

Making Your Mind: Molecules, Motion, and Memory
Lecture 2 – Building Brains: The Molecular Logic of Neural Circuits
Thomas M. Jessel, Ph.D.

1. Start of Lecture 2 (0:15)

[ANNOUNCER:] From the Howard Hughes Medical Institute. The 2008 Holiday Lectures on Science. This year's lectures, "Making Your Mind: Molecules, Motion, and Memory," will be given by Dr. Eric Kandel, Howard Hughes Medical Institute investigator at Columbia University, and Dr. Thomas Jessell, Howard Hughes Medical Institute investigator also at Columbia University. The second lecture is titled "Building Brains: The Molecular Logic of Neural Circuits." And now to introduce our program, the Vice President for Grants and Special Programs of the Howard Hughes Medical Institute, Dr. Peter Bruns.

2. Welcome by HHMI Vice President Dr. Peter Bruns (1:10)

[DR. BRUNS:] Thanks for joining us for this year's Holiday Lectures on neuroscience. Before we get to the next lecture, let me make a brief commercial message here. Our speakers are wonderful and the lectures are exciting but they can't cover everything, so let me draw you attention to a website, one of our websites that helps supplement some of this. It's called www.biointeractive.org, biointeractive.org. if you go there you'll find streaming videos of all the Holiday Lectures and animations and click and learn activities, and podcasts. That's biointeractive.org. Our next speaker, Tom Jessell, is interested in how nervous systems function and here he's going to talk about such things as the ability to move which is called motor control, and he recognizes that critical to this is how the right connections are made between neurons and their partner cells. Tom, just as Eric, has been showered with a variety of prizes. This year he won the Kavli Prize in Neuroscience which is a new global prize, again representing the very peak of this science. In his lecture he will describe how the nervous system develops and how the intricate and complex connections are made within the nervous system. So before the lecture here is a brief video introducing Tom.

3. Profile of Dr. Thomas Jessell (2:51)

[DR. JESSELL:] One of the remarkable things about doing neuroscience at Columbia, and I've been here for almost 25 years now, is to observe the way that the science community has changed over that period. One of the enjoyable things about being in a neuroscience community like Columbia is that one is surrounded by colleagues who are thinking about very similar problems but approaching them in completely different ways in completely different systems, and who you can observe how those problems are being approached at many different levels and that becomes important in shaping the way that one designs and thinks about the nature of the problems you want to approach. Neuroscience is a much broader discipline than many others because it builds on traditions in psychology and in a clinical sense psychiatry and neurology but at the same time combines biophysics and now molecular biology. So when one talks about neuroscience as a discipline it covers many, many different approaches and philosophies, and these lectures I think provide a rare opportunity to convey both the intrigue and the excitement about a particular field of science, and arguably there is no aspect of modern science that is more enigmatic but more challenging and exciting than understanding how brain function works, the nature of cognitive processes, and so what I would hope is that through a selected set of examples one can convey some of that enthusiasm and at the same time try to explain how advances in the last decade or two really have transformed the way that we not only think about some of these problems but can begin to approach them at a practical level.

4. Assembly of neural circuits and behavior (4:46)

So good morning. Peter thank you for that introduction although after Eric's lecture I feel I hardly needed any introduction, at least visually. So I'd just like to begin by saying that it really is a special thrill to be able to be here today to join you in these Holiday Lectures on brain science. I think, I hope you'll agree with me that Eric in his first lecture gave a really remarkable, wonderful introduction to modern theories of mind and the way in which different regions of the brain control our thoughts, our memories, and our actions. I'm going to continue, pick up on some of these themes but in two slightly different ways. I'm going to look at the brain at a higher focus and I'm going to move backwards in time and ask about the origins of mind and the building blocks of behavior. In this lecture I'm going to try and introduce to you the way that nerve cells assemble themselves into interconnected networks that we call neural circuits, show how the workings of those circuits and the precision with which those circuits are assembled really define the repertoire of animal and human behaviors, and in the way, on the way I'll try and give you a glimpse of the way in which errors in this developmental assembly line underlie certain brain disorders of cognition, of function.

5. Human development from egg to adult (6:18)

But to begin let's get to the heart of the problem and really focus on the mystery of brain development. So all of the remarkable features of the brain that Eric introduced to you really have rather humble origins in initially a single cell but then a small group of cells soon after fertilization. And with the passage of hours and days this small undistinguished group of cells acquires the shape and form of an early human embryo and then with the months and years this embryo becomes what we see here, an inquisitive and a diffident child. And I wonder if any of you can hazard guesses as to the later fate and identity of this young boy. Anybody want to hazard a guess? It becomes no other than our Holiday Lecturer Eric Kandel. And so what I would like to try and do is really understand the origin of Eric Kandel's laugh, the essence of Kandelness. So this is the behaving Kandelian brain here. And what we want to do as developmental neurobiologists is to try and understand not only Eric's behavior but all aspects of animal and human behavior.

6. Comparing the brain to a cell phone (7:37)

And so to some extent what developmental neurobiology tries to do is to make this analogy perhaps between the brain and this familiar object, the cellphone. So both of them, you admire their performance, you come to rely on them, sometimes they even govern your lives, but do you really know how they work? So perhaps what one needs to do is to disassemble the cellphone or the brain into its component parts, and if we can identify those component parts and understand their individual functions and the way that they're assembled then perhaps that would give us a better insight in the way that not only this evil machine but also the brain operates. So that's what I want to try and do to, for you in the next few moments. And so

7. Zooming in on circuits and single neurons (8:30)

what we need to do is to move not just from the sulci and gyri that exist within the brain but look in greater detail at the component parts. So what we're doing here, what I'd like to do here, is begin to dissect the brain and take a region of the cortex that Eric introduced to you as the sort of seat of cognitive function, and then look at higher magnification, place it under a microscope as the great neuroanatomists of the past century did, and examine what one sees at this higher level of resolution, and what you see is shown on this slide here which is a neural circuit. So we're looking at the cortex and there are many thousands of neurons each occupying its own position, it have differences in cell shape, but it's this interconnections of thousands of neurons that presumably control all of the behaviors that Eric introduced to you. But the level of a circuit itself is not enough. We need to understand the

properties of individual nerve cells or neurons within those circuits. So we're going to focus now on one individual nerve cell which is shown in the left in brown, and look at this in slightly higher magnification. So you can see that the nerve cell is a highly specialized cell type. It's not like a heart cell or a liver cell. It has many processes as well as a cell body, so we can see here the cell body which contains a nucleus in the genetic information, and then many processes radiating from it. So moving up from the cell body is one of many dendrites. The dendrites are long processes that serve as the receptive end of the neuron, receive information that imposes on that neuron from other nerve cell types, and then you can also see that one of these processes has a different name, the axon. That is the means of output of the nerve cell or communication. And so the axons seek out other neurons with which to form points of communication, and those points of communication are known as synapses.

8. Synapses are the points of communication between neurons (10:30)

And so here at higher magnification is the dendrite of the cell that we're looking at in brown receiving a synaptic input from another nerve cell in this circuit here. So synapses are the points of communication and information transfer in the nervous system, and I will talk today about how they develop, and then in Eric's next lecture tomorrow he will indicate some of the secrets of synapses. They have another altered secret life which he'll reveal to you. So there are many things that one can say about synapses but the first thing that is important to note is that there are a lot of them in your brain, in fact in each of your brains there are some 10 to the fifteenth synaptic connections. So that is way more than the number of stars in our galaxy, albeit not as many as the grains of sand in the Sahara Desert. But it gives you some sense of the scale and the number of connections that are driving each of your behaviors. But the synapse is not like a grain of sand which sits there inertly. The synapse is a small compact means of communication, and in order to try and indicate how information transfer flows across synapses I thought it might be useful to see these electrical impulses moving between neurons in circuits across synapses in something resembling... it's not real time but it's a slowed-down view but nevertheless it gives you some indication. So if we could have the first animation.

9. Animation: Molecular mechanism of synaptic function (11:58)

We're going to be looking at these electrical impulses, these flashes, which are called action potentials, are crossing three neurons in a circuit here and as we look in higher power you can see it crosses the synapse and moves onto the next neuron in the circuit, crosses another synapse, finally activates the next neuron in this chain. And if we begin to focus at slower speeds and at higher magnification we're going to focus on the information transfer at that single synapse. And so there is the dendrite on the right, the synaptic terminal on the left, and as the impulse information comes down, these little green disks are channels in the synaptic terminal that open. They let calcium ions into the nerve terminal. Calcium ions then activate small packets of neurotransmitter which release their contents into the gap of this synapse. Some of them diffuse away but others activate receptors on target neurons. They open yet further channels which let sodium ions in and so this information transfer propagates from one neuron to the next along these connections.

10. How do neurons differentiate during development? (11:07)

So in order to understand how behavior depends on circuits, depends on information transfer in synapses, we need to approach these problems from a developmental perspective at three different levels. First how neurons become different. There are vast numbers of neurons in their brain, there are probably at least 5,000 different neuronal classes. How do those neurons acquire their distinct identities? And then, how those identities have been established, and neuron knows how to project its axon over long distances to find the vicinity of its potential target cell, and finally once it reaches the vicinity of its target cell, how it forms a synaptic connection. So let's start at the beginning and first of all ask the

question of how neurons in different regions of the brain in fact become different and Eric gave you a wonderful classical history of the idea of regional localization. That history is evident at very early embryonic stages. So on the left-hand side here we're looking at an embryo perhaps at an early stage of neural development and what this small group of cells has to transform itself into is the brain that we saw functioning from many of Eric's descriptions here. And again this phase of transition from embryonic nervous system to adult brain is one of enormous change, their cells are proliferating to give rise to all of the neurons of the brain, the shape of the central nervous system is changing and again perhaps the best way of visualizing this is actually to look again in accelerated time at this process of cell division and shape change during the central nervous system formation. And we're going to see that now in this developing brain animation.

11. Animation: Development of the human embryonic brain (14:56)

So we're introducing ourselves into this process about one month after fertilization when the entire embryo is smaller than the size of a dime. And we're going to look in at the nervous system where relatively small numbers of cells have been allocated and then monitor over the nine months of human gestation the increase in the number of cells and the change in the shape of the central nervous system. So by three months we have something like a million cells, the nervous system is beginning to exceed the size of the dime. You can see that the future forebrain and the cortical region are expanding disproportionately to the rest of the nervous system. By six months we have hundreds of millions of neurons and the brain is beginning to take now a familiar shape. You can see the beginnings of the indentations, the sulci and gyri that Eric described, so that by the time we approach the eight and nine months this is now a familiar picture and we have tens of billions of nerve cells present. After birth there's yet further addition of cells to produce the 100 billion or so nerve cells that occupy the human brain, and one remarkable feature is that even in adult life new neurons are being added, and there is evidence that the rate of new neuronal production depends on the degree of richness of your environment. So every time you come to the Howard Hughes Medical Institute headquarters here you're going to be producing neurons at a remarkable rate and you will leave with more neurons than you came in with, and so this process of neuronal generation is really, drives all aspects of behavior.

12. Specific gene control early patterns of brain development (16:36)

Now during this developmental process not only are you producing more cells but cells are acquiring particular identities that we saw in the mature brain from Eric's work. So the brain at this stage becomes regionalized so this is a very schematic view on the right-hand side of the different regions of the brain that Eric described. Many of those regions are apparent even at this early embryonic stage. And here they're coded in convenient colors, blue for the future cortical region, red for the spinal cord region, and we know that these regions are different not just because of this simple color coding but because genes make these regions different. And so for example if we focus in on that blue region, the future cortical region, we can recognize this even at an early stage of development by the fact that that region and no other region expresses a gene which we are visualizing in this blue stain here on the right-hand side. So this is a gene that defines early cortical identity, and there are equivalent genes that will define other regions, the hindbrain, the midbrain, the spinal cord. These genes, I'm showing you one here called *Foxg1*, fall into a class of proteins that are termed transcription factors, and these are genes that define many early aspects of development and you may ask what in fact is a transcription factor. So this shown here is a protein as you're looking on the left-hand side that binds to specific target DNA sequences in regions called promoters and by binding to those target sequences in genes, activates programs of gene expression. So in a sense the early regionalization of the central nervous system reflects the differential expression of these transcription factors which begin to make cells different in a way that anticipates the regional functions of the brain. So one of the early puzzles of neural development really is to understand how the brain acquires these regional specializations. And so this question in a sense is "how the brain

gets its stripes," because here for convenience I've changed the complicated shape of the early embryonic nervous system and depicted it as a single, simple tube where at the front end you have the cortical region in blue, at the back end the spinal cord in red, and so how do the early neural cells acquire these identities which we can recognize by transcription factors.

13. A young neuron's location determines its ultimate identity (19:10)

Now the very earliest stages of neural development these identities don't exist, the neural tube is, if you like, a *tabula rasa* where all of the cells are equivalent. And what we now understand is that the way in which these cells begin to acquire their differences is by signals that are provided to the early nervous system from other cell types in the developing embryo and so this just depicts that idea that the initial pattern, front-end to back-end is established by signaling molecules, secreted protein factors and these factors act at different concentration thresholds. They establish a gradient so in this case the factors are a specialized set of proteins called Wnt proteins so cells at the front-end of the nervous system are exposed to low concentrations, cells at the back-end of the future nervous system are exposed to high concentrations. And in this way cells respond to these concentration thresholds of secreted factors by expressing different transcription factor domains. So the general problem of early development can be reduced to this simple question of how cells convert their position into their eventual identity which will drive the organization of circuits, and because this is such a core essential feature of early brain patterning I want to look into it in a little more detail and ask how this mechanism of signaling operates because we see it operating again and again throughout neural development. And so we need to understand how cells really interpret the graded signals to which they're exposed.

14. Animation: Signal molecules trigger transcription factors (20:49)

So let's look for example just at three cells here. They're equivalent in potential. They could give rise to any region of the nervous system but by chance they occupy different positions and that position exposes them to different concentrations of this graded signaling molecule. So you can see three cells exposed to three different concentrations of this signaling factor. These factors interact with cells by binding to receptors on the cell surface, and then by virtue of the amount of signal you activate receptors to different amounts. And then as a consequence of that you initiate the activation of a first set of these transcription factors that are sitting in the cell waiting for the signal. So these cells, these factors are present but they need to be activated either by addition of a phosphate group or clipping off a bit of the protein, and so as a function of the concentration of signal, you activate different levels of these transcription factors, the purple triangle shown here. And these are proteins then that, once activated, will seek out target DNA sequences with which to bind. And we see at this point that there's a difference between these three cells measured in the different concentration of transcription factor. So the cell on the left has a low concentration that is sufficient only to bind to gene A whereas the cell on the right has higher concentration and can activate genes A, B, and C. And as a consequence these cells begin to acquire different profiles of gene expression in the way that we saw. And these target genes by and large are themselves transcription factors so this is a cascade of signal to first transcription factor to second transcription factor that then, these genes really start to mediate the process of establishing cell differences. And finally through their secondary actions cells acquire in this case different colors but different identities.

15. Studying simple motor circuits in the spinal cord (22:53)

And so this core principle accounts for the diversity of neurons you see, or cell types. We're seeing it initially in the regionalization of the brain and are now begin to focus in a little bit more detail on one

of these regions to ask how cells within a region, let's say the spinal cord because we're going to use that as a canonical core circuit which controls movement, and ask how the neurons in the spinal cord really become different, now that we've seen how the spinal cord acquires this identity distinct from the cortex. So we need to take a closer look into the spinal cord and ask about the neurons that are generated in response to this early step of regional specification. So I want to introduce to you a simple motor circuit that we're going to talk about through much of the rest of this lecture and through my lecture tomorrow, and introduce some of the key neuronal players. And so what we're looking at here are sets of neurons that are generated within the spinal cord that control movement because if one thinks about it, movement is in a sense the overt expression of all aspects of behavior, so understanding the circuits that control movement will be critical for understanding the expression of behavior. No neuron is more important in movement than a cell called the motor neuron which is the cell shown in yellow here which is generated in the spinal cord, sends its process, its axon shown in yellow to innervate target muscles and we'll see in the second half of this talk that process of target muscle activation for real. But there are also other classes of neurons shown in different shades of blue, red, and green which are interneurons and these are neurons that form local circuits that control motor output.

16. Motor neuron type derives from position in the neural tube (24:41)

So in order to understand this developmental program we need to understand not just how the spinal cord becomes different but how these neurons within the spinal cord acquire their identity. And when one looks at this image you can't see any obvious pattern in the organization of these cells but if we move back a little bit earlier in development and ask where each of these neuronal cell types originates from we see something quite remarkable, that is, that each of these neuronal classes is generated from a fixed position along the top to bottom or dorso-ventral axis of the neural tube. So motor neurons shown in yellow always appear at this relatively ventral position wedged in between the green and the red interneurons.

17. Sonic hedgehog (SHH): An important signaling molecule (25:28)

So we see this rainbow-like pattern of neuronal generation that looks not dissimilar to the pattern of regionalization that we saw earlier to divide the nervous system into its regions and because of that you might expect that this process of neuronal specification might also be established by graded signaling molecules and that turns out to be true that this pattern of the neuronal cell types in the ventral spinal cord is controlled by another graded signaling system which is shown in this diagram here. So this blue circle has Shh written on it, so the signaling system here has a strange name, it's called Sonic hedgehog signaling. So you might ask why a gene that controls many aspects as you'll see of nervous system patterning acquire such a strange name. and there's a long history to that but the recent account is that a scientist at Harvard, Clifford Tabin, when he discovered with others this gene decided to call it after Sonic the Hedgehog which had videogame notoriety at that stage. So what I would just say to you is that, is there a better reason for becoming interested in neuroscience and maybe even becoming a neuroscientist than the possibility of discovering a gene that encodes some important brain function, and naming it after your favorite videogame. So I will leave Sonic here in a reclining state just to remind you of the excitement of gene discovery in brain science. So Sonic hedgehog controls all of these neurons and it does it in an interesting way through this gradient, so these red cells require high concentrations of Sonic hedgehog signaling while the blue V0 cells require a low concentration of Sonic signaling. So this gradient acting at concentration thresholds makes the difference between these neuronal types.

18. Eye position depends on SHH signaling (27:25)

Remarkably this graded patterning signaling system is true not just in the spinal cord but throughout the central nervous system. Sonic hedgehog controls the pattern of half the cell types in the central nervous

system. So if we look in this image we're now showing in blue on the right this stripe of Sonic hedgehog that you saw and if you take a section through the level of the forebrain region which contains the neurons that are going to give rise to the retina which control vision in the eye you can see that the position of the eye field is furthest away from this Sonic hedgehog gradient, whereas other cell types are much nearer to the Sonic hedgehog gradient. So you might imagine that given this graded signaling system that defines a different concentration threshold, twofold differences in concentration, that this signaling pathway would be extremely sensitive to perturbation. If you get the concentration wrong then you would have a consequence for differences in cell fate,

19. Demonstration: SHH concentration and eye position (28:25)

and I just want to give you with a demonstration if you like, a little demonstration how one might think about changing the Sonic hedgehog signaling concentration and then I'll talk about the consequences of that. So here we're looking at this same image where the eye field is generated in a hedgehog-dependent way but far from the source of hedgehog here. So imagine what might happen if we start to strip away the concentration of Sonic hedgehog for some reason or another, then the cells that are most dependent, that need the highest concentration, would disappear first and as you gradually decrease the Sonic signaling concentration those neurons that are generated at more ventral positions would disappear and there would be a progressive shift in the position of cells generated at more dorsal regions and eventually the limiting extreme of this would be that in the virtual absence of Sonic hedgehog signaling you would lose perhaps all but the most dorsally-generated cell types, which in the forebrain is the eye field. And so this raises the question, are there conditions in which these concentration-dependent hedgehog signaling pathways really are affected and do they have an influence on brain organization and development?

20. Blocking SHH signaling can create a cyclops (29:47)

And I will show you just one example that this is in fact the case from a set of animal studies but then I'll talk briefly about human genetics that argues the same thing is true. So this slide may be a little bit shocking but it's... perhaps I won't leave it up for long, but this is what we're going to be looking at, is an example of cattle or sheep in the wild who eat a certain plant, the corn lily or hellebore which contains a Veratrum alkaloid, a chemical which blocks hedgehog signaling through its receptor and as a consequence what happens is most of the ventral cell types, so the progeny of the animals, the newborn lambs or calves from parents that have eaten those plants show this sort of defect. They show this cyclopia here. So on the right we're looking at a single midline eye in much the same way that I showed you on this panel here. So this is an example in the animal kingdom of blockade of hedgehog signaling leading to brain patterning abnormalities. Now one also sees this unfortunately in human cases, where there is not ingestion of an alkaloid from a plant but genetic lesions in the hedgehog signaling pathway, and those patients suffer from a syndrome called holoprosencephaly which again is associated with a progressive loss of neurons from these ventral regions as a consequence of the blockade of hedgehog signaling. So in this way what I've tried to do in the first part of this lecture is give you a sense of how one goes from a uniform population of cells, begins to diversify those cells, to make the 5,000 or so cell types, neuronal types that we see in the human brain. And after the break we'll discuss how those neurons continue the process of forming connections with their target cells.

21. Q&A: Do signal molecules have to be present throughout cell life? (31:37)

But I'll stop here and be happy to take questions on this part. So there's a question over in the back there.

[STUDENT:] I was wondering when you were talking about signal molecules you mentioned that they influence the transcription factors that regulate gene expression, correct? So does that mean, like, signal molecules have to be present throughout the cell life?

[DR. JESSELL:] Yes, so this, that's a good question of how long does the signaling molecule have to act? How long does a cell have to be exposed? So what we understand is that there can be an initial brief period of exposure, so the early history, like with you, your early history determines in a large part what you are. The early exposure of a cell to a signaling molecule sets in place this molecular program that is mediated through transcription factors, and then the cell weans itself away from its dependence on that signaling molecule, because many of those signaling transcription factors activate downstream target genes which consolidate their identity, so in just in the same way that your character as a mature individual is no longer dependent on your early experiences, but it's shaped in that way. So cells behave in the same way.

22. Q&A: Can cell type be changed after initial signaling? (32:52)

[STUDENT:] After... you were saying how the cell kind of weans itself from the gene signaling concentration, is it possible to alter the cell type after which by changing the concentration of the gene signaling?

[DR. JESSELL:] Yes so that's a good question so it's again, can you in a sense do an end run around development and change the fate of cells that have gone through some aspect of their normal developmental program? And by and large the answer is no, that if you're a neuron it's extremely difficult to persuade that neuron to become an alternative cell fate but the answer may not be an absolute no and one of the interesting aspects of stem cell biology is the idea that perhaps you can take cells from one tissue or organ by understanding their developmental program, redirect them at a later stage to a different cell type or tissue type. So this is an area of active research at the moment and I think the answer will depend exactly on which cell and at which time you really study that question.

23. Q&A: How do cells secrete the correct amount of signal? (33:59)

[STUDENT:] I have a question. When proteins are secreted by other cells, so, how did these secreting cells know what, like, the correct amount of protein to secrete so that you get the correct grading?

[DR. JESSELL:] Yes, so this is a great question. So we are talking about the nervous system which is really a comparatively late aspect of the entire developmental program. If one thinks about it the entire central nervous system comes from a single cell. So at the point that we've intervened in this developmental program there's a long developmental history. So other signals have made the source of the cells that secrete the graded signals know that they're supposed to secrete graded signals so there's... the simplest question you can ask is how one cell, a fertilized egg, becomes asymmetrical but gives rise to two different progeny daughter cells. And to some extent the nervous system is only intervening halfway through that developmental program. So these are great questions but unfortunately we have to move on.

24. Demonstration: The electrical activity of Dr. Jessell's arm (34:58)

And so what I would like to do now is continue this developmental story and don't, don't be worried here. So what I'm going to try and do is really focus on this question of neuronal identity having been established, how do we now start to innervate the target cells? And we're going to stick with the motor system for a moment and consider the motor neurons that innervate their target muscles, and that's what you're looking at in this image here, and so the job of a motor neuron is to extend an axon out of the

central nervous system to find particular target muscles — not all muscles are the same, some flex, some extend the limb — find the right muscle targets, and then form a functional synaptic connection. So I just want to give you an indication of those synaptic connections between motor neurons and muscle in action. And so, unbeknownst to you before this lecture started I was wired up with electrodes, so, to monitor the state of muscle contraction. So these are surface electrodes which are monitoring the electrical potentials on my biceps and triceps muscle, a process called electromyography. And so what they do is record the ability of motor neurons to activate muscles in a coordinated manner, to flex and extend when Eric is playing tennis, or when you simply take a step as we'll see in the next lecture. So let's see if we can actually demonstrate nerve-muscle connectivity in action. I hope what you can see is that the amplitude of the discharge here is... we're looking at these electrical signals in muscle as I first flex my bicep, triceps, biceps, triceps, this is not just pre-recorded, this is real physiology in action here, because I can alter the rate of these contractions and activations or I can maintain biceps activation, probably not for as long as some of you, but at least for a period of time. So here in this simple demonstration we can record the endpoint of the developmental program that I'm now going to discuss further with you.

25. Neurons use growth cones to find partner cells (37:13)

So you've just seen motor neuron muscle target in action, and if one thinks about it, what you were watching is a neuron in the spinal cord that was extending down my arm and could have been controlling my fingers there, so a distance of almost a meter, or a neuron in the cortex that Eric described going down and activating a motor neuron in the spinal cord. So these cells are tiny but they have to send their axons over long distances to reach their targets, and so that is a considerable challenge for a neuron, unlike a heart cell which sits next to its neighbor. So how do neurons do this? And the way that they do this is that they've invented a specialized sensory and motor apparatus that sits at the end of the growing axon and that is called a growth cone. So here on this diagram we're looking at a neuron and the tip of that axon has this elongated expanded structure of the growth cone, and you can see the shaft of the axon is shown in red but that the growth cone itself, this spread region, can be marked by small spikes or we call them filopodia which are filled with actin filaments. So this structure serves both as sensory apparatus and a motor, a motor function. It actually permits the axon to extend.

26. Video: Growth cones in action (38:25)

And I just want to give you some idea of both initially its sensory and then its motor functions in a set of videos. So the first video was prepared by a scientist, Paul Forscher, which just indicates that unlike this spiky appearance this is a subtle and ruffling set of behaviors and so we're looking at two growth cones here contacting each other and you can see in almost real time, slightly speeded up, the way in which these filopodia are palpating the environment, seeking out in this case each other's growth cones.

27. Neuronal pathways are like a subway system (38:59)

So in this case we can see here that these are highly dynamic regions at the end of axons. So these regions have a motor function to guide neurons from the site in which they're generated to their eventual target. So this is a process called neuronal pathfinding and really is a remarkable journey and just one way of sort of illustrating this is, how many of you actually arrived at Howard Hughes today by using the D.C. Metro system? A good number, that's great for public transport. And how many actually started out at the Greenbelt Metro Station? Is there anybody here? Yes, excellent. Over there. So to get here you would have had to travel along the Green Line into central D.C., change direction onto the Red Line, not get distracted by all of these other side routes or tracks, and finally know where to terminate your journey at Bethesda and arrive at the Howard Hughes Medical Institute. This is exactly on a smaller scale what growth cones and axons do,

28. Video: Long-distance neuronal path-finding (40:04)

so the next animation or video we're going to see is in fact two axons from the eye coursing across the brain to find their eventual visual system targets, and you can see the growth cones, this is from work by Christine Holt and Bill Harris. And you can see they're leaving a trail of an axon in their wake. Eventually these two growth cones get their wires crossed but undeterred by that they continue to grow in precise direction towards their eventual targets here.

29. Neuronal path-finding by attraction and repulsion (40:36)

So here is a depiction of these two neurons and that behavior that you've just seen raises the question of, are they navigating to their target by some internal compass or global positioning system or are they dependent on the cues that they find in their environment: "no entry" signs, "obligate right turn" signs, and what neuroscience has shown us over the last 10 years or so is that most aspects of axon path finding and neural guidance depend on signals that are acting on the growth cone as they undertake this migratory path. And those signals as we'll see come in two forms, they're sort of lures or they're deterrents so we can call them carrots and sticks if you like. And so these two neurons are really guided in a positive way by attractive cues and those attractive cues can act locally, so the neuron on the top is crawling along a highly desirable substrate to get to its path, and the neuron underneath is attracted by again a long-distance attractive cue secreted by distant target cells. So one set of cues that guide axons to their targets are positive in nature, the second set are repellent or inhibitory in nature. So these same two neurons are influenced by the desire to keep away from that block of red cells, which provides a repellent factor and to move as far away as possible from this source of long-distance repulsion. And these different types of cues operate for all neurons on all stages of their trajectory, and so this becomes complicated, and to simplify it I'm just going to focus on one class of cues and give you a sense of how these cues operate during the guidance of one set of axons.

30. Video: Contact repulsion of a growth cone (42:26)

So I want to demonstrate contact repulsion, the ability of one set of cells to repel the growth cone of a growing axon. And again we're going to see this in one of the Jonathan Raper's videos. So the cell at the top has the growth cone you can see, and it's approaching this innocent-looking axon that lies in its path, and you can see again the growth cone is a highly motile structure, it's sampling, it contacts the axon and then you can see the growth cone collapses, and further back in the axon, we'll see this again just so you can see it, is a re-attempt to grow in a new direction as a consequence of contacting that one axon, the axon provided a repellent signal that caused the growth cone to collapse, essentially a no-entry zone. It learned not to go in that direction because its entire morphology was collapsed by contact, and it starts again off in a different route. So this contact repellent system is important in many aspects of axon guidance within the developing nervous system.

31. Repellent cues and motor neuron path-finding (43:31)

And since we're focusing on the motor system I just want to give you one example of the way that these repellent cues guide motor neurons to the muscle targets whose activity we saw demonstrated for you. So we're now looking at a cross section of the spinal cord with two sets of motor neurons growing out into the developing limb, one set labeled in green, the other in red, and as they approach the early limb they're forced to make a decision. They can't continue to go straight and they have to decide whether to grow up as the green ones do dorsally or down ventrally as the red axons do. And this is important in innervating flexor and extensor muscles with precision, and the picture on the right is just a real section through the spinal cord demonstrating the branching of these axons. So a repulsive system very similar

to the one that I showed you in that growth cone video establishes the precision of these axon pathfinding choices in a way that I'll try and demonstrate for you now.

32. Animation: Repellant ephrin signals guide limb intervention (44:31)

So these are the two sets of axons approaching the base of the limb, and the upper dorsal region of the limb contains one of these repellents, it's called Ephrin B but the name doesn't really matter. What's important is that it will repel motor axons and growth cones that express the receptor EphB whereas ventrally there is a different repellent, Ephrin A, which is capable of binding to its EphA receptor. So in work that Arthur Kania and Ed Lauffer have performed this repellent system which is mirror-symmetric ensures the growth of these axons into the right halves of the limb. So let's see how this happens. So initially axons are growing along, they reach the base of the limb, and initially the red axons will encounter the dorsal mesenchyme, the dorsal cells in the limb. As a... and the green axons will encounter the ventral source of Ephrin A signaling. This is a productive engagement that leads to the collapse of the growth cone in just the same way that we saw, and so no matter how many times the red axon tries to innervate the dorsal half of the limb, that growth cone will be collapsed and it won't be able to grow any further. If by chance the red axon samples the ventral half of the limb and the green axon the dorsal, then that productive receptor engagement doesn't occur, there's no collapse and so the axons are free to pursue this path in a perfectly unimpaired way. So in this way this repellent signaling system ensures the initial pattern of motor innervation of the limb.

33. Finding the right partner in a very crowded brain (46:06)

This is a simple binary choice, grow up or down. But you can imagine that once these axons have chosen up or down they're faced with further sets of cues that then define their trajectory in progressively more complicated ways, and so the picture you're seeing on the left are the secondary choices that are also guided and influenced by these environmental signals. So in this way, motor axons arrive at the vicinity of their muscle targets and are capable of innervating the target to produce coordinated movement. So this is a relatively simple system for establishing connectivity. If we think about what happens in the central nervous system with many more neurons and many more potential targets, this problem of how synapses form becomes much more challenging, and so we're now looking, for example, you're an arriving axon and growth cone arriving in this cortical circuit, you just have to choose one or a very small subset of the thousands of potential neurons. So this is analogous in a sense to this issue of, there's a large crowd, you have to find a face in the crowd because you have important information to communicate with one of these individuals. This is actually a typical picture around Columbia of people trying to get into one of Eric Kandel's lectures, with the doors closed but nevertheless it presents the problem here. You have important information and you don't want to give it to the wrong person, so you have to find one individual in that crowd. This is analogous to the problem that a growing axon has as it finds its target.

34. Binary choices produce specific neuronal connections (47:40)

But what we'll see is this complicated problem can again be reduced by focusing on detail, on simple binary yes-or-no choices in many cases. So for example consider this issue of two neurons. The neuron in light blue has to connect with target cell A and it's important that the neuron in dark blue doesn't connect with cell B so you have both to establish connections with the right targets and ensure that you avoid connections with the wrong targets. The little that we know about this system at a molecular level in the mammalian nervous system, say that we use many of the same principles that we saw with axon guidance: positive cues and negative cues conspiring together. So there are examples where the positive connectivity depends on adhesion molecules and the negative, the absence of connections depends on repellent molecules.

35. Gene knock-outs reveal importance of repulsive signals (48:35)

And I just want to give you one very brief final example coming back to the motor circuit, this time considering not the motor neuron itself but the sensory neurons that provide important feedback information to motor neurons that controls movement. How do those sensory neurons form selective connections with their motor neuron targets? And so this is a diagram now of a more complete aspect of the circuit, where the sensory neurons are shown in blue, the motor neurons in green, and what's interesting is that the light blue neuron has to form a connection with its light green target motor neurons, but it's equally important that the dark blue neurons fail to form connections with that set of dark green motor neurons. And it turns out that a repellent signaling system ensures that the dark blue sensory axons fail to connect with the dark green motor neurons, and this is work from my colleague Silvia Arber. There's a different class of repellent, it's called a semaphorin. The name doesn't matter. But from this diagram on the right you can imagine that you could change the pattern of connectivity by changing the expression of that semaphorin which is only expressed by the dark green neurons. So let's use the power of modern mouse genetics either to inactivate the semaphorin gene, remove it from the dark green neurons or instead express it in the light green motor neurons and see the consequences for connectivity. And I'll just schematize that here. So when we knock out the sema gene, shown by that red X under the CM, now those neurons are perfectly capable of receiving synaptic input from those dark blue corresponding sensory neurons, and in a converse way when we introduce the repellent into the other set of neurons we prevent those connections from forming. So in this way we've seen that repellent molecules guide not only motor neuron muscle connections but sensory feedback connections onto the motor neurons themselves, and build to construct this circuit.

36. Some synapses are stabilized and others eliminated (50:38)

So this defines the target initially but is this the end of the story? Well, there are further developmental events that have to occur in order to produce a functioning refined circuit. It turns out that many additional things have to happen after initial synaptic contact. So we've seen how target recognition ensures the connection between a growth cone and the target cell. There are two fates of those initial contacts. Some of them can wither away and die and fail to be maintained, in a process called synapse elimination, and remarkably about half the synapses that initially form in your brain fail to be maintained and disappear over subsequent developmental time. The remaining half mature to form the synapses that you've now seen structurally and functionally. And so this suggests that there must be many additional mechanisms that take that initial contact and ensure a stable functional connection. And we now know something about the genes and the proteins that act at this last stage in defining mature patterns of connections.

37. Synaptic stabilization proteins implicated in autism (51:46)

If we focus in on the synapse in a little bit more detail then we can see that two of those proteins are these sticks shown in blue and orange, a protein called neuroligin that is found in the target side of the synapse and a protein neurexin that is found on the synaptic terminal. And they are important in maintaining many connections in the developing brain. These two proteins are important not just because of their developmental role but we now know that these two genes can be affected in a neurodevelopmental disorder called autism. So if you have a mutation in the neuroligin gene or in the neurexin gene or in other proteins involved in synaptic maturation you acquire this developmental disorder and as many of you know I think autism is a dramatic disorder that affects young kids, their interpersonal relationships, their ability to perform tasks without becoming repetitive, and the prevalence of autism has increased dramatically in your lifetime. So this indicates that genes that control synapse formation not only produce large developmental defects of the sort that we saw in the first half

of the talk, but also these more subtle effects that influence cognitive function and many more sophisticated aspects of behavior.

38. Summary (53:10)

So I'm just going to finish by reinforcing and reminding you of some of the aspects of neural development that are trying to explain the behavioral functions that Eric introduced to you. We now know that genes, neurons, and circuits really function as the building blocks of behavior. We've seen how a relatively small number of signaling molecules produces the vast diversity of neurons. We've seen how axons form connections with targets and form synaptic connections, and this is really only the beginning of trying to understand this mystery of development.

39. Q&A: What causes synaptic elimination? (53:43)

But I will stop there and would be happy to take a few quick questions.

[STUDENT:] What causes synaptic elimination?

[DR. JESSELL:] Yes, so this is a great question that I didn't have time to talk about. So, one of the interesting aspects of elimination is that the activity of the synapse itself in relation to its neighbors helps to determine whether a synapse is maintained or eliminated, so if a synapse is used in a coherent and perhaps functionally important way, that synapse will tend to be maintained. So as you saw from the animation, synapses are firing all of the time, so the pattern of activity influences whether a given synapse or its neighbor will be maintained or eliminated. So there's many aspects of neuroscience are probing this problem to find, try and establish the link between activity and gene expression.

40. Q&A: Connection between synapse activity and intelligence? (54:43)

We should move on to another...

[STUDENT:] Is there, like, a direct connection between synapse activity and overall intelligence?

[DR. JESSELL:] We as humans have more synapses than a simple marine mollusk like an *Aplysia*, which Eric will talk to you about tomorrow, but I would be the last person to say in Eric's presence that we are really more intelligent. Eric began to address this. Organisms evolve to meet their particular niche, so if you look at the social behavior of an ant or a colony of ants, it's really a remarkable set of behaviors which is suited for that individual. So intelligence to some extent is in the eye of the beholder. Presumably you need large numbers of neurons. The reason the human cortex is so big is that many aspects of human function depend on large numbers of neurons. But it's a complicated issue and I think you can't relate it simply to individual synaptic activity.

41. Q&A: When does most axon formation occur in development? (55:43)

So I'm going to take one more question, yeah.

[STUDENT:] When does most of the axon formation occur in development?

[DR. JESSELL:] Yes, so different regions of the brain have their own developmental schedules, so the axons that connect motor neurons to muscles are established way before many of the connections in the cortex, perhaps because the motor system is so important for the survival of the animal early on. So

axons begin to be extended almost immediately the cell is generated, but that time will depend on different regions of the brain. We should unfortunately stop there. Thank you.

42. Closing remarks by HHMI Vice President Dr. Peter Bruns (56:29)

[DR. BRUNS:] So, thank you, Tom, for an amazing lecture. It's amazing how a series of simple cell interactions can lead and sum to such a complex nervous system. You have more questions, not only in this room but the people out there in web land and DVDs in the future? If you do, we have another website that you should know about. It's cleverly called Ask a Scientist, one word, askascientist.org. If you go there you can post a question and get an answer from our group of volunteer scientists who will, who will answer that and there are other things on there too. There's for instance a place, a link for resources such as homework help. So Ask a Scientist, an interesting website. So, come join us tomorrow when we will continue these discussions and talk about various aspects of neuroscience, including the complex choreography of movement and the molecular basis of memory. Thank you for joining us.