a lot of regeneration occurring, we assume that that axon is literally pulled back, retracted, just like we saw in the tissue culture pictures. We just saw the axon was able to retract some distance. That's what's happening apparently here.

So they end up forming their arbor in one location. And they might form more than one arbor. Like, they might form an arbor here in this colliculus. They might form another arbor-- I showed it going in the opposite direction because this could be in the thalamus, or they're terminating in one of the geniculate bodies.

And then when they first arborize here, they tend to arborize throughout the layers of the geniculate body or the colliculus. But then they become more focal. They end up only in the one layer. So they're maturing now. They're doing more pruning.

And then, they go through a slower process. This is even after the eyes open, is right here, which in the hamster is about 15 days old. Then they undergo changes, the ending matures, they form synapses, and so forth.

In these earlier stages, they're not forming mature synapses yet. But it doesn't mean they don't form contexts. There are immature synapses that they do form, and many of them are not maintained in the adulthood. Yeah?

AUDIENCE: [INAUDIBLE].

PROFESSOR: No. No, good question. There's no myelin yet in these earlier stages. The myelinization, the oligodendrocytes don't appear until the animal is a couple weeks old. They appear in these later stages. When we talk about arbor maturation, that's when you're beginning to get the oligodendrocytes starting to wrap axons.

Very important point. The myelin appears after the initial formation of the axons and its connection. Thank you for that question.

OK. So this is just about the rate. And I point out, we were able to estimate the arborization rate in the geniculate body especially. When they start to arborize, how long does it take for those arbors to form? And in the geniculate body, they have to grow from the surface all the way into the deeper part of the geniculate.

It's about a tenth of the rate of elongation. So it's much slower. But remember, they now are supporting multiple growth cones instead of just that one, or just a few. And that might be all you need to account for how much they slow down. Because

remember, in order to grow, they have to get membrane from the cell body, and it's transported down the axon in the form of these little vesicles that then join to the membrane at the growth cone area.

Oh, and then I also point out arborization, they never fasciculate. But the axons, when they're growing, even though they're not really following each other, they do become more and more tightly packed. And they end up in fascicles. Because when they retract from each other, they're retracting very short distances. But in the arborization period, when they retract, they're retracting much further. So they tend to stay further apart.

These are other molecules, the ephrins, the ephrin receptors. Do you have any idea what those do? Yes?

AUDIENCE: [INAUDIBLE].

PROFESSOR: That's right. There are gradients of ephrins in the tectum, and there are gradients of the receptor for those ligands in the retinal cells, meaning all down the axon from the retina. And this just shows the picture summarizing the gradients of different ephrins. Some of them, it's a more gradual gradient. This is ephrin-A2. And here's A5 that's not found at all in the more anterior parts of the tectum. This is the retina here and the tectum here and these diagrams.

And they know that these play a very particular role in the formation of the map, at least the nasotemporal map. But then later, they discover there were often greater gradients in the other direction too. So even though the axons are organized when they come in, there's a greater organization that results from these ephrins.

So how does it work? The major mechanism seems to be a selective repulsion. And it's known from experiments like this where they form-- they force the axons to grow down these little channels in culture. They have axons coming from temporal retina or from nasal retina. And they create in these channels a distribution of tectral membranes that are drawn from the more rostral tectum or from the more caudal tectum.

AUDIENCE: [INAUDIBLE].

PROFESSOR: Yeah. I guess I didn't name it, but that's what it's called.

And you can see here that if they're axons coming from temporal retina, they won't grow when they get to the membranes from the caudal tectum. They repel there. They will only grow over the axons from the more rostral tectum. Whereas if they come from the other end of the retina, from the nasal retina, then they will grow all the way through.

And some of them-- there is a gradient. Some of them are stopping here. Some of them are stopping here. Some of them will go all the way to the back, just depending on where those members came from. So that's apparently how the ephrins work. Depending on where the axons-- the amount of the ephrin receptor, they can grow various distances into the tectum.

So, that's all about normal development. Mostly the visual system, where it's been studied most extensively, but there are good studies of the growth of the corticospinal tract. There are other systems that have been studied, but that retinal tectal system has been the major model for the study of map formation and early development of the axons.

I want now to talk about plasticity. We'll start with plasticity in those maps. I just described that these chemical gradients are sort of determining where they're going to end. Is that a real rigid determination, or is there some plasticity in it? We find that there's considerable plasticity.

I just sketched here the tectum. Let me show you here, you're looking down on the top of a hamster midbrain. That's the superior colliculus. This is inferior colliculus back here, where those axons don't go. And here's another picture like that.

And then I show a picture of the retina. And I've labeled them according to retinal coordinates here. The coordinates that we use are based on the attachment of the eye muscles, because it's always the same. And if you just go by the position with respect to the head, then you're dependent on, well, exactly how were those eyes

rotated when you were measuring in the eye.

And of course, this is often done with physiology, getting these maps. But you still don't know the exact position. But if you go by the eye muscles, then you know.

All right. So now, let's do this. Here's the normal map, temporal retina. Here's the axons. Let me indicate axons coming in here. Here's the optic tract. Just draw a few of the axons. But that's the course they follow. The geniculate body would be up here. They're crossing over the geniculate body. And of course, most of these axons have crossed in the optic chiasm. So most of them are coming from the left eye, and they're coming into the right superior colliculus.

So now, very early, when these axons are just starting to grow in, let's just make a lesion. We'll just eliminate the whole caudal tectum. What happens? Do you just get half a map forming? That certainly was one of the possibilities. Sorry?

AUDIENCE: [INAUDIBLE].

PROFESSOR: It does compress. The temporal retina is still represented there. But now the nasal retina is represented here. Upper and lower retina are still upper retina over here, lower retina over here. So you get a compression of the map.

It's not totally linear. There's quite a bit of variability in it. There's even the stakes made, depending on how nice and neat your lesion was. But in general, we've done this both physiologically and anatomically. There's a pretty good compression of the map. Yes?

AUDIENCE: [INAUDIBLE].

PROFESSOR: Well, these were done on hamsters that don't really have good color vision at all. They do recover their behavior, but that does not mean their orienting movements were totally normal. What would you expect?

Well, this front of the tectum is normally controlling movement in the nasal part of the field, the nasal half of the field, where the center of their eye is looking 60

degrees out here. So basically, now, when they turn to a stimulus affecting that front of the tectum, they never make these really big turns. But then, animals normally make their turns in a series of small jumps anyway. But no, they can't make a normal rapid turn to the more caudal field.

AUDIENCE: [INAUDIBLE].

PROFESSOR: Well, there is some color representation in the tectum of animals that have good color vision, like the squirrel, for example. But people have not studied color very much in the tectum. It's mostly in the cortex that it's been studied.

OK. So now, what about map expansion? Now, the way that has been studied is very early, we put a little electrode into the eyeball, through the sclera. We poke an electrode in, and we make a lesion, usually with heat or radio frequency.

So let's say we've done that. We've made a hole. So what happens in the eye? Normally, that would correspond to an area like this. What happens is the axons up here that would normally terminate here, where I'm putting the star, will move. And they will spread into that hole.

So you'll get an expansion of the map there. It's not part of the retina that's missing. There are exceptions to this. If the lesion here resulted in damage throughout the retina so there was some loss of cells, all the cells where you see the red color, but just partial loss out in the rest of the retina. And then they don't spread so well. So the density seems to make a big difference.

That was the discovery of Douglas Frost, who is now at the University of Maryland, at Baltimore. He's been working for a number of years. But in his thesis here at MIT, that was his discovery. And of course, to do that work, we had to work out all the details of these maps that we were describing earlier.

All right. Now I want to talk about collateral sprouting. We know it occurs after certain adult lesions. It happens in the periphery, in muscle tissue and in the skin. If you lose one of the small nerves coming into your hand-- or let's say you lose 2/3 of the axons in the nerve coming into your hand. The remaining normal axons will

sprout. They will sprout collaterals and make up for a lot of that loss-- very important in clinical neurology that this happens, because it results in functional recovery.

But let's talk about the factors that affect that collateral sprouting and what could modulate that collateral sprouting. It is described in the chapter, but I'm not going to press you to do it. But it is a good question, once you have gone over it. The collateral sprouting can be so marred, it can just violate norms rules of development.

For example, the axons of the optic tract don't terminate in the medial geniculate body. But they can sprout into the medial geniculate if you remove the normal innervation there, and that is promoted by blocking their ability to terminate in the tectum. The major factor for that projection apparently is just getting rid of the normal projections to the medial geniculate.

The reason is, the axons of the optic tract cross right over the medial geniculate, the very caudal part of the tract, representing the lower visual field. And they do have some capability to terminate there, but normally the inferior colliculus projections are already there, and they won't terminate there. But if you suddenly get rid of all that normal innervation, then they can show this kind of collateral sprouting.

So these are pictures of the-- these are side views of the hamster. We just see the optic chaism here. It's just the uppermost brain stem. So here's the diencephalon, hypothalamus down here, thalamus there. These are the geniculate bodies where the retinal axons terminate. There's the medial geniculate body. And there's the colliculi, superior and inferior colliculus.

This is not really a schematic drawing. It's accurate in that they really are in this arrangement. I'm basing it on reconstructions of the hamster brain. And I'm showing one little bundle of optic tract axons and showing how the axons can sprout collaterals. This is normally now, not after a legion. They send collaterals into each of the two geniculate bodies, ventral and dorsal.

The one we just called the lateral geniculate, we usually mean the dorsal one,

because that's the one that projects the visual cortex. But that ventral one which is part of the subthalamus also gets retinal input.

And then you see them going on and terminating in their largest terminal area in the superior colliculus. And then I'm showing that the colliculus neurons, those very neurons receiving input from the retinal fibers, they send axons into the thalamus, into the diencephalon, into this nucleus next to the lateral geniculate, the LP, the lateral posterior nucleus, and to that ventral lateral geniculate body. So that's just all the normal projections.

So now we do this. We make an early lesion, wiping out the whole normal terminal region in the midbrain, the superior colliculus. We can do that with heat, applying it to the-- the bone is just cartilage at the age where we do this. Get rid of all that terminal area.

What happens? Of course, you lose this projection from the colliculus into the thalamus. And these axons which are just growing, but many of them you've destroyed, some of them are still growing in. They now don't have a place to terminate.

Here's what happens. Some of them do grow into the deeper layers of the tectum, where they normally would not terminate. So they regrow into that area and they form some abnormal terminations. But they also sprout into these areas that have lost their normal input from the colliculus.

That's collateral sprouting. They sprout abnormal collaterals there and there. And these are very dense if you do it early in life-- very dense abnormal projections. And if in addition to wiping out the colliculus here you also eliminate all those auditory system fibers into the medial geniculate, now you not only get those abnormal projections described in the previous slide, now you get axons growing right into the medial geniculate body.

And this has been studied first in the hamster, but we didn't do the behavioral work on that. Then [INAUDIBLE] did it on the ferret, because there he could do

physiology. He could do detailed physiology better than we could do in the hamster. And he even did behavior. He has behavioral evidence for their functionality. It's like now the auditory cortex becomes a visual cortex, and he's recorded from it. We'll come back to that near the end of the class.

And you can record from them here too, when you find the visual input in the auditory system structure. So that's what I mean when I say they lose their normal specificity. They can grow into areas that are denervated. It does not mean they'll grow anywhere. They grow heavily into the LP, they grow into the medial hemisphere, also quite densely, but we've never been able to get them to sprout like that in the hypothalamus. So some places are chemically more compatible where they're at in the [INAUDIBLE] than others.

So when we talk about collateral sprouting, sometimes it's more of a spreading, as you see here. Here I'm just showing you the concept. Here we have two axons terminating in a cortex-like arrangement, or it could be in the colliculus, but in a laminar pattern.

If we get rid of one of them, like this, the other one here doesn't really have to increase the number of terminals. It may just spread its terminals over a wider here. And in the case of the optic tract in the tectum, that appears to be exactly what happens. They don't really increase the total number of terminals. They spread over a wider here. So they are showing collateral sprouting, but they're losing some of their terminals elsewhere. So often, you get a very thin layer all the way across the tectum if you eliminate a good part of the retina.

All right, we talk about-- we say they sprout because axons compete for terminal space. What does that mean? What are they competing for? They're either competing just to get those synaptic sites that our cells regulate, so they're fairly fixed in how many of them there are, but they're also competing for chemical factors that they take up from their regions where the terminate. So they're competing for growth factors, they're competing for synaptic sites.

They also compete in another way, while they're growing. We know that when they

contact each other, they pull pack, they repel each other, because sometimes we know that specific molecules are called collapsing molecules, and in tissue culture, we call that phenomenon a contact inhibition of extension. They contact each other, they pull back, change direction slightly, and then keep growing. So that's what we think competition is. It's multiple things.

But there's something else too. Axons, when they're growing, can vary in how vigorously they're growing, how vigorously they will form terminals. We call that the growth vigor of the axon, and that is something affected by various factors, including these chemical factors for many axons NGF, but other neurotrophins as well.

So let's talk about things that can modulate that collateral sprouting. The chemical factors that we just mentioned, but they can be modulated by activity. There's very good evidence for this in the visual cortex, because you have axons that represent the right eye and the left eye terminating in adjacent columns in the cortex.

But if you reduce the activity of one eye so only the activity of the other eye is present in an early developmental period, what happens is the axons representing the open eye will spread further. They'll form larger arbors. And the ones not getting as much activity because the input has been bought, they grow much smaller.

So now you get, we call them ocular dominance columns. The columns representing the closed eye will be much smaller, much narrower, than the ones representing the open eye. But you can manipulate it in other ways too when animals have damage. This just shows the effect of NGF on the amount of growth, really drastically affecting the growth vigor of the dorsal root ganglion axons.

And here's a diagram of how we conceptualize this. Here's the axon just starting to arborize. It has very high growth potency. Then it forms more and more. It doesn't maintain its growth potency. The more terminals it forms, the less that growth goes down, until it just stops. It loses its growth potency, even though there's no other axons competing. That's because many axon systems can only grow so many terminals. This has been measured in some systems like in the sympathetic ganglia.

This has been measured in England by Geoff Raisman and Pauline Fields, that he worked with. They actually used an electron microscope, and they could estimate the total number of terminals. They were always regulated. So if they eliminated some, it would sprout, but they could only grow so many terminals per axon, and that's what we think is happening here.

So now let's do something else. Here's the normal arbor. It's formed. Let's just eliminate some of them. What happens? Well, now you're below the normal number, so the growth potency goes way up. It goes up here, but it will also go up for a collateral area, say, back in the thalamus, until it forms the number that it regulates.

AUDIENCE: [INAUDIBLE].

PROFESSOR: If we block them here, we've eliminated the cells they like to terminate on. And the cells down here that are still there, they've got their terminals already. So terminal space is not available.

All these factors have to be taken into account. You've got to consider what synaptic space is available and how many terminals these axons can actually form. And there are differences in different axon systems. We know, for example, motor neurons can grow a lot more terminals if you just give them more muscle tissue. So you can grow big muscles, and you'll still get the whole thing innervated, because motor neurons don't strictly regulate the total number. They're capable of forming more terminals or fewer terminals. And that's of the norepinephrine axons and dopamine axons and so forth. Although that's not been as well studied, the phenomena we know about indicate that that's true, what I'm saying.

So in other words, there's two different kinds of factors that affect these. One of them, you could say, is an extrinsic factor based on the other axons around, competition among axons. The other is really intrinsic, and I call that a conservation of terminal quantity. They tend to conserve the total quantity of axon terminals they form, or the total amount of arbor.

Sometimes we don't know exactly if it's always the number of synapses. It's only been counted in that one system. But that's why I use the term terminal quantity. They tend to conserve. They can only form so many.

Now, I will make some notes on these remaining slides. They're pretty straightforward, the concepts. And what I will do in the next class is we'll start by going through some phenomena of axon regeneration. I'm going to show you what happens if you totally cut the optic tract at various ages.

And I can tell you just in brief summary, if you cut it when it's really early, you don't need to do anything else. They'll just regenerate. But after a certain age, they won't anymore. So the question is, what do you do to get them to regenerate in the adult, and I will show you two methods that have worked and allowed us to get vision back in the hamster after transecting the optic tract.