GERALD SCHNEIDER: So we're going to continue some factors. We're going to continue the discussion of external growth today, talk a little more about what's guiding those axons when they're growing. We talked about the two modes of growth last time, so you know a little bit about how they develop.

There's many different aspects to the idea of axons competing with each other during development. One thing they compete for growth factor, for example, in some cases. If they don't get enough growth factor, they can undergo apoptosis, which is a kind of cell death. Sometimes it's called cell suicide, but it lacks something to get some signal. It will kill itself.

The first growth factor to be discovered was NGF, the Nerve Growth Factor. And that was found later to be a member of a family of important growth factors, the neurotrophins. And I've just listed some of them there.

These growth factors actually play a number of roles. They are not just survival factors, but they're trophic factors that promote growth. They're also tropic. They influence the direction of axon growth.

So for example, I think I have a-- let's talk about one experiment, the first one, really, that directly showed in tissue culture that NGF has ionotropic effect. If this is a Petri dish, and you have-- get a smaller pin-- and say here's a cell, and it's growing an axon. And here's the growth cell. It's growing there in the culture. There'd probably be a lot of cells, but we'll simplify it and just have one.

This was done at Harvard by Jonathan Hardin. He put a pipette in the tissue culture, a pipette that contained a solution of NGF. And let's do it a little more like he actually did it.

So let's say he put the pipette-- that's not drawing. Here we are-- like that. He put it near the growth cone. So what's it doing? It contains a solution of NGF, so it starts leaking the NGF. It comes out of that. And what happens there is that the growth [? column ?] [? turns, ?] starts growing towards it.

So then, he would move it, get rid of this one. He moves it over here. And what happened was this thing turns again and grows towards it. So he can basically direct that growth cone around the Petri dish by where the pipette is leaking the NGF.

The very simple experiment there were up until this point, all the evidence for chemoaffinity of any was pretty indirect. Then they began with NGF to find out that these were-- they directly influence direction of growth. Ramon y Cajal had postulated chemical effects on axon growth around 1900, and then later, Roger Sperry developed his theory of chemoaffinity that he thought could explain neuronal specificity and development in general, that is, axons grow to specific places in the nervous system because they're guided by chemical cues.

And he explained, with that idea, that axons will follow specific tracks. And then when they reach their terminal areas, they will form their specific connections, which could be a map. So we'll talk more about that today, also.

Let's talk about his experiments on axon growth, which he did using the phenomenon of regeneration in non-mammalian animals. Now over the years he did this, he used both frogs and goldfish. Most of the experiments are on frogs that we'll talk about.

We'll go through these three things here. I think my computer just locked up. Oh, there we are. So let's talk about that experiment.

Many of you know this experiment already. First of all, if you crush the frog optic nerve, it will regrow. How do you know it will regrow? Well, before it was known anatomically, they just did it behaviorally.

And all of Sperry's initial experiments were done with a behavioral assay. So he would crush the optic nerve initially, without any eye rotation, just crush it. And the animal was fine.

But then if he keeps testing him, eventually, his vision comes back, and not only comes back, but it is pretty accurate. That is, he would orient to the worms presented or flies presented in various parts of the visual field accurately again, indicating that the axons must have grown back to the correct position. So Sperry wondered, well, why can they grow back to the right positions?

So he tried an interesting experiment. He crushed the nerve and he rotated the eye 180 degrees. So if here's the frog and here's his eyes, how do you draw a frog? And here's what he's doing-- initially, he's presenting, say, puts a worm out here. A normal frog, if a worm starts to move, anyway, the frog will make a sudden turn and flick out his tongue, grab the worm. And he will do it accurately.

And of course, if the nerve for that eye, for this eye, has been crushed-- and they usually crush it just behind the eye-- if the nerve has been crushed, he'll be blind in that eye. And if he recovers, he'll start to orient again. But now he's rotated the 180 degrees. So what happens?

What happens is if the worm is here, the frog turns that way. He turns in the wrong direction. Or if you place it on the floor in front of him, he'll orient as if it's up there.

If you place it up here, he'll orient down there. He's 180 degrees disoriented. So what does that mean?

It means that the behavior, the reward the animal's receiving, isn't affecting. He keeps orienting wrongly. He simply has a mis-wired brain because you had rotated the eye, and the connections reform according to the retina, not according to the visual field.

So it's as if there's a program there set by properties of the retina that will determine where those axons grow and detect them. So he postulated there was a specific matching of chemical tags, tags on those retinal ganglion cells and their axons, with matching chemical tags in the tectum. That was a strong form of the chemoaffinity theory. Later it was argued that he required too many genes and so forth. There were a lot of arguments about that. The theory is basically, though, been borne out, and not exactly in the form of Sperry's initial work, but--

However, many other experiments were done on regeneration and growth phenomenon. And some of them were done on hamsters here at MIT in my lab. But many of them were done on frogs and on goldfish.

And the work on frog or goldfish and the work on hamsters indicated that you could get distortions of these maps. And that raised questions about exactly how the chemo affinity is working, if it's working at all. What do we mean by "map compression" or "map expansion?"

Let's say here's the tectum . We'll deal with the right tectum. And let's say you do this-- do you eliminate the whole caudal half of the tectum with a lesion on the right side.

So the question then is, what happens when the axons grow back and only half the tectum is there? Well, when that was done in the fog or in the goldfish, initially they found that if here's the retina, and this is nasal retina and temporal retina, superior and inferior retina. Actually, why don't we do it for visual field?

It's very confusing when you deal with both visual field and retina. I convert them quickly and automatically in my mind, but it's not easy when you begin to talk about it. So let's deal with it the way the frog experiments were done.

They did the visual field. They were mapping receptive fields electrophysiologically. That was the way they did it.

So if this is the visual field, nasal field and temporal field, superior, inferior field, and here's the tectum, normally, this would be the nasal field, temporal field. And this is superior would be-- oops. Superior field and inferior field would be lateral. That's the way you would expect it to be mapped on both sides.

And here, they're dealing with the field of the left eye. And initially, they found that if they had only half the tectum, they got basically recordings, spots placed, in this half of the field only, which could be found in the tectum. So they would record there, and then they could record the responses here.

Sorry, I don't know what I'm doing here. Hitting the right button, I think. So you see what they're doing-- they're recording systematically across the tectum from different positions, and then they map the receptive fields.

But then, give it a little time, and the thing reorganizes. And they start getting receptive fields now throughout the field. The temporal field, they're finding now, is represented here. So these spots here, these three spots in the temporal field, would be like that. Normally, they would be back here.

So we say the map has been compressed. The nasal field, here, they find represented up here. And similarly, superior is near the midline, and inferior is lateral.

So the whole map is compressed into the small tectum. You understand the experiment? So that's field compression.

So now, they tried another experiment. I'll draw here another field. And I'll just draw the retina here or the field corresponding to the retina. And they basically ablated half the retina.

So for example, they could do this. They could eliminate half the retina. They eliminated the whole temple retina, then the nasal field would disappear, the nasal retina, the temporal field would disappear. So that was another way to do these experiments-- they would ablate a good part of the retina in the fish, and then do the recording later.

What happens when part of the retina is projecting into a whole tectum? And the result was, again, that if they gave it enough time, the field expanded. So the half retina would expand its representation over the whole tectum.

Those things were done in the hamster, also. Yes, question first.

AUDIENCE: [INAUDIBLE]

GERALD SCHNEIDER: Good question. Is it always perfect? In other words, can you get mistakes? They didn't pay too much atten-- it wasn't perfect, but it was pretty good. And within the limits of their recording, and so forth, it wasn't clear just how degraded it was. It didn't appear to be very degraded. It did go through a transient period, though, where it was more disorganized. That's true.

Now, we did those experiments also in hamsters, but the hamsters don't regenerate. So what do you do? Well, if you make the lesions very early in life when the system is still developing, the retina-tectal connection is still developing at birth.

At birth in the hamster, the axons are still at the end of their elongation period. They're just starting to sprout these widespread arbors. So we could take the caudal tectum out of the hamster, and then we let them grow up.

And now, rather than record, we did anatomical experiments. Now, how do you study topographic organization with neuroanatomical methods? There were some advantages over physiology.

That was one of the disadvantages of the frog and goldfish experiments-- that they hadn't done the anatomy. So what they're getting through the recordings doesn't tell you, necessarily, what the axons are doing. Of course, you make the connection, assume that you know what they're doing.

So we did it in the hamster. And we discovered two things. First of all, if we took off the caudal tectum as they had done in the goldfish here, as you see there at the left, we eliminate the caudal half tectum, and now we made, for example, a little lesion in the temporal retina-- we were using degeneration methods-- trace the degenerating axons from a little piece of the nasal retina, temporal field, something that should go here to a-- something that, say, should project back here, what happened?

Well, that part of the tectum is missing. What do the axons do when they come in the tectum? What we found is they do generally go here, indicating that there was a compression of the field. But in addition, we found inaccuracies.

We found little extra projections, also, but the main projection went to the right place if the map had been compressed. And we did other parts of the retina in such animals. We also, in some cases, ablated the rostral tectum instead of the caudal tectum.

So for example, if here's the tectum now, and we ablated the whole rostral tectum, now the axons are coming in like this. Here's the optic tract. So now they have to cross the area of damage, and they did that.

If we were tracing a group of axons that were supposed to be going to the caudal end of the tectum-- let's say we were tracing axons that we're supposed to be going here, we did find that they would go there. But in addition, we found mistakes, occasionally found projections clear up in the front. And they seem to be related to a derailing of the axons when they were crossing the lesion area.

The organization of the axons, the order of axons as they are entering their terminal area, seemed to make a difference, also. That isn't something that was predicted by the chemoaffinity theory. But I can say, in general, that the-- insert it in front-- that the map was accurate.

There are other experiments there that we could go through. But it was later that they discovered that-- well, before I go through this data on the chemoaffinity, what other ideas were there around that could explain the map formation? Might it be possible even without chemical gradients to explain it?

Well, for one thing, the axons had an order when they came in to this. These axons here represented the superior field. They came from inferior retina. Let's just indicate the field. These represented inferior field.

So the axons were already orderly in one axis to the field when they were entering. And that's true for all the mammals. Anytime you see something in the upper visual field, it's following this edge of the optic tract.

If you see something in the center of your field, comes in the center. You see something in the lower part of your visual field, it comes in laterally into the tectum. Well, we did studies of that order. And we found out that they become orderly by the time they reached the geniculate bodies.

What about the nasal temporal axis? Well, studies of some species indicated quite a bit of order. But in the hamster, it turns out that the nasal temporal axis, axons from nasal and temporal were mixed in with each other.

So there had to be some factor sorting them out as they came into the tectum. Some people thought it might be time of arrival. And there is some difference in temporal and nasal in time, probably not accurate enough to explain some of these effects.

Then later, it was discovered that there are specific gradients of molecules in both the retina and the tectum. The initial gradients that were described turned out not to be the important ones. But then, it was discovered that these two groups of molecules, the afferents in the tectum and these tyrosine kinases in the retinal ganglion cells and their axons-- the Eph family-- were distributed in gradient fashion.

There's not a single molecule like that, there's several distributed across the nasal temporal axis of the retina. And there's a matching gradient in the rostral caudal axis of the tectum. And initially, they just discovered the rostral caudal axis and temporal field, not the upper, lower.

But they did then get evidence that they do play the kind of role postulated by Sperry, in that the first experiment showed that axons from the temporal retina don't like caudal tectum. They repelled from it. And that was done in tissue culture by taking membranes from the caudal tectum and from the rostral tectum, and making little channels on the Petri dish, little lines where you deposited the membranes, and find out, then, where the axons from temporal retina or nasal retina will grow.

And axons from the temporal retina simply don't like membranes of cells from the caudal tectum. They're repelled by it. And that's specifically due to these molecules.

Later, other molecules were found that could provide the guidance for the upper and lower field, as well, even though that axis is already represented in the tectum in the axons by the time the axons get there. But in general, the order of axons in the tract is not sufficient to explain the precision of the map. That seems to require the chemical guidance.

So when we talk about whether these molecules can explain all aspects of map formation, the answer is no. There are some aspects that they can't explain. They can't explain mistakes that are made when you create lesions.

They probably don't explain the initial formation of the rough map. But they are the major explanation for the topography. There are some later effects of activity that also play a role, that probably play a role independent of these molecules. But basically, the Sperry ideas were borne out.

Not only these mistakes in the retina tectal map were made, but they seem to be relatively minor as far as function goes. But we found with these early brain lesions we were making, there were other kinds of violations of neuron specificity rules. So first of all, we have to discriminate between regional and topographic specificity.

Topographic specificity is what we've been talking about. One part of the retina goes to one part of the tectum, another part of the retina goes to another part of the tectum. So what is regional specificity? That simply means that axons from the retina go to the tectum, but not to the inferior, go to the superior colliculus but not the inferior colliculus.

They go to the superficial layers of the superior colliculus, not to the deep layers of the superior colliculus. They're regionally specific. Axons from the retina don't project into the somatosensory system or the auditory system. They seem to know where to go. That's regional specificity.

Let's draw a little map of the midbrain here. I can keep my-- I'm obviously hitting something here. This is inferior colliculus seen from the side, superior colliculus, and here's the thalamus. Here's the lateral geniculate body where the retinal axons terminate.

So let's draw the course of an axon here coming into the superior colliculus. It's terminating in these two structures. And it's not terminating in the adjacent thalamus here. The medial geniculate body, LP, gets only a few.

Let's put in green here an axon coming from inferior colliculus that goes here. Now, what if we do this-- put in the red here. Let's take out superior colliculus, but we'll also damage that tract.

If we did that, we found out that-- I'll use the heavy pen here-- these axons not only went into the lateral geniculate body, but they also invaded the medial geniculate body. So that's a sprouting axon. The key thing was we had to remove the normal input to the medial geniculate body.

The optic tract goes right over the medial geniculate body at the caudal edge of the tract. And they were always axons from the caudal edge of the optic tract. So then we could have the retina projecting into the auditory system, but only when we made these lesions, and only when we made them early in life. We took away the normal terminal area. And when you do that, axons tend to sprout other places, but they tend to sprout in the places that have lost their normal termination.

So it looks like chemical specificity, then, might represent kind of relative preferences for the axons. There may be some places they cannot terminate at all, but there are some places they can terminate if you give them the space and if you don't let them terminate in the normal places. And that phenomenon, the retina projecting into the auditory system, has been studied a lot in the ferret by Mriganka Sur and his students in this building. And we will probably mention those experiments later in the class.

There have been a number of experiments on how regionally specific axons grow during their elongation period. And I want to go through just a few of those experiments. First of all, growth of axons in the grasshopper leg, which gave evidence for specific guidance by glial cells. We call those glial cells "guidepost cells."

And then we'll talk-- I may not finish today-- spinal cord studies that gave some new evidence for chemo specificity, not in the topography formation, not in termination patterns, but in the course of the axons in their growth, course of axon growth. And then we have additional data on specific glial cell barriers to elongation. First of all, the grasshopper leg.

This is a reconstruction of the leg in the embryonic grasshopper, so it's sort of stubby-- here, you see the ganglion that they're calling CNS or central nervous system. And this shows the leg as if it were transparent. And the cell bodies of these neurons are in the epithelium, so they're a little different from the dorsal root ganglion cells in mammals.

But they send an axon, then, that grows according to a specific pattern towards the nervous system of this animal. And they can reconstruct the pathway. And they find it's quite consistent in its pattern. You see the reconstruction here.

And they notice that at the points where it made these turns, there were specific cells that the axons could be responding to. And to test whether that were true-- whether those cells were acting as guideposts-- they tried, for example, ablating this one particular cell. And when they ablated a particular cell along the path, they could alter the pathway, as you see in these experiments.

So they were calling those cells "guideposts." How were they working? Well, when the axon starts to grow here, it sends out long filopodia.

The filopodia are so long that they could reach all the way to a guidepost cell. That seemed to provide some point of adherence, and the whole growth cone would move towards that cell. But once getting there, they didn't just stay there. They sent out the long filopodia again and reached the next one, and then they would grow to that one, and so forth, from cell to cell until they reach the center of the system.

So it's like a connect the dots game, going from one cell to the next. And that was the theory of how these axons grow in the grasshopper leg. Where are we here? And the later growing axons would follow those initial ones that we call the "pioneering axons" if they go first.

They then asked, well, could they ablate the pioneer cell? Would the later ones do the same thing? And the answer is yes, but normally, the later-growing axons fasciculate along the first one. They seem to follow it, but any of them are capable of following that same course.

But when we looked at the optic tract in mammals, we found that the later-growing axons actually don't grow on the surface as the earlier ones. In fact, they space themselves out in between the pioneers. So they don't seem to be following the same kind of rules that you get in the grasshopper leg. So that's just a cautionary note, there-- that what's discovered in one system may not be what you discover in every system. I can see the time. I think I'll start with this next time.