PROFESSOR: I just got these from [INAUDIBLE]. Now, some of you wanted to see those simulations using screen characters sort of watching these robotic characters with motivations that have dynamics similar to what is suggested first from ecological studies but also from studies of hypothalamic neurons. So I will post these. I should post them under the hypothalamus chapter, but maybe I'll just send them by email to all of you so you can take a look.

All right. We finished with the Chapter 26 material, which was the introduction to hippocampal and other limbic system pathways, particularly the Papez circuit. And we'll come back to that right after this, a little section here on hormonal influences. Because I want to deal with both hippocampus and then amygdala and the more anterior parts of intra-striatum before we go on to corpus striatum and neocortex.

So the major topics in this little unit are sexual differentiation of the-- especially the human hypothalamus, emergence of male/female brain differences, and determinants of sexual orientation. Studies have been done of that. And then a little bit on bird song, because of the importance of hormonal as well as other determinants and the discoveries that have been made in that field.

Other relevant topics concerning actions of estrogen on the brain, the use of [INAUDIBLE] cultures, usually slice cultures, with a look at effects of hormones on differentiation of neurons. That's how we know that it's probably not just hypothalamus, not just a few areas that have been-- things that have been discovered so far, but they discovered that, for example, the catecholamine systems in tissue culture are influenced by sexual hormones as well. All right.

A related topic is the effects of thyroid hormones. And here, this is an important one,

because thyroid-- particular thyroid hormones are known to be very important in brain development with abnormalities in thyroid, for example, consistently leads to deficiencies in cerebellar development, but other brain areas can be affected as well. Mostly t4.

And one reason that in recent years there's been more attention to this is because of pollutants that mimic-- that bind to thyroid receptors. And when this happens in the nervous system during development, it can cause brain abnormalities. There's some evidence from animals. That was the topic the led me to work with Eric Montie on the brains of sea mammals, particularly the Atlantic white sided dolphin.

And also we did some work on California sea lion. California sea lions, not because of thyroid hormone, but because of their exposure to pollutants-- natural pollutants-- that they encounter in their environment in Southern California that cause brain abnormalities. They can practically wipe out the hippocampus, for example.

This is Montie here. This was his PhD thesis. I was one of his committee members, and he worked closely with me for part of his project because he needed to-- he was basically doing the control studies for his work on pollutant effects and he needed help in identifying various structures from the MRI images. So I went down to Woods Hole a number of times, and he also sent me images to help him with the identifications.

And that resulted in some publications. If you look up Eric Montie online or Montie and myself, and there were a couple other authors, too. People at Woods Hole that were involved in some way in getting the brains that we used. And I just mention this one website because it's particularly good for the thyroid hormone information.

When we-- if we're interested in sex differences between male and female brains, particularly in humans, what are the three factors that are likely to play a roll? What are the things that we look for? Hormonal effects, genetic effects, and environment, learning of some sort. And we know from many studies that although all three have effects, when it comes to sexual orientation, environment doesn't seem to have-- isn't a major determinant at all. It's genetic effects and hormonal effects.

So the neuroanatomical studies of sex differences received a big impetus when there was a neuroscientist who was also a gay man working at Harvard. A lot of his work was with Hubel and Wiesel. Began working on this topic, and he was able to obtain results of homosexual males and normal males and started doing comparisons. And of course, he was concerned with male/female differences as well.

And that led to a lot-- a whole series of studies, not just-- this was not just by him, but by others as well. So I just want to review some of that here. His name is Simon LeVay. Very good neuroscientist. L-E-V-A-Y. I think capital V-A-Y. Simon LeVay. Very good neuroscientist and good friend of a lot of us in neuroscientist. OK, that just summarizes what I just said.

I believe I reproduced this table in the book because this is from Schwab studies. Martin Schwab has done a lot of work on this topic, and his studies are often referred to because he was one of the earlier ones to really put emphasis on it in his work. And he collected all these data. These are references he refers to in his paper, if you look it up. His Trends in Neuroscience paper back in '95.

And he lists these different diseases because they were-- and for all of them, they were-- the frequency of occurrence was different for males and females. Look how asymmetric it is for, like, anorexia nervosa. 93& female, 7% male. Or bulimia. One particular type of schizophrenia, whereas most types of schizophrenia only 27% females, 73% male. But for that one type, it's different.

And then look at the ones that males are dominant. Dyslexia is very well-known for that. Sleep apnea. Tourette syndrome. Yes.

AUDIENCE: [INAUDIBLE].

PROFESSOR: That's a very good question. I don't know the answer, but that's-- I mentioned when I listed these extra credit that if you can come up with something like that, a question that you can find something, please do so and we'll give you extra credit for it. Be useful for the whole class.

But because of these kinds of differences, I mean, it becomes obvious that there has to be differences in male and female brains. And one of those differences could actually be structural. Of course, we're more concerned with the structural differences, but with any differences, it could cause functional differences in the brain.

So a place to start is, well, when are-- when are there differences in gonadal hormone levels? When are they at a peak, that is, during human development. There's actually three different periods when they reach a peak. One is the early prenatal, first half of gestation, during the formation. That's the period when genitalia are forming. Then perinatally, there's another peak. And then the levels are lower again until puberty, of course, when the differences we're most familiar with appear.

But if you look for structural differences in the hypothalamus, the studies that have been done indicate that, at least for the sexually dimorphic nucleus of the anterior hypothalamus, the one that's received the most attention, the differences don't appear until about age four. So that's when the hormonal peaks are going down. And so this is-- first of all, I should show you where it is.

This is a section that is about at this level or just anterior to this level in these diagrams. These are black/white versions of the color ones I used in the book. But here are the curves, and you can see there's some scatter, but the males are in the solid points and the female brains are in the open circles.

And for the males here, the squares indicate a homosexual man, and you know that when this nucleus is concerned, the homosexual brains are-- not that homosexual males don't show female brain characteristics at all. They fit the curve of changing sides of this nucleus-- it's size, number of neurons. The fit the male curve.

And the differences you can see here, up here, this is-- if we take this as when they appear, that's about four years old. Before that, there were no differences that have been seen in hypothalamic areas. It's called the sexually dimorphic nucleus, the

preoptic area of the human hypothalamus. So it's pretty anterior.

What is a problem with doing this kind of work? It's a major technical factor. The problem is if you're looking at volume of brain tissue, there's a lot of factors that can affect that, and one of them that will affect it is-- in fact, even your ability to differentiate the nucleus can be affected just by the tissue fixation. It varies so much in human tissue. It's not like doing a study of rats or monkeys, where you can control that.

There are laws. The laws differ in different areas. But you don't just profuse human beings. The only really well-fixed human tissue we have that appears in the textbooks is from a guy that committed suicide by drinking formaldehyde. And he has a beautiful tissue in his digestive tract. Sorry. So it's a big problem for brain. I don't know anybody who's committed suicide by perfusing his whole body. OK.

And of course, it varies with the tissue-- the fixative used as well. But mostly just time before fixation. So The solution is to do cell counts and use only brains where you can actually identify the structure, not just have to go by gross location, but actually identify it from the [INAUDIBLE] architecture.

The problem is that's a lot harder to do than just looking at volume. If you do a series of cross sections and take the area of a nucleus of each point and integrate over the structure, you will get a good estimate of the volume. But these factors make cell number to be much better. But there are good methods for estimating number where you don't have to look at every single section and you count. One of the problems there is you want to come only cells, say, that the nucleolus so you don't count the cell twice, because it's cut through more than once in your sections.

OK, so let's look for cell groups that are different. Additional structures besides that sexually dimorphic nucleus, the preoptic area had been found. And there is a nucleus that-- first discovered in rats, verified in humans-- that does differ according to sexual orientation. And then there are sex differences in the CNS outside the hypothalamus, too. These have been discovered particularly by magnetic resonance imaging methods.

So this is the one I showed you. This is that-- it's also called the sexually dimorphic nucleus now, but the earlier name was the interstitial nucleus of the anterior hypothalamus. Interstitial because it's among the fibers of the [INAUDIBLE]. But here is another nucleus that's been found to be different in gay men from normal men.

So I have some of those. That's the suprachiasmatic nucleus, which we talked about before because it's the site of some of these neurons have that roughly 24-hour rhythm and represent the biological clock. So here was the initial report, which was a paper in Schwab and Hoffman in brain research back in 1990. They reported an enlarged suprachiasmatic nucleus in homosexual men. 1.7 times as large compared to controls. It contained 2.1 times as many cells. So it's a pretty striking difference.

The sexually dimorphic nucleus doesn't differ among homosexuals and normals in either volume or cell number. It basically doesn't support the original idea that they were testing, that homosexual men have a female hypothalamus. That's not true at all. But of course, among homosexual males, some of them are certainly more male-like and some of them are more female-like, and they don't-- these studies have sort of ignored that. I'm not sure why. Seems to me that would be important.

They did find male and females do differ in the suprachiasmatic nucleus just by shape of the nucleus. Also changes with age and with Alzheimer's. But this was their first report that said homosexuals don't have a female hypothalamus. And then how would you answer this question now? What appears to be a major cause of variation in sexual orientation in humans?

You could say hormones, but in fact there's more data to indicate its genetic. Dean Hammer of the NIH was the first to get clear evidence for genetic differences, and that's been supported by others. But we'll talk more about genetic differences in a few minutes. There are other sex differences in the brains reported that are harder to study.

I remember a study in rats and in hamsters that shows that-- I think it was first in hamsters-- where they show a difference in dendritic length in certain nuclei of the hypothalamus. What does that mean, if the dendrites are longer? There are also differences in spines, like spine length and spine density.

Well, what are dendrites doing? That's where axons are making synaptic connections. The spines generally won't exist unless those connections are made. So differences in spines and in dendritic length implies differences in connections or numbers of connections.

So even if the cell number is the same, there could be differences. And that's what the studies of dendritic length indicate-- that we can't-- very difficult. You can't get that kind of data on human brains-- adult brains. Golgi doesn't work well, particularly in poorly fixed tissue, but it doesn't work very well in mature brains at all. It works better in younger brains. So there's probably a lot more sexual dimorphism than we're-- than we've really found so far.

OK, let's go to a different area where there is a very clear sexual difference. This has been found by a totally different group of investigators-- people that were initially studying rats and looking at the spinal cord. They found a sexually dimorphic area in the sacral region.

The nucleus of the bulbocavernosus or bulbospongiosus, it's sometimes called. This is the muscle involved in engorgement of genitals in both males and females. But the nucleus is considerably bigger in males, probably because the organ is bigger. The muscle is bigger. And there are other differences, too. I mentioned imaging methods have confirmed what was known from brain collections earlier, that the size of the corpus callosum is larger in females.

The fact is the larger-- the smaller corpus callosum size in males indicates that the male brain may be more compartmentalized, less integrated, between the hemispheres than in females. And in fact there is behavioral data to indicate greater left hemisphere dominance in language, for example, males. That's why the frequency of severe aphasia after lesions of the left hemisphere, the aphasia tends

to be worse in males than in females. Females are much more likely to have some language in the right hemispheres as well.

Yes?

AUDIENCE: [INAUDIBLE].

PROFESSOR: Yes. There are various structural details that differ, too. For example, if you look-- especially we know it for the planum temporale. This is right around the auditory region of cortex. There are a number of differences.

If you look under Galaburda in the literature-- another good homework project. Al Galaburda has worked for years at the Beth Israel Hospital here in Boston. Has done a number of studies in human brains.

In more recent years, he's done less than humans, but in fact, I think he made his biggest impact by his studies of human brains. He worked under Norman Geschwind for a long time, a very well-known behavioral neurologists in Boston. He died a number of years ago, but Al was much younger and I've met him fairly recently. I know he's still active, but he's doing most of his experimental work on rats now.

But he discovered, for example, that in dyslexic males who died, it was very common to find abnormalities in the left hemisphere, little collections of cells that appeared not to have migrated normally. He found-- not in normal males, but in severely dyslexic males. And we think that because of the hormonal differences between males and females during development, that happens more in males. You don't get it as much in females.

And then I just note here there are brain size differences that are not dependent on body size. Male brains being larger from early in development. I thought at first it had to do simply with body structures in females who don't-- are not related to brain size and innervation patterns at all.

But in fact it seems that these-- the differences appear, according to the imaging

work, anyway, most recent ones I could find indicate that there's little change after about age five. I expected it would occur more after sexual maturity, but that's not the case. So that's a difference we don't fully understand.

How many of you have heard of Fernando Nottebohm?

AUDIENCE: [INAUDIBLE].

PROFESSOR: Sorry?

AUDIENCE: Before or after reading the chapter?

PROFESSOR: Oh. No, I want to know if you knew about him before you encountered him in the chapter. At least for the older neuroscientist, he's very well known, because the findings made such a big splash when they came out. And it's led to a lot of the work using birds as models of plasticity, behavioral change, and because of its relation to language. There are a number of parallels in the way human language is controlled [INAUDIBLE] with singing birds and the way bird song is controlled.

There were a couple of major findings of that lab. This is one of the pictures. I think this one's in the book, where there's-- shows sections just from the left hemisphere and there are big hemispheric differences. They have left hemisphere dominance for bird song, just like we have left hemisphere dominance for language.

So this is from the left hemisphere of a canary brain. The male on the left here and the female on the right. See that-- the very big difference in that nucleus. It's pretty easy to quantify. And then the same here for zebra finches where it stands out even more. But again, very big difference. And you'd find the differences in numbers as well as in the size of this particular cell group. This is the nucleus robustus, or robust nucleus of the acropallium. It appears to become homologous to the amygdala or part of the amygdala in mammals.

AUDIENCE: [INAUDIBLE].

PROFESSOR: Sorry?

AUDIENCE: Is there a reason why the [INAUDIBLE] looks like-- I know you said that it's not that there's difference in the number of cells, but is there a reason, even though it's smaller in the female, the cell population looks denser than it does in the--

PROFESSOR: Yeah. There are still a lot of questions about the females. Of course, they've concentrated on the males, the singing species, but I'll mention some of the things where they find, for example, the cycles of these cell numbers. It doesn't affect just this nucleus, but other areas, too. And it occurs a lot in females as well as males. And they don't-- it's not as well studied.

But, of course, in evolution, what's the sex that's orchestrating the whole thing? It's, of course, the females, because they are choosing males according to their singing. So they have to be able to perceive the details of bird song. So what we need is more studies of details of the auditory system and auditory perception in the females. OK.

This is one of the Scientific American figures that Fernando Nottebohm published back in '89. He shows here a drawing of a syrinx-- like the vocal cords of the bird. And the major brain pathways, the higher vocal center is here. The diagram here makes it a little easier to see.

It doesn't show the auditory pathway, but it shows field L, which is the equivalent of the auditory cortex of the bird. The thalamic nucleus gets the auditory input from the caudal midbrain. The equivalent of the inferior colliculus in the bird projects field L. It's got three main layers. And that nucleus projects to this area, and the mesopallium.

The higher vocal center, often just called HPC, it's sexually dimorphic. As is nucleus robustus here on the output side. It gets output directly from the higher vocal center. The nerve pathways, then, that descend either directly to the hypoglossal nucleus, which enervates the syrinx. Particularly a particular part of the hypoglossal nucleus does that. But also there's a less direct pathway through the most intercollicular nucleus. This is basically the bird's inferior colliculus area.

It doesn't look like a little hill, so they don't-- we don't call it the inferior colliculus very much. But some of the terms are still used, the mammalian terms. OK, well there's also a less direct and even more interesting pathway, and it's the one that's involved most in learning of singing. These are the two sections, dimorphic structures, we talked about.

But there's another sexually dimorphic nucleus here in the striatum just called Area X. It gets the projection from the higher vocal center, which, remember, also projects directly to the nucleus robustus. They leave that one out here. There just showing the less direct pathway. It goes to the striatal area here, which, as in mammals, the striatum has a pathway through its output neurons to the thalamus.

And the thalamic structure it goes to projects to a kind of motor cortical area. It's called LMAN. It's the lateral magnocellular nucleus of the anterior nidopallium. The nidopallium is that area this is the neocortical equivalent, but it's subpallial. The neurons don't migrate the same in birds and in mammals. But it is equivalent.

So that would be most like the motor cortex, like [INAUDIBLE]. And it has outputs to the nucleus robustus, which then, as these two pathways reach in the hypoglossal nucleus. And that we know, that lesions in these structures can-- basically, the bigger the lesion, the more syllables the bird loses through his singing. Very dependent on those structures. It's a kind of-- you can say it's a kind of habit learning. It's a striatal type of learning. And I'm sure much of our speech is just like that.

So in one of the papers Nottebohm wrote was called "A Brain for All Seasons," because these morphological differences in these structures vary from season to season. And as does-- as do the levels of the male hormone. And here, they're plotting, basically, the syllables that the animal and the volume of these two structures, the higher vocal center and nucleus robustus.

Now, this, of course, when the bird's very young, the hormone levels haven't appeared yet-- hormone differences. But when they do, you get the growth of these structures. And it's during this period when you see the rise in the size of these

structures that the bird is learning the syllables of this song by listening, basically, to his father. And it reaches a peak. And then it doesn't change during the period when the song is not changing. So it's no longer plastic.

And then when you enter a period of plastic song again, the size of these structures goes way down again. So they're largest when the song is stable-- when the animal has learned the songs, and he's using those structures to control his singing. And this is another way it's been studied, where they've plotted the changing levels of testosterone and then the number of syllables.

See them increasing during the period of plastic song. And then as testosterone levels rise again here, he loses the-- he loses-- no new syllables appearing, and the cycle repeats. The cycle you see here, this is from May to May. So that's a full year, and that cycle is repeated.

But then the other big discovery was birth of neurons that migrate just like in development. Here, you see migrating neurons here, where they're showing wheel processes stained with the [INAUDIBLE] protein and then neurons moving along them. And this is from a tritiaed thymadine study from a *Scientific American* article. It has particularly nice illustrations, and here he shows you inject the thymadine, so you label the dividing cells.

If you look at the brain just one day later, you see where you'd expect them. They're all-- you've labeled the cells in the ventricular layer. Now, remember, these aren't babies anymore. This isn't when the brain first formed. And this is happening every season. Look at them after eight days. They're beginning to migrate. After 15 days, they've moved out into much of the endbrain. At 40 days, they've not only moved well away from where they were born here at the ventricle, but they're-- the ones shown in red are differentiating into neurons.

And as I said, this is actually-- the total emphasise was on males at the beginning. But it turns out there's quite a bit of cell birth like this in the female, too. And even though we know that, it's simply not been studied as much because we can't correlated it with the singing. The female isn't doing much singing.

So a couple of things that indicate that there's a lot of sex differences that we probably haven't even found yet, and certainly haven't-- one is that if you look at most of this was in vitro work. Look at the differentiation of the midbrain dopamine neurons that's affected by sex steriods in culture. And she found an interaction between the estrogens and [INAUDIBLE]. They're affecting the growth hormones directly.

So in fact, there's hormone-- sex hormone receptors in many parts of the brain, including many areas that we've not seen nuclear structural dimorphism, but that doesn't mean the neurons aren't being affected, even though they may not change in the growth structures that we look at more anatomically.

So what in addition to hormonal factors may explain male/female differences? Is it only hormones that we need to look at? And that is all people thought they had to look at for many years. They assumed that brains of males and females are similar. They're basically all female, and then the male brains differentiate because of testosterone. And if you remove the gonads of a male, then the brain just stays female. And that was the picture that everybody learned.

And then gene expression studies came on, and this fellow, Eric Vilain, out at UCLA started publishing his results on gene expression studies. And they were doing-- they could compare the expression of over 12,000 genes with their screening method, comparing male and female brains using embryonic mice. And they started long before the animal developed sex organs.

And they found that 54 genes were expressed in different amounts in male and female mouse brains prior to any hormones influence. And 18 of them were expressed at higher levels in male brains. 36 were expressed at higher levels in the female brain. That was published back in 2003. Yes?

AUDIENCE: [INAUDIBLE].

PROFESSOR: No. No, they're not all-- the differences appear-- I'm not sure. You could look here in these papers to see if they tried to be more specific. But you see, when they first

discovered these, they didn't even know what proteins these things are coding for, and they-- it was this large scale screening that they were using. But that's just the first step in trying to understand what's going on.

In 1990, they did identify one on the Y chromosome. They call it SRY. It's a sex determining region on the Y chromosome. It's also known as the testis determining gene. That same gene is found being expressed in the substantia nigra in rats. It's involved in dopamine secretion.

In fact, if you reduce the gene's activity, you get a Parkinson's-like syndrome, which is interesting because it could explain why men are more susceptible to Parkinson's disease than women, but still women get it. And women produced plenty of dopamine even if they're not expressing that gene. So obviously, there are other determinants. So that's--

And here is the problem-- I just wanted to note here, it's a disturbing part of modern molecular-- cell molecular work that often, you hear about something this early and then you stop learning about it. The reason is people delay their publication because they want to patent everything. People get greedy, and the drug companies are often dominating the game. So I think we would know a lot more now if this weren't-- people weren't so convinced they were going to make a lot of money from it. Sorry to say.

AUDIENCE: [INAUDIBLE].

PROFESSOR: Well, you can't-- basically, they won't publish it until they're sure that they've patented everything or initiated the patent. There's a whole procedure that follows. Then yes, you can publish, but if you publish too early, then everybody else will get started on it, and maybe they can work faster than you. So people just delay. They keep it secret.

It's true even in the field I was working in for so long, the regeneration work. Again, people are so convinced they've got some big finding that's going to make a lot of money. All right. I don't claim to be lily white either, because I got involved in that for

a little while, but then I stopped. I decided it was destroying the way I did science. I didn't want it anymore.

Some of the differences in sexual orientation might be explained in gene expression differences. They found in mothers of homosexual men, there's some skewing of X chromosome inactivation. But it would only explain results for only a subgroup of gay men, and it's even been reported that such skewing is not transmitted to the offspring. So that's been up in the air.

Perhaps the-- and you can look under Vilain, you'll see the more recent things that he's been publishing. And this is Dean Hammer of the NIH, by the way, who first discovered the genetic differences in--

AUDIENCE: [INAUDIBLE].

PROFESSOR: Well, the X chromosome is inactivated in-- during normal development. And this skewing can vary a lot in how asymmetric it is. That's where there was an abnormal, they claim, to get this extreme skewing in mothers of homosexual man. But I report the finding here in case some of you may want to see what's happened to it. And I didn't follow it up this year. I didn't look any further. I was looking at a lot of other things instead. And I don't think I put this in the book. If I had, I would've done a lot more looking at it.

There's a lot of other quantitative variations in human brains, including sex differences. And they've been found to have both genetic and environmental origins. They vary a lot from one part of the brain to another. For example, the cerebellum seems to show a lot of variation, and they think it shows more variation than other structures because experiential factors make a big difference.

That was Rapaport's suggestion. She's given a lot of talks on this. She's given one talk here, but not recently. She had very large numbers in her studies, so we think it's pretty reliable, a lot of the things she's reporting.

They find differences between-- this has been known for a very long time. There's all kinds of studies in differences between schizophrenic brains and normal brains.

But in fact, they're quite variable. And there's so many studies of schizophrenia that indicate this, that, and the other kind of thing that explains what schizophrenia is that you begin to realize after a while that it's because there is no explanation. There's just a lot of variation. So we don't want to have a final answer there, except perhaps for certain schizophrenics.

But one thing that really surprised people was this. Young people with attention deficit hyperactivity disorder, they find region-specific differences between people without it and people with it. And of course it varies with severity as well. So the fact is, there's a lot of individual differences in brains, and some of it-- the fact that it's correlated, remember, doesn't prove anything about cause. It only makes a suggestion that it may be related to the causes.

Don't be too quick too-- things appear in the newspapers all the time, usually published by medical residents who find some correlation or other and they publish it. There's a lot of pressure to publish in medicine when you're early in your career because you get better jobs if you're published. But people go too far in interpreting correlations. In other words, there's no experiment done to prove any causative relationship.

So I just-- these are the kinds of things you should review here. And if you like Rumi, there's one of his poems. I think we're at the end of the hour. So we'll be talking now about the hippocampal formation next time. And then finally, we'll say a few things about the amygdala and ventral striatum before we go on to the dorsal stream. striatum.