

Episode #51: Interview with Seth Grant

SYNAPSE PROTEOMICS

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INTRODUCTION

GC: This is episode 51 of the **Brain Science Podcast**. In recent episodes we have been talking about brain evolution. We've talked about how the human brain is bigger and has more neurons than the brains of other animals. There are still things we don't understand about why we have intellectual abilities that other animals don't have. One of the things we do know is that as brains become more complex the number of synapses per neuron doesn't really seem to go up. However, researchers who are studying the proteins that make up receptors in the synapses are discovering evidence that these proteins become more complex. So now there is an hypothesis that synaptic complexity might be part of our ability to have more complex memory and learning.

Today I'm going to be talking with Seth Grant, who is the principle investigator for the Brains to Cognition (Correction: this should be [Genes to Cognition](#)) project at the Wellcome Trust Sanger Institute, which is at Cambridge University in England. Earlier this year Dr. Grant published a paper in Nature Neuroscience, which was featured in the New York Times, and caught the attention of several **Brain Science Podcast** listeners. They blogged about it and also they asked me to try to get Dr. Grant to come on the **Brain Science Podcast** as a guest. So I'm happy to have him on the show today. (...)

(Music)

INTERVIEW

GC: My guest today is Seth Grant from Cambridge University. Seth, I'm really glad to have you on the **Brain Science Podcast** today.

SG: It's very nice to be here Ginger, and I'm very happy to talk to you and your listeners about our research.

GC: Great. In June of 2008 you published a paper in Nature Neuroscience, which was featured in the New York Times, and caught the attention of several of my listeners.

Before we talk about that paper and why it is important, can you tell us a little bit about your background and how you came to be in your particular area of research?

SG: My own background was originally going to University of Sydney in Australia, where I started a medical degree, and after the first two years of my medical degree I had the opportunity to do one year full time in a research laboratory. I joined the laboratory of professor David Reid and studied the biology underneath the Sudden Infant Death Syndrome, and in that I was doing recordings of nerves that control the way breathing is regulated during development, and I became very interested in neurophysiology, and developmental processes, and the regulation of breathing. This was something that really caught my enthusiasm and I thought research was a fabulously interesting thing to be doing, but I went back to medical school, and after finishing medical school I had another research opportunity to work at Cold Spring Harbor Laboratory in New York, and in 1985 I went there to work with Douglas Hanahan, and with Douglas Hanahan I started work on transgenic mice in cancer research. And this was a very exciting period – it was the first time transgenic mice or genetically manipulated mice were being used or created, and it opened up an entirely new world of understanding the complex aspects of mammalian or vertebrate biology.

During the time I was doing that research, which really was a time at which cancer... molecular cancer research exploded, I thought, well, you could do this type of work and study the complexity of the nervous system and study fundamental processes such as learning and memory and try to work out what the molecules might be. That was something that there was very, very little known about in the late 1980's.

With this sort of idea in mind, Jim Watson, who was the director of Cold Spring Harbor lab at the time, was incredibly supportive and enthusiastic about doing this research, and he introduced me to Dr. Eric Kandel who was at Columbia University, and Eric invited me to come and work in his laboratory, which I did, in 1989. And a couple years after that we published some of the first examples of mice that had been genetically modified, and were found to show abnormalities in learning and memory, and abnormalities in fundamental properties of the synapses, which are the junctions between nerve cells. And so this really opened up the ability to discover the genetic or molecular basis of higher mental functions such as learning and memory. And so this was really how I got into learning and memory research and I've been working on it ever since, and the most recent work is on the evolution of the synapse, and the mechanisms of learning and memory.

GC: So you've really come to this with a good perspective on why it's important. We've covered quite a bit of Dr. Kandel's work on earlier shows – in fact I just met him last week at the Society for Neuroscience meeting, very briefly, but I managed to get him to agree to come on my show next year so I'm excited about that.

So, before we talk about your paper, could you talk a little bit about the difference in connections between the terms, “genetics,” “genomics,” and “proteomics”?

SG: Yes, these are all very trendy terms these days, and I’ll just take you through each one of those in turn.

Genetics is the study of gene function, or the function of the biology as revealed by genes, and typically involves the study of cells or animals where there has been a mutation or an abnormality introduced into a gene, and as a result of that, the function of the cell or the animal has changed. And of course, the readers will understand this, but a mutation in a gene effectively means a change in the DNA sequence that encodes that gene.

Genomics is a different thing. Genomics is the study of the organization of all of the DNA, or the genome. And of course the genome encodes roughly 20,000 genes in mammalian systems. Therefore, when one is studying genomics of man or mouse we’re studying *all* of the genes – typically in genetics you might only study one gene at a time in many cases.

So that gives you a sense of the large-scale features of genomics and the somewhat small-scale features of genetics.

Proteomics is the study of the sets of proteins or all of the proteins that perform biological functions or are found in cells or tissues. Proteome is to proteins what genome is to genes. Again, proteome is dealing with large sets of molecules.

In our case we were particularly interested in the proteome, or all of the proteins found in synapses. But you might be interested in all of the proteome of red blood cells, in other words all of the proteins that are found in a red blood cell.

GC: Are there any other terms you think we might need to define before we talk about your paper a little bit?

SG: I think one of the key terms to describe is a neurobiological term, which I’m sure your readers will be very much introduced to which is simply the word, “synapse,” meaning “to clasp.” A **synapse** is the junction between nerve cells, and it’s really the most important unit within the nervous system. And I think it’s a key definition to understand.

GC: Another term that you used in your paper that I was a little uncertain of the meaning of was “orthologues.”

SG: Yes. “Orthologue” and “paralogue,” slightly unfortunate sort of technical words... but in many ways they’re really quite straightforward. An **orthologue** of a gene is the identity of a gene between two species.

We quite commonly give a name to a particular gene – sometimes it has a nice easy to remember name like *fin* or *sark* or something like that – sometimes it has an unfortunate sort of acronym, like the *dlg* or *psd95*, and these names that I just told you about would be a typical name of a gene that you might find in a mouse. Then I would say to you, “What is the orthologue of *sark* (in a mouse), what is its orthologue in humans?” and effectively what you’re asking there is can you show me the DNA sequence in the human genome that corresponds to the *sark* gene in the mouse genome?” In other words it’s really just the same gene in the other species.

The other term was paralogue. And **paralogue** is a related gene in the same family, sort of like brothers and sisters. They have originated from the same sort of “parents,” in this case they’ve come from a common ancestral gene, but the paralogues are families of genes that are very, very similar in their structure.

GC: So understanding the orthologues is very important to tracing the evolutionary history of the synapse...

SG: Yes that’s right, and in general one of the most interesting aspects of studying molecular evolution has been simply to ask, if I know a gene that I would like to study the history of it, I would like to find the orthologues for it in other species. And by doing so you can ask questions about what other species have that gene or have that orthologue, which species do not. And by tracing back in time, into very ancient organisms, such as single-celled animals or insects for example, you might be able to find the point at which you can no longer *find* an orthologue. And then you can assume that that animal that is most far back in time or that has a furthest back in common ancestor, with an orthologue, is a point at which that gene actually arose.

GC: OK. So I think we’re ready to talk a little bit about your Nature Neuroscience paper. Do you want to just give us an overview of that and why it’s an important paper?

SG: Sure. Well, I think this is a really important paper and I might say that from somebody who has been studying synapses and cognition and behavior for quite a few years now, that this was really one of the most interesting, exciting projects we’ve done for a very long time.

But let me tell you why I think it was so nice to take it on as a project. It’s really because the synapse is the most important functional unit of the nervous system, and despite the fact that synapses have been described anatomically by Ramon y Cajal more than a century ago, and electrophysiologically by Sherrington and others since the early part of

the twentieth century, almost *nothing* is known about the evolution of synapses. Yes, they have been observed and found in the nervous systems of many, many animals – there's no question about that. That doesn't really tell you about the evolution of them, because simply, when you look under a microscope, you can say, oh look, there they are – they exist – but it doesn't tell you anything about the similarities or differences of them, and certainly not at the molecular level.

In the literature today you'll find very, very little on the evolution of synapses. Why would that be? Well the answer is, I think, really quite simple: nobody has had a good way to look at the problem. With the advent of proteomic methods, we came into the position of being able to take proteomic and genomic information, and using that, and I'll explain how we unravel that – to understand the evolutionary history of the synapse, and I'll tell you in this story why it's so exciting – because it has led us to a completely new insight into the origins of the nervous system. In other words, when and where did the brain first evolve? And it has also led us into a new insight, into how the brain, not only from where it has evolved and originated but how it has changed during that process of evolution and has led to some of the fundamental aspects of the complexity of mammalian nervous systems.

GC: So, how do you do proteomics in the synapse?

SG: Well, I'll tell you in a very simple sort of historical way, because then you'll see how really quite simple this all is. It is certainly quite straightforward, starting with nervous tissue, which can be obtained from nerve cells or brain tissue any number of different species. In this case we started working with mice.

It's possible to take a piece of brain tissue, and in a test tube, effectively, to isolate synapses as discrete sets of physical particles. In a solution that is containing these synapses, you can separate those away from all other tissues and axons and dendrites and glial cells and all these other parts of the nervous system.

And in the year 2000 we published a paper, where we isolated from synapses a large set of proteins which are bound together into a micro machine: it's a set of proteins bound to a neurotransmitter receptor. We found 77 different proteins all assembled together into this molecular machine. And we know this molecular machine, which happens to be called the NMDA receptor complex, or MAGUK associated signaling complex - all very unfortunate complicated names, but it's a micro-machine that sits within the synapse.

Now, we became very interested in this set of proteins in this micro-machine, and then we decided we would like to find all of the proteins embedded in the synapse. And we took the entire synapse, and took all of those proteins that were found in it, and we put them into a mass spectrometer, which is a device, which can fragment the proteins into small pieces, and measure the atomic weight of those small pieces. And by using DNA

sequence from genomes, you can identify with absolute certainty which gene made those protein fragments.

And so what I've really tried to tell you is that by using these instruments you can identify all of the proteins and all of the genes that make those proteins in the synapse.

We did that over the period of the year 2000 to 2005, and as a result we had a list, or a catalogue, of sets of proteins that make up the synapse. Now, I'll tell you how we go from there to evolution in just a second, but I'd just like to reflect on a very interesting point – in the year 2000 where my ex-collaborator and mentor Dr. Eric Kandel – that was the year he won the Nobel prize – and he received that Nobel prize for his important work on signaling in synapses - since the year 2000 the number of known proteins that have been found in synapses that have been found through the methods of proteomics as I've just described to you has increased the number of proteins more than 10 times. In other words, in the last few years, as a result of proteomics, there has been an enormous amount of discovery of new molecular constituents that make up synapses.

GC: So they're a lot more complicated than we ever imagined.

SG: *Far* more complicated than anybody ever imagined. And this leads into what I think will be the new and important area of neuroscience, which is the study of complexity at the level of molecules in the synapse and of course at the level of circuitry and anatomical complexity at the level of the whole brain. And what I'll tell you in this podcast a little later on, is that there appears to be a very interesting connection between the molecular complexity of synapses, and the anatomical complexity of big-brained mammals such as ourselves.

GC: Right, because we've been talking on previous episodes about the whole issue of our big brains. So, how do you go from these lists of proteins that you discovered to studying the evolution of the synapse?

SG: This is surprisingly straightforward, at least in the very first steps; all you need to do is to have a computer. And if you have a computer, you can log on to genome databases, and these genome databases consist of the full DNA sequence of many, many different organisms and species. And, since we're starting with the mouse, that is to say that the proteins that I've been describing to you were discovered in the synapses in the brains of mice, and we know that the genes that encode those proteins, a very simple question we can ask is this: for each of those proteins, in which species can you find the gene that makes those proteins?

And so, we looked at initially 19 different species. Of course, we had humans, and we had different primates within those, we had different rodents – apart from the mouse there

was the rat, but we also looked at birds, reptiles, and fish of various kinds, and all of those animals that I've just mentioned now are vertebrates, or chordates.

We also looked at invertebrates. And we looked at the genomes of fruit flies and worms, and bees, then we also, for reference, we looked at the genome of animals that don't even have a nervous system at all. And we used one of the most well studied animals, which is yeast, *Saccharomyces cerevisiae*. In other words we studied animals that are unicellular eukaryotes such as the yeast – and these are representative of the sort of ancestral or ancient unicellular animals. Then we studied simple metazoans, these various insects and marine animals and so on. And then we studied chordates and amongst those many mammals and indeed other vertebrates. And we simply asked, can we find these synapse genes in these different animals? And we could count them up.

The first, perhaps not surprising, finding was, if you look in all of the mammals, humans for example, and other mammals, they essentially have the same genes that make synapses as are found in the mouse. In other words, we've got the same synapses, or at least the synapses are made from the same sets of proteins amongst all mammals. This was also true across all of the different vertebrates. So all vertebrate species basically have the same molecular machinery for building synapses.

The first real surprise was that invertebrates have about half as many of these proteins. Now, you might ask, well why would you be so surprised about that? Well the reason we were surprised about that was, in the neurobiological field in synaptic biology, it is the dogma that synapses of invertebrates are the same as synapses of vertebrates. But what I've just told you is that there's roughly half of the number of synaptic proteins that are used to build invertebrate synapses than there are vertebrate synapses.

This is already telling us something very interesting in that it seems to imply therefore, that a lot of the proteins in mammalian synapses are really not necessary – they're some sort of vertebrate-evolved specializations. And I'll come back to that a little bit later on. But I'd just like to go back in time a little further if you'd like, back to unicellular animals and yeast – and this is something which is really quite surprising, and caught a lot of peoples' attention: 25% of the proteins that you can find in human synapses, you can find in animals that don't have any synapses at all – in unicellular animals.

In other words, synapse has its ancestral origins - the mammalian human synapse -has its ancestral origins, in an ancient set of proteins, which predate the first nervous system and are found in unicellular animals. We looked carefully at those proteins, and we asked ourselves, what is it that they do? What's so interesting about those proteins?

Well, this is really I think quite enlightening, because what we find is that those proteins that are found in unicellular animals are used by those animals in their response to their environment. Environment for unicellular animals, a change in nutrients, pH, stress and

other external stimuli, and the unicellular animal will respond and change its pattern of gene expression, its growth rate and other kinds of features. And that's precisely what these ancestral synapse proteins were involved with. In other words they're involved with behavior of this ancient animal.

So, this set of proteins that we find in this ancient unicellular animals are what we refer to as the proto-synapse or the set of molecules that predates or is the precursor to the kind of synapse that we all know and love, that is, the one between nerve cells, but actually the molecular machinery is truly very ancient.

I think that what this is telling us is that the very origins of the brain, the evolutionary origins of the brain, are not in animals like jelly fish and other very simple animals with a few neurons in a very simple brain, but the origin of the brain is much earlier than that – it is right back in unicellular animals, and that ancient molecular machinery was allowing that animal to make decisions and respond to its environment.

GC: Are you talking about the receptor proteins or also the signaling proteins?

SG: We're talking principally about the signaling proteins... let me just sort of break down a little for your listeners a bit about the sort of architecture of some of these post-synaptic proteins. In general when I'm referring to these proteins that are on the post-synaptic side of the synapse.

Now, just to be clear about what that is, synapses are at the end of an axon, and an action potential spike travels down that axon, causes release of neurotransmitter, and that place from which it is released is known as the pre-synaptic terminal. It releases the neurotransmitter onto the other side of the synapse, the post-synaptic side, and that's the neuron that is receiving the information.

Now, within that post-synaptic side of the synapse are many of these proteins that I'm talking to you about, those that were in the NMDA receptor complex, as well as those in the post-synaptic density. It was those that we characterized in our proteomic experiments.

But within that set of proteins you could think of it in a very, very simple and nice way: if you think of the surface of the membrane of that post-synaptic side of the synapse and you think of the receptor's membrane proteins that are embedded in that, therefore looking into the outside world, those are the most upstream proteins.

The ones that are just beneath and inside the synaptic terminal, down to those are intermediate proteins, and at another level downstream further again would be the very much downstream proteins.

So I hope I've sort of portrayed for you, you have upstream and downstream proteins in this post-synaptic architecture. Now the reason that I took some trouble to explain that to you is because these upstream and downstream proteins have a different evolutionary history.

It is these downstream proteins, very much the internal signaling proteins, the ones that ultimately control, say for example, protein synthesis or gene expression, which everybody knows are important in aspects of learning and memory. Those tend to be the most ancient proteins – they're, the ones very much found in yeast. The upstream proteins, these receptors and so on for which there's very few of them in yeast. But we discovered that those are the ones that have expanded very much both in invertebrates and expanded yet again to increase this number of orthologues that I was telling you about in the vertebrates. So there's a differential expansion, differential evolution of these particular synaptic proteins.

GC: Glutamate, which is a major excitatory neurotransmitter, was actually in existence way back in the days of the yeast?

SG: Not in the form that it is – the glutamate receptors as we know them in the mammalian systems have actually changed quite a bit during molecular evolution. And in the invertebrates we have fewer glutamate receptors than we do in vertebrates. Vertebrates have increased numbers of those. But also the structure of the receptors in the invertebrates is different. And in a separate study to the Nature Neuroscience paper, we characterized the differential structure of these invertebrate glutamate receptors, which turn out to be simpler and don't seem to have as many points of protein interaction within them. This is consistent with the discovery from the molecular evolution of the synapse, that there are fewer molecules for it to interact with.

And you'll recall I said earlier that there are the glutamate receptors such as the NMDA receptor are bound to other proteins as part of a molecular machine. Imagine sort of like the motorcar today with its sort of super sophisticated engine with its many hundreds if not thousands of component parts all assembled together into a unitary device – that would be something like a mammalian NMDA receptor complex. But the invertebrate version of that receptor complex has many fewer components, a bit like a motorcar from the 1930's, which has many fewer fancy bits and pieces. Fundamentally it's doing much the same job but it's not doing it with such sophistication, elegance or subtlety to it. So in that sense the glutamate receptors and the proteins are present but the details are really quite different.

But you even go back further into unicellular animals like yeast they do not have a glutamate receptor in the way that synapses have them, invertebrates and vertebrates. They have some of the components, like the ability of some proteins just to bind

glutamate, but they're not hooked up to the transmembrane proteins and other components in the same way that they are assembled in these other animals.

So again this points to the fact that the very specialized elements of synapses, they evolved most in the most recent animals.

GC: And you think that there's a relationship between these proteins and synaptic plasticity and learning?

SG: Yes, there's no doubt about that. In part of this analysis, what we asked was really a very simple question, because we now knew which genes had evolved in which organisms and when and where. There is an extensive knowledge over the last 20 years, again developed from much of this mouse genetic work that I referred to, whereby we can ask, is this gene involved with learning and memory in a mouse, through gene mutation? Or indeed in a human, is it involved with learning or memory or mental illnesses by virtue of being involved in a gene mutation? So we have a catalogue of genes involved with learning.

And if we have a catalogue of genes involved with learning from mammals, we can apply the same sort of evolutionary analysis to it, and we can ask our simple question to it, and say, "Well, when did genes involved with learning and memory evolve?" When did they first come about? And I can tell you that it's quite an interesting story. There are some genes that are involved with learning and memory in humans and in mice, and they're also involved with behavior of flies, and learning in flies, and they're also in behavioral responses back in yeast. In other words, the same gene is being conserved throughout evolution and its involved in behavioral responses in all kinds of different organisms.

And then there's other genes which are highly specialized for mammals, and invertebrates don't possess these genes, they happen to be what's called paralogues, and some of these genes are very, very important in mammals. I'll give you a nice example.

There is a gene which is on the x chromosome, and in males who have a mutation in that gene (and in humans this will be boys), they have particular kinds of learning disabilities or a kind of mental retardation. And this particular mutation is in a gene called SAP102 – it's a key component protein in this NMDA receptor complexes. And we've created mice with a gene mutation in that. We found that they too have learning deficits. But this gene is exclusive to vertebrates. Invertebrates don't have that gene and certainly yeast do not have that gene. In other words what I've told you is that there are some genes that are more recently evolved which are involved with learning and memory, and although I did not go into this, this particular gene I just told you about is very much involved with specialized aspects of learning and memory. It's involved with how animals choose the right strategy to solve a problem.

Strategy choice is a very, very fundamental aspect of behavior. When you're confronted with a problem as we are constantly throughout the day, we can choose different strategies to solve that problem. And it turns out that that SAP102 gene is a key gene involved with strategy utilization. So these genes have evolved, and we believe that they've given us behavioral complexity.

(Music)(...)

GC: So, the synaptic complexity that's seen in the mouse, is the human synapse more complex than the mouse?

SG: The difference between the mouse and the human is really a very interesting question, and it's something we're actively studying at the moment. It's too early to give you a definitive answer to your question. But I can address it at the level at which we addressed it within that paper we published in Nature Neuroscience. The genes make synapse proteins in the mouse - we have virtually the same set of genes in the human. It's really not significantly different, nowhere near as different as it would be between a mouse and say, a fruit fly.

So, just because we have the same genes doesn't mean that our synapses are necessarily the same. What we know is that the genes make the proteins. But we also know that it's the levels of the proteins in the synapses that are important. Let me try to explain that: Imagine now, in the mouse brain, there are two synapses that you are interested in. And I should tell you that there are billions of synapses in the mouse brain - I'm sure your readers know that. But consider two types of synapse: One might be in the cortex, and another one might be say in the hypothalamus. If you looked at the molecules that are found inside those synapses, they would not be the same. They would be of different levels. Let's say the NMDA receptor subunit number 2 might be found at very high levels in one of those synapses but very low levels in the other synapse. In other words, it's not just having the proteins that matter; it's the levels of proteins that matter.

So coming back to your question now, we're actually trying to study the synapses of humans and ask, do they contain the same proteins, and do they contain the same levels of those proteins? Now I can tell you that we do know directly by studying the proteome of human synapses that the general composition is really very, very similar to the mouse, but we think it's quite possible that there's quite a lot of fine tuning or subtlety to it which means that the synapses in the mouse will be somewhat different to those of the human.

GC: And you expect to find possibly differences in the synapses according to what region of the brain you look at?

SG: Most definitely.

Let me just return to that general question about the brain regions by telling you again about our synapse evolution story because so far what we've talked about is really what you would call the origins of synapses and the evolution of molecular complexity. And I'll just repeat that once again in a simple sort of way: the origins of the brain appear to be in a proto-synapse or ancient set of proteins found in unicellular animals.

When unicellular animals evolved into metazoans or multicellular animals that proto-synaptic architecture was co-opted and embellished by the connection of new proteins onto that ancient proto-synaptic set. And that set of new molecules was inserted into the junctions of the first neurons or the synapses between the first neurons in simple invertebrate animals. When invertebrates evolved into vertebrates around a billion years ago, there was a further addition or enhancement of the number of these synaptic molecules and that has been conserved throughout vertebrate evolution where they have much larger numbers of synaptic molecules.

Now here is the very interesting and initially sort of subtle point that we didn't appreciate, but one day it dawned upon us that the following must have happened: the expansion or evolution of synapse proteins between the invertebrates to the vertebrates - that occurred as a result of genomic events, creation of new genes, genome duplication - there was an expansion of the number of genes making synapse proteins.

Imagine now the earliest vertebrates and that evolutionary event of expanding the number of molecules occurring. That actually occurred in the small animals before any of these animals with big brains such as humans, primates, dinosaurs or other giant animals actually evolved.

So I'll just say that again: The large, complex synapses evolved before large anatomically complex brains.

GC: And this was in the early vertebrate...?

SG: Yes indeed. The very earliest chordates or earliest vertebrates would have been very small creatures, not unlike invertebrates, but they had had this large synaptic proteome machinery and all of the large-brained vertebrates, such as all those animals I mentioned a minute ago, they all evolved millions of years later. In other words, big synapses, molecular synapses, evolved before big brains.

So this raises the intriguing question, was the evolution of big synapses a pre-requisite, a necessity, or something absolutely required for big brains to evolve? And I have to say we simply don't know the answer to that question. But we can ask a different kind of question and say, could this big synaptic architecture be any in way relevant to the evolution of the large complex brains of all different mammals and including humans,

and I think the answer to that is most certainly yes, and that is part of that study that we published in June of this year.

GC: And this challenges the dogma that basically a synapse is a synapse is a synapse.

SG: Well at some level it certainly does but it doesn't take away the interest in the synapse. I like to think of it in a very simple way, and I would like to call it the "synapse first" hypothesis. Synapses I think have only become more important. What I just told you a moment ago is that first part of the brain to ever evolve was the proto-synapse. In other words, synapses came first.

GC: Right.

SG: Now what I've also told you is that before big brained vertebrates evolved, and I'd like to spend some time talking about those, and I'm sure in many other programs you talked about the evolution of the frontal cortex and all these different parts of the nervous system that characterize humans as interesting differences between primates. But big anatomical brains arose after the evolution of big synapses.

So synapses have led the way, not only in the origins of the brain but they've led the way in terms of the overall size and complexity of big-brained animals. And I think I should take a minute and explain to your listeners how we can make a connection between big molecular synapses and big brains. Shall I just take you through that?

GC: Sure.

SG: So, this is how it works: imagine now the following situation. We have two data sets. One data set is the list of all of the synapse proteins that you have in a mouse, and we also have which I've already told you, the evolutionary history of each one of those proteins, and we can tell which ones rose back in ancient single-celled animals and which ones arose in invertebrates and which ones arose in vertebrates. We have those three broad categories.

Now: put that to one side and I'll tell you about a completely different set of data and then I'll tell you about how we put the two of them together.

So this other set of data, is a set of data, which is data from looking at the expression or where you find these proteins in the brain, and we studied the mouse brain again because it's a good model system for studying mammalian brain, and we did something rather simple. We looked at the levels of those proteins in different types of nerve cells or neurons, in different regions of the brain, and we looked at many different regions of the brain, and in a very tedious study, we catalogued well over a hundred of those synapse

proteins, and we documented, if they're very high in this part of the brain in levels of expression and very low in another part of the brain, and so on.

So now we have these two data sets. I've told you we have the phylogeny or evolutionary history of the molecules, and we have the expression data or the levels of those proteins in the different parts of the brain. And we simply said this: is there any relationship between those two sets of data? And effectively we overlaid them, and did a statistical analysis.

And immediately a highly statistical and very strong result came out.

And it's really quite straightforward. It's as simple as this: the ancestral proteins that are found in unicellular animals are the proteins that are found in more or less all of the different synapses in the brain of the mouse. The most recently evolved proteins, the vertebrate proteins; those are the ones that are the most diverse in the brain regions of the mouse. So some of those proteins are very high, for example, in the frontal cortex, others might be high in the hippocampus; others might be high in the cerebellum. In other words, they're very variable like that.

So what that is telling us then, and I'm just returning now to this ancient vertebrate synapse that arose before big brains, it tells us that when this big synapse evolved, what the vertebrate brain then did as it grew bigger and evolved afterwards, is it exploited the new proteins that had evolved into making new types of neurons in new types of regions of the brain. In other words we would like to put forward the view that the synapse evolution has allowed brain specialization, regionalization, to occur. And we know from many, many studies that the regionalization of the brain, those parts of the brain involved in learning, those parts involved with fear, parts involved with some aspects of mood and so on, those parts involved with motor function; that all appears to be built on the template of molecular evolution of the synapse.

GC: What kind of professional feedback have you gotten about this?

SG: It's been remarkably well-received I have to say, and many people particularly outside of neuroscience, other professional scientists who might be experts at yeast biology, experts at signaling and so on... think that it's really quite fascinating because it has a simplifying effect on this extraordinary complexity of the nervous system – that's one point, but it also has an interesting effect on other people and I think to us too, and that is, it somehow makes sense. And I think it does make sense in many interesting ways, so I like to think that we may have shed a little bit of light on the very complex nervous system and how it evolved in different species particularly in mammals. I think it's quite striking to think that much of the answer to this can be found by inspecting the synapse.

(Music)

I think what we can see here is some general, not just the origin of the brain idea, but also how synapse evolution has led to neuron diversity and complexity – it opens up a lot of fascinating questions in all sorts of different directions and naturally, upon those sorts of questions experimentalists can address all sorts of different types of problems.

Maybe I'll just illustrate one or two for you. It points to something that I think is really very major, but unanswered question: How many different types of synapses are there? In a traditional kind of way, electrophysiological pharmacological way, you could easily categorize synapses in the brain into excitatory, or inhibitory, - that would be a very elementary kind of classification.

But you might then want to go to another extreme – you might want to say, well how many different types of synapses are there, if you categorize them by the different types of molecules that you can find in them?

Now we don't know the answer to this, but it's quite possible that in fact no two synapses in the brain are the same. There could be extraordinary diversity within the nervous system. Now I don't think it will be quite as diverse as that – but there might be very large numbers of different types of synapses, and the way they would all come about is by being built on combinations of these synaptic proteins, which have evolved.

So what I'm trying to tell you is that, again, returning to this sort of synapse protein evolution of complexity model, it is a beautiful way of generating diversity in types of synapses simply by using different combinations of those proteins.

And with a combinatorial model in mind, you can not only ask questions about what's different between two parts of the nervous system as we have already talked about, but you can say, well what's the difference between a mouse, or a rat, or a bird, or a monkey, or other sorts of species?

It may be that some of the most important behavioral features of those animals are not because of the size of its brain or how big its frontal cortex is, but it may be more of a reflection of the chemical properties of those different kinds of synapses.

(Music)

GC: I would like to ask you a couple of questions that one of my listeners, Diane Jacobs submitted, if it's OK with you.

SG: Of course.

GC: A question that Diane asked was, “Do you think that the single cell organisms with their ability to sense chemical gradients by means of receptors on their cell membranes, do they represent the first step toward the evolution of the nervous system?”

SG: Yes, I think that’s an excellent interesting point which also I think could and should be experimented upon. And what I think Diane has mentioned, is that the single-cell animal is capable of distinguishing different concentrations of some chemicals in its external environment. And that ability requires sensory transduction and signaling within the cell. I think there’s all likelihood that some of these proto-synaptic proteins will be contributors to that process, so I like to think of this sort of ancient molecular machinery as sort of decision-making machinery, decision-making in the sense that it allows a cell to make a choice or change its response in relationship to the environments affecting the whole organism. I could think of all sorts of nice ways to experiment on that particular thing – for example you could take different yeast with different mutations in some of these proto-synapse proteins and ask how well do they respond to the chemical gradients.

GC: Do you think that the old single cell membrane itself could have involuted to become the beginnings of the nervous system?

SG: Yeah. I don’t think it’s the shape of the membrane that matters so much; I think it’s the molecular constituents that are either in the membrane or just beneath it. And I think the molecular machinery, to which the receptors are bound to, form a molecular computer.

I haven’t talked about some ongoing research that we’ve been involved with but we’ve been trying to work out the molecular wiring diagram that’s in the synapse. In other words when you activate a receptor all of these other proteins that I told you about all these hundreds of proteins that are there – which ones get turned on and when, and what is the sort of circuitry of that?

We’re used to sort of thinking of the brain as a circuit of nerve cells, this one turns on this one and that one turns on the next one and so on – that’s all quite sort of easy for us to understand but it’s no less complex within the molecular world inside the synapse. It’s a highly elaborate super-sophisticated molecular computational device within it, and I think that’s the kind of machinery that one looks at in a very simplified form in yeast.

GC: So even the yeast, in a sense, makes choices.

SG: Absolutely. In a very sort of romantic sort of phase of mind I would like to say that maybe yeast can think. They are in a sense thinking, but they’re not having the same thoughts as us.. They do happen to be using some of the same molecular constituents as us though.

GC: I'd like to try to summarize what we've talked about so far to make sure that I have all the ideas straight in my mind. The major signaling components were present long before nerve cells evolved. Synaptic complexity evolved long before big brains evolved. And synaptic complexity is important to the evolution of more complex learning and memory. Is that accurate?

SG: All correct.

GC: And this challenge is as I said before the challenge is the current view that the key to more sophisticated thought is just having more neurons or more synapses.

SG: Right – let me just say a few words about this standard model of behavioral differences between species and the origin of the nervous system.

The standard model is that nervous systems first evolved when one could first see neurons.

And as I mentioned earlier that can be in simple kinds of invertebrates. They have neurons, there's nerve cells firing and they are connected together. And that would represent in many peoples' minds first brains. And then really the difference between different species is more in the number of nerve cells they have and perhaps the number of connections that make them.

And it's on that sort of foundation of neuron numbers and circuit size that we've also considered the differences – subtle differences really between different species, for example between chimpanzees and humans in the relative proportions of let's say frontal cortex and so on.

So, all of that kind of thinking which I think everybody is familiar with is really all based around the assumption that the fundamental unit is the neuron, and it's all about how many you've got. I have oversimplified that a little bit and I'm not trying to say that that isn't correct and that it's not a major component of differences between species.

But I do think that it's the case that the molecular machinery that is core to the synapse evolved before neurons, so we would like to sort of revise the thinking and say that the brain really did first evolve in unicellular animals. I realize it's a potentially controversial standpoint though nobody seems to have really argued with it so far. I think that's where the origin of the nervous system lies. And I think the point is that we've discovered that there is really major differences in key aspects between the major complexity in invertebrates and vertebrates, and therefore neurons are not all the same, certainly synapses are not all the same between these different animals and this really allows us to make the general statement that molecular synapse complexity is another yardstick by which differences between species can be measured. It's not just about neuron number.

GC: When you isolated the synapse proteins from the fruit fly your group was the first to do that is that correct?

SG: Yes, to the best of our knowledge, we were the first people to ever isolate the synapse protein from any invertebrate and we chose the fruit fly because it's such an outstanding model system.

GC: And then showing that its proteins are simpler than those of a vertebrate would be an important piece of evidence in your basic argument about the changing complexity of synapses as the animals evolve.

SG: Yes, it's quite an important experiment because as I mentioned earlier when we first looked at this difference between species we did so in an inferential way, because we looked at the DNA and the genes that make synapses, so it really was important to formally measure the proteins directly from the brains of the animals, and it very much was in line with our genome analysis. With these two lines of evidence it's unequivocal that there are major differences in the complexity between invertebrates and vertebrates.

GC: I just bring that up because one of the things that I like to emphasize on my show is just the importance of experimental data in making hypotheses and testing hypotheses, how important it is to test them.

SG: Yes, absolutely important to test them. I mean that's a fundamental basis for making scientific progress and I can assure you that in this story as I've mentioned one or two times that one of the most exciting things about being a scientist is when you attempt to test these things you come up with a result that is surprising and wasn't even one of the ones that you thought you might have attained. This happens a lot in science – I always get a little bit suspicious if everybody finds what they think they're going to find, because surprises are telling us that nature is more wonderful than we can imagine.

GC: What questions do you want to explore next?

SG: I am particularly interested in the following question: Where does behavior come from? And what is it that allows an animal to have the diverse repertoire of behaviors that it manifests?

Just to sort of shed some light on that view: in 1973, I think it was Tinbergen and Lorenz and others who got the Nobel Prize for their research on behavior of animals. Tinbergen and Lorenz described the ethogram, which is a catalogue of behaviors that an animal shows. And you can imagine now if you go out and look at a horse in a field that it does a variety of different actions and movements. And these different activities or responses that an animal shows could be catalogued in a naturalistic way. It's very interesting to

ask, how can it be that a mouse can have motor learning, or fear learning, it can be interested in exploring novel objects, it can do many different types of behaviors and have a repertoire of behaviors. You can then also say in humans and in primates we also have a repertoire of behaviors and actually some of that repertoire looks very similar to that of the mouse.

And I would like to know, from the point of view of the molecular organization of the synapse, how the repertoire of behaviors is built up for an animal, and in what way do the different molecules of the synapse control those different aspects of behavior. So that's one of the things I would like to work on.

GC: That sounds like a question for a whole career.

SG: (Laughter) Actually we hope to answer it by this time next year.

GC: Oh, then you'll have to come back on the show and tell us the answer.

SG: (Laughter) Let me tell you the second thing I want to talk about, a study in which we're working on.

GC: OK.

SG: And I do think this is really, really deeply important. And it is the fact that in this research that I've described to you on these many molecules in the synapse and how they originated and evolved, there are within that set of proteins, many genes and proteins that are intimately involved and causally involved with psychiatric and neurological diseases. And I'm sure your audience is well aware that the burden to the health system certainly in western nations, the biggest burden is on diseases of the nervous system, and a very large amount of those are a burden within these synaptic molecules.

So what I've been telling you about, about the evolution of the synapses is fascinating and interesting, but I want to tell you also that the approaches that we're using here also feed directly into our understanding of how genes and proteins involved with mental illnesses act and how those genes evolved in the first place and I think that this sort of fundamental research is quite possibly leading us into whole new ways to investigate and potentially derive new therapies and drug targets that are going to be important for those diseases. This area of research I talked about tonight is intimately linked into fundamental medical applications.

GC: Cause it would be nice if we could invent drugs that we actually knew how they worked instead of discovering that one works but not really having a good idea what it's really doing.

SG: It's not only nice, it's absolutely vital and essential – if any of your listeners has a member of their family who's had a major psychiatric illness or a neurological illness they will know how terrible these things are. And they'll also know how number 1, they're not curable, and even when they're treatable, the treatments are not very good.

GC: Yes. But your work offers hope that that can improve.

SG: Certainly, I'm confident that it not only offers hope but I can see ways through it – by studying these new molecular targets within these sets of evolved proteins how we can actually even develop assays for new types of medicines.

GC: I'm really glad that you came on the show tonight and I've really enjoyed talking with you.

SG: Well it's a pleasure Ginger and it's a pleasure to have the opportunity to explain this science to some of your listeners.

(Music)

GC: I'd like to thank Dr. Seth Grant from Cambridge for being on this episode of the **Brain Science Podcast** and I'd also like to thank listener Diane Jacobs for bringing his work to my attention.

This episode has been a little bit unusual in that we really got down into some of the basic science; however, I think that Dr. Grant is also very unusual in his ability to take a large view of his work and to think about the implications of his discoveries of what's going on down at the level of the synapse. There are a lot of people working on various things about how the synapse works but I don't know how many of them really are thinking about the evolution of the synapse, and what it implies with regards to the evolution of brains in general. And of course, some of what Dr. Grant talked about today is clearly speculative, but I think it's really exciting to talk to somebody who really is on the leading edge and who is willing to, shall we say, stick his neck out for his ideas. The excitement of science is not having all the answers – it's exploring the questions, and exploring the possible answers. It will be interesting to see in the next few years what happens with these ideas.

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Transcriber's note: Listeners of this episode on synapses may wish to review [Episode #8](#) on Neurotransmitters: also, a [transcript of Episode #8](#) is now available.

Closing announcements have been omitted from this transcript. For detailed [show notes](#) and links please go to <http://brainsciencepodcast.com/>.