Brain and Mind
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The Assembly of Neural Circuits in the Developing Brain

Introduction by Gerald Fischbach

Gerald Fischbach: Thanks, Rod. One of the groups that Rod thanked was the National Institutes of Health, and I want to put in a plug for this, for everyone here, understanding the huge role that public funding for our research has played, and how now at a time of leveling-off of the funding I hope everyone in this room will realize the need to support the NIH. To support fundamental science in this country, and in the singularities of investigation like you just heard, basic research that will improve the health of this country. We should know about spiders and toxins if we're going to understand epilepsy and thought disorders and a number of others. So I want to add my thanks to the NIH.

Tom Jessell, I believe, is the leader in developmental neural science in this country and around the world today. He's been interested in motor neurons through much of his career, almost all of his career, and he has focused on the embryo much like Ramón y Cajal focused on the embryo to simplify things and to understand how neural circuits get assembled. It was thrilling a week ago to hear Tom take part in this symposium, which really originated with studies done in the '50s and '60s . . . fundamental questions of how one nerve cell recognizes another and forms a synapse on it. To see the culmination of this work in the molecular studies Tom will describe in synapse formation within the spinal cord. And you will see that his work has immediate implications for devastating diseases of motor neurons, including amyotrophic lateral sclerosis and spinal muscular atrophy. Tom also has a number of very well-deserved honors, being a member of the Royal Society of London, and as associate of the National Academy of Sciences. Very few people with foreign citizenship are elected to our national academy, and Tom is one of them. Tom?

Neural Circuits and Behavior

Thomas M. Jessell: What Gerry didn't mention is that I was a student of Dr. Fischbach 25 years ago, and if he was in a less modest mood he would tell you—and who's to deny it?—that he taught me everything I know. And it's a pleasure actually to be reunited with Gerry in his new position as dean as Columbia moves into an exciting new phase in the neurosciences.
So human beings, as with all other forms of animal life, display a remarkable capacity to interact with and respond to events in the world that surrounds them. These actions and reactions to a large extent underlie and define our behaviors. And the repertoire of behaviors that is exhibited by an individual organism, a single animal, is extraordinarily diverse. And some of these behaviors are relatively simple. The ability to move and to act in response to sensory stimuli, and others are more complicated and involve cognitive processes and influence mood, affect, and thought. But each of these behaviors, whether simple or complex, depend[s] on the functions of nervous systems, and in particular depend[s] on sets of neurons that form connections with each other, so-called neural circuits.

And so one of the challenges in neural science is to understand the relationship between the organization of neural circuits and the emergence of behavior. And over the last fifty years or so, in large part through clinical studies, we've come to understand that circuits in different regions of the brain are assigned to different specific behavioral functions. And one striking example of that was shown by Gerry in the use of deep-brain stimulation to affect motor and movement disorders. But there are many, many examples of this sort.

But despite this progress, I think it's reasonable to say that we have still a very poor and unsatisfying understanding of the relationship between the detailed workings of the neural circuit and the emergence of the behavior from that circuit. So in many ways one of the challenges that faces neural science today is shown in this first slide here. And so if [what] one is trying to do is establish the link between the properties of individual neurons, the way they assemble into circuits, those circuits are embedded in the brain to produce certain specific and idiosyncratic behaviors.

And in brief, if we understood how circuits in a sense accounted for the essence of Eric Kandel then the problem of neural science would be solved and we could all go home. But unfortunately we don't understand this link. And so one of the challenges is to try and make that link between circuitry and behavior. And there are many ways of approaching this problem, but being a reductionist what I'm going to try and do today in this morning's discussion is really make an argument that perhaps we can gain insights into the link between circuitry and behavior by studying how these circuits are assembled during embryonic and postnatal development.

If we understand the organization of circuits during development, perhaps we can understand and glean new principles about the way those circuits function in the mature state. And a better understanding of the structure of these circuits may give us insights into the way that behavior is reflected in changes in circuitry during experience, learning, and in disease states.
Assembly of Neural Circuits

So what do we know at the moment about the organization and assembly of neural circuits? Work over the last thirty to forty years has told us, I think, that the assembly of a circuit occurs in progressive stages with, perhaps, two major components. The first is simply by observation we've learned that circuits are assembled from embryo to embryo from organism to organism in a highly stereotyped reproducible fashion. Neurons don't grow out at random to innervate any number of potential targets, they begin to develop in a highly organized way. And I think this is interesting because it essentially makes an argument for a hardwired state of neural circuit formation. And the implication of that is that perhaps there are genes and genetic programs that underlie that hardwiring that leads to the first aspect of circuit formation.

But the final pattern of circuits that we see in the central nervous system, in the brain and spinal cord, is simply not controlled by these genetic hardwired programs, but then the experience, the environment that an organism [is] exposed to then, in an important way, refines and modifies those circuits. So one can think of development as first initially being a genetic program, which then gets later modified by experience and activity.

But what I'd like to do today is try and point out that in trying to understand the progression of circuit assembly perhaps the important key, at least at a reductionist level, is the idea that the driving force for circuit assembly is the assignment or the allocation of neurons with particular identities. Now neurons cherish their identities because what the identity of a neuron is doing in early stages of development is, for example, defining where those neurons are positioned within the brain, and by implication who their neighbors are. It's defining how neurons are capable of communicating with other classes of neurons through the process of extension of axons from the site of generation to distant target regions, and then neuronal identity also determines the formation of stable and meaningful interrelationships with other classes of nerve cells through the process of synapse formation that Gerry has indicated.

So the brain contains hundreds, perhaps thousands, of different classes of nerve cells, and probably an equivalent number of neural circuits. So in trying to approach the problem of the assembly of a circuit, one could choose, and people are choosing, many, many different regions to study this general issue. What I'd like to do this morning is to illustrate the way in which genes, neurons, and circuits might influence behavior by focusing on one particular set of behaviors, motor behaviors, the control of movement. Because if one thinks about it, motor systems, the control of movement, is in fact the manifestation, the expression of all aspects of animal behavior. Without movement, despite what you think, there's no way of expressing your behavior to others. And one particularly graphic way of bringing this point home deals within a clinical context: patients, for example, who've had cerebral infarcts or strokes who preserve mental and cognitive functions but have
no ability to communicate that information, their thoughts, with the outside world. And Jean Bauby some time ago wrote a very graphic description of what it feels like in this book *The Diving Bell and the Butterfly*, where after a stroke he was paralyzed and had the inability to communicate in any way other than moving his left eyelid. So this illustrates the importance of motor systems. And the central nervous system commands its motor systems through circuits that reside within the spinal cord.

And the spinal cord is an interesting region of the nervous system to study general problems of circuit formation for a couple of reasons: The first is that one of the big problems, how sensory information is transformed into motor output, is occurring in a relatively immediate way in the spinal cord compared to many other circuits that are found, for example, in the brain. But the spinal cord also gives one, in a sense, a nice reflection of this interplay between genes and experience in the control of circuitry. No one who has observed a newborn calf walking within minutes after birth I think could argue against the idea that there must be innate programs of circuitry that control movement in the absence of learned experience. On the other hand, the ability to play a musical instrument clearly indicates that the motor system is acquiring learned behaviors. So perhaps by studying circuits in the motor system one will begin to be able to get insights into this balance.

And within the spinal cord one class of nerve cells, the motor neurons, which Gerry has introduced to you, play an essential role in communicating the central nervous system's interaction with the periphery, with target muscles. And one of the motor functions performed by the spinal cord that is particularly evident is that of locomotion, the ability of animals to translocate from one position to another. And a somewhat fanciful view of motor control but which illustrates some of the points I want to refer to is shown in this slide from the Italian futurist Giacomo Balla, and so this is the dynamism of a dog on a leash. But what I want you to focus on is the coordinated limb movement that occurs in a conserved manner across mammalian evolution. And so in order to take a single step or to walk or to run, several things have to happen in terms of motor control, muscle coordination. So, first of all, individual muscles in the limb have to be activated in a precise choreographed manner in order to take even a single step, and then the activity of programs of the limb, motor programs, on the left and right sides of the body have to be coordinated in order to achieve this locomotive behavior.

**Diagram of a Core Motor Circuit**

So what I'd like to do this morning is really take you into the workings of the central nervous system from a developmental perspective and see to what extent genes can explain neuronal identity and how that drives the circuits that control motor behavior. And so in order to do this, we're going to look now at a diagrammatic view of a core motor circuit that resides within the developing and mature spinal cord. And that circuit is shown here. So now this is a cross-section through the spinal cord, through your spinal cord, through any vertebrate's spinal cord, and
what we can see here are the positions of motor neurons, which as Gerry showed are located in their cell bodies within the spinal cord and then they send axons out into the periphery over long distances to reach individual muscle targets. So some motor neurons are dedicated to the control of extensor muscles, other motor neurons are dedicated to the control of flexor muscles. But motor function cannot exist through motor neurons alone, there have to be important additional neuronal elements that control motor output, control motor behaviors. And I'm going to be discussing two classes of neurons that impinge and influence the activity of motor neurons.

So the first are sensory neurons, and the job of the sensory system is to convey the state of muscle contraction and relay that information from the periphery back into the central nervous system. So sensory neurons send axons, which monitor the state of muscle contraction, project via a second set of axons into the spinal cord, and in some cases this sensory motor transformation is remarkably direct through the formation of direct monosynaptic connections between sensory and motor neurons. And it's these connections that drive the so-called monosynaptic or knee-jerk reflex, which all of you can experience as the activity of this particular synapse in the central nervous system. So sensory feedback is important in fine motor control, but then in addition the phasic firing properties of motor neurons that are involved in the locomotive behaviors we've seen depend in addition on sets of interneurons that are actually buried close to motor neurons within the spinal cord. So there are sets of interneurons allocated to the distinction in firing of motor neurons that project to extensor and flexor, and also ensure the coordination of motor output patterns on the left and right side of the body.

So how, in a sense, does this circuit arise during development? So in order to approach this issue we have to go back from the details of the mature circuit and move back into embryonic development and begin to address how this circuit emerges. This picture is actually a real image of a developing mouse embryo about midway through gestation in which motor neurons located in the ventral spinal cord are shown in green and sensory neurons in red and blue. And the main point here is to illustrate that even at this early stage, many of the cardinal features of motor innervation of muscle have been established. Motor axons have emerged from the central nervous system, begun to project into different peripheral target sites, and the question of motor neuron identity can be inferred in part from looking at the different trajectories of the axons, these green processes here, of different sets of motor neurons.

So as we approach the problem of motor neuron identity how can one begin to organize in some practical form? And so here is a diagrammatic view of the spinal cord, which illustrates some of the challenges that motor neurons have to undertake in terms of acquiring identities that determine their target projections. So this is looking along the rostrocaudal axis of the spinal cord, the head-to-tail axis here, so some motor neurons are formed at limb levels, those neurons will
innervate muscles in the limb; others are formed at trunk, at thoracic levels, and will innervate different motor neuron targets.

So one can think about the problem of allocation of motor neuron identity in several subsequent steps, progressive steps. So first of all the developing embryo has to decide to make a motor neuron as opposed to the many hundreds of other classes of neurons that are found in the central nervous system. Because of those thousands of neurons it's only motor neurons that have the capacity to extend axons out of the central nervous system and communicate with the periphery. And then if we're trying to innervate limb muscles to control locomotion, motor neurons have to acquire a limb identity that ensures that they innervate the right peripheral targets. And then each individual muscle is controlled by one set of motor neurons, so-called motor neuron pools. And so we need to understand the identity of this fine degree of motor neuron diversity because that is the core unit, in a sense, of this circuit. Motor neuron pools innervate muscle targets. They receive selective sensory feedback information, and they also drive the specificity of interneuron connections.

**Signals Establishing Cell Identity**

So this is already looking rather complicated, but what I'd like to try and show you is that, in fact, one key developmental principle addresses or is relevant to each of these steps of neuronal diversification, of neuronal identity. And this is a development principle that applies not just within the nervous system but of the shaping of tissues and organs throughout developing embryos. And that molecular principle is shown in this slide here. And so this could be a neuron or a neural precursor cell, a neural stem cell, and this cell acquires its identity through a relatively simple molecular program. Its identity is influenced by signals to which it's exposed at early stages of development, often protein factors that change the fate of that cell, divert it from one potential fate to an alternative fate. And the way that these extracellular extrinsic signals signal to this neuron or this neural precursor cell is typically throughout transmembrane receptors, similar but importantly different from the sorts of transmembrane proteins that Rod has described to you. That signaling pathway is then transferred into the nucleus of that cell where it induces the expression of a set of genes called transcription factors, and these are proteins that have the capacity, once expressed, to bind to DNA elements with high specificity and to induce the expression of certain target genes. And these target genes in this context are the proteins that drive neuronal circuitry. So one can think of this developmental principle in two important steps, how extrinsic inductive signals control transcription factor expression, [and] how these transcription factors direct the downstream expression of proteins that control neuronal circuits. And so I'm going to try and illustrate how these two principles shape motor neuron projections, shape sensory innervation into motor neurons, and control interneuron diversity that influences motor output.
So the inductive signals, if we begin there, within the spinal cord, operate along two major axes within the developing embryo. So if this is the early spinal cord, you can see that the spinal cord is positioned within the embryo along two axes. One is the dorsoventral axis, the top-to-bottom axis here, and a second is an anterior-posterior, the head-to-tail axis. So the position of a motor neuron, in a sense, can be described by its position within these two coordinate axes. And different signaling factors, different inductive signals, contribute in a collective way to assigning motor neuron identity. And I’ll just illustrate what we currently understand about the way these inductive signals control motor neuron identity.

So the first set of pathways I’ll talk about along the dorsoventral axis, so here is a cross-section through the early spinal cord, the neural epithelium, and you can see individual neuroepithelial cells before they’ve made the decision to give rise to motor neurons. And at this stage, all neuroepithelial cells are essentially identical. And they acquire their different identities in part through their exposure to different environmental signals. And the key signal that controls motor neuron and different classes of interneuron identities is a secreted protein known as sonic hedgehog, which derives from these two ventral midline structures. It has this bizarre name because it has a counterpart in fly patterning in embryonic development, and fly geneticists have a propensity to naming the genes that affect developmental processes in rather more exotic ways than their mammalian geneticist counterparts. But nevertheless sonic hedgehog is secreted from these two cell types, and establishes a protein gradient within the neural epithelium so that the position of a cell determines its identity by virtue of exposure to this concentration gradient of extrinsic sonic hedgehog, ensuring that motor neurons generate in one position and different classes of ventral interneurons that contribute to the motor circuit are generated in adjacent spatial domains.

So in turn this sort of observation raises the issue of how a single cell can respond to a gradient of this extrinsic signal and acquire an all-or-none identity, because these cells make up their mind with unerring precision. So how is that step achieved? It turns out that, as I mentioned, the way in which this extrinsic signal establishes cell identity is by setting up spatial domains of transcription factor expression, and along this axis it turns out to be a set of homeodomain transcription factors. But the main point is that different dorsoventral domains have different molecular identities revealed by differences in transcription factor expression, and that an individual cell is forced to choose one or other of two alternate identities in a winner-take-all strategy because these transcription factors talk to each other within the cell, so one transcription factor cannot coexist in the presence of an alternative transcription factor through repressive interactions. These cells force an identity on a progenitor cell to give rise to a motor neuron as opposed to an interneuron.

So this gets you to a generic motor neuron state, but how do motor neurons, for example, destined to innervate the limb, acquire their particular limb level identity? And a very similar developmental principle operates along the dorsoventral axis to
the one that I've just described along the rostrocaudal axis. So here there is
another graded signaling factor. It turns out to be a member of the FGF class,
fibroblast growth factor class of secreted proteins, which establishes a gradient
along the anterior-posterior axis. That gradient is then read out, as Jeremy Dasen's
work in the lab—together with Serena Liu—has shown, in the patterned expression
of Hox proteins, another set of homeodomain transcription factors. So limb-level
motor neurons express one Hox gene, shown in green, thoracic level express a
different Hox protein. And again this intracellular fight between transcription factors
ensures a unique identity. So in this way inductive signals in the environment
gradually induce programs of transcription factor expression that lock cells into
particular neuronal, in this case motor neuron, states.

The other remarkable thing about this patterning mechanism is it is not the
particular consign of the spinal cord in a vertebrate embryo. The Hox genes were
first discovered for their ability to pattern the entire embryo in insects, such as a
*Drosophila* embryo here. And in fact the same is true for Hox genes within the
mammalian embryo. So it turns out that Hox genes influence not only where motor
neurons are generated but the position of limb formation, thus ensuring that the
right set of neurons and the right target are organized in register in the developing
embryo. So through this progressive set of interactions we can begin to see how
extrinsic signaling factors control transcription factor expression and gradually
allocate neurons with particular identities.

**Transcription Factors Controlling Innervation**

And the same is true when we move further along to the pool identity. It turns out
that different Hox genes correlate with particular motor neuron pools and that these
Hox genes then drive the expression of yet further classes of transcription factors.
So here we're now going to move from the first of these developmental principles
about [how] extrinsic signals control transcription factor expression, and now begin
to look at how these transcription factors that are allocated to particular motor
neuron pools in fact control later aspects of motor neuron development, the ability
of a set of motor neurons to innervate on target muscle and not another.

And one way of thinking about that is just to focus on what are the tasks of a single
set of motor neurons as they emerge from the spinal cord and project to their
muscle targets. And we can think about this in three sequential steps: First of all
motor neurons have to get out of the spinal cord, but that is not good enough, they
have to know to innervate one target muscle, an extensor muscle for example, and
ignore the ability to innervate a different functionally antagonistic muscle. So this is
a process of axon pathfinding and target selection. Then once the axon reaches
the vicinity of the muscle it has to know to branch and form effective synapses with
that target muscle. And then in addition motor neurons, although initially scattered
within the spinal cord, cluster together into so-called motor neuron pools, and that
the reason they cluster is that this allows motor neurons to communicate with each
other through ion channels of a family related but different to those that Rod
MacKinnon has talked to, but these ion channel communication steps ensure that all the motor neurons that project to a muscle fire in a phasic coordinated manner, which is important for locomotion.

So it turns out that these different transcriptional modules that have been defined in motor pools have different jobs. The process of axon pathfinding, reaching the muscle target, is the responsibility of one set of transcription factors, the Nkx6 proteins, as Natalia de Marco's work has shown, but that these later steps, the branching and innervation and the clustering of motor neurons into pools actually are the job of the ETS transcription factors. And just to illustrate how transcriptional identity can drive later aspects of circuitry, I'm going to focus on this one class of transcription factors of DNA binding proteins.

So first of all I'll just show you some evidence that the innervation and the clustering of neurons depend on these transcription factors, and we can get this evidence simply by eliminating through genetic means these transcription factors from mice and examining the development of motor axon projections. And so this is looking at the mice, which are either normal or mutant in a particular ETS gene, focusing on the branching. This is one muscle target. We've used a genetic trick to light up motor axons in green, and you can see a perfect innervation of both the proximal and distal regions of this muscle in the presence of this ETS transcription factor. In the absence of this gene what you can see is that vast regions of the muscle are deinnervated here. So it's this type of evidence that indicates that late aspects of innervation of muscle target depend on this set of transcription factors. It turns out that the same gene that is controlling muscle branching in the periphery is controlling the clustering of motor neurons in the spinal cord, as work by Silvia Arber and Jonathan Lin and Chris Henderson showed. So here is one of these so-called motor neuron pools, a tight cluster of motor neurons within the sea of motor neurons that innervates the limb. If you eliminate this one ETS gene then instead of being tightly clustered these motor neurons are now scattered at random throughout this set of motor neurons. So in this way then transcription factors not only define terminal branching but also the organization of neurons within a motor pool.

But these are transcription factors, these aren't the proteins that really [are] the workhorse that are controlling these developmental events. So presumably the transcription factors are, in turn, controlling cell-surface proteins that recognize the environment and mediate these different motor neuron behaviors. And in some cases we know the targets of these transcription factors that actually control these behavioral properties. So I'll just give you one example, which is how ETS transcription factors ensure motor neuron clustering. And it turns out that these transcription factors control a set of cell-surface recognition proteins called the cadherins. And so the cadherins, from work by many groups, has revealed that what these proteins, which are sitting in the cell surface membrane, like to do is bind cells together, and they bind cells through so-called homophilic, like-recognizing-like reaction. And what that means is if you have two groups of cells,
which express different cadherins on their cell surface, even though initially mixed, these two cell populations, through cadherin recognition, will sort into distinct clusters. And that is, in fact, what is going on in terms of the role of cadherins as we think in motor neuron sorting, so the selective expression of cadherins under the control of ETS proteins by one and not another set of motor neurons ensures that they cluster together to form discrete pools.

So that in this way genes, transcription factors that are regulated by environmental signals and control cell surface proteins, ensure that particular sets of motor neurons are organized within the central nervous system and innervate different target muscles in the periphery.

**From Individual Neurons to Circuits**

So how can this transcriptional logic move from the properties of an individual neuron to begin to reconstruct a circuit? So I want to begin to talk now, very briefly, about the role of sensory feedback information from primary sensory neurons. The job of these sensory neurons is to relay information from the periphery to innervate particular sets of motor neurons in the central nervous system. So what sensory neurons are doing is giving the motor system, if you like, a sense of place, it's telling the central nervous system about limb position. So whether you're Michael Jordan or Margot Fontaine the ability to control one's limbs in a precise pattern depends not only on the motor system but depends on sensory feedback information that is monitoring limb position and the state of muscle contraction.

So one of the remarkable things is that the transcription factors that I've described to you, the ETS proteins that assign motor neuron identity also are expressed by the sensory neurons that provide this feedback information. So that it turns out that not only are these genes, these red and yellow nuclei or red and green nuclei, here defining individual sets of motor neurons, but if we look in their position of the sensory cell bodies, they're defining sets of sensory neurons, so that introduces the idea that, in fact, elements of this circuit that are going to functionally communicate sensory and motor neurons are defined genetically by the matching expression of one set of transcription factors. And we know that these transcription factors are important not only for motor neurons for also for sensory neurons, because again if we use mouse genetics to eliminate these genes we find dramatic defects in the ability of sensory neurons to communicate with motor neurons. And I'll just show you one example from a mouse mutation in another of these ETS genes. So in diagrammatic form what the sensory neuron has to do is grow into the spinal cord, grow down into the ventral region, and find and form synaptic connections with motor neurons. Here are these sensory projections, and here is the synaptic potential recorded physiologically. If we get rid of this ETS gene, then the sensory neurons are still there. They can make it halfway into the spinal cord, but they completely fail to project into the vicinity of motor neurons, and not surprisingly in the absence of this contact there is no synaptic communication between these two cell types.
So this begins to suggest that transcription factors are good not only at controlling neuronal identity but beginning to build up aspects of circuit formation. The problem doesn't stop for the sensory neuron in just innervating the motor neuron, you remember it has to innervate the right type of motor neuron in order to produce functional output. And so what this system offers an opportunity to approach is the problem of synaptic specificity, why some sensory neurons innervate some motor neurons, ignoring others. And with new anatomical methods developed by Julia Kaltschmidt, we can actually see a motor neuron and can see the synaptic terminals in this region on a motor neuron. So we need to understand how this particular set of sensory synapses chooses this motor neuron, ignoring another motor neuron. And again these transcriptional cassettes that I've been describing may provide some insight into this problem. Because it turns out that not only are the ETS genes expressed by sensory and motor neurons, but the cadherin proteins, which we know are targets of the ETS genes in the motor system, are also expressed by the sensory neurons, and there is a correlation between gene expression by sensory and motor.

So this leads to the intriguing, although still untested, idea that perhaps recognition between a sensory axon terminal and the motor neuron dendrite that accounts for the functional specificity in the system is mediated by these cadherin homophilic recognition interactions. And so genetics should allow us to probe this circuitry in more detail.

**Interneurons and Control of Motor Output**

So this gets us from a sensory to a motor system, but now for the last few minutes what I'll move to is the important role of local circuit interneurons in driving the phasic firing patterns of motor neurons. And we can think of these spinal interneurons in two sets, those that are involved on one side of the spinal cord in controlling flex and extensor movements, and those that communicate information across the spinal cord, ensuring the left-right phasing of motor output. And one graphic way of illustrating how these interneurons control motor output comes from actually watching the activity of motor neurons in the spinal cord as an animal steps during a walking movement. And so what we're looking at now is a top-down view of the spinal cord where each of these colors is actually a cluster of motor neurons, the so-called motor neuron pool. And the colors indicate the state of activity, of firing of these motor neurons—blue, cold colors, no activity, warmer, red colors a high level of activity. This is a static image. What happens if an animal begins to walk, what happens to the firing patterns of these motor neurons? And what I think you can see here is that the firing patterns change dramatically but in an organized way. You can see that motor neurons on the left and right side are never firing in phase, and you can see that motor neurons at different levels of the spinal cord are also firing out of phase, which corresponds to the innervation of flexor and extensor motor neurons.
So can we use this information and begin to pick apart through genetics the ability of interneurons selectively to control, for example, left-right coordination or extensor-flexor coordination? And in studies performed together with Martyn Goulding's lab at the Salk Institute, we've taken advantage of the fact that in just the same way that motor neurons have a transcriptional identity, interneurons have a transcriptional identity. So mutating the genes that control interneuron identity should permit us in a way that is not easily possible through other routes to selectively inactivate or eliminate one of these sets of interneurons and examine the consequences for coordinated motor behavior. So there are two classes of interneurons, as I mentioned, the so-called V0 interneurons, which mediate communication across the spinal cord, and a different set involved in flexor-extensor. One of the remarkable things about this motor activity is it doesn't require sensory feedback, it doesn't require descending information, and you can isolate the spinal cord and see these patterns of phasic motor activity. So here we're recording from the ventral route the axons of motor neurons, a burst of action potentials, from a set of motor neurons that innervate a flexor muscle. And the important thing, if we concentrate on this burst is that, for example, on the right-hand side if we look on the left-hand side the bursting is perfectly out of phase. Similarly flexor and extensor bursting shows alternating phases. And this is completely in vitro, implying that these local circuits operate in the absence of sensory feedback information. So this is the normal situation.

What happens if, for example, we eliminate this set of interneurons through genetics, what happens to locomotive behavior? Then in the absence on these crossed interneurons what you find now is that the left-right normal phasing is completely degraded and now motor neurons on the left and right side of the body fire in bursting, so you've eliminated the left-right coordination while preserving the flexor-extensor coordination, which is presumably the function of this set of interneurons. So in this way we can begin to dissect out through genetics local circuits as well as sensory feedback as well as the motor innervation of muscles.

Unresolved Issues

So what I've tried to do is to indicate in this very simple system a core circuit that controls many aspects of motor behavior, involving motor neurons, sensory neurons, and local circuit interneurons. And at least from our perspective the key to understanding the development of this circuit goes back to the simple view that inductive signals control neuronal identity, neuronal identity is established by transcription factors, which bind to DNA targets and induce target proteins. And these target proteins, as I've mentioned, control neuronal position, axonal trajectory, the formation of synapses, and that collectively this is what constitutes a neural circuit. And these neural circuits, as we've seen, control simple motor behaviors.

But there are several unresolved issues, I think, that relate to this sort of even simple circuitry. First of all I've described a core circuitry that controls vertebrate
locomotion. But in fact if you look across vertebrate organisms, and there seems to be a consistency of showing slides of snakes in this symposium so far, what you can see is that different vertebrates have evolved different ways of using this core motor circuitry to suit their particular behavioral requirements. And it seems likely that these different adaptations of this core motor circuit are in fact innate, are genetically programmed, so one of the things that genetics and circuitry has to try and do is understand how you modify this core genetic circuit to produce the specialized behaviors that different vertebrate organisms display.

And the second challenge is to come back to this issue that so far what we've been talking about is solely this early hardwiring component, and upon that we have to understand how experience and how activity and how interaction with the outside world modifies this core circuitry.

So to end as we began with a slide from Balla, the hand of the violinist, here you can see that this fine degree of motor coordination is not acquired de novo, has to be learned. Where are the circuits that control that learning process? We know that some of those circuits exist within the central nervous system in the brain. Are there changes in circuitry in the spinal cord that also contribute to these learned motor behaviors? So there are many challenges, but nevertheless the hope is that by understanding the details of circuitry in this one system we can extract principles that are going to be relevant to the formation of circuits in many other regions of the brain that control much more complex diverse behaviors. And I think the next ten to twenty years is going to see an increasing ability to meet those challenges.

And I will stop there, but again just emphasize the people in my lab who've done much of the work that I've summarized here. The work on transcriptional identity and motor neurons is that of Jeremy Dasen and Serena Liu. Much of the work on motor axon outgrowth and synapse formation is from Natalia de Marco, [and] Ivo Lieberam. The work on sensory motor synapses is that of Julia Kaltschmidt. And the work on these ETS transcription factors and cadherins are that of Silvia Arber, Jonathan Lin, [and] Stephen Price. And the work on local interneuron control is that of Alessandra Pierani. And we've enjoyed many collaborations with other groups during the course of this program.

Thank you very much.

**Question and Answer**

I've been informed that there's time for a couple of questions, if people have burning issues.

[Question inaudible.]
Yes, I'm going to rephrase the question. Are there other proteins that might be involved in these developmental process related to motor neurons? And the two that were mentioned were particular proteins, neuregulin and agrin. What I've given you is really the tip of an iceberg here of a very complicated molecular program, so this is not to say that these are the only genes that are going to be controlling aspects of sensory motor development. And one of the tasks is to work out how this complicated array of genes fits together, and I think this is one of the things for the future.