GERALD SCHNEIDER: We have a question from a student at the beginning here asking me to clarify what I meant by fixed action pattern. Do you remember what a fixed action pattern was? We were talking about behavior.

We said it was an instinctive movement. Basically, it's an ecological term of-- from zoology. The ecologists in the field of zoology originated this study of inherited behavior patterns, OK?

Humans have many of them, and all animals. Their behavior patterns there are pretty similar from one member of the species to the other because the basic pattern is inherited. Brain structures develop under genetic control and form the circuits necessary for these patterns.

It would include, in humans, things like crying and smiling and frowning, and looking down when you're a little shy, and things like that. There have been cross-cultural studies of human. There's a very nice book on human ecology by [INAUDIBLE], if any of you are interested. And there's also a very well-known book like Darwin, Charles Darwin. And he pictures a number of these behavior patterns like in dogs.

All right. We have a little more to say about these primitive cellular mechanisms. I've been talking about secretion. This is from the last class.

Now, you should know from the quiz, if you didn't remember, since I stated it in the question. How are these things discovered? They were all discovered by electron microscopy, and I drew pictures of them. We've talked about all seven of these.

OK. What is exocytosis? It's just a term for secretion by a cell when a cell puts something out. What is endocytosis? A cell drinking-- cell drank something in.

Both of these things happen at one place more than any other, in neurons, and that's at the terminal, the axon terminal, OK? Exocytosis, that's how it secretes neurotransmitters and the vesicles join to the membrane, and so forth. How do we know endocytosis takes place? Yes?

AUDIENCE: There are other [INAUDIBLE].

GERALD SCHNEIDER: OK. The reuptake mechanisms involving our receptors can take up neurotransmitter. How could we test it experimentally? It doesn't only take up neurotransmitter, we can inject some substance, some protein, some enzyme.

It might be fluorescent-- might be a fluorescent dye. We can inject it and some tracer substance that's taken up by the cell. And then if it gets taken up by the cell and moves within the cell, we'll be able to see it.

So we do that with things like horseradish peroxidase. I'll show you pictures of that later today. It's taken up by the cell terminals.

It's also taken up by the cell body. But if you put it in a region of termination-- say we put it in the optic tectum where the retina terminates, we're in the lateral [INAUDIBLE] body, any place the retina terminates. If we put a substance, a tracer substance, it'll be taken up by the terminals, and then it gets transported. How does it do that? Well, there are active transport mechanisms, remember?

It's moved back to-- all the way to the cell body, and so we can look later if we give it a little time to happen, it'll show up in the cell [? blot. ?] OK. Remember what the molecules are involved in retrograde and anterograde transport? There's two of them I mentioned-- kinesin and dynein.

Dynein is the one involved in retrograde transport, the process I just described. OK. These processes can move not just molecules, but they can move organelles. So organelles get transported from the cell body down to the terminal, little vesicles containing various things.

OK. And, usually, when we get retrograde transport, also, the substance is encapsulated in a little vesicle, in an organelle. And that's what actually moves back to the cell body. OK. So I'm going to now talk about how these cellular dynamics are used in experimental studies.

The CNS, mostly anatomical studies where this process of sectioning the brain is used so that we can look at it with a microscope. Now, in order to section the brain, you've got to fix the brain, OK? In order to see anything in it, you've got to put-- do something to the tissue, because the brain by itself, you're not going to see too much.

You can see things if the brain has been fixed, because myelin, for example, will bend the light differently from the non-myelinated areas. And so you can make out myelinated fiber bundles. But you won't see the cells well unless you stain them.

So we have various ways of staining them. Or we can inject things in them. We'll talk mainly about things we inject in them today so that we can trace accents.

But, first of all, we have to fix the brain, which means we usually do that in the process of killing an experimental animal, replace his blood with a fixative. OK. It's an embalming procedure. What the fixatives first used were things like alcohol, OK, and certain acids.

But the fixatives that have made the biggest difference for non-anatomical work are the aldehydes, formaldehyde, initially, and then for electron microscopy and other things, as well, glutaraldehyde. So after the brain is fixed, it will harden to some degree, depending on the fixative. And then it can be sectioned.

In order to section it, we can freeze it and cut frozen sections. That doesn't allow us to cut very thin sections. We can cut down to maybe 15 or 20 micron thick sections if we freeze the brain.

There's a couple of different ways of doing that. You can freeze it with powder dry ice on a sliding microtome, or we can freeze it in a cryostat with a whole blade, and brain, and everything is kept at a low temperature. But there are other ways still without causing any freezing.

We can embed the brain in something. We can embed it in wax, for example, paraffin-embedding. And there's other things like celloidin that we can embed in our natural cellulose.

But let's say now that we've injected something-- my computer is-- it must be asking me something, if that's mine. I don't know. Maybe it's one of the others nearby computers here.

OK. Now, we mentioned the Golgi stain before. This is a picture of a section that's a little thinner than is usually done for Golgi stain. The process was that a block of tissue was prepared. The brain is blocked into very thick sections and stained with the silver procedure that will mark certain cells. And that's how these black cells were produced with the Golgi method.

This particular Golgi method that was modified by Cox-- so the Golgi-Cox method-- in order to get a lot of cells and their dendrites. And then after that was done, then the brain was cut into thinner sections, and it was counterstained for cells that didn't work too well. But you can sort of see some cells there in the background.

It just points out that you're staining only a small percentage of cells. And you can see the cell bodies in the dendrites, and you can see the pyramidal cells with the apical dendrite. You can see a little nonpyramidal-shaped cell there, a granule cell.

You can make out dendrites. In this particular stain, we can sometimes see the axon, like there, we can see the axon beginning. Other times, it's a little more difficult. There's an axon beginning.

OK. And Cajal was the anatomist that used this the most, so it's been used by a number of other people. He was particularly prolific, perhaps, because of an ability he had that he didn't actually have to trace the axons the way most people will do now, and it's rather difficult and tedious to trace in detail these accents. Well, if he didn't trace them, how did he do it? Does anybody know?

He had a photographic memory-- very unusual. He would study the brain for a long period of time. He would see huge numbers of cells, but studying them he would pay attention to what he most wanted to learn that day, and then he'd go for a walk. And then he'd come back and realize what he really wanted to draw and he would draw them with remarkable accuracy.

When people redo the things he studied, they'd pretty much find the same things. And that's what he's doing here. He's not looking through the microscope, he's just drawing.

OK. Now, one of the methods that-- the first method that was widely used for experimental tracing of a pathway, it was the degeneration technique. And there's more than one of them. Now, when I say experimental tracing of a pathway, that's to be contrasted with looking at material where nothing's been done to the brain, but looking at a normal brain that's then been fixed and stained, say, for axons.

There are stains that will stain axons, and you can see major fiber bundles and so forth. But you can't-- there's so many accents, you can't really find-- discover for sure where they're going, or even where they came from. In some cases, like axons coming out of the eye, it's pretty obvious they came-- where they came from. They came from someplace in the retina.

OK. But when those axons reach terminal areas in the brain, you can follow the tract to the main terminal areas, but it's-- even there, it's difficult to see all the places they terminate without some experimental tracing method. And those are what we call the tract tracing methods, OK? The initial method was developed by Marchi. It was for staining degenerating myelin.

OK. Marchi method-- sorry, that's a C-H-I there. There was a problem with the Marchi method. It could mark a degenerating myelin, OK? That's what it was good for.

Degenerating myelin pathways could be specifically marked. But it had a major problem, in that the myelin usually disappears in the terminal region. So the myelin doesn't go all the way to the terminal [INAUDIBLE]. So you can never be absolutely sure.

I mean, if you were skillful and you had also done Golgi studies and studied Cajal, and so forth, many people that use the Marchi method turned out to be right. Degeneration methods have other-- this is using the technique of anterograde generation, which I should explain here first. What you see in the picture here is this-- the yellow cell there is connected by an axon to another cell.

It all has input, the cell over here, and it shows the site of injury, OK? Now, if that's injury transects the axon, OK, you will get degeneration then in two directions. Anterograde degeneration will be the degeneration of the axon distal to the cell body, OK?

And we'll go-- it will involve degeneration of the entire axon. Usually, the first thing that starts to show signs of degeneration actually is the terminals, OK? But then the entire axon will degenerate, it will fragment, change its chemical properties. And, eventually, it will disappear by processes of phagocytosis.

Now, the myelin stains will stain only the degenerating myelin, so it won't reach all these terminals. You also have degeneration in the opposite direction. Now, the breakup of the axon is much slower. And in some cases, doesn't even occur.

There are then changes in the cell body. And, usually, when we talk about retrograde degeneration, we mean changes in the cell body. You'll get chromatolysis. The chromatin in the cell will change its distribution, so the cells will start looking different. In some cases, the cell will gradually degenerate.

In other cases, it will just atrophy but not-- won't die, OK? We still call it retrograde degeneration. Sometimes we call it retrograde atrophy if the cell doesn't die.

OK. But there are then changes throughout the cell taking various amounts of time. Here, you have the antegrade degeneration pictured, and they're also pointing out that sometimes at the site of where the cell was cut, rather than dyeing the cell, the initial response is actually an attempt to regenerate, OK? That occurs, apparently, in every cell, but cells vary a lot and how much they can regenerate.

OK. Most of them in the [? CNS, ?] the majority of them will not be able to regrow their axon. Many of them will show some sprouting in the region of the cut, OK? And we'll talk about that later on.

Nauta, who was an MIT Professor for the last part of his career, before he came to MIT was realizing the problems with the Marchi method, that you can never be sure of tracing tracts to their real site of termination. He investigated various silver staining methods, because they could stain rather thin axons very well, and just normal axons. One of them was a method developed by [? Dostoevsky. ?] He liked it particularly well.

He studied its chemistry, and began to work with a man in Switzerland on modifying the [? Dostoevsky ?] stain, so it would stain the degeneration better. They initially came up with a stain we now call the original outer stain, which did stain degeneration very well, but it stained all the normal axons pretty well, too. So it was still pretty difficult to pick out the degenerating ones.

His work with Gygax in Switzerland-- sorry, this was the original out of stain, I think, was before Gygax, OK? But then with Gygax, he worked on a method for suppressing the staining of the normal axons. And this was a huge breakthrough in tract tracing techniques. Because when you got that technique to work well, you could do the stain and all the normal axons would stain very lightly, and the degenerating ones would keep staining darkly.

Now, the way he did that was they applied an oxidizing agent. The normal tissue would oxidize faster than the regenerating tissue. So the length of time they exposed it to the oxidizing agent was very critical, and they often did trials using various periods of time in the oxidizing agent to see when they could get the optimal stain.

That stain did not-- he wasn't convinced. It always would stain the actual terminals very well, so he kept working on it. But it was only when he came to MIT, OK, that he was able with Robert Fink, working as a technician in his laboratory, he developed a stain that would be specific for the axon all the way to the terminals.

And Leonard Heimer, a Swedish anatomist who had just come to work with Nauta, had also been working on these stains. He had a slightly different way of doing a similar thing. His method-- Heimer's method worked better for hypothalamus, limbic structures.

Fink's method worked better for sensory pathways and motor pathways. So they published it together and that became the standard method for tract tracing for a number of years. Question.

OK. What's the advantage of staining degenerating axons. It was a method of marking one particular tract-- that's a critical question, OK? So now, think of the experiment you could do, OK?

We can take out an eye, destroy all the axons coming from the retina. They all start degenerating, OK? We let the animal survive for a few days, so we get the generation but the axons have not disappeared yet.

Now, we apply these stains. Let's say we were applying using the Fink-Heimer method. We would stay in the brain and we would see standing out in dark black that axon pathway. They would look like they're degenerating. It would be fragmented.

OK. And we'd learn to recognize the degenerate axon. But they would very-- they were very easy to see, because now we have a stain that's specific for the degenerating axons. You could do other things. You could go into the brain with a little electrode and electrocute a few cells in one little region.

OK. And then you would trace the degeneration from that area. There's always problems with the degeneration techniques, because when you're making a lesion in the brain, you're also killing the axons going through, unless you have some chemical method that will only kill cells and not axons. And there are such methods.

But by the time those methods were developed, things that could kill cells and not fibers, other methods were beginning to be developed, methods using the intracellular transport properties of cells, OK? So, for example, it was found that the enzyme horseradish peroxidase, or HRP, as I mentioned before, if you put in the region of the terminals, will be taken up by the terminals of axons and transported back to the cell body.

Here you see a cell here and cell here, all-- everything in dark black there. Cells in the retina, retinal ganglion cells, that have transported HRP from a terminal area in the brain. Now, in this particular case, another label has also been put in another part of the brain. No clear yellow.

It's a fluorescent molecule, which is also taken up by axon innings, and it gets transported retrogradedly. Nuclear yellow binds primarily to the nucleus. And here, you see a double labeled cell.

So the two areas that were injected, both contain axons from this one cell. And that's the advantage of a double retrograde staining technique. You can find out, get experimental knowledge, about axon branching patterns. Here we have nuclear yellow in the nucleus and HRP in the cytoplasm.

Here's another retrograde tracer, Fluoro-Gold. Some of these tracing substances are rather expensive cause it's-- it is gold. OK. But it marks cells very beautifully, more than nuclear yellow or HRP [INAUDIBLE]. A little more like HRP, but even better.

You can see it's got the entire cell body plus axons and, at least, proximal dendrites. These are, again, in the retina. And you see this, the marking is varying in intensity. And that's because the brighter cells we injected more of the terminals, they took up more of the substance, than others.

Now, in this case, in two different terminal regions of the retina, we injected some-- in one place, we put fluorescent beads. In another area, we put the Fluoro-Gold. OK. And then we can-- because the molecules fluoresce at a different wavelength, OK, we used different filters in our microscope.

We can see the fluorescent beads with one filter, and the Fluoro-Gold with another. And we see here a cell in the middle that's got both labels. Other cells that have only one as these, especially, the area injected with Fluoro-Gold.

OK. So then we know that at least some of these cells-- and we can chart their size, and so forth, and their locations-- have axons going to both structures. Others have an axon going only to one of the two structures. Now, horseradish peroxidase is frequently used because it not only goes in the retrograde direction-- it was discovered later-- it's also a pretty good antegrade tracing technique. It will get from the cell bodies to the axon.

That was discovered when the methods for visualizing HRP became more sensitive. And here, this is from work in my lab. We put HRP in an eye. And we're looking at the optic tract here in the [? tween ?] brain. And you see the axon [? carborizing ?] in two structures, the ventral and dorsal lateral geniculate bodies-- a little bit here, also.

And you see them actually going right into the ventrobasal nucleus, which in the adult wouldn't do this. But in the baby, they're a little more widespread in their connections. And that's what we were studying here.

OK. All of these methods that I've used, I find vary a lot in their sensitivity. The degeneration methods were pretty good, but they were very good for some structures. Other structures, it was difficult to see, for sure, whether there was really any axons, particularly, areas of very sparse projection.

That was also true of HRP, although, HRP was more sensitive. And I didn't mention another method that was used around the time of HRP, also, very commonly and had one big advantage. It avoided that axon of passage problem, and that was radioactive tagging of proteins, of-- I'm sorry-- amino acids.

So they would be taken up by cell bodies and synthesize into proteins. And the proteins would be transported down the axons. So why did that avoid the fiber passage problem?

Well, if you inject the amino acids into a structure in the brain, it's taken up by the cell bodies. It's also taken up by axons going through, particularly, at nodes around [INAUDIBLE] you will get some. But it doesn't get synthesized-- no proteins are made except in the cell body.

OK. So to avoid the fiber passage problem, and that was tested experimentally, you could prove that it didn't label axons of passage, and only labeled the cells in the region of your injection site. But then, how do we find the radioactivity? We can tag with tritium, say, we could use tritiated proline, or tritiated leucine. These were the common ones.

OK. And then what do we do? Well, we have to wait a little while and leave the animal alive so the amino acids are taken into the cells, made into proteins, and then we have to have time for transport. But, say, you wait a few days, five days, maybe.

And then we had to use a procedure called autoradiography where you section the brain, put them on the slides-- put the sections on slides. Then you go into a dark room. And in the dark, you coat the slides with photographic emulsion and leave them in the dark for a long time. The radioactivity exposes the photographic emulsion.

We would usually counter stain those sections for cell bodies with a nissl stain, usually, so we could see the cell bodies. And then, when you would see the silver grains in the photographic emulsion over the tissue. So we could localize where those proteins went that had been transported from the cells we injected.

Now, that method had one other advantage that some molecules, like proline, if you leave them in-- put a lot in and leave them long enough, they would actually cross the synapse and you would get transneuronal transport, OK? And so we could map out the termination pattern in the visual cortex from injecting the eye, even though there was a synapse in the thalamus, OK? So there are a number of advantages to these techniques.

Later, it was found that HRP will actually go-- transfer only two. I still found that the sensitivity was a problem. The most sensitive method I found uses another method called immunohistochemical staining, or marking, OK?

Well, we're using an antibody for the molecule we've put in. The one I like the best for tracing in the visual system is a fragment of the cholera toxin molecule. We often use pretty dangerous stuff in the lab. We don't get the full [INAUDIBLE] toxin, so we're pretty safe.

[INAUDIBLE] subunit of it. This fragment of cholera toxin is taken up. And when we use immunohistochemistry to find that molecule, we use an antibody against cholera toxin.

I find that it's extremely sensitive. I've been able-- here I'm showing you a bright field picture where we've bound the antibody-- the secondary antibody that we could mark with HRP. If we look at it in dark field, you can see it here in part of the geniculate body.

This was so sensitive because it could stain the entire axon right to the terminals, not just the terminals. Didn't stain them in a fragmented way, so they look like Golgi pictures. And I was able to show that the retina-- with students I was working with-- the retina projects to many different structures at the base of the brain, hypothalamic areas, that were not originally believed to be part of the projection area, the retina. We still don't know what some of those projections do.

Now, immunofluorescence can be used to mark several things at the same time. In this case, you see a cell's dendrite, and you see two different markers. One that's marking the inside of the dendrite, and another that seems to be marking something on the surface.

I think they were using a synaptic protein specific to synaptic regions. And you can see that if you look at higher magnification where they're also marking the nuclei of cells, or the third antibody. OK. In each case, they're linking the antibody to a fluorescent marker.

OK. And for that, you need a secondary antibody. And these procedures have been a well worked out, and they're the most common methods now for getting sensitive marking. And, of course, that last method, immunohistochemistry, can be used for more than just axon tracing. You can find-- as I showed you in the last picture, you can use it to find the specific location of various molecules in the brain.

OK. Let's talk now a little bit about specializations of the membrane. And if we have time, a little bit about endogenous activity. This is a different order in-- than your printout, but they're the same topics.

I put them in a different order, because we've already talked about the axonal uniqueness, the uniqueness of the axonal membrane with its voltage gated ion channels, particularly, for sodium that could result in the axon potential. This is this a very-- this is a specialization for irritability postsynaptic membrane. Receptors are another example of a specialization.

We haven't talked yet about transduction mechanism sensory transduction that result in receptor potentials, if they're specialized receptor cells, generator potentials in the dendritic part of the neuron. And I've given you this picture, because it's a nice classification of major receptor types in organisms. These are not the only types, OK?

For example, we have chemoreceptors that can detect oxygen, for example, in the blood. We don't think about those very much because we don't sense oxygen. But it's part of our autonomic nervous system.

You think more, though, about taste and smell where you have specialized receptor cells and taste buds in the tongue. And these are connected to primary sensory axon terminals, actually, the dendritic part of the primary sensory neurons. The receptor potential is generated in these receptor cells in response to particular chemicals that we-- in our mouths, OK?

And that causes changes in the properties of the membrane here, and it leads to action potentials beginning here, the axon hillock, and then going into the central nervous system. In the case of smell, it's a little bit different, wherein, the olfactory epithelium lining our nasal cavities, the neurons themselves act as the receptors. OK. The dendritic portion of the primary sensory neuron is embedded in the mucosa of the nasal mucosa.

And molecules that we take in through the air dissolve in that mucosa and specifically stimulate particular receptors in the primary sensory neuron, which then generates axon potentials, which reach then into the central nervous system, the olfactory bulb cranial nerve one. Then we have various mechanoreceptors, receptors that are specialized for detecting mechanical stretching and poking, OK, or vibration.

This is a axon ending dendritic part of a cell in the skin with a little specialized structure around it. The pacinian corpuscle is good for detecting fine pressure changes. We also have free nerve endings in the skin that respond to deformation of the skin.

We have endings, sensory endings, in muscle that detect muscle stretch, and others in joints, tension in our joints. These are all examples of mechanoreceptor. And another one is in the ear, OK?

Where there are specialized cells in a membrane, the basilar membrane in the air, which when basilar membrane vibrates, the vibration is detected by these sensory neurons, as the sensory cells, rather than not neurons, especially, sensory cells, which then cause a receptor potential that affects the primary sensory neuron endings in the cochlea.

And, finally, we have-- in the visual system, we have the ability to detect photons, which is a different kind of thing, and can show us this is a characterization of what kind of thing is happening with chemoreception, mechanoreceptor-- receptors in photo reception.

In the case of photo reception, which I didn't finish previously, you have a specialized membrane inside the cell that responds to photons and results in a molecular release that affects a membrane receptor, which then in turn affects the ion channel. All it does is change the amount of current flowing through the membrane. It doesn't cause any axon potentials.

OK. In our retina, the primary, the routes and cones, receptor cells change in their membrane potentials in response to protons, but they don't actually generate axon potentials. And that's true, also, for other cells in the retina. It's true for some cells in the olfactory bulb.

In the case of mechanoreceptors, it-- in response to deformation stretching, or movement of some sort of the membrane, you can hear they simply show an ion channel being stretched open. It's probably not what actually happens. They're probably-- the mechanical change in the membrane results in changes in the proteins.

And then when these proteins change their conformation, you get ion flow across the membrane. Case of chemoreceptors, usually, it appears to be like the metabotropic receptors we talked about before. The binding is to a specific molecule, which then, in turn, affects a nearby receptor. I want to say a little bit more about endogenous activity, but I'll have to do that at the beginning of the next hour.