GERALD SCHNEIDER: OK, what were we talking about last time? Neuronal migration.

And we talked about the shepherd's crook cell. Let's just-- I won't load that slide. Why was it so important to find that cell that could be identified at such early stages? You see, some people didn't believe that you could get nuclear translocation in the midbrain when the thickness of the neural tube was fairly grayed. They thought you would need radial glial guidance.

So what other explanation could there be for those pictures? Well, with the Golgi method, it's possible that they could be staining glia and then seeing bumps on the glia, misinterpreting the pictures. They had to be able to reconstruct the entire sequence. And remember what they did, they took-- if this was the surface of the tectum, this is the ventricular layer.

And here's one of these elongated cells. Here's a cell body. Then very early on they saw that-- sorry, I should start here, and where they started. Let's do that. The adult shepherd's crook cell is a neuron here with an axon that goes out like that. It always has that characteristic.

It's not clear why it goes up a little bit before it goes out. But it always has the shepherd's crook. I'm not drawing the dendrites and everything. Because what's critical about identifying that cell was the shape of the axon. So that was the adult, OK.

And then they went back successively to earlier stages [INAUDIBLE]. And they saw that a cell with the shepherd's crook. But then they saw the connection there. It might have disconnected from the ventricle already at that stage.

And then they went back a little bit earlier and they still of the shepherd's crook. And then the cell body was down here and it was connected here. And then even earlier, it looks like the first one I drew there. So they went backwards in time. They couldn't have been always mistaking this for a glial cell. So that was the idea.

In other words, the mechanism of migration in the chick midbrain for that particular cell was nuclear translocation. OK, so that's where we left off. Now let's talk about cell migration in the neocortex in the cerebellum where nuclear translocation is not the mechanism. There are many similarities though in what the cells are doing.

But it's pretty clear now that in the neocortex when cells migrate, they move along radial glial cells. And many of them move along the same radial glia. They go up to a structure called the cortical plate. We'll look at pictures of that. And finally you get the six layers of the neocortex.

And they show an inside out pattern of movement as seen with these labeling methods. They were actually known before it was really verified. I put verified rather than demonstrated initially, because these patterns were known before the autoradiography methods.

But they were more tedious methods. They used, for example, vitamin A poisoning at different stages. And vitamin A in high doses will kill the immature cells. So they could wipe out whole layers of neocortex when they were developing. You can do the same thing with heat application. It's not intense enough to kill every cell. But it'll kill the developing ones.

But it was these labeling methods that were the clearest. So this is a picture from the [INAUDIBLE] graduate textbook. It's just depicting an early stage of cortical development in the middle of the cell migration period. The hamster is born when this is still happening. In fact, this is about the stage they're born at.

OK, then they're showing a column there through the neural tube in the left hemisphere of the brain. They're showing that neurons can be seen moving along those blue cells, which are the glial cells, OK. So here you see this cell.

And here you see the blowup. And then you see the blue cell there which is attached here at the pia. And it's-- I'm sorry-- here at the ventricle and here at the pia. The cell body for that radial glial cell is down here in the ventricular layer.

OK, now that mitoses are happening here at the ventricle. But not only right in the ventricle, but also in a layer just deep to the ventricle where cells have, even when they've detached from the ventricle, you get another layer of mitotic cells there. That's called a subventricular layer.

The artist just couldn't-- he just assumed, well, this is above this. This must be the subventricular layer. He wasn't-- because he didn't know, and somebody didn't check it. So I'm pointing out, a lot of textbooks make mistakes in anatomy.

OK, this is from a study at Columbia University where they were first able to see migration in tissue culture with movement in the cell lung and glial cell. And here you have, see that it's moving along. In the 31 minutes they were looking at it here, it moved that far.

OK, so this now is showing different stages of development in the mouse or rat. I think this is probably rat, although they're very similar. So VZ here means ventricular zone. So the neural tube is very thin here. It's basically a ventricular zone in a little bit of marginal zone there. And you see they're depicting mitosis happening there at the ventricle.

Then a little later you see some fibers appearing here in an intermediate zone and some cells appearing up here. There's just a few. They call that the pre-plate period. There's a few cells right at the top. But still, the main thing going on is a lot of mitoses here. The cell division is symmetrical. So one stem cells becoming two stem cells, becoming four stem cells, and so forth.

And the longer that happens, the bigger the area of cortex. So in species like us, when we have such a large neocortex, that stage is going to go along a lot longer and the cortex will become-- increase in area. OK, now when you get asymmetric division and one cell becomes post-mitotic, it attaches to an elongated cell that has now been, it's been shown pretty convincingly is not a neuron but a glial cell, just like they showed in the previous picture.

Here you see they're depicting a glial cell with a cell migrating along it here and another one up here. There's not one radial glial cell for every neuron. There's a lot of neurons are using the same cells. But basically all the cells that will end up in one column through the cortex are moving along one of the radial glial cells.

The intermediate zone is acquiring more axons here. And that will become the white matter of the cortex of the ventricular layer. The cells migrate up to this bunch of cells here. And that's called the cortical plate. That's the developing layers of the neocortex.

That's a little later going to end up like this where you just have a thin ventricular layer-- they're not even showing the cells here-- then the white matter, then the layers of cells in the cortex. They're showing largest pyramidal cell layer 6. It would actually be in layer 5, smaller cells here in layer 4, more pyramidal cells and granule cells in layers 2 and 3, and very few cells up in layer 1, but lots of dendrites and axonal connections. But they occur throughout all the layers.

OK, now the other interesting thing about this is that when these cells move up, they don't end up-- they're not dropped off down here. They move past all the other cells in the cortical plate and they end up at the top. So the latest born cells are more superficial. And that's why we call it an inside out pattern of migration.

The cells on the inside here of the cortical plate were the first ones born, the first ones to reach the cortical plate. And the later ones pile up on top. That's what we mean by the inside out pattern. So that means in the cortex here, layer 6 cells where the first born.

And we could see that in audioradiographic pictures by labeling, injecting tritiated thymidine to label mitotic cells [INAUDIBLE] inject the pregnant female or inject her fetus with the tritiated thymidine at different stages. And if you do it very, very early and then just wait until the pups are born and they grow up, you will see the radioactive cells here in layer 6.

And if you do it very late, in the hamster would have to be postnatal, you would see them up here in layers 2 and 3. Excuse me? MZ means marginal zone, the most superficial layer of the neural tube.

OK, so now let's look at a slightly different thing that happens in the cerebellar cortex. We'll look at the proliferative cells and the post-mitotic granule cells. Now, this is-- and they have taken these-- we mentioned this briefly from-- OK, this is the new slide. Then I'll repeat pictures from an earlier lecture.

The cells move from this specialized region of the inner plate. I call it the [INAUDIBLE]. It's a thick area, looks like a lip when you look at it in dissection. And they migrate into the roof plate. And they form a proliferative layer there. So that's different from the [INAUDIBLE]. It's a little bit like the cell ventricular, where cells move away from the ventricle, but they remain proliferative.

But they're there right next to the ventricular layer. Here they're moving further away. But they keep undergoing mitosis. And they really proliferate. I mean, those granule cells end up-- enormous numbers of them, they're probably as many granule cells in the cerebellum as there are in the rest of the brain.

OK, so this is the earlier picture. If you look here, this is the earlier picture, the neural tube that developed early in development where you see the inner plate and basal plate here, and the stretched out roof plate of a-- typical of hindbrain. The layer of proliferative cells that we call [INAUDIBLE] is there. And cells are migrating into the roof plate, and other cells are migrating down.

The ones that migrate down, remember, become cells of the pons. They also become the inferior [INAUDIBLE] structure. There's several structures that they become. We're concerned right now with these that move into the roof plate, OK? This is the location that we're talking about, the rostral end of the hindbrian.

And when animals like hamsters are born, the whole cerebellum is very small. I should show you some more pictures of that, because most of the development is postnatal. Now, this is a picture from Cajal. And here's one of the things he saw.

If he looked at the cerebellar cortex real early on, it was not only much thinner, but he saw a dense layer of cells at the surface. And he knew from studies of the cerebellum that those weren't there in the adult. This was something peculiar to the developing animal right around birth and after birth in the mouse.

And he saw in using Golgi methods and other methods-- what happened here? OK. He saw these cells with various shapes. Some of them were just sit around cells, some of them had processes. The deeper ones tended to have two processes like that.

And then he also saw some with a third process going down. And then especially later in development he would notice that the cell body appeared to be moving down, the process that was going ventrally. So the P is up here. This is the layer of proliferative cells. He's just showing you a few of them. It's packed very, very densely with cells.

These big cells down here are very large cells of the proliferative layer of the cerebellum, the Purkinje cells. Those did not develop that way. Those came from the ventricular layer down here. And they moved up, much like we saw in the spinal cord and neocortex. They're large cells.

So those cells are all there already. They developed early, just typical of larger cells. Many small cells develop late. And that's what's happening here. So here's the picture he put together. These are the cells that move from around the clip. They grew processes when they became post-mitotic. And many of the cells that are undergoing mitosis, [INAUDIBLE].

But when they became post-mitotic and begin to differentiate, they grew lateral processes. Now, this would be the way they would look in a frontal section. Because these cells look different depending on the plane you look at them in. The lateral processes here become-- both of them are axons. The cell has axons going in two directions. And we call them parallel fibers, because there's enormous numbers of fibers all parallel with each other, all doing the same thing.

Now, what about this? There's a process going down. Well, that's an axon too. But the cell doesn't stay there. It doesn't stay there with three axons coming out of it. Instead the cell body moves down the axon. This process is always a little further down.

So here you see a cell let's move down further. Here it's moved clear down to the level of the Purkinje cell. And then as it moves past the Purkinje cells here, it begins to differentiate. And finally you end up with cells like these.

The one furthest to the left would be the more mature granule cell of the cerebellum, always located below the Purkinje cell layer. And it's now growing these specialized little dendrites that will receive input fibers just like the spinocerebellar tracts or the axons from coming from the pons. So there are the major input layer of the cerebellum, OK.

The axon then of the mature cell goes up to the superficial layer above the Purkinje cell and forms parallel fibers, each cell forming one parallel fiber. So this just summarizes what we were talking about. The cells migrate away from the pial surface. They leave their axons behind. It's like a spider moving down its web.

They move past the big Purkinje cells to the adult location. That's where they grow their dendrites. And the cerebellum is an amazing structure. Because it's a very large cortical sheath. And yet no matter where you look at it, you'll see the same structures. No matter where you look in the cerebellum, this same kind of thing going on. It's more uniform in structure than the neocortex.

So there at the end we were starting out to talk about migration. We saw the migration, how that happens. Then we talk about differentiation when the cells are growing their dendrites and axons. We know that the axon often develops first. We saw that for the shepherd's crook cell on the tectum. The axon grew out even before the cell body reached its final position.

We saw the same thing for the granule cells. Even though the movement was very different, the axons grew earlier, long before the cell was in its final position. Now when these processors are growing, they have an active tip that's broader than the trunk of the axon. We call it the growth cone. I'm going to show you some pictures of those, including video clips.

And where they go depends on a number of factors. One of them is we call selective adhesion. The active growth cone, which is you can imagine like my hand here. These little processes extend out. If you imagine now that the tips, the tips of the fingers here are more adhesive to something on the surfaces they're growing on.

And they can't grow except on surfaces. They don't grow through fluid space. These grab on to the more adhesive substrates. And then they get pulled along. That's how the axon grows. So this is one of Cajal's pictures of stages of nerve fiber development.

So you see the cell layer beginning to grow its axon first. So you can see it only has an axon. There you see his picture of the growth cone. And Cajal described this just from Golgi methods without seeing them in tissue culture.

And then when it reaches its adult location, it begins to mature, forms its terminal arborization, can also grow collaterals. Those can happen at various stages. And then the cell body will be changing too, growing its dendrites. They usually grow somewhat later than the axon. So that's Cajal's picture.

And here's a particular kind of-- it's a freeze fracture picture of a growth cone showing you that there's a lot of things going on inside. In fact, there's many actin filaments there which are contractile proteins. There's many organelles, including mitochondria and vesicles. And these little extensions are what we call filipodia, OK. Filipodium would be the singular.

I haven't finished all the flash cube terms for the development lectures, but I'm working on them. So I will post that. But the thing to remember about these is that they're transient structures. These little structures extend, and then they pull back, and they extend and pull back. So it's a very active structure. And you'll see that in the pictures. They're volatile structures.

And remember that at their tips, they're more adhesive. And when they contract then if the tip is adhering to a substrate, the growth cone will tend to be pulled in that direction. You say, but if there's a lot of them pulling in every direction, how can they move at all?

Well, it seems-- we think it moves as the result the vector sum of all the tensions on it. And we know some of the molecules involved, cell adhesion molecules. One of them we call NCAM neuronal cell adhesion molecule. But there's a number of adhesive molecules like that.

OK, now one way that this is studied, it's very difficult to see these things if we want to look at them in the live action in the real brain. That has been done, but with great difficulty. Only recently if we have methods we can do that. A lot of the work has been in tissue culture.

And this is just a picture of cultures from-- this could be either a dorsal root ganglion or a synthetic ganglion. It's probably a dorsal root ganglion placed in culture. And in this particular picture, they're contrasting a culture where there was no particular growth factor, and another one where they added this growth factor, neuronal growth factor, the first growth factor to be discovered, NGF we call it, the first member of the family of growth factors we call the neurotrophins.

And so here with the right medium and with growth factor, you can see a proliferation of axons coming out of that clump of cells. And we can study the growth cones and other properties of growth in tissue culture in a flat plate. And there have been many studies like that. And I'll show you now some video clips.

OK, this is just-- the first one just shows the large growth cone. We might even have to turn out all the lights here, because it's the little dim there. It's that shadowy thing in the back there. Here's the trunk, and the axon has got some little processes coming out. And it's moving across the screen. Can you see it there?

This is the end of it. And you see there's a filipodium, there's a whole bunch of filipodium. So let's watch it again. It starts here. And just look at what's happening there. You'll see them extend, and then they disappear, and they extend again. And the axon is gradually elongated.

So what's happening there is it's-- we're seeing the action at the growth cone. There's a lot of things going on in the cell, too, of course. And one thing is it's adding membrane. Where's the membrane come from? Well, it comes from the cell body. And it's being transported down this axon in little vesicles. The vesicles contain things too. But the membrane of the vesicle becomes the membrane of the axon, all right.

OK, let's just look at the next one. Like I said, I don't have to go back through all this. OK, this time you're going to see two axons that don't belong together because they don't occur together in the real brain. One's from the retina, one's from the dorsal root ganglion. See we're magnifying in 2,400. But that, of course, it probably much more than that because of the size of this screen, then the 60X acceleration, OK.

OK, one here, one here, watch what happens. This one is retracting. You'll see it's still hanging on there a little bit. But it is going to withdraw those in the cell. This is called growth cone collapse. Perhaps calling it retraction of the growth cone is a little more descriptive. They're both nice descriptive terms.

But that's very common. When two axons are growing in culture and they meet each other, the growth cone of one touches the growth cone or the axon of another. They either can cross over or one of them will retract. The growth cone can retract. It's when it's filipodia touch the other.

And we find that depends on which cells they are. Now, if you have retinal axons meeting a dorsal root axon, you usually get retraction when they touch each other. If it's two dorsal root axons that touch each other, they still often retract. But they retract more than 50% of the time. But they often also cross over each other.

Now, when they retract, what happens? They pull back. And then after a very brief pause, they'll start growing again. And usually when they start growing again, they change direction slightly. That's the most common thing to happen.

So now we'll look at three of them growing. This one here, there's one there, and there's one there. Can you see them? This one is going to branch. It's seeing a lot of adhesion here and here, less here, and it ends up branching.

These two are touching each other. And one of them is retracting. This one's just pausing. And then it keeps going. And this one is continuing to retract. You see the body, the thickest part of the growth cone, is pulling way back. It's lost all its processes here. And this, the distance of retraction can vary quite a bit.

STUDENT: [INAUDIBLE]

GERALD SCHNEIDER: It's properties of the membrane. But we also now know that there are secreted substances around, that these things can be secreting molecules. One of the molecules that causes that, they call collapsin. Because it literally affects the membrane of the other cell and can induce the retraction.

Now, in the literature it's commonly believed that there's no way to predict how far they're going to go. But in fact, I've studied that literature. And I found out that it's actually somewhat systematic, that depending on the axon properties, you'll get various-- not only different frequencies of retraction, that is the probability it'll retract varies-- but also the distance it'll retract varies.

And I found out that that-- and I did this by computer simulation. I didn't bring any of my simulation pictures. But I showed that the distance that retracts makes an enormous difference in the pattern of axons you get in the culture. The short distances will give you axons that are growing like they're glued to each other, fasciculated. And the large distances, you get non-fasciculation. I'll show you pictures of what those things look like, yes.

STUDENT: [INAUDIBLE]

GERALD SCHNEIDER: This is just artifact debris.

STUDENT: [INAUDIBLE]

GERALD SCHNEIDER: That they're moving on a substrate, OK. They're moving on a plate that's coated with polylysine or something. And the substrate you grow them on will affect how they grow. That's important. OK, this is, I think this is the last one. It shows a growth cone coming from the left. And it's contacting a fibroblast. That's a peripheral cell.

Now, it's a sympathetic neuron, a sympathetic ganglion neuron. And this could be happening in the natural situation. These are peripheral cells contacting each other. So here's the axon. There's the growth cone. Here's the fibroblast. And it's met a barrier.

And what it does is it keeps-- stays very active. And, see, it's moving along the surface. It feels itself along the surface. And, in fact, it's turned here now and it's growing along the surface this way. Yes.

STUDENT: [INAUDIBLE]

GERALD SCHNEIDER: From the cell body. That's a very important question. These growing cells are metabolically very active, OK. And it is shipping by anterograde transport mechanisms the vesicles down the axon, OK. Does it happen in [INAUDIBLE] is the question. And the answer is yes.

We will talk a little bit about regeneration soon. In the peripheral nervous system, especially when you have an injury, you will get a lot of growth like this. Axons will attempt to regrow. In many cases, they will reform something approximating their original connections.

Let's see how we're doing on time here. OK, so now I'm going to describe two different kinds of growth [INAUDIBLE] that was discovered in the optic tract. But now we know it happens also in the fibers growing into neocortex, a number of other systems where you get similar things.

Growth that looks different grows at a different rate, has different properties. So the properties of these growth cones must be different in two different stages of the same cell growing it's axon. And then we a little bit about what they're doing. They compete for space to terminate. We know they're influenced by growth factors. And these growth factors can play more than one role.

I'll describe a little bit of that in this class. When we talk about it, we spend sessions on that in the second term. And, of course, if they're coming from the retina, they have other problems to solve. They need to terminate in a very specific way. In fact if the're coming from the retina, they terminate in a topographic way.

The same is true for those axons, say, the spinothalamic, or the medial lem-- spinothalamic or medial lemnicus, they have to terminate in the right place, because there's a topographic map, a map of the body surface for the retina.

And when they're elongating, we know they're very specific. And now we know there's different kinds of guidance. There's chemical guidance of more than one kind. There can be diffusing chemicals that can attract them or repel them.

And you'd have to write this down. We'll go over it later. They can be attracted or repelled by a diffusing molecule. Or they can also-- they just by this, there can be molecules on the surfaces they're contacting. And those molecules can attract them or repel them also.

We know the glial cells play specific roles in axon growth. We saw the importance of the glial cell in neuronal migration. Well, they also play very important roles in the growth of axons. The main environment that the axons are growing through is a forest of glial cells.

So when the axons grow into the optic tectum coming from the eye, for example, what do they meet? They meet processes of neurons too. A lot of those processes are growing through, it's a forest of radial cells, some of which will become glia, others will become neurons.

Then I'll stay a little bit more about competitive interactions among axons and the various factors. I've mentioned a few of them already, influencing, growing, or regenerating, or just axons growing through collateral sprouting. OK, so here's a picture from our research, about two months of growth in the optic tract.

First, we talk about the period of elongation. And the cells have this kind of appearance. Then they have little enlargements along their way, little tiny processes, and then an active growth cone. They don't branch very much. They might fork. But generally they won't form much branching at all.

And then in a hamster, they're born at this stage in the optic tract. And then the axons start sprouting collaterals. And they don't just sprout collaterals where they're going to end up with an arbor. They sprout them all over the place. Even axons growing through half the tectum, it'll sprout little collaterals all along, OK you see there.

But then the axon will start to focalize. It'll pull in a lot of those collaterals. And one of them will grow. It might not be the one at the end, like this one I'm showing growing along the way. And then finally we'll withdraw the other collaterals. it might form more than one.

As I show here, it's forming collaterals in another place also, as many axons have more than one place where they terminate. And then the differentiation continues. And axons might end up, for example, terminating in one layer and not others. So it simply withdraws its branches in some places and continues growing them in others.

When it withdraws, sometimes we call it a pruning, a self pruning it's going through. We can also prune them by making lesions, damaging them. And then the hamster, this is all happening when the eyes are still closed. After the eyes open, there's a maturation of arbors that continues.

And we know from various studies that that maturation can be affected by visual input. We don't know much about it in the tectum. But it's well known in other parts of the visual system. So finally you end up with the mature arbors. So these are the two modes, elongation and arborization.

We know that there are different proteins found in the cells during these two modes of growth. I did some initial studies of this with Ken Moya, who's been working in France since then. But he's an American citizen. He came to MIT to do his graduate work. He worked with me and [INAUDIBLE] at Harvard Medical School.

And he looked at different stages of the development of these axons and found out that the cells were making protein and transporting different proteins at different stages. So then elongation, for example, it was transporting a lot of NCAM, neuronal cell adhesion molecule.

There are other proteins related to synapse formation that begin to appear during arborizaiton. We know also receptors can change and respond to various growth factors. We weren't able to specify that. But more of that has been found since that time. We know that a particular molecule, BCL2, is very important early on, prevents the cell from dying. It's [INAUDIBLE] apoptotic factor.

And now we also know it plays a role in axon outgrowth. And there's many other proteins too. So with any elongation, what we study was the anatomy. We saw that they grew rapidly, 80 to 100 microns in an hour. That might not seem so rapid to you. It's about 2 millimeters a day. But for a little cell, that's pretty rapid.

In case you-- that's a micron. It's a Greek letter mu. But because I didn't bother to put the Greek letter, I'm not-- so I just use u. So 80 to 100 microns an hour, about 2 millimeters a day. And at that stage of growth, when more and more axons appear in the tract, they grow up against each other. They form a bundle or ribbon.

We call that fasciculation. If they're fasciculated, they form fascicles, they group together. And they're largely unbranched. And they have this-- during elongation, they have these swellings and filipodia that occur. It's almost like the whole axon is a growth cone. Because they have-- although there's the more active growth cone at the end, they have many little filipodia along the way.

OK, so that we're talking only about that top, this area. That's the elongation. And then when they aroborize, they slow down. And my calculations for the hamster optic tract, and the [INAUDIBLE] body when they arborize, where they grow at about a tenth the rate, now that's the average rate for a group of exons.

So [INAUDIBLE] the actual rate of an individual exon is not what I was able to measure. We also know that when they grow like that, they don't bundle up. They grow separate. They stay away from each other. They space themselves out so they're non-fasciculated with their branching. They're undergoing a lot of branches.

So whereas one-- in elongation, they're supporting one growth cone. When they're arborizing, the same axon can be supporting many growth cones. Maybe that's why they slow down. And then we know that it's widespread, a lot of branching. But they're branches are rather rudimentary. And then later they vocalize. They grow mainly in one place, and they lose the others, OK.

And in that period of early branch formation, you can get branches appearing in places where the axon will not terminate later. And sometimes they even seem to be growing arbors there, but then they're withdrawn. For example, the optic tract will grow into the ventral posterior nucleus. That's a somatosensory structure, but they don't terminate there normally. They grow in, and then they pull out.

Lateral posterior nucleus gets a lot of axons in development, many fewer in the adult. It does get a few. We used to think they didn't get any. But with the sensitive methods, we do see a few there, but many more in development. And then it was discovered by Inocenti, an Italian neuroscientist working in Switzerland, that this happens in transcortical connections, in the cat, and probably all mammals, where you have whole transient connections forming, and then they disappear.

Sometimes they actually form synaptic connections in structures that they then withdraw from. And other times, they're just these little transient collaterals, as I showed you for the optic tract. And when that vocalization is occurring, that's when the topographic map becomes more precise. There is a crude topography even at the beginning when they first start to arborize. But then it becomes more precise as they focalize. We'll start there next time.