OK, we have to finish talking about axon growth today. Mostly about regeneration of axons. But first we have a little bit-- we were talking about sprouting, and the factors affecting collateral sprouting after damage in the brain. And I wanted to talk about the ability of axons to compete. Their growth vigor can be affected by various factors. For example, chemical factors we've already mentioned. Growth factors like NGF. And remember this figure that shows NGF effects on a dorsal root ganglion, or a synthetic ganglion would be very similar.

But growth figure can be affected by activity as well. So for example, when axons representing the right eye and the left eye, parts of the retina that represent a similar part of the visual field, grow into adjacent areas. If one eye is shut for a while, the more active eye will take over more space. That's because the axons are more able to compete for terminal space there. And that happens in the cortex, it's also been observed in the tectum of some animals.

This illustrates a growing axon early in development where all its endings have a lot of ability to compete. But when they extend, and the arbor reaches a certain size, the growth figure goes down, and now the axon won't compete anymore. But we can alter that also by damaging the growing axon.

So for example, here I show an axon that's reached an arbor of full size, but now we do partial damage. The best evidence for this has been obtained in the olfactory system. When you do that, the arbor will grow in other places, further than it normally grows. So this part of the arbor will sprout additional terminals. But it might also develop terminals more proximally. This has been demonstrated both for the optic tract and the lateral olfactory tract.

So it appears that axons will grow until they've reached a certain size, and then they lose that ability to compete for terminal space.

So keep in mind the two kinds of factors, extrinsic factors, like chemical factors from
the outside, or activity. And intrinsic factors, which is this conservation of terminal quantity. That is, it has to be due to a factor inside the cell. The cell is programmed to form a certain quantity of arbor. Once it's formed that, it won't try to grow additional arbors.

You observe it in the scan, for example. You observe it in innovation of muscle. That's, in fact, where some of these factors were first discovered, but then now it's been discovered in central nervous system in a number of different systems as well.

So now we can think of this term, plasticity, very broadly in terms of these different developmental stages, and of course, learning. We now know that there's some of these developmental stages, like proliferation of cells, cells undergoing mitosis, migration of cells, differentiation of the cells grow to their axons. And then all of these things happen to some degree even in the mature brain.

It's only in recent years that we found out about that continuation in the mature brain. In fact, for a long time we doubted whether any structural changes happened in the mature brain at all. But now, it's been seen as a correlate, at least at the level of synaptic terminals. And [INAUDIBLE] arbors as a correlate of many types of learning.

And this factor, I'd like to say a little bit more about now, and we'll return to some of those other things later, the phenomena of neural depth. I've mentioned what a trophic factor is. That many cells have to obtain a certain amount of trophic factor from their terminals in order to survive. If they don't get enough, they will kill themselves. It's a form of cell suicide. We call it apoptosis.

Cells early in development, of course, aren't in contact with their terminal, so how do they survive? They apparently survive because there are intrinsic factors that are present when they're growing. The cell with the growing axon that disappear later. And that has been found in the visual system, for example, and there's evidence now for these two different factors that play such a role.

Let's look at some evidence for trophic effects that keep cells alive. These are
experiments on the motor neurons that innovate the limb muscles. And I've brought pictures from the Purves and Lichtman chapter, which some of you have read. Look at these pictures at the bottom. Here you see a chick with two legs on one side. Here's a frog with three legs on one side, one real leg on the other side. His front legs haven't been tampered with in development. But simply by transplant techniques, you can produce animals like this.

Why would they do that? Because of the phenomenon of cell death during development. When motor neurons develop in the ventral horn of the spinal cord, and similarly for brain stem motor neurons, there is a super normal number of neurons produced initially, and then some of them die. And they die after the axons have grown out and they've established terminations. They've innovated their target.

So the question was why? If it's that some of them simply can't get enough growth factor, well then if we provide them additional places to terminate where they can get that growth factor, like additional muscles, as in these cases, put an additional leg there. Then many of those motor neurons shouldn't die. And that's exactly what happened.

So if you look at the spinal cord of these animals and count the number of motor neurons on the side with the additional legs, on the side with the normal single leg, you'll find different numbers of motor neurons-- there are many more motor neurons where there's the additional muscle tissue being innovated.

Well of course, that means that population size matching could be a major reason why this mechanism exists. You don't have to determine in the genome the exact number of neurons needed in a population. You can produce more than what's needed, and then when the axons form their terminals, they occupy all the terminal sites, the ones that don't find a place to terminate will simply die off. So we get a matching source of, the source of a cell, cell number, and the terminal size.

But another thing it does possibly is correct for errors, like when we get a topographic map. But I've already mentioned that we know about other factors that
determine topography. But what I didn’t mention is that when a topographic map forms, like the map of the retina in its growth to the midbrain tectum, it forms a topographic distribution, so one part of the retina’s represented one place in the tectum, another part of the retina’s represented in another place.

The map often has mistakes in it. It's not a perfect system. And there is a disappearance of most of those mistakes during the period of cell death in the retina. Because in that system, just like with motor neurons innovating muscles, there’s a super normal number of neurons in the retina, and then some of them die. And during this period of cell death, there’s some error correction. So we think there is evidence for both of these kinds of purposes of naturally occurring cell death in the nervous system.

Neurotrophins are believed to be a major trophic factor that explain some of these phenomena. We know that, I mentioned that activity increases growth bigger. Activity also is associated with release of growth factors in the neurotrophin family. It’s also been associated with certain forms of learning, and it’s been found more recently when new neurons are produced in the adult brain. So some of these phenomenon may come up later in the class.

What I want to say now is a little bit about another form of plasticity, the regrowth of axons after damage. It’s an area that I’ve worked on for a number of years. The general problem of axon regeneration is this. When axons-- axons, of course, when they’re growing seem very plastic, but can they regenerate if you cut them? The answer is yes, if you cut them really early when they’re still elongating.

But after they enter their terminal area and they start to arborize, they go through a transition to a different state where very few of them can regenerate anymore. In fact, many systems we can't get any of them to regenerate. And the ones that do, don’t grow very far, or they aren’t able to establish permanent connections.

So something changes in the adults. And the question is what is it? How much of it is intrinsic, the intrinsic ability of the neuron changes, but simply can't elongate anymore? Or could it be there's just inhibitory factors in the environment?
The dominant theory of people working on the problem of regeneration has been that inhibitory factors appear, and there's a lot of evidence that growing axons are inhibited in the adult tissue environment when they're not inhibited from growing in the embryonic tissue in [UNINTELLIGIBLE]

Now, when axons can't-- but there's additional evidence that intrinsic factors also play an important role. Maybe not for all neurons, but many neurons, probably the majority in most systems, can't regrow after the animal reaches a certain age.

And then we know that if an axon's cut off from its termination site, it's not going to get those growth factors from the termination site anymore. So what's going to happen? Well the cells will gradually die. In fact, when the animal's pretty young, they die pretty fast.

But let's say a person suffers damage here in the occipital cortex, the visual cortex. It gets its visual input from a cell group in the thalamus called the lateral geniculate body. Many of you know about, and if you don't, you learn about it later in the class.

So a person who suffers a damage as an adult to the visual cortex, over time-- and this happens fairly slowly in the human-- the cells in the genicular body will shrivel-- they look like they're losing weight-- and then they'll start to die. Well as they die, now what happens to the axons coming from the retina that are terminating on those thalamic cells? Some of them die, but not all of them. Some of the cells in the retina start dying too.

Why don't all of them die? Well, because those same axons that terminate in the genicular body, some of them at least have terminals in other places too. So they survive because of what we call sustaining collaterals.

So when we want to induce regeneration, our first problem is just to preserve the damaged cells. We have to keep them from dying, because they're not going to be able to grow axons if they die off. Another problem is to provide a permissive environment. We know they don't like to grow in adult tissue. We have to give them a substrate to grow on.
So you might have to provide a bridge. They don't like to grow through scar tissue that forms in the brain due to both connective tissue invasion, and glial cells. We call that a glial scar. In the spinal cord, it's both a glial scar and connective tissue innovation from the meninges. So we have to provide a permissive environment.

Now, if the cells have lost their growth figure when they're older, we might have to use chemical factors to promote that growth. That's a separate factor. And then when they grow their terminals, they might reach terminal regions that have been invaded by other axons. So now what are we going to do?

After all that, we get regeneration, other terminals have formed. And that happens too, due to collateral sprouting, for example. So that might require various forms of plasticity of the regenerative connections. So sometimes even after regeneration takes place, the functional recovery does not occur, or it occurs very slowly.

Rutledge Ellis-Behnke who's now a professor at University of Hong Kong wrote a paper on this. He called it "The Four P's--" because of each of those words starts with P-- "Four P’s of Regeneration." To correct a notion in the literature that there was just one factor we had to worry about. Cells lose their ability to regenerate in other animals. And there's actually multiple factors.

I'm going to tell you a little bit about using peripheral nerve as bridges. This is based on the discovery that axons in the brain that won't grow in the adult brain environment, will grow into a peripheral nerve that's artificially transplanted into the brain. Like you can take a nerve from the leg or the eye. From the same animal so you don't get an immune response. And you put that in the brain and axons will grow into it, whereas they won't grow in the brain.

Then we've developed some new materials that can used to form bridges. And for this we've collaborated with bioengineers with some nanotechnology applications. And then in addition, there's chemical methods for inhibiting scar formation or for breaking up scars, and also the possibility of genetic transfections to alter the ability of axons to grow, cells to grow their axons.
This has been much more difficult because the techniques for genetic transfection don't always work very well, so we've focused on the first two here.

Now in this picture, you're looking down on the brain stem of a hamster. This is the coddle into the midbrain, rostral into the midbrain, thalamus. So the hemispheres I've taken away just to show you the top of the midbrain. Spinal cord's back that way. Here comes the optic tract fibers. Most of these came from the other eye. Most of them crossed over. What I'm showing here is the position where I made a knife cut. Cut all the axons entering the tectum.

And then across-- here's a side view now where I'm showing the position of the cut there. And I'm showing how I can take a little piece of peripheral nerve from the leg of the animal. And it's a very tricky procedure because I have to-- I can't just plunk the endings in on either side. It doesn't work. I have to de-sheath little bundles of axons. They're all in these little bundles inside separate little packets of axons.

I get them out and I do some preliminary work on the endings. And I get all those little bundles coming out. And then I take a tiny glass pipette, wire that nerve across and poke those endings just below the surface in the superior colliculus or optic tectum here. And right into the surface of the optic tract in front of the cut. And I can fit up to three of those pretty easily across a single cut in the optic tract.

So I'm going to show you-- I hope I have it here. Here we are. Well, if that doesn't work-- that worked. This just shows you behavioral test where we show the animal-- the reason he doesn't actually look up, even though the stimulus is placed up here, he's not looking up because the projections that we've generated isn't totally accurate. But he does turn.

Let's just show through the labeling of retinal fibers, you see some of them coming through a bridge here. It's one of three bridges, and these are terminals in the tectum. They've all regenerated. The tectum had no terminals before a procedure. This is just a close-up. This animal is 5 and 1/2 months old. So he's a pretty old hamster. Hamsters only live around a year. Sometimes a year and a half or two
years. It's sort of a middle-aged hamster.

Now, then we went to another procedure initially on young animals, but now we've done it on older animals, where we make a deep cut in the midbrain, cutting all the fibers going into the coddle tectum. So where else, we make the cut up there.

But now we're not going to use the peripheral nerve bridge at all. We're not going to mess his leg up by taking a large nerve out of the leg. Instead we're going to inject that site with a self-assembling peptide solution. It's an engineered material that's not available naturally. It looks like a little water in the vile. It's a very dilute solution. But it has a wonderful property. As soon as it contacts a salt solution, for example, the cerebrospinal fluid will work very well.

It forms, it assembles, it self-assembles into this microfiber mesh work. And we got interested in trying that.

It was developed at MIT by Shuguang Zhang in biological engineering, and fellow from this department he was working from, Todd Holmes. He was working with Dick Wurtman. And the initial discovery was if we look at that cut, if we don't put the material in there's a gap. Axons can't grow across a fluid gap. No axons can regrow in these brains. But when we did that injection, most of the gap closed up. And that was very consistent.

So here's the model now. I'm showing the eye connecting mostly to the opposite side of the midbrain, and then controlling by means of this cross tectospinal pathway that we've mentioned in the class, and caused the animal to turn right for something, something shown on the right eye. I should say in the right visual field.

So now here's the brain. In this case, it's a photograph, and this is the superior colliculus here, and this is where I'm making my cut. Sorry, I don't have the journal file. So there's the lesion again. So if we make that kind of a lesion, we discover the gap has axons that grow into it. There is regeneration.

In fact, I should go to the journal here so I can-- there's a picture of where the hemispheres would be if I left them in. I drew them in on the picture here. And this is
what I did here in this animal. I made this kind of cut. Cutting all the axons. The axons are coming in like that over the thalamus into the colliculus behind.

Now, if I do this biology on the brain and label all the retinal accents, so I get them to pluress. Here is the position where my cut was, but you can see, the axons have crossed and they've innovated the tectum behind them. So we're getting a lot of regeneration in these cases. Much more, in fact, than we had expected.

So let me now show you first of all, a blind animal. Look in here. If I can get the video to go on. He's blind on the right side. This just proves it. He's showing the stimulus on one side, no response at all. Unless you touch the whiskers, of course. But as soon as you move into the other visual field, he turns.

So now this animal has the same lesion as the other one. The only difference was the first one, the control, just had saline injected. This one had the self-assembling peptide injected. It doesn't turn quite as fast as normal, but it's pretty good. He's got vision and he can find his reward. He won't start for lack of vision.

So that'll give you just a little bit of flavor of some of the research in that area. It's still going on at the University of Hong Kong, and I'm often asked by students whether I'm doing it here because they'd like to work on that. But getting support proved to be much easier in Hong Kong, so we moved that project over there.

Let's talk about, just give an introduction to the motor system now. This chapter's been posted. I'm sorry I didn't post the list of readings and questions until today. I just forgot about it. But I did post the chapter. So if you haven't read it yet, please read it. And I'll get the next one up by tomorrow.

So now we've looked at all levels of the central nervous system in an introductory way. And now we’re going to begin looking at specific functional systems, and we’re going to start with motor systems. I'm not going to start with a motor cortex, which you often hear about in discussions in the department here, are motor systems. It came relatively late, invertebrate evolution. I want to consider first, the evolution of motor control, the major functional demands, which we've mentioned before. They
certainly preceded the vertebrates and the neocortex.

We'll look at what structures for motor control are present in all of the vertebrae. Then we'll look at what are the elaborations of that system into the mammals. And when we start talking about specific organization, that'll be tomorrow, we'll start with motor neurons and see how they're organized.

So these are the three major types of movements that we find represented in membrane, remember, we've talked about before. Locomotion for approach and avoidance movements. Escape from predators so you can stay alive. And of course, all the things we need to approach things about-- foraging for food, exploring, seeking a goal object of various sorts. It could be a nest, it could be a mate, and so forth. These are very basic for all the different motivational systems.

The next one is orienting of the head and body. You need to be able to do that to accomplish the goals of these drives that we mentioned already. And then finally, to finish the action, acquiring the object in some way, you need to grasp it in some way in most cases. You can grasp it with the mouth directly, or grasp it with the lips. We call these general purpose movements because they're needed for many different actions patterns.

So just to review, the control of these three types of movement from the midbrain, we know they're descending pathways from the midbrain locomotor motor area. And depending on how you simulate that region of the coddle midbrain, you can get what looks like escape behavior, or a simple locomotion that could be used for approach.

We find that tectospinal tract, which we just mentioned-- we saw it on my diagram in the previous slides there-- controlling orienting by turning movements. And then the rubrospinal tract controlling limb movements, reaching and grasping. And for oral grasping, you don't need the limb movements at all. There are connections from the tectum also into the hindbrain for that.

This is a picture we saw before. The origin of the tectospinal tract from neurons
here, like this one, crossing to the opposite side and descending into the medial midbrain and hindbrain. And then this neuron in the rubrospinal tract, important for controlling distal muscles of the limbs.

Remember the midbrain was the connecting link between membrane structures, or forebrain structures in general, not just the endbrain, but the tweenbrain, as well. The only way those forebrain mechanisms could control movement was through the membrane, when in the earlier period of forebrain evolution, which covers most of the period of evolution of animals. Midbrain also controls the visceral nervous system. And remember, in our discussions, we were using the blue color for these movements. We were using the red color for the visceral nervous system, and control of motivational states.

And in addition, the midbrain’s involved in various kinds of fixed action patterns, like predatory attack. We usually associate these things with the hypothalamus, but in fact, they can be elicited from the midbrain directly. The hypothalamus is simply a slightly higher controller of those same movement patterns.

So a little more about approach and avoidance first involving locomotion. It was a major importance in the evolution of head receptors. These functions played crucial roles in the evolution of olfaction and vision. And I’m just summarizing for you here the role of olfaction in enticing approach or avoidance. And I summarize here the output pathway from the olfactory system, how they got from the olfactory input to the output. Going through the striatum and hypothalamus to reach the midbrain.

Going through the medial pallium, which we now call hippocampal formation, again, to reach these midbrain mechanisms. Similarly, for vision, these are the two forebrain sensory systems. They didn’t exist without forebrain. You have various links to motor control. The importance of the striatum and medial pallium, for vision was that it allowed those links to be plastic. And there were also inputs that reached the midbrain from below, taste, somatosensory inputs and auditory inputs. And all of those, of course, also went up to the striatum as it was mentioned here.

I also want to mention something that’s often neglected in these kinds of
discussions that we don't initiate locomotion only by inputs from the outside, and initiate it from inside. For example, from hypothalamic neurons, these are initiation of activity by an intrinsic motivational state. In this state that builds up we call a drive. And Konrad Lorenz gave it a more specific, theoretical term, his actions specific potential.

And all of his discussions were based on the model of motivational control that was not a neural model, but that has been bodied now in more formal models involving computer simulation. Some of that has been done right here at the media lab in Bruce Blumberg's lab. And he was explicitly inspired by the kind of model that Konrad Lorenz developed.

Now, you've had some readings from Larry Swanson. This is a picture on page 111 of Swanson's book. Just talk about his motor system hierarchy. Here at the bottom of the hierarchy we have the pools of motor neurons mostly in the spinal cord, also in the hindbrain.

Above them we have the pattern generators. Now, those are in the spinal cord or in the hindbrain. That's where the activity originates that's driving the motor neurons. They are the interneurons in the cord, in the networks of neurons connecting to those interneurons as well that generate patterns. How do we know that?

Well we can eliminate all the higher inputs to the spinal cord, and we can still get, for example, a stepping pattern, alternate stepping, if we stimulate the feet in the right way. Can an animal walk if he doesn't have the brain connected to the spinal cord? What would he be missing, besides vision, and audition, and olfaction? He doesn't need those things to walk, right? But he would have some problems because he would lack his vestibular input. He'd have a little trouble standing up.

But actually, do you need vestibular input? Am I using only vestibular inputs to keep from falling over? Now, I'm rather top heavy here. Why don't I just fall over? If we eliminated my vestibular system, I would still get input from my feet. And so I would have some ability to balance just with spinal mechanisms. Although actually, I almost don't do too well with just spinal mechanisms.
Above that, above these pattern generators, and Swanson shows that to be a lot of neural apparatus. But in fact, it's all in spinal cord and hindbrain. Above that, you have the pattern, central pattern initiators. And above that, central pattern control. The initiators are cell groups like those in the midbrain that we call the midbrain locomotor area. When you stimulate them, it initiates locomotion.

And when certain lesions are made in the midbrain that cut off higher mechanisms from the midbrain locomotor area, you can get an animal that just persists and walk and walk and walking because there's nothing to shut it off anymore. And that's been seen with the neurological syndrome.

And then he calls even higher mechanisms, for example, those in the hypothalamus, central pattern controller. So let's look at that just for locomotion. And I've doctored his slide a bit here. This is the same model on the right side in black there, but now it's specifically applied to locomotion. And he shows his little schema there of various orders of neurons in the locomotor pattern generator just to indicate that that contains some hierarchy itself.

So here would be a neuron which when stimulated would trigger a whole pattern. What do you call it in a-- we're studying a little animal and we find a single neuron that we can stimulate and initiate a whole pattern of movement? What do you call it? What do you call that cell? You call it a command cell. A command neuron. Do we have command neurons in the mammal? Actually, probably not single cells, but there are groups of cells that when you stimulate will cause a pattern to be generated like that.

And then here above it, like the locomotor pattern initiator of the midbrain locomotor region. That would be this region. And then above that, locomotor pattern controllers. He puts here hypothalamic locomotor region, and I'm indicating that that's true for any of the drives that can initiate locomotion. They would be represented here in the hypothalamus. So there you have that hierarchy.

But now what controls that? Why does the animal starts to walk well. For example,
very early in the evolution of the forebrain, olfactory input reached the midbrain and the hypothalamus through this, what we now call the ventral striatum structures. And just behind the olfactory bulb in the basal forebrain. So I’m showing that here, and I made it darker because that was really an evolution.

They reached the hypothalamus. In fact, there were some olfactory fibers that went right to the hypothalamus in, say, the hagfish or the sea lamprey, which are pretty similar to those ancient animals. And then some of the fibers go from corpus striatum to the midbrain, the midbrain locomotor area.

But then other inputs came into the corpus striatum, remember, through these, what we call the old thalamus-- those are still there. Striatum is still getting such input from the visual system, the auditory system, and the somatosensory system. They’re coming in, reaching that same means of controlling locomotion.

Well we have pathways reaching neocortex that do the same thing. Again, getting into corpus striatum, controlling locomotion, it’s input that comes into the thalamus reaching the neocortex.

But here, something new has happened. Once you get neocortex, and the neocortex gets very large, and neocortex starts to bypass the striatum, it can now project directly into the midbrain, and to some degree, even directly to the lower motor mechanisms we’ll call them, the pattern generators. And in the animals with the largest neocortex, including us, all the way to the motor neuron.

So that gives you an introduction of what we’ll be talking about. We’ll say a little more at the beginning about these locomotor controllers and then we’ll go on with the introduction to the motor system.