2015 PAGET LECTURE: FOUR STORIES ABOUT UNDERSTANDINGTHE BRAIN - TRANSCRIPT

Lecture given by Professor Sir Colin Blakemore to an invited audience at the Understanding Animal Research annual Paget lecture / Openness awards event.

Can I say what a pleasure and honour it is to give this lecture. Stephen Paget founded the Research Defence Society, a courageous person who's been an inspiration to the many scientists and others who have at times suffered as a result of the criticism of animal research, but who on balance have made an enormously important contribution to our understanding of physiology and of medicine. It's a humbling honour to look at the list of previous lecturers; the first lecture was given in 1926 by Julian Huxley.

I want to talk about the question of the brain. I think whatever area of science you're in, whatever area, not just Biology, there is a wide recognition that the question of understanding the brain is a central issue for science. In fact an issue that raises quite deep questions about why and whether human beings have the capacity to understand everything that goes on around them. After all, the organ of understanding is our brain; it's an interesting philosophical conundrum to think whether we've been endowed with a device that has the capacity to understand itself.

So I'm going to consider the role of research on animals but not just research on animals, in the growing understanding of how the nervous system works. And to do it by telling four little stories about pieces of research, two of which I've been involved in, my lab has been involved in, two of which I haven't worked in but I think there are some interesting conclusions that come from these four little stories.

This is a view of the human brain; this is a photograph of the human brain. Some of you will recognise it; it's from Wilder Penfield's classic

studies during preliminary examination before surgery for epilepsy, trying to determine whether removal of the epileptic focus would cause catastrophic effects, particularly for language. And he did it by stimulating the surface of the cortex around the suspected position of the focus in conscious awake patients, asking them what they felt or listening to what they said or didn't say, looking at the twitches and movements that were produced and so on.

Now I would imagine that most of the audience are fairly physiologically sophisticated but for those of you who are not I should point out that the human brain doesn't come with little numbers of it. [Laughter] Wouldn't it be nice if it did, because a large part of our task is trying to find out what is done where in the human brain but actually much more interestingly how it's done.

Before I plunge off into nice stories about science, let's just set it in the context of the clinical need to understand better the nature of the brain and the disorders associated with it. A fairly recent estimate of the total economic burden of brain diseases, and I'm including psychiatric disorders of course in that, the total burden in Europe was estimated in 2011 as nearly 800 billion Euros. Many brain disorders, both psychiatric and neurological are of course age related. We have to keep in mind the demographics; 14 million people in the UK will be over what used to be called pension age by 2030. Even more so I think it's fair to say that no neurological or psychiatric disorder can currently be cured. Most quite frankly, clinicians in candid moments would admit are not really adequately treatable in the conventional sense and worst of all we don't even understand the pathological processes that underlie most neurological and psychiatric conditions. The pathogenesis is very poorly understood. So this is a huge challenge, both scientifically to understand what's going on in the normal brain, to understand the pathology that causes brains to go wrong and then to move on to developing more adequate treatments and cures. And just to establish the scale of the problem – you know some of the numbers

– the human brain has of the order of the same number of neurons as there are stars in our galaxy and when you consider that each of these neurons has on average 10,000 connections from other neurons, then the total number of connections – and it's of course connections that matter, the total number of connections, a thousand million million, is simply staggering. In fact since – that's 10^{14}, 10^{15}, since human life span is about 10^9 seconds it means that on average over the whole of our lifespan we are creating about a million neurons every second. And one of the most interesting discoveries in my lifetime in science is that that creation of new connections isn't all happening very early on, as was thought when I was a medical student 50 years ago, it continues through life. And one of the most interesting challenges is to understand how that property of adaptation, of change, of reorganisation, or plasticity, plays in both to normal function and in some cases to the development of disease.

So the four topics I'm going to talk about very briefly each, are these: development of the cerebral cortex, the vast folder mantle that seems to be primarily involved in doing the cognitive things, the high level things of perception and the consciousness and the decision making and the high level control and decision making about movement and so on; language; Huntingdon's disease, as one example of a neurodegenerative condition and also an example of an autosomal bonded genetic disorder and finally stroke: the commonest of all neurological conditions, responsible for an enormous burden of disease throughout the world.

So first of all development of the cerebral cortex. Well the cerebral cortex in human beings is vast, but it has grown as it were, through evolution gradually. There's been a progressive process and the general organisation and layout of the cerebral hemispheres, of the layers of the grey matter of the cortex are very similar in human beings and other mammalian species. This is a picture of, an 18th century picture actually, of a human brain and you'll know that it's divided into

lobes, the parietal lobe, the occipital lobe, the temporal lobe and frontal lobe. The general layout of those areas is similar in all mammals and moreover the disposition and function of major areas responsible for sensory processing and control of movement are very similar in their arrangement in mammals. The precentral gyrus is responsible for the control of movement, connecting directly to motor neurones in the spinal cord. And the body is laid out as you know, from the feet here, to the hands and face, lower down in the gyrus and running parallel with that is the region of the post central gyrus, which receives input from the body, from the tissues of the body, from the skin and the deep tissues of the body, laid out in the same topographic arrangement and to a large extent interconnected with the motor context. There's a visual area at the back, and an auditory area here at the top of the temporal lobe. Now that picture could have been drawn by a neurologist at the turn of the last century, 1900. This was broadly known from the effects of damage to the brain in human, the deficits produced by local damage, local damage stroke and so on in the human brain before any of the modern research involving micro-electrodes looking at the characteristics of individual neurons and how they function. So this much was known and moreover from comparative studies in animals it's clear that that general pattern of disposition of the sensory and motor areas was established right at the beginning of the mammalian line and conserved through the whole of mammalian evolution. So if you look for instance at let's say a hedgehog as a representative of early insectivores at the beginning of the placental mammalian line, the disposition of the somatic sensory, the touch areas here, the visual areas here at the back. This is the back, that's the front. And the auditory cortex, the green area. The basic arrangement of those is similar to what one finds in a cat or a sheep or a monkey and in a human being. But the sizes of course are not to scale here, but the human cortex is hugely disproportionately large compared with that let's say of a hedgehog. But what is clear is that a much larger fraction of the whole surface of the cortex is occupied by those basic sensory processing areas in a hedgehog than

in a human being. What's happened during evolution to a large extent has been the addition of this extra stuff, what – I was going to say 19th century neurologists would have called association cortex or even in some cases silent cortex. As if it was uncommitted in its functions and was somehow perhaps receiving signals from the committed areas and processing clever ways and perhaps responsible for thoughts and intelligence and those high level things.

Of course we know that in reality the rest of the cortex in higher mammals is filled with a mosaic of committed, computationally committed areas, many of them actually distinctive and recognisable by fine detail of their histology. Each probably responsible for processing a particular aspect of an incoming sensory signal or a particular aspect of an outgoing motor command.

Well if the human cortex has evolved progressively and gradually from some kind of early skeletal arrangement then there is hope that the conservation of the control mechanisms might mean that one can legitimately look at those mechanisms in lower animals, in lower mammals. And that has of course driven a great deal of research on the development of the cortex, because there is very little one can do in human beings to look at processes with the precision that modern techniques give in animals.

This is a mouse, this is a beautiful video made with optical projection tomography, a method developed in the human genetics unit in Edinburgh and it shows a mouse embryo at as you can see, 10 and a half days, post conceptual days and the embryo has been selectively stained with monochromal antibody staining to reveal two transcription factors, *Soc 6* and *Pac 6* which were expressed very early on in the development of the nervous system. And you can see that they're differentially expressed, very precisely differentially expressed within the nervous system, defining territories within which gene expression is being regulated differently, already partitioning up the brain into committed regions.

The general arrangement, the way in which the cerebral cortex develops its layers has been studied not only in rodents but in other species and there's every reason to believe that it's basically similar in human beings. The forebrain starts as a vesicle, telencephalic vesicle, the walls of which are made largely from stem cells, from neural precursor stem cells, which are proliferating rapidly, symmetrically proliferating, not yet producing neurons as the forebrain vesicle grows in size, the telencephalon grows in size. And suddenly at a crucial stage those stem cells start to produce differentiated postmitotic committed cells, some of which become neurons. They migrate upwards, here are the stem cells here at early ages in the so called ventricular zone, the wall of the telencephalon, which will become the forebrain and then they start to produce neurons, which migrate upwards. And the first of those, the earliest of those neurons, this is based on relatively recent work in mice and rats, the first of those neurons are not mature type neurons which are going to participate in later circuitry, they're a so called pre-plate, they're a transient population, many of them die and they probably largely play a role in organising the rest of the development. At a certain stage the stem cells, the same stem cells probably in many cases, start to produce other classes of neurons which are genuine cortical neurons, which form a kind of sandwich, they migrate upwards along the processes of the neural precursors, to take their place splitting the original pre-plate into two layers, the so called marginal zone, which becomes layer on of the mature cortex and then the sub-plate region below. And gradually these cells accumulate as more and more of them migrate, the later arriving ones moving up towards the top of the cortex in an inside out sequence and that forms the familiar six layers of the neuro cortex. Again, every reason to assume that's similar from the crude methods that had been applied in human beings until quite recently.

But a crucial question of course, in knowing how the brain works, is to know how connections are formed and for the cerebral cortex a crucial part of the connectivity is that which brings sensory information in to

those distinct specified regions, the sematic sensory cortex, the visual cortex and the auditory cortex. And it's known that in all mammals, including humans, that general topographic arrangement of those areas is determined by projections from different nuclei within the thalamus, the sub-cortical structure which has co-evolve with the cerebral cortex, to which the sense organs project. So here's the thalamus hidden down below the cerebral hemispheres and it consists of a number of nuclei receiving information from the ears, from the somatic sensory service, in this case the whiskers of this mouse, and from the eyes to different regions of the thalamus. And for each region of the thalamus there's a relay, a somatic relay and the thalamic cells then project up to the correct regions of the cortex. So each bit of the cortex, in the marmoset and in the hedgehog and in the human being, each bit of the cortex that's going to become a particular sensory area receives its sensory input from a particular area of the thalamus.

So how is that achieved? And I'll just describe very briefly some work that Zoltan Monna did in my lab starting many years ago, in which we asked questions about the possible molecular control of the process of ingrowth of fibres into the developing cortex from the thalamus. And we chose to approach that initially not by studying it in the whole embryonic brain but by trying to produce some in-vitro reduced preparation. And I'm glad to say that part of this research was funded by a foundation which supports research on the replacement of live animals. We were using tissue culture; tissue culture it must be said or fragments of neural tissue which of course were retrieved from living animals but the main part of the experiment was done in vitro.

What we wanted to do was to see whether we could produce a model of the way in which axons from the thalamus invade the embryonic cortex and then use that to define molecular mechanisms that were controlling that process. So we took samples of very early developing cerebral cortex, usually at around the time of birth in mice or rats - early experiments were in rats, and combined them in tissue culture, in

organotypic culture, with small fragments of the thalamus, with distinct regions of the thalamus taken either at birth or before birth. But we knew from the living animal that axons are growing in the cerebral cortex from the thalamus a few days before birth so we could look at the timing, the age, the effect of the age, of those different components and circuitry. What we found to our great pleasure was that fibres would grow from the thalamus into the cortex in these conditions and we could label the thalamic block with a carbocyanine influorescent dye and therefore look at the axons and here they are, influorescent microscopy growing into the slice of cortex, there's a slice of cortex lying in culture and growing in a manner that looks very similar to the normal ingrowth of fibres that you see in a living embryo.

But an interesting feature emerged when we combined slices of cortex taken at birth with thalamic fragments from just before birth. The thalamic fibres grew in but did not stop growing and you see here they ascend to the surface, here's one that is just turned through 90 degrees near the surface growing off horizontally through the cortex in a way that you never see in vivo - they normally grow in and then stop at the fourth layer of the cortex which is the classical receiving area where the neurons have synapses on them from incoming thalamic fibres.

So one of the things that we showed by taking slices of cortex at later and later ages was that the cortex suddenly turns on the signal associated with the developing layer four, what we call a stop signal at around three days after birth in the rats, which terminates the growth of thalamic axons, it causes them generally to bifurcate and then for the growth cone to collapse and they form synapses. Earlier the cortex turns on a growth permissive factor that allows thalamic fibres to invade. They don't invade before a couple of days before birth and begin to invade very shortly afterwards.

So we were able to reveal a cascade of factors that seemed to control the ingrowth of the cortex. Well an obvious question then is is the specificity of interconnections between different thalamic sensory nuclei

and the appropriate receiving area of the cortex, is that somehow predetermined by some kind of molecular tag or key that's appropriate for that connection alone? What's going to be the visual cortex has a kind of chemical tag on it which attracts axons from the visual part of the thalamus? And to look at that question we did this very simple experiment and we grew a single fragment of thalamus, in this case from the visual part of the thalamus, the part that would receive information from the eyes in association with two fragments of cortex, one the occipital cortex, the appropriate bit of cortex to which it should project and then another bit of irrelevant cortex, the frontal cortex, to which it would never connect. And what we found to our surprise was that connectivity was indistinguishable. So connections simply depended, the ability to form connections just depended on the proximity of thalamic axons to any bit of cortex available. What mattered then was how the thalamic fibres are guided to the appropriate region and delivered to the appropriate region, because they will connect to anything.

We looked at that by a technique of labelling that had just been developed and has been very influential in developmental studies, the application of these carbocyanine dyes, which you can get in different colours and these dyes are lipid soluble, they incorporate into the membranes of fixed neurons, so this can be done in fixed tissue, not in living tissue and they slowly diffuse along the axons and you can use them to trace connectivity even in dead tissue.

So here we are looking at a cross section, this is a coronal section through one half of the brain of an E14 rat, gestation's about 21 days in the rat. So here is the cerebral wall and at this stage it's just starting to develop neurons, it started a couple of days before in the lateral part here and it's just at this stage just starting the middle part to producing neurons which are moving up to become those early pre-plate neurons, the transient population. Here's the dorsal thalamus, those are the neurons that have the task of sending their axons up the appropriate

region of the cortex and it's already a pretty tortuous route. Those cells only arrived a couple of days before from the place where they were born and they don't form distinguishable nuclei at this stage.

Now the techniques that we used involved implanting tiny crystals of carbocyanine dyes either into the cerebral wall at different points or into the thalamus at different points to examine the interconnections, whether axons are produced in one direction or the other. And in fact the first projections that you see within the pathway are produced from the cortex, from those very early born transient pre-plate neurons, which are going to die. Here they are presumably doing part of their role in guiding subsequent connections, so here a little crystal's been placed on the surface of the cortex, it has labelled stem cells by diffusion here you see, the so called radial glial cells which are all precursors. But here at the surface are pre-plate cells which only migrated into position a few hours before, already spinning off axons, growing down in a sort of parallel nicely organised bundle towards this region, towards what will become the internal capsule between the telencephalon sub cortical structures. (23.00 min)

If you put a crystal into the dorsal thalamus at the same time you find that axons are growing upwards from the dorsal thalamus and if you get the two placements right, if you put it into the correct region of the cortex, the one which is supposed to receive from that thalamic nucleus and the correct region of the thalamus, you'll get beautiful pictures like this. Here the downward fibres are stained in green and the upward fibres are stained in red and they meet each other and the thalamic fibres then grow over the surface of the cortical fibres, guided towards the appropriate region which they then after waiting for two or three days invade and innovate.

Well of course we worked for 10 or 15 years on this in rodents and we thought well we're really interested in mice, they're super animals but our real curiosity was to know what happens in humans. And we made the broad assumption that things would be similar, we could learn all

the lessons from mice, find a few corroborating steps in studies in humans and that would sew up the issue. Well we started to work, and this is work with a post doc still in my lab in Oxford, Irina Bystron, looking at human embryos. A much harder task than mice of course. We obtained embryos from surgical abortions, from the MRC embryo bank here in London and in Newcastle. The quality of the tissue is crucially important using antibodies to stain selectively, to stain different proteins, different gene expression products.

Here we are looking and we can use these techniques to look at embryos as early as four weeks post conception. So here is an embryo at about four weeks post conception. This is a picture of one of the embryos that we studied. The whole embryo is about 2.5 mm long. And you can see this curious structure at the head end, that's the neural tube, the inward folding neural plate, which is going to become the brain, the spinal cord and the brain, which hasn't completely folded yet. You can see here an illustration at this stage, there's still an opening in the neural tube at the head end and here it is. Well we've done a great deal of work and much of it I'm very pleased to say does indeed correlate very well with what's happening in mice. These are very preliminary results but here we are looking at carbocyanin dyes revealing axons growing downwards here from the cortex at very early stages, Carnegie stage (18) about five weeks post conception. Growing downwards towards the internal capsule. And here in another example where a small crystal's been placed in the thalamus just a little later a bundle of thalamic axons growing upwards towards the cerebral wall. So it looks as though a very similar process is going on. We haven't yet examined in detail that handshake process of guidance but it seems very unlikely that it's happening.

However, one of the visually dominant features of early embryos, which we had never seen in any other species or read about in any other species, was this population of neurons. Here we are looking at the

cerebral wall at Carnegie stage 13. That's around about the 32nd day. And it's stained with an antibody for a neuronal marker, so these things that are stained heavily here are neurons. They're in the surface of the cerebral wall in the pre-plate, that region which neurons invade from the local ventricular zone, from the stem cells. However, these neurons don't come from the local ventricular zone and they arrive before there are any neurons being produced locally. They come we know now, from a region of the ventricular zone which will become the future hyper thalamus and they spread out over the whole of the surface of the telencephalon. They're a very curious population. We call them predecessor neurons; here's one in more detail. Here's the cell body, the cell body migrates through a long forward process by sematic translocation. This is not an axon it's a process, they don't produce axons, although they express neuronal markers they don't produce axons. They anchor themselves in on the peel surface here where they make contact through tight junctions with the apica processes of neural stem cells. We think what they're doing is performing transcriptional control of neuronal genesis in other regions of the brain by communicating through tight junctions with them. Wherever they arrive local neurogenesis turns on very shortly afterwards.

This has been described in no other species. We and others now have looked extensively even in monkeys and have not seen neurons of this class. Whether it is a unique adaptation for the very large human brain for some reason we don't know, but an obvious warning here is that the lessons learnt from animals are not necessarily entirely transferrable to humans.

Just a moment about language. Language of course is one of the most important things we do, it's a defining characteristic of human beings. Again, 19th century neurologists could tell us well actually not as much as we know about it but a lot of what we know of language now. They already knew that strokes in two regions, of course Brocka's classic observations, the effects of lesions in this region of the frontal cortex

close to the face and mouth representation of the primary motor cortex, the region known as Brocka's area which produces an aphasia in which the patient is still able to understand what's said to them, they're still able to read, but they can't produce speech. They can't produce organised speech – huge difficulty in finding words, very, very primitive syntax, they just can't put things together, even though they can understand them. On the other hand lesions here at the junction of the temporal, occipital and parietal lobes, the Wernicke's area, produces as it were, the symmetrical condition, aphasia, of understanding. People with Wernicke's aphasia can't read, they can't understand what's said to them. They still pour out a sort of language with neologisms which look sort of syntactically constructed even though it's usually nonsensical. So this disjunction between understanding language and expressing language is beautifully demonstrated by not animal research – animals don't speak and there's no evidence that any species has a fully developed syntactical communication system like human beings.

Those observations are entirely derived from very simple clinical observations and to be absolutely frank we have not got that much further in understanding how language is done, even though it's so crucially important for understanding what human beings are. We know a little bit about the connectivity, so here between Brocka's area there is strong interconnection with the face, mouth, tongue, larynx area of the parietal cortex, not surprisingly because it's involved in controlling – it's a kind of pre-motor control system for speech. Equally the Wernicke's area has strong inputs from the temporal cortex, from areas involved in the analysis of speech sounds, but also from visual areas. And interestingly this connectivity, even from a primary visual cortex forward through areas moving upwards towards the parietal cortex can be and has been studied by functional magnetic resonance imaging. Now I'm sure so many of you have been wondering when I would raise the value of functional magnetic resonance imaging and some of you might have expected that I would say of course these

neural imaging techniques are completely displacing research on animals and will answer all of the questions.

FMRI is incredibly important, a large fraction of neuroscientists make use of it. But there are two key factors you have to keep in mind if you ask what it could contribute to understanding the brain. One is its relatively poor spatial resolution, a few millimetres at best. And the other is the long-time constant of vascular responses on which FMRI is based, lasting a few seconds. So although it's useful for knowing pretty crudely where things are happening and roughly when they're happening in relation to stimuli, it will never give us the detail of knowledge that one can gain from the study of individual neurons in animals.

But I'll show you one example of the way in which it can be used in the study of language mechanisms and their development for instance. And we don't have for language the kinds of tools that can be applied so effectively in other areas where animal models are more appropriate.

This is a nice study done by Bedny and colleagues a few years ago, in which they used three different stimuli, they put people into the scanner and looked at localised activity here, here and here. The yellow thing in the middle is simply the overlap between the purple bit and the green – three different stimuli producing activity in this region of occipital and inferior parietal regions. But what were the three stimuli? We know that the primary visual cortex is at the back here. Well the stimuli were to begin with, well yes this is the primary visual cortex which responds very well to static patterns, just black and white or coloured patterns, even when they're stationary. But if you move those patterns, either with just straightforward motion like this or using complicated things like transparent motion, you produce activity selectively in this group of areas here shown in blue, including this region V5 or MT, which seems to be very – from animal studies, from monkey studies we know is very, very committed to the computation of visual motion. The green area

was selectively activated by another form of motion called biological motion and it looks like this, it's this sort of thing. Okay, which all of you will instantly see I'm sure as a moving figure. It's computer generated, consisting of a number of dots which are basically doing the same things as these dots – just making local movements. But it's the relationships between the movements which tell you that this is an image of a person moving. Similarly you could distinguish between a man and a woman or different animals simply from the pattern of movement of the articulations of the joints. And we are crucially sensitive to that and it's a pretty important biological signal to us – very, very useful and it turns out we have an area of our cortex which is committed to it, there. So you can imagine that during evolution as the additional cortex has been added, starting with the hedgehog type brain that just has very early simple visual processing, these extra computational bits added on to do clever things like recognising and analysing motion and even biological motion.

Well then what about the purple bit? Is that just another evermore elaborate form of motion which is being analysed? Well the stimulus that generated the response in the purple region of the brain was verbs, spoken verbs or written verbs. Now all verbs are verbs of motion of course but probably early verbs, the sort of verbs that would have been used by Palaeolithic people, probably were largely verbs of motion – carry, stab, hunt, whatever. So is it so surprising that the system for analysing verbs might have grown out of some kind of sequential change of evolutionary development associated with the detection of movement? And this area here, it's the right hemisphere it's true but this area here on the other side is bang in the middle of (Wernicke's area), the area responsible for analysing and understanding language. There is a similar area on the right but it's less dominant in most right handed people.

So that's a kind of Just So story based on FMRI. Wouldn't it be wonderful to know in the kind of detail that we have about the

processing of visual information in this area, coming from two decades of exquisite work on monkeys, wouldn't it be wonderful to have that kind of knowledge of what's going on in this area? And we may never have that.

Huntingdon's Disease briefly: Huntingdon's Disease is a neurodegenerative disease, it is interesting and different because it's an autosomal dominant disorder; if you've got the gene you will get the disorder. It's also distinctive in the region of brain that principally degenerates. Huntingdon's Disease is a progressive neurodegenerative disease, although it's usually of quite late onset. It has both cognitive and motor symptoms, the dominant ones are motor, the uncontrollable movements, career form movements and so on, but there are cognitive symptoms as well. It affects about 1 in 10,000 of the population at least in Western countries. 95% of cases are late onset, that is after having children and that's in some respects very sad because it means that the genetics is transmitted and it's associated particularly with selective degeneration of the corpus striatum, the sub-basal ganglia structures, sub-cortical structure, closely involved in the control of movement. And here we are looking at a cross section through the brain of a normal human and here one with hugely enlarged ventricles because of the collapse of the corpus striatum in a person suffering from Huntingdon's disease.

The gene for Huntingdon's was the first gene identified for autosomal dominant neurological disorder, it was found, cloned in 1993. The nature of the gene is fully understood, it's a triplet repeat gene, it has an expanded polyglutamine sequence in it, so it generates a protein that has a long sequence. This means that the gene is understood, the protein is very well known, the expression patterns in a human are quite well known and there is in these terms a perfect animal model. In fact there are a number of them and I think the most convincing of them was developed in London by Gill Bates, a variety of mice in which the axon of the human mutant gene was inserted into the mouse genome

so it produces mutant human mutant protein – Huntingdon protein as it's called. And that generates a mouse which dies young, develops motor disorder, also as we've shown in some of our work has cognitive difficulties and a variety of other conditions. It is the most comprehensively compelling mouse model of any neurological or psychiatric condition that I know of. One thing – my lab worked on Huntingdon's for several years and one of the things that we discovered which was nice, because it turned out to have a previously unknown clinical correlation, the mutant gene has associated with it a reduction in neurogenesis in the adult brain. You'll know I'm sure that there are certain small regions of the adult mammalian brain that continue to produce neurons throughout life. Principally the dentate gyrus of the hippocampus, which pushes neurons into the hippocampus, replacing neurons, and that probably plays a part in memory processes and actually in the forgetting processes it's thought. And there's an anterior stream of neurogenesis which shunts new neurons into the piri cortex and into the olfactory bulb, part of the olfactory system. And it turns out that the mutant gene in the mice we've discovered, reduces neurogenesis dramatically in both of those sites.

So here we are looking at neurogenesis and this is the percentage of cells that are dividing in the dentate gyrus and pirifom cortex in mice carrying the Huntingdon's gene compared with wild type mice. Huge differences in both cases, this is a dramatic reduction. So we thought well if there's a reduction in neurogenesis then there are going to be problems with memory and problems with olfaction. Well we've certainly demonstrated the problems with memory and that correlates well with the cognitive disorders that are detectable in clinical patients, diagnosed clinical patients, because you can genotype before the onset of motor symptoms. But what we predicted was that there'd also be problems with the discrimination of smell and the detection of smell.

This is in a mutant mouse, doublecortin staining for immature neurons in the peripheral cortex, here in the wild type mouse lots of newly born neurons and here in the Huntingdon's mouse very, very few. We tested olfaction discrimination in the mice and it was very poor and olfactory detection and moved into a very small clinical study where we showed that patients diagnosed, genotyped with the Huntingdon's gene but with no obvious other symptoms had difficulty in discriminating odours and also in detecting odours, the detection threshold. So it's quite likely there's similar defects happening in human patients.

Now this is a preamble to a lesson about the value of commitment to the 3Rs frankly. While we were doing this work, one of my then graduate students, a Rhodes Scholar and neurosurgeon from South Africa, Anton Van Dellan, wanted to run experiments on improving the quality of life of laboratory animals. Because after all the normal cage conditions for lab mice like this are really pretty unimpressive, a couple of mice, two or three mice living in a cage and that's it. So what he did was to introduce randomly within mouse cages in the animal house, enrichment, odd things just put into the cage changed every couple of days to give the mice something to play with. It turned out that the mice that he was doing this with were also involved in the study on Huntingdon's; we were producing Huntingdon's mice from heterozygous parents, so some of the offspring were carrying the gene, some were not, some were wild type and we didn't know which were which until we broke the code at the end of the various studies that we were doing. So this was an unplanned, a nice unplanned double blind experiment, because some of the Huntingdon's disease mice and we didn't know which ones were mutant, were being exposed to the enriched environment and others were not and lo and behold when we carried the normal kinds of diagnostic testing that we did on the mice, we found a separation between the two and we broke the code. So here for instance and this was confirmed in every test, cognitive and otherwise, that we looked at with these mice – here's a simple test in

which the nice are put onto a wooden rod and they walk out to the end of the rod, there's bedding and so on underneath, they play around with the rod and then eventually when they get a bit tired they fall off. At least they do if they're suffering any motor disorder. Wild type mice can stay there for a long time. So we had a test of coordination ability and here we are looking at the non-enriched, the normal mutant mice and this is the percentage of the group of mice that were failing this rod test as a function of age. And here even from about 6 days on some of them were already failing at 100/120 days, all of them were failing that very simple test, they were in really bad shape by this stage. But here a matched randomly allocated group with enrichment, very few of them had detectable symptoms even out to 160 days. So this is the equivalent if you like, to translate it in simplistic terms to human beings and the equivalent of delaying the onset of the motor disorder by many, many ears. And this is an autosomal dominant condition which had previously thought to be just absolutely autonomous and inevitable and progressive. There was absolutely nothing that one could do about it. It also rescues the degenerative changes in the striatum. In the mouse the striatum collapses but here we are looking at Huntingdon's the mutant mice living in an enriched environment, not statistically distinguishable from wild type mice, but if they are non-enriched here there's a significant collapse even at this quite early stage in the volume of the (corpus) striatum8. So enrichment slows down or to a large extent prevents for some considerable time, the degeneration of the striatum.

Well the nice thing about this discovery, and by the way that has now been demonstrated in virtually all other neurodegenerative conditions in animal models, that enrichment in the environment delays the progress of the disease. But what we realised was that enrichment now provided us with a tool to separate trivial consequences from the mutation from things that might be critical in pathogenesis. Because we could for any particular molecular change, anatomical change, behavioural change, we could ask is this change modified by enriching

the environment? Because we knew that enriching the environment significantly delayed the progress of the disease and perhaps the most interesting work that came out of that was a study by Tara Spires, now in Edinburgh, now a Chancellor's Fellow in Edinburgh, as part of her DPhil in my group, looking at the expression of a growth factor, an important neuronal growth factor, BDNF, brain derived neurotrophic factor, in different parts of the brain. And what she found was that in mutant mice using quantitative western blotting, in mutant mice there's a significant reduction in the amount of BDNF specifically in the corpus striatum. And that's interesting because BDNF is a growth factor, it helps to support and sustain neurons. So was it possible a reduction in BDNF expression or availability in the striatum was causing the degeneration and therefore the disease.

One wouldn't know without a control and a control is enrichment, because enrichment delays the disease, the question is does it also delay the decrease in BDNF expression? And it does. So there's a nice precise correlation between those. So what we've suggested on the basis of this and a large amount of other work was a hypothesis for the pathogenesis and frankly the pathogenesis of Huntingdon's disease was not, even now, is not fully understood. But our suggestion was that BDNF produced by cortical neurons and we knew that expression even in the mutant mice was normal in the cortex, is transported along cortical striatal neurons, particularly from frontal cortex, into the striatum. And that transport is interfered with by the mutant Huntingdon protein. There's other parallel evidence that that protein is involved in vesicular transport, so this isn't so stupid, and I understand entirely irrational idea. So the mutant protein might reduce the amount of BDNF reaching the striatum, therefore causing degenerative change and hence precipitating the cascade of symptoms.

Well there it sat, the last line of our paper said this might offer a new approach to therapy, but ah yes, agonists of the receptor for BDNF don't cross the blood brain barrier, I mean there are big problems with

BDNF agonists. However, the FDA has very recently given approval for a clinical trial, an early clinical trial, in the University of California, in Davis, directed by Jan Nolter, who proposed to us genetically modified mesenchymal stem cells derived from donors, from human donors, from bone marrow, genetically transformed so that they over expressed BDNF directly injected into the striatum, into the corpus striatum of patients, genotyped patients. And the basis of this which has convinced the FDA is the fact that such stem cells, mesenchymal stem cells, harvested from bone marrow, transformed genetically and then injected into the mouse model of Huntingdon's disease delays or virtually prevents the onset of symptoms, in a way very similar to environmental enrichment. So their plan and here we are looking at a section of the striatum, stained for neurons, the blue cells are neurons, the green cells are mesenchymal stem cells over-expressing BDNF. So the clinical trial moves in this case to humans with inter-cerebral injection into the striatum of the modified stem cells in the hope that this will alleviate the condition and we're keeping our fingers crossed but it would be very nice to think that a discovery stimulated entirely by fundamental research questions might eventually lead to translation of that sort.

Finally, and briefly, stroke: Stroke, a very common condition and very debilitating and very expensive, caused of course by the occlusion of blood supply to a region of the brain or by haemorrhage. Here for instance these are scans that show the blue penumbra of subsequent bleeding around an area of initial stroke. And a lot of changes which go on around a stroke in which associated cortex dies far beyond the region that's been immediately impacted by the ischemia or by the bleeding associated with the stroke. And the drug companies of course have been very interested in whether one might prevent in some way the additional damage, the neurotoxic damage in the region around a stroke. (50.00 minutes)

This has been a graveyard frankly, for pre-clinical studies as many of you will know. Here are just a few of the papers - trouble with animal models do, stroke models, model stroke and so on and the reason is 500 neuro protective strategies, drug strategies, have been developed with very encouraging pre-clinical results. But as fairly recent paper concluded, only aspirin and intravenous thrombolysis have any certain clinical value. It's been a catastrophic failure of the transition between pre-clinical and clinical, seized with glee by some of our opponents of course as being typical of the failures of pre-clinical work on animals, its non-transferability to humans. Just a quote though from the same article, exactly the same article, in a review of animal studies published in seven leading journals of high impact. About one third of the studies, pre-clinical studies, did translate at the level of human randomised trials and a tenth of the interventions were subsequently approved for use in humans. Glass full or glass one tenth full or 90% empty, I don't know, but it seems to me that 10% isn't bad when one recognises the difficulty of transfer from animal studies to humans.

The fact is that we wouldn't need animal pre-clinical studies if we had the ability to do everything, to develop and to test on humans. But an alternative way of putting that is human studies are essential to discover whether therapies that appear to be effective in animals can be transferred to humans and are safe. There's an essential interplay between the two. What has come out of – largely of the analysis and the agonising about the failures of the pre-clinical stroke studies is a lot more scrutiny in the design of pre-clinical animal studies, even with suggestions such as this in a recent paper that they should be subject to very similar designs, protocols, controls, management as clinical studies, with different study sites being involved in pre-designed animal studies, with much more scrutiny about numbers and power and statistical design. There should be a steering committee, very similar to that for a clinical trial and so on, that pre-clinical CROs might be responsible for this kind of study. There's certainly a lot of re-examination of the validity of animal research at every level because of

criticism of inadequate numbers, inadequate power, calculations, inadequate comparability and in some cases poor experimental design. I think that's something that we have to accept and do better, it's not a reason for saying that animal research can never work and should be abandoned, because abandoning this crucial phase in the development of new treatments would be catastrophic for the patients for instance helped by the 10% of drug studies that do transfer directly right through to clinical benefits.

So finally to conclude – what can we learn from all of this? Apart from lessons about those individual areas of research? What I've tried to do is to give a picture of balance. Balance of the value of studies on animals when there is no alternative, when the work simply cannot be done on humans and where the animal model is appropriate for understanding human beings, which is not always. The great difficult I think, and it's not unique but it's certainly true in this area, is the continuing polarisation within this debate about the importance of animals. Opponents to animal research often certainly employing exaggerated and sometimes factually incorrect arguments, but equally the supporters on our side are often equally exaggerating the benefits, exaggerating the difficulty of conducting parallel studies or equivalent studies in human beings and so on. Avoiding that kind of polarisation by admitting the difficulties in some cases as well as the advantages in others, I think is absolutely crucial.

Just to give you an example of one of the most problematical issues, which is selective quotation and drawing upon the opinions and evidence of others, what did Darwin think about animal experimentation? Well he said this 'You ask about my opinion on vivisection, I quite agree that it is justifiable for real investigations on physiology.' But he also said this 'It is a subject which makes me sick with horror so I will not say another word about it, else I will not sleep tonight.' And interestingly he said both things in the same letter. This is the full text of a letter to Ray Lancaster. 'But not for mere damnable

and detestable curiosity.' So he was making a distinction between invaluable research and useful investigations in physiology perhaps applicable with just tinkering for no benefit at all. So one should be very careful with quotation.

So I wanted to raise just some questions which I think we all need to address. Do we really have adequate ways of assessing the validity of the animal models we use? It seems to be crucial particularly if they're part of a pre-clinical study. Is there more scope for developing alternatives? The kinds of technical advances being made in human study are making things possible in humans now that we're not in the past and we must recognise that and those of us who have been committed to animal work not deny it. Yes and is there sufficient funding available for the areas of new opportunity? And on the other hand is medical progress being impeded by excessive bureaucratic regulation? And by excessive I mean not demonstrably valuable in terms of maintaining ethical standards or the correctness of procedure. I think those of us who deal with the problem of applying for animal licences, not that I do any more, but those that I know who still deal with it, do wonder from time to time whether the benefits that are gained by the enormous amount of bureaucracy with the process are matched by benefits to the animals and benefits in terms of the value of the research. And how effective is the evaluation of evidence from animal research before it transfers into clinical trials? And particularly of course, how justified is the use of primates, how justified is genetic modification and especially how justified are we in using techniques that are almost bound to cause suffering?

So with those thoughts I'd like you to think about the brain, it's a crucial issue because of those dilemmas that I raised right at the start. It's a wonderfully exciting area of science, it's incredibly important clinically because of the magnitude of the problems that need to be solved. But at the heart of that whole, those driving forces towards science and the

brain, there is the conundrum that it is the organ that makes us think, that makes us know and that makes us suffer.

Thank you.