GERALD SCHNEIDER: All right, let's get started. We have a few slides from the previous class. So that'll be on-- for those of you who have paper, that'll be on your previous handout. If you have a tablet, I included that in the notes I put on the web.

We were talking a little bit about the factors that affect axonal growth. And we mention that chemical specificity in the formation of the optic tract connections and the midbrain tectum. Let's just review briefly chemical guidance in the spinal cord with the discovery of the netrin molecules.

These molecules-- this might not be on your printout-- they were homologues of axon guide molecules found in other animals-- invertebrate animals and nematodes, C. elegans, and fruit flies. Let's draw a picture of the embryonic spinal cord. Remember what that looks like? So there's the ventricular layer. You see the walls of the neural tube. This is the floor plate region here.

They found that these cells in the floor plate secrete netrin-1. And it diffuses through the neural tube, so it's more concentrated in the ventral part of the neural tube than in the dorsal. So when axons start growing out from the neurons, there on the alar plate, there are neurons there that have an axon that specifically responds to netrin.

And initially, the molecules down here attract it so the axon grows down into the floor plate. Now, what are these axons going to do, if they are, say, developing spinal thalamic tract axons? They're going to cross over. They're decussating. But they were attracted to the floor plate. We don't want them to stay in the floor plate.

But when they reach there, a change is induced in the receptor in the axon. So it changes its response to netrin-1. And it now becomes chemorepellent. And the axon then can grow to a position roughly here. And then all these axons will collect, form the spinal thalamic tract by that mechanism. That certainly doesn't give a complete explanation for how the axon's guided, but it's a major factor-- getting the axons to grow down and cross over.

All right, so we're not going to go through this whole slide. I just want to emphasize that these effects of such molecules depend on the receptors. The receptors express, and we saw the example of that. If the cell changes, the receptors expressing-- it's the receptor that determines the action on the cell, that a molecule will have that binds to a receptor on the surface.

And these various names are various molecules that have tropic effects. What does tropic mean? Provides direction. Tropic would mean it effects growth.

Now, in addition to these kind of chemical effects of attraction and repulsion, we have specific barriers to elongating axons in the developing nervous system. First of all, they have to have surfaces to grow on. They won't grow through fluid spaces. They have-- so where those surfaces are and how they're arranged will affect the way axons are growing.

In addition, there's some very specific things that have been discovered about these barriers. One of the ones discovered a while back was that there are glial cells at the midbrain, at the tectal midline. So let's just-- we'll draw down here a picture of the midbrain's-- the top part of the midbrain, and here's the ventricle, down here. Oops.

So that would be-- we're just looking at the top part of the neural tube in the midbrain. And right at the midline there, if we had a cell-- a stain that could stain it, there are many cells that all look like this. They're elongated cells that extend from the ventricular layer to the pia.

Now, in the developing nervous system there's many more. They're all over the place at the ages when axons are growing into the tectum. However, those ones at the midline are different, because the retinal accents grow in up here. I'm drawing them this way because they're growing at right angles to the section that I've shown.

But they can't-- the ones from the left eye, if they're growing into the right tectum, here, can't do that. They can't cross over there. There's a barrier. How do I know that? Well, I could do something that could induce them to grow over there. I could get rid of the input to the left side, just by removing an eye. they won't cross over unless I damage those cells.

So one thing a student of mine did is, he inserted a knife here, from the side like that. Just cut the tops of all those cells off. Didn't kill the cells, because the cell body is down there by the ventricle. If he did that, and he removed an eye, then the axons would spread across. He just had to remove that midline barrier. In fact, it's possible to damage only the cells right at the midline and get that effect.

This is just a picture from one of his experiments. You can see my-- this is the little bit of the ventricle showing here. And here he's labeled these radial cells. And then you can see the path of the knife here. Because all those cells are cut off. And then with a different marker, he's labeled the retinal ganglion cells and their axons. And here, early on after that cut, you begin to see axons crossing over. They would never do that if he didn't remove the barrier.

We'll talk about oligodendrocytes and how they also can inhibit axon growth. The next class is this class. We're going to talk about plasticity in axonal development, where you can get regeneration. You can get a lot of collateral sprouting in the developing organism. You can also-- we'll mention collateral sprouting again, in the adult.

These are two kinds of glial cells that play particular roles in axon growth and development. Do you remember what Schwann cells and oligodendrocytes are? What are Schwann cells? They're the glia cells in the peripheral nervous system that surround the axons. OK? And when axons regenerate, say after an arm injury, if you damage a nerve, the axons will show anterograde degeneration. Everything distal to the cut will die, but not the Schwann cells. In fact, the presence of those Schwann cells is very important for getting axon growth.

They secrete growth factors and they provide surfaces that axons can grow on, very supportive of regrowth. However, in the central nervous system, we have a different cell that makes myelin, the oligodendroglia, or oligodendrocytes is the other name. It's not supportive of regeneration, and in line with that discovery that they don't support regeneration was the discovery by Martin Schwab in Switzerland that on the membrane of that cell, there's a protein that inhibits axon growth, so he called it Nogo.

This is just to remind you what myelin is. If this is a motor neuron and we make a longitudinal section of it, remember that the Schwann cells, a series of them will be along that axon. And each Schwann cell will wrap its membrane around one segment of the axon, and then there will be a little gap, the node of Ranvier, and then another Schwann cell, and so forth, all the way down the axon. OK? Whereas in the central nervous system the oligodendrocyte does its work a little bit differently.

This is a reconstruction of an oligodendrocyte in the central nervous system, and you can see it has these little branches, and each branch goes out and contacts an axon, and then its membrane expands and wraps around. So here you see a cross-section through an axon, there's the axon inside. OK? And there you see the Schwann cell membrane, it's wrapped around and round. This is just an enlargement to see how it does end.

It's just like wrapping a piece of paper around a noodle. OK? And this is an electron microscope picture, a cross-section of myelin. So here you see the axon in the lower right, and it shows the mitochondrion and the axon. There are microtubules and filaments inside the axon and a few other organelles. This is the axonal membrane. And outside the axon, you see the membrane of the Schwann cell if this is in the peripheral nervous system.

There's a little bit of cytoplasm at the end there. Almost all the cytoplasm gets squeezed out from most of the myelin. Now this is a rather large amount of myelin that would be present in a larger, thicker axon. So these Schwann cells, then, not only form myelin, but they also secrete growth factors. They're very active when the nervous system is damaged, but they do this during development too.

The particular surprise occurred some years ago when we believed that there was almost no regeneration in the central nervous system, and it was found that if you take a piece of peripheral nerve transplanted into the CNS, you can get axons to regenerate into the peripheral nerve.

Remember I said oligodendrocytes don't support axon growth, but the Schwann cells do even of central nervous system cells. It Doesn't mean that all of them will do that, but at least some of them will, and that was a major discovery and it's enabled us to get some functional recovery in our studies here at MIT of regeneration in the adult central nervous system.

This just summarizes what I've said about oligodendrocytes. Now I said you don't get regeneration in the central nervous system very much unless you use these axons via the peripheral nerve.

Why don't you get it? We found that this protein, the Nogo protein, doesn't fully account for the [AUDIO OUT] you're working with. It gets some of them to grow if you provide them an environment that's not just the central nervous system oligodendrocytes. So you give them something abnormal, for example, a piece of embryonic brain. Very supportive environment for axon growth, of course, and it works when you transplant it into the central nervous system.

I mentioned the peripheral nerve with live Schwann cells, and there's another cell that will also do that, its olfactory and sheathing cells. It's the cell that ensheaths the primary sensory axons of the olfactory system, so they're like Schwann cells anyways. I want to say now a little more-- we want to focus on both development and the injury effects here. I'm going to talk a little more about collateral sprouting, it's not the same as true regeneration.

How many of you remember I said something about collateral sprouting before? Do you remember that? If you don't, I'll try to define it again. I want to describe to you this phenomenon of invasion of vacant terminal space. Sprouting seems to be induced by loss of axons. So if you end up with terminals with no axon ending on them, we don't know all the molecular factors that occur to induce the sprouting.

We know one thing that happens in the periphery and that is the Schwann cells become very active, actually sprout branches and induce growth of their axons. The basic phenomenon in collateral sprouting is this, you get one group of axon to degenerate and another grows into the vacant terminal space. So for example, if you have-- your hand is innervated by a number of peripheral nerves and they overlap a little bit in their distribution, but mainly you could find nerves, say, that innervate the left or the right half of your hand.

Now let's say you suffer an injury. OK? You suddenly lose sensation in part of your hand because the axons are missing. Over time, and this doesn't take very long, you will get collateral sprouting of the remaining intact axons. They will sprout collaterals and grow into the denervated space. It also happens in muscle. If you have a muscle, say of the leg, and you lose one of the major nerves containing the motor neuron axons innervating that muscle, the muscle will become weak.

It's now innervated by many fewer axons, some of the fibers may not be able to contract at all, they've lost all their innervation, but now collateral sprouting will occur as long as there's some intact axons innervating that muscle. This has been studied even more than what happens in the skin. And again, Schwann cells become active, induce growth of-- exactly what means, we don't fully understand, but they induce growth of the remaining axons. And strength will come back, not because of regeneration, but because of sprouting.

There was a question. How long does it take, you said, to regenerate? How long it takes to regenerate is one thing, how long it takes to get collateral sprouting is another. OK? Let me describe something that happened to me, I'll put it in very direct personal terms. OK? I went to sleep on my arm one time. I have diabetes, my blood sugar was high, that makes you pretty susceptible to nerve compression damage. OK?

I learned not to go to sleep like this because what happened was when I woke up, not only the same thing that would happen to you, my hands were asleep, both of them-- they felt tingly and everything, and I was moving them around getting the circulation back. Everything came back in my left arm, in my left hand. But on the right hand, I couldn't raise my fingers. I was partially paralyzed, it was very scary, but then I realized it's probably partial.

And if I'm patient, maybe collateral sprouting will-- and sure enough, over two weeks time, within two weeks, I had regained the strength. I could now raise my fingers again even though if I had compressed the nerve here, and regeneration happens at most two millimeters a day, the fibers could not have regenerated in that time. OK? So before the regeneration occurs, the intact fibers were sprouting, re-innervating the muscles, and that gave me this return of function.

There were probably sensory effects too. I did notice sensory changes. They tended to go away at about the same time, but that would be due to the different nerve fibers in those peripheral nerves. All right. That phenomenon was known for skin and muscle since the late 1950s, and there were a number of articles published and review articles published on that phenomenon that long ago. And then people began to wonder, well, what about the central nervous system?

Is there anything like that in the central nervous system? Remember when I talked about sprouting before, I mentioned studies of the spinal cord where there was a transection of descending axons. OK? This will happen on-- even a partial spinal transection can cut off a lot of descending axons in the cord. And then I pointed out that even reflexes disappear if they're controlled by a level below the spinal injury because of diaschisis, loss of function because you've removed a lot of excitatory connections.

But then, over time, the reflex connections are all intact, and over time, the reflexes come back and one of the major reasons is collateral sprouting. The same reason I could raise my fingers again with collateral sprouting in the muscles of the hand and forearm, so this happens in the spinal cord. So it was discovered-- and then they verified with anatomical experiments and electrophysiological experiments, studying the dorsal root axons in the dorsal horn of the spinal cord that this indeed does happen, you get collateral sprouting. OK?

I want to talk about a demonstration deeper in the CNS, not of primary sensory axons now, but of axons in the CNS. I won't talk about the optic tract here. But in general, this phenomenon explains some of the functional recovery and functional change. It's not always recovery, sometimes it's not good. OK? I don't have a blowup of that, so you'll have to look carefully at that diagram here. This is the one we want to look at.

Here you see a diagram of cells in the septal area. OK? Now if you remember your dissections, the area in front of the thalamus on the midline, called the septal area. A lot of you were dissecting the fibers coming from the hippocampal formation and we followed axons into that area. It goes right through the septum of many connections there. Now the septum's part of the limbic forebrain, the limbic [INAUDIBLE] brain, so it gets many connections from the medial forebrain level.

Now look at the normal cell here. This cartoon is showing that the cell gets inputs from the hippocampus on its dendrites. It also gets inputs from the hypothalamus. They go not only to the dendrites, but also to the cell body. Now that was the anatomy that this experiment was based on. To find out if you could get collateral sprouting of those connections like you can get in muscle and skin, Geoff Raisman, this guy in the UK tried the experiment where he either cut the hippocampal fibers going into the septum or he cut the medial forebrain bundle axons coming from the hypothalamus. OK?

He cut one or the other, and then he looked with the electron microscope at these cells, and he already knew that the hippocampal axons terminated only on the dendrites whereas the axons from the hypothalamus terminated also on the cell bodies. And over time, he saw a couple of very interesting things. If he waited long enough after the hippocampal damage, he saw these funny little double synapses that you never see in the normal.

So these synapses, the terminal boutons had expanded, they had sprouted and formed additional terminals. And he quantified that and showed that they increased and increased over a couple week period, and I think he followed up to a month. He got some changes up to a month and it tended to stabilize. OK? And if he cut the-- made a lesion in the hypothalamus and got rid of these connections to the cell body, he now began to see connections from the hippocampus appearing on the cell body, indicating those axons had sprouted.

OK? So this is the sprouting part here and here. So those have appeared, they weren't there before. Now if you look very carefully at different periods of time, at short intervals, at what was happening in the terminals-- look down here. Here would be a normal synaptic connection with a terminal bouton, you see the synaptic vesicles terminating on a dendritic spine of the dendrite.

Now when he induced degeneration with the hippocampal lesion, you would see that terminal being engulfed by a glial cell, an astrocyte. OK? Eventually, the astrocyte would be occupying acting like its replaced the axon. OK? That's what's happened here, and then you begin seeing the astrocyte sharing that space with a new axon. And then finally, you don't see any of those in between configurations, you see only synapses on those dendrites again.

So just by looking at various periods of time, he was able to reconstruct something of the dynamics of sprouting in the CNS, and his studies with Dr. Field in England became very well known, the first one was 1969, next one 1973. He did a number of those studies. OK? Now in the developing nervous system, you can get even more drastic types of collateral sprouting. I mentioned before how you can get the axons to grow right into the medial geniculate body if they're coming from the optic tract just by getting rid of the normal input there.

And right where the optic tract crosses right over that auditory system structure, you'll get some sprouting, probably encouraged by a lesion of the tectum where these optic tract axons are missing some of their own terminal space, but we think that terminal space availability is the major thing. You can even get them to terminate in the somatosensory system by a similar kind of manipulation. Just get rid of the normal input there, you can induce growth into the somatosensory system. OK.

You get a lot of this kind of sprouting within the tectum where I've studied it the most and I diagrammed the result this way. If you have different axons terminating, they often terminate adjacent to each other with terminal harbors that can be fairly large. Now if one of them gets damaged here, the other one will spread into that vacant space. And we talk about sprouting and spreading, and they're not always easy to distinguish what would be the difference.

If this arbor simply sprouted new connections as I've shown here, it would end up having a supernormal number of terminals. That isn't necessarily what happens. There have been a few studies that indicate that at least some types of axons will form a certain number of terminals and that's all. OK? So in that case, they have to spread the same number of terminals over a wider area and that is what appears to happen in the midbrain tectum to the optic tract axons.

They will spread or sprout, but in fact, the number of terminals seems to not become supernormal. OK? Now let's just go back and review. We'll come back to a little bit of that. Let's just review the factors of influencing growing, or regenerating, or sprouting axons. Remember they were studied first in regenerating animals. It was harder to study development until the techniques became more developed.

Then with better anatomical techniques, it was studied directly in developing animals, and then we could look at the time course of sprouting as well. There's two major types of chemical specificity, positive attraction for the axons or negative repulsion, or barriers to the axons. And each of these types can be by contact or can act at a distance by diffusion. OK? The same thing for repulsion.

They can be something on the surface, molecules that cause axons to be repelled. You can also retract from the surface or they can be diffusable factors also. Then in addition, we have competition among axons. And finally, there are different ways we can modulate the tendency of axons to grow, so I'll call that modulation of the competitive growth figure. It's an intrinsic property that we know applies to arborizing axons.

So let's just summarize a little more about this. This is a diagram that was published that just says the same thing I just said about chemical factors. It shows here a couple of cells growing axons. They're growing away from one source of molecules here that our chemo-repulsive. So they're-- action by diffusion here and they repel the axons, they grow away from it. They're growing towards another source here, molecules being secreted that diffuse there, and here's the growth cone.

Just like we remember the growth cone turning towards NGF, the growth cone of the dorsal root ganglion turning towards NGF in a Petri dish, that's what they're showing. Then they also indicate it growing through a channel here where it's growing through a channel with surface molecules that act as contact attractants and it's avoiding areas of the surface that repel the axons, both surface and diffusable factors, and it mentions what some of those are.

The netrins that we talked about, like netrin-1 in the spinal cord, can act in either way. They can attract axons or they can repel them. OK? Contact repulsion, I'm going to mention some [INAUDIBLE]. I mentioned a molecule called collapsins, that's one of the semaphorins that we know will cause the growth cone to retract if fat molecules are being secreted. So this just summarizes-- maybe it's a little bit of what I've said.

We talked about adhesion, that's, of course, the contact effect. We know laminin, for example, is a molecule that certain axons like, they like to grow on it. We mentioned the neuronal cell adhesion molecule that tends to make axons pull together. That's also an adhesive effect, and then the diffusable factors. These are all positive effects. And here are the negative effects, the midline barrier that I talked about, contact repulsion, the oligodendrocyte factor, the Nogo protein that repels axon growth.

And then we mentioned, when the map forms in the tectum, there's [INAUDIBLE]-- in the tectum, there's axons from the temporal retina are repelled from the caudal tectum because of the molecules that they carry on their surface and the molecules they encounter in the tectum. And then finally, we mention the netrins and the growth of the spinothalamic tract axons. And these are the types of competition we talked about, and I will cover these last few things at the beginning of the next lecture.