GCPH Seminar Series 6 Seminar 1
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'Nature and Nurture? The intergenerational risk of transmission for chronic illness

Michael Meaney

Harry Burns, Chair:

Okay, ladies and gentlemen and welcome to the talk tonight. I'm Harry Burns, I'm your Chair for this evening. As those of you who have been regular attendees at this series of lectures will remember, the lectures given by Chris McEwan and Liz Gould, who introduced to us to the idea that the way in which young animals and young humans experience the world very early on in their life shapes the way their brain is structured. From that flows a number of consequences for physiology, for their psychological, social and physical health in later life. And tonight we're very honoured to have with us Michael Meaney, who is the James McGill Professor in the Department of Psychiatry, Neurology and Neurosurgery, wow, at McGill University in Montreal.

Now, that of course, gives him a strong link with Glasgow because James McGill was a student at Glasgow University. He only ever lasted a couple of years and I wondered if he flunked. But he certainly made a great success of his time in Canada. The other interesting thing is that if you search Michael on Google the first name that comes up is Michael Meaney. The next thing that comes up is really very interesting. It says 'Michael Meaney, contact Michael Meaney at SuperModels.com, available for commercial and glamour photography'. [laughter] I thought 'here's a very interesting guy', because it then goes on to say more photographs can be found at such and such a place. I didn't follow that link. But clearly Michael is a name that's much more versatile in North America than it is here. So Michael's going to talk to us tonight about terminal care and the interactions between genes and environments.

Michael Meaney:

Thank you, can you hear me? You can? No?, no, no, it's... I guess, you don't need the microphone. You're going to regret this. [laughter]. Harry, if you'd gone to those pictures and there's actually a couple of secretaries with invitations for seminars like this who have done that. And they've been horribly disappointed to find that the first Michael Meaney and the second Michael Meaney are two distinct human beings. The second Michael Meaney features in nude modelling. Well, as you can imagine, it's not me; much that my CV would be tremendously more interesting and the invitations more plentiful if they were the same person.

The other thing about James McGill that might go to addressing the question at what makes it interesting being a James McGill Professor is that on the campus of McGill University James McGill is a drinking song [Laughter] that might feature in the history of James McGill. It's a honour and a pleasure to be here and thank you to Harry and to Andrew for the invitation. What I'm trying to do with this lecture is to leave you with something a little bit different, something which you may not have been aware of when you came through the doors. In particular, what I want to do is pick up on a conversation I had with Andrew that I had yesterday. We were talking about where trends in public health

have gone and how people understand and interpret not only secondary knowledge but themselves and that was of course the old nature/nurture debate.

The nature/nurture conflict had two manifestations. I think one of the manifestations was the issue of hereditary parental inheritance. Probably the oldest manifestation is for this to simply be used in some way to justify the passage of resources from one generation to another independent of the individuals who are actually on the other end of the inheritance. And so we spoke about birthrights and we basically justified that in some way through inheritance we came to an understanding of the gene as being a biological rationale. What is interesting in the history of our chemistry was that we had to find the gene long before we ever discovered DNA. Which is very interesting because what it means today is that many of us walk around with an understanding of what a gene is that has actually no relationship to our real life. Instead, our notion of what genes are and what they do are informed by the pre-conceived issues. And the nature/nurture conflict in fact embodies that. Because what people do is they speak about genes and environment in some ways as if they were two independent forces that could in fact, biologically, realistically operate independent of each other. And that we could then think of the construction of the individuals on our planet as being to some extent influenced by this force and to some extent influenced by that force and that whole notion has been reinforced by some of the unfortunate research trends where people say, 'your ability to lightly brown chicken on a barbecue is 13% environment and 80% **.....**'.

Those notions, as I say, are interesting because they live and breathe with our science and our culture without really any reference to what genes are. Now the second way we can think about gene environment to actions of course is that we can also try to disassociate biological forces and whatever you might mean by that, from the social forces. And so, for example, I know one of the questions that people speak about here in Glasgow and which Harry and Andrew and I spoke about of course is the influence of poverty. Here, for example, there was a great resistance to think that biology could have any reference whatsoever in this debate because clearly we were talking about forces and influences that were social and economic. So when Andrew and I were trying to hold that conversation we were trying to understand what has transpired with that discussion. How have we managed in any way to move beyond those rather stultifying versions of defining who we are?

And what we came to and I think Andrew put his finger on it when he said that the groups of researchers that he leads in concepts are people who take the psychosocial biological approach. And clearly this is implying that there's some integration that's occurring here between the social understanding of the world and the biological understanding of the world. And that's what I want to try and underscore tonight. I want to try to suggest that in fact I want to believe the more people understand about genes and how they operate the better able you are to appreciate how critical their environmental influences are in the understanding of biology.

What I want to start us off with is really just some basic principle, a basic principle about health. When we think about development the best word in understanding the world I'm telling you about is that of adaptation. Understanding biology, everything component when we're talking about the development of the muscle cell, fat cell, or the main cell, think of it as a process of adaptation. It is a manifestation of our gene pool. Our genes are being activated by environmental signals and the dialogue between those events is actually adapted to nature and determining the outcomes. So muscle cells develop in relation to nutrient supply. That if you experience between premium cell development and poor cell development; as do bone cells, as do fat cells and brain cells of course adapt to a far more diverse array of events. In part, we can characterise these events as being economic. They're economic in the sense that they require access to nutrients, they

require shelter and safety and security from infections. They are economic and that is more or less just as true for a developing planet as it is for a human being.

And they are social, in every organism on this planet there is at least a maternal influence. And that influence is going to obviously being manifesting in very different ways depending on the species that we study. But nevertheless we can always think of adaptation as a pretty instant part to a social event, to a parental influence in some way. Now we can take that very simple axiom o of biology and see if living and breathing within many of the studies that are emerging from social genealogy. We know, for example, that the chances of us developing any of these particular chronic diseases is statistically associated or predicated by the quality of our life. And so we know that individuals who undergo trauma in the form of physical or sexual abuse, emotional neglect, partially inconsistent discipline, each of these individuals is more likely to develop these forms of chronic illness.

Yet intuitively, of course, you're going to think well that makes sense when we think in terms of psycho pathology. The interesting thing to consider is it's no less true if you're talking about diabetes, heart disease or in fact obesity. Indeed for those who thumb through the pages of journals on medical studies the odds ratio where this might be greatest is obesity. So our health is shaped, at least it would seem, in part by the social forces that define our environment. And a question we can ask then is how does this happen? How could the quality of early family life influence the developing organism so as to bias them either in favour of these particular diseases or a better quality of life? And one of the emerging hypotheses is the following. This hypothesis, which is very much a work in progress at this stage suggests that one hypothesis is that early in life the quality of the family environment influences the nature of individual differences in the way that our brain and endocrine systems respond to stress. And that these individual differences in defensive responses, if you will, in turn predict individual differences in obesity scores.

Now this model is based in part from studies of years of clinical medicine and physiology, which have suggested that the hormones our body produces during periods of stress can actively promote each of these particular diseases. What is also emergent in human studies, as well as the studies with non human species is evidence that, in fact, these forms of early stress shape these individual differences in stress reactivity. The likelihood of our body activating these stress responses in relation to our physical health in everyday life. And it's this part of the hypothesis that I really want to focus on in the course of tonight's lecture.

But let's take this model one step further because I think if you take it logically and pursue this there's a real way we can fit this model into many other discussions that are primary to those individuals who responded. And that is, of course, that when we look at this particular relationship we understand right away that knowing that neither these forms of chronic illness nor these forms of early family dysfunction are randomly occurring across the population. They are both more likely to occur in individuals who have grown up in conditions of poverty. And it is not at all hard to imagine how the distress of poverty problematises the health and wellbeing of parents so as to encourage this form of interaction between parents and children and thus to initiate this particular cascade. Now that, as I said, is very much a working hypothesis. I'm going to show you some evidence in favour of this in the following slides. One of the things I really like about this particular model is that it provides a bridge between individuals who look at the social determinants of health and those who study its biological underpinnings because both can be found encompassed within this particular working model.

Okay, the other thing that has emerged in the course, I'd say, about the 1990s and then more recently in more intensive studies is the idea that in fact we can indeed understand the affects of poverty with respect to many of these particular elements, by appealing to

the idea that the effects of poverty are mediated through the functioning of the family. And this has come from studies where people …..[and others, who have suggested family mediation. And I'll just show you one example of a slightly more recent study that I think clearly shows this. This is a study by …..published a few years ago in …… which they used statistical modelling to understand the link between poverty and child behavioural problems. And what they found out in their modelling was that in fact the relationship between poverty and childhood behaviour was mediated not only by individual differences in parent-offspring interaction but more proximally by the level of emotional distress suffered by the mother. And, in fact, at that stage in time our reviewer published a report showing that the cortisol, a stress hormone in children was actually predicted by exactly the same model. That essentially what is prevalent is that the distress of poverty works through the emotional and mental health of the parent to influence parenting, which in turn then describes the effects in the behaviour of the child with behavioural problems.

If you start looking at other dimensions of child development you see some interesting features here. In fact the relationship between poverty and child cognitive development was not described necessarily so strongly by differences in parent/offspring interaction. Rather it was described by a difference in the quality of the home environment; the degree to which the home environment provided cognitive stimulation for the child. But in any case, in essence what we're talking about are a raft of studies that suggest that environmental adversity imposed on home environment leads to changes in parenting as well as in the quality of resources within the home and that these factors in turn lead to changes in child development.

Now none of this I suspect is a surprise to any of you, right? The interesting feature to all of this is that we can take exactly the same theme and find it living and breathing within the literature of evolutionary biology. And this is not a literature in which people are studying on the development of human children. It is the literature in which they are studying the development of insects, reptiles and simpler organisms. And the model that emerges here is one that speaks to a phenomenon known as 'maternal effects' or 'parental effects'. This model, which describes patterns across an enormous range of species, suggests that the quality of the prevailing environment in terms of nutrient supply, levels of affection and predation, that all of those signals alter the level of parental investment in the young. That's true of insects, that's true of reptiles and that's even true of plants. This level of parental investment then signals the developing offspring and shapes these developmental elements. Again the interesting thing here is that's the same model we're talking about and discussing. In other words, when we actually appeal to these type of mediation models, we're not suggesting something that's esoterically unique to human psychology, we're talking about something that's a common principle across all forms of development on the planet. It is, if you will, a biological reality.

Now in each case what we're talking about in studies that describe this work is how exposure of the parental offspring to some form of adversity and in the examples I'm going to show you this is risk of predation. This somehow alters parent/offspring interactions in ways we don't fully understand that programmes individual differences and defensive responses. Let me give you the first example. These are skinks, small reptiles, very common in Australia and also very popular amongst the local snake population as a source of food so the rattle snakes prey heavily on these skinks. The skeets that are the most frequently preyed upon are those that are physically small, have shorter tails which are easily ingested and are less reactive to the cues of the presence of snakes so they're not as quick to run away and evade the snake. That's the profile, if you will, of the skeet that's most likely to meet its demise by way of predation. The interesting thing is, if you take the mother skeet and you simply expose her to the scent of a predatory snake then the offspring are larger, have longer tails and behaviourally are more likely to evade and

escape from snakes. You've changed completely the development of these skinks by simply exposing the mother to the cue of predators in her life.

Take you to another example and this one takes us even further into this model because it speaks about trans-generational effects. These are water fleas, and no, even in Canada water fleas do not emerge to this particular size, okay? [laughter] And this is a scanned enlarged electro micrograph of what you would almost imagine a water flea to look like. Under benign optimal conditions, that is, in a laboratory with no predators, these water fleas take this ovular process spread. However, if you then transferred a water flea out of this aquarium and put him into an aquarium, into water which has previously been occupied by a predator then what happens it responds to the sense of this predator. These water fleas form these horned and longer spiny tails. It makes them harder to ingest. And physically what happens is these guys are more likely to evade a predator or to escape predation than these more delectable tit bits on the right. [laughter] So you've got an inducible defence, it's a great idea, right? So mums don't have to worry about the metabolic costs associated with this defence. But if the predator scent is there, they show this change, a massive change in morphology. So what ……did in Germany is that he took adult female water fleas that were all born in these benign aquariums with no predators around and transferred half of them into an aquarium that had been previously occupied by a predator. Eventually, they developed this massive helmet and long, spiny tales. Then he took them and transferred them back into the controlled environment and allowed them to reproduce. What he found was that the offspring of those mothers who had been exposed to the predator were born with larger helmets than with those born to mothers who had never been exposed to the predator. As they matured into adulthood in the absence of a predator their helmets started to shrink in both cases but remaining substantially larger in this group. But then if he exposed them to the sense of the predator these guys showed much greater reproducible effects. In other words by simply exposing the parental organism to adversity in early life you had changed the development of the offspring.

So we're looking at a very common theme here. Our question really here is simple. We want to try and understand how it is that this might occur? How is it that the context within which parenting occurs can biologically come to defend itself so as to alter the development of its youth and thus their health and productivity over the course of the lifespan? What I'm going to suggest to you through a series of studies is that, in fact, the variations in parental care that derive from environmental adversity alter, literally alter the structure and activity of genes in the brain of the offspring that regulate the way in which they respond to stress and that these same effects also feature in part of our own development. The way this environmental force operates on the genome involves a level of plasticity at the level of the DNA that is referred to as an epigenetics.

So let me tell you just a little bit of background as how you might want to think about this. For most of us we don't walk around thinking regularly about how it is that the molecules inside our cells behave. Right? We think of them as somehow molecules. They exist within cells, they do their thing, right? So you make a particular hormone like ribosomal peptide, it does its thing and off it goes. And, of course as you can probably appreciate the bio chemistry of that is very different. That molecules, including DNA, have a structure. So they can exist in a linear form or folded up. And that structure has an awful lot to do with the way they behave. What I want to tell you is that these epigenetic modifications that refer to plasticity forms an influence that alters the structure and thus the behaviour of the genes. And what I'm going to tell you is that that in fact is can be signalled by quality of life.

Okay, so this is really a very interesting problem in a sense and in a way we can all anticipate that some process like this has to occur, even at the level of DNA. Because we

know that even when we talk about the monozygotic twins who shared exactly the same genome, is that they can actually turned out to be rather different. So you have these two characters who are in fact perfectly identical monozygotic twins who share exactly the same nuclear genes. Well, life has somehow impinged upon Fred and Phil in a way that has caused them to become notably different in matter. And in ways that exceed something that simply exceed that of holding a beer can differently. We imagine that somehow environmental forces have produced this level of diversity and what we want to try and understand is how that can actually happen.

So what I'm going to do is spend a little time introducing you to the topic of what this means. What it means to structurally modify genomes and then I'm going to give you some experimental examples of how we think that can virtually happen. So, first of all, how do we think about epigenetics? Well, we're all familiar with the particular manifolds that refer to DNA. In fact we commonly refer to DNA as a genetic code. Now there are weaknesses associated with that particular metaphor. But in large measures what we're actually saying is that the nucleotide sequence that is characteristic of the genome determines the products of the genome. It determines, for example, which R & As and which proteins are produced from that particular series. That information is defined by nucleotide sequence. That's only on one level of information contained in mature DNA. The second level of information is epigenetic engagement. That is a level of information that is chemically altering and it is superimposed on our DNA. And so what you're looking at here is the one form of epigenetic mark that is going to be the subject of this lecture. That is simply the addition of a methyl group into studies. It is in fact very much a scholar's topic as since the real leader on this topic is actually Deirdre Burke at University of Edinburgh.

So for many years we've known that in fact you can modify the activity of DNA by adding particular chemical marks onto various sites. Let me tell you that first of all one thing to understand when we're talking here is that we are in fact speaking about two specific forms of information. For example, binding these methyl groups onto DNA does not change the sequence. The underlying sequence remains unchanged. What changes is the level of activity of that region of DNA. So epigenetics by definition is any functional change in the genome that does not involve the alteration of sequence. So what we're talking about again is a chemical sequence superimposed on the DNA.

Okay, so here's where things get a little into detail. And obviously it's not my intent to burden you with the details of this information. Rather I'll simply walk you through a little bit about how it is that DNA operates and why it is that environmental signals are so critical to the operation of the genome. Okay, so epigenetic effects refer to the modifications of the chemistry of the chemical environment in which the DNA operates. Epigenetic alterations alter the activities of the gene. What they do in fact is determine how active this nucleotide is in producing proteins for our kids. The DNA methylation that we're speaking about right here is simply the addition of methylation on to particular nucleotides. DNA methylation is chemically very stable, potentially. And so the interesting thing about this is that if you modify the DNA in this way that potentially can actually last for the entire lifespan of the organism and DNA methylation alters the activity of the gene.

And now what I'm going to do is tell you a little bit about how that works. So again, I mentioned to you that structure in biochemistry determines the operation of molecules and I'm going to give you an example of this particularly with relation to DNA. So if we pick up commonly on the nature of DNA it's often graphically portrayed as if the DNA molecule is simply linear and passive and it has these various signals impinging upon it. And the truth of the matter is very, very different. Here is the way, this is simply….. This is the way DNA exists in the real world. What is have is the DNA helix, literally 145 to 150 base pairs

and it's wrapped around the protein core, okay? And in the default condition this is a very tight configuration; that DNA is wrapped tightly the histoproteins.

Now why the hell should you care? We should care in terms of this discussion because when it's in this configuration DNA is really inactive. It's not doing very much. In fact, if you want your DNA to do something you need to remodel this configuration. The reason for that is that DNA is activated by particular protein signals known as transcription factors. Basically they prescribe the transcription of the DNA. They cause the DNA to …….. If you have the DNA tightly wrapped around the histomes these transcription factors cannot physically interact with the DNA. What you need to do then is to somehow open up this configuration so the DNA sites can then interact with these transcription factors. Where those modifications occur is actually on these histoproteins not on the cores that reside inside but rather on these amino acid tails which protrude outside of the DNA itself and they are subject to all kinds of chemical modifications.

The most commonly known modification is that called acetylation. You simply take a lysine, an amino acid on this histome tail, you apply acetylene to it and what that does is to neutralise the positive charge of the histome proteins, opening them up and now transcription factors that interact with the DNA and the gene can be actively transcribed. How's that? Does that seem reasonably clear? It can't be that hard….biochemistry without a doubt but for the most part the thing to keep in mind is you've got to work on the DNA if you want it to do anything that's sensible. The way you do that is by opening up these histomes and by settling these histomes. Now the question you might then pose is 'How does the methylation of DNA interfere with this particular process?' Well, what happens is that when you methylate regions of the DNA and add these methyl groups onto it you attract a protein. The protein is known as the methylated DNA binding protein. It literally interacts physically with the DNA at sites that are methylated, and it brings a whole bunch of friends to the party. One of these is known, a classic protein, is known as histone deacetylase. What histone deacetylases do is they kick off the acetyl groups and they prevent them from re-attaching on these histome tails. What that means is that you have now bio safed this whole process to a closed configuration because if you cannot acetylate these histome tails you cannot open a recombinant and the transcription factors cannot bind to those cells. So these histone deacetylases mediate the effect of DNA methylation. Methylated DNA attract these enzymes, they prevent chromatid from opening up and they bias you toward silencing the gene transcription.

If you've made it through so far you've made it through probably the technically most difficult part. I think for the most part the thing to keep in mind primarily is to think of DNA as an actively regulated molecule. In part, its own methylation status is part of that sequence.

So, finally we get to some studies… this in fact has been a working hypothesis. We have in fact proposed the idea that apparent differences in parent/offspring interaction lead to changes in epigenetic marks in specific regions of the DNA. In particular, we suggest these changes involve DNA methylation. But the changes in the epigenetic marks then lead to changes in the ability of genes to actively transcribe particular proteins and thus to differences in the more complex levels of phenotypes or traits, such as stress responses. For the most part, the research I'm going to take you through has been done with this wonderful organism here, which is a rat. The reason I make that distinction is that these are intensely social animals who are used to growing up within an extremely complex social network. Of course, the lynchpin for this social development is that of the mother. What you're looking at here, in fact, is the mother nursing her many offspring and what you see right here is this licking behaviour that the mother performs on the offspring during a…... And what we do first and foremost is to simply try to characterise naturally occurring individual differences in mother/offspring interaction. To do this we spent 6 to 8 hours per

day watching mothers interact with their offspring, characterising the frequency of this licking behaviour. What we find, and this is where it can really boggle your mind, is that these naturally occurring variations in maternal care. This licking behaviour in particular, programmes the expression of genes in the brain that regulate stress responses, thus giving rise to stable individual differences in stress reactivity and the way in which maternal care regulates the expression of these genes is through these epigenetic modifications.

So, maternal licking. Why would we focus on the licking behaviour of the mother? Well, we've known for many years that in fact physiology and the cardiovascular activity in the developing pup is in fact socially regulated. It's regulated by the mother's licking of her offspring. When mothers lick their pups it stimulates the activity of the pituitary synatotropic system which produces hormones like proforma. It also suppresses hormones which would otherwise inhibit growth, such as glucocortoid, so the mother's licking is actively driving the physiology of the pup in favour of greater synaptic growth; that's what's occurring in an immediate context. Our question is, do these individual differences in the licking behaviour have anything to do with the long term outcomes in terms of these individual pups?

So, we look at variations in maternal care, we study daily the first ten days of life and we find that the frequency of this licking behaviour is actually normally distributed across the population. In that in fact some mothers lick their pups about 2 to 3 times as frequently as do other mothers and those are the ones on which we focus our studies. We statistically define these high and low licking mothers and what we do then is to study their offspring well into adulthood. So we're doing what many developmentalists have done before us. We are characterising a certain difference in the level of environmental experience in one stage in life, in this case involving maternal care, and studying its outcomes in adulthood. And the particular angle I'm going to focus on in tonight's talk is how it is maternal care shapes and develops individual differences in stress responses. Now to understand that I'm going to give you a little bit of background in how it is the brain of a rat , much like the brain of a human, activates the stress response.

The critical brain region probably in the activation of the stress response is this area here known as the amygdala. The amygdala is largely a brain region that exists in the limbic system, the older part of the temporal cortex, in which the region is specified to alert the system when any suggestion of threat is presented to the organism. So the activation of the amygdala coincides with the activation of the stress response. Fortunately, the activation of these responses is subject to the influence of brain regions, like the hippocampus and the prefrontal cortex, that can modulate the activity of the amygdala and thus modulate the degree to which the organism responds by normalising the stress response. When you talk about individual differences in stress reactivity, whether you're talking about the rat or the human, for the most part these individual differences lie in brain structures like the hippocampus and the prefrontal cortex then they modulate and generally tend to inhibit these stress responses. In talking about the stress response for the most part, in the initial part of the talk, I'm going to focus largely on the endocrine production in stress response; the way it is that our body produces 'stress hormones' as we call them during a period of stress. Now, I'll give you first of all just a little bit of the anatomy and then a more systematic description of the system.

The way we activate a stress hormone response is basically mediated through one of the oldest areas, it's the oldest area of the living brain, known as the hypothalamus. So stressful signals have impinged from the amygdala down to the hypothalamus where they cause the hypothalamus to release a hormone known as Corticotropin-releasing factor. This is CRF and it's probably the master stress hormone. It is fact, when activated, responsible for increases in cardiovascular activity, changes in digestion, changes in emotional wellbeing as well the activation of stress hormones. Corticotropin-releasing

factor is then transmitted to the pituitary where it causes the activation of the intermediate peptide hormone known as adrenocorticotropic hormone or ACTH. ACTH then acts on the adrenal glands and the adrenal glands release corticoids, which are the signature of a stress response; they are the major stress hormone.

So in essence, what occurs then is that stressful signals impinge on the hypothalamus, the hypothalamus releases this corticotropin-releasing factor to the pituitary, the pituitary gland releases ACTH which causes the adrenals to release gluco-corticoids and 'boom', we have a stress response. Now one of the ways in which this system is modulated is a classic feature of endocrinology that we call negative feedback, that is, the end product of the system. The glucocorticoids travel in the bloodstream, they act very quickly on the pituitary and the hypothalamus to arrest these signals, that is, they mute or dampen them CRF and ACTH…..Tonically, over a longer period of time, these same hormones act outside the hypothalamus on brain structures that then regulate the sensitivity of the system. So glucocorticoids, for example, will interact with the receptor protein on the hippocampus brain cells and the net effect of the interaction of the steroid with the glucocorticoids receptor is to inhibit the reception of CRF. The more you inhibit the production of CRF you more you dampen ACTH response to stress.

Had enough, sure? Ok, I've tortured you enough.

Here's something that's interesting. When I was training early on in science, we discovered that the more glucocorticoids receptors that are made within the hypothalamus, the greater the ability of this negative feedback signal to inhibit CRF. So animals that produced more of these glucocorticoid receptors have a more efficient ability to inhibit CRF and thus to dampen the pituitary adrenal responses to stress. Animals that produce fewer of these glucocorticoid receptors the inhibitory signal is not as effective and these animals show greater production of CRF and thus a greater stress response. So all were saying here is that when the signal is greater, the inhibition of the pituitary stress response is greater and that's important because it is exactly this difference which describes the adult offspring of mothers who are high lickers versus those who are low lickers. If you take an adult male or female offspring who was raised by a high licking mother they produce more of these glucocorticoid receptors, they are better able to constrain the production of CRF and thus they produce more modest stress responses. In contrast animals that are raised by low licking mothers produce fewer of these glucocorticoid receptors, they are less capable of inhibiting CRF and thus they show a greater response to stress. The key here is the difference in the activity of the gene which produces the glucocorticoid receptor. It's more active in offspring raised by high compared to low licking mothers and thus the stress response is different and just to show you that graphically, this is the glucocorticoid response to stress in an animal raised by a high licking mother, in the black circles you see the stress response in an animal that was raised by a low licking mother. They differ in stress reactivity. And the reason they differ in stress reactivity is because the gene which produces the glucocorticoid receptor in the hippocampus is more active in animals reared by high licking mothers than in animals reared by low licking mothers. You have a social modulation of a gene that produces a receptor that regulates the way in which the animals respond to stress.

And of course you can ask all kinds of questions about this particular finding. One of the questions that you can ask, for example, is that, well, is this really due to the mother? Is it really the mother's behaviour, the licking behaviour per se, that's driving the differences in the glucocorticoid receptor gene? And we can answer that question by doing what is a fairly simple, albeit labour intensive experiment, we can do adoption studies. We can take animals that are born to low licking mothers, the biological offspring of a low licking dam, and transfer them right after birth to an animal that we know to be a high licking mother and vice versa. We can take animals born to high licking mothers and foster them onto

lows and when we do that study we completely reversed the findings. So we know that it's the mother's behaviour and studies from our labs and from other labs have shown that in fact the tactile stimulation associated with the mother's licking is most pertinent to gene markers. So it is in fact the mother's licking that's producing these differences in the cell.

The question then is how does that happen? How does a social event regulate the activity of a gene? First question. And second question, how is it that once these pups have grown up, weaned, moved away from home, don't write, don't call (expect for when they need money) that somehow that effect persists? That they continue to produce those differences in the glucocorticoid receptor? What I'm going to do is to walk you through just a little bit of how this happens, and again the details here are critical. Essentially what we want to know is how can licking behaviour, which is a social event, alter the reactivity of a hippocampus brain cell so that it produces more of these glucocorticoid receptors?

So the first challenge is that you want to find that critical signal that links the behaviour of the mother to the cell itself, this extracellular signal. What kind of neurotransmitter or neurochemical event is directly modulated by the care of the mother? Once you get inside of a cell you then want to know how the signal is actually transduced into a set of molecules that directly interact with the DNA of the cell. Here is the answer to how that occurs. Essentially what happens is that when the mother licks the pup it increases sympathetic activity, so increases the activity in output from sympathetic nerves systems so that you get more adrenaline produced by the pup. The adrenaline acts on actually stored fat, brown fat tissues, and it causes the brown fat cells to take up a particular hormone, thyroid, and to produce higher levels of the more active variant of the thyroid hormone known as trigli thyroid or T3. Thyroid hormones affect almost every aspect of development, including that of the brain. And what they do is they act on specific nerves in the brain to cause an increase in the release of a neurotransmitter than can deploy a chemical called serotonin. Serotonin has multiple effects, particularly within the brain structures such as the around the cerebral cortex and we know the licking behaviour of the mother causes an increase in the release of serotonin. We can then also describe exactly what receptors or intracellular pathways are effected by the release of serotonin. That part we can define.

Now what we really need to do is to discover what is the transcription factor which links this extra serotonin signal to the DNA? That was, if you will, is the major obstacle. This was overcome when we identified one particular transcription factor known as NGFIA. That's the transcription factor that is effected by serotonin and activated by maternal care. What makes that a really important event is that we know that this transcription factor, NGFIA, has the ability of directly interacting with DNA. Ok, so it is potentially is a single factor which could link with a social event and we've shown, for example that we know that serotonin itself is capable of not only activating the NGFIA but of turning on the glucocorticoid receptor. What we also found is, in fact, the effects of serotonin are mediated through NGFIA. So we took some hypothalamus cells and put them in a culture dish and we simply mimicked the effects of maternal care by training those cells with serotonin and 'whoops' we can cause an increase in the production of the glucocorticoid receptor and that effect is completely blocked disable the cell's ability to produce…..So NGFIA is the signal that links the mother's licking and the production of serotonin to the increase in the glucocorticoid receptor and we also found through the study of pups that the mother's licking directly increases the production of NGFIA so we know this cascade of events and signals is occurring within the hippocampus neurones when these pups are being licked by their parents.

The next question we have to ask is where in the DNA this NGFIA signal being transmitted from? We can stem the mother's influence up to and including the production of the NGFIA. Now we want to get to the DNA itself so the gene can be transcribed. So, in fact, there are studies we are doing in collaboration with a good friend……Sable from University of Edinburgh where what we did was to look at that part of the gene that seemed like the most likely place to look. It is that part of the gene that regulates the activity of the glucocorticoid receptor gene. So when you think of a gene in the simplest of terms you can first of all think of a region of DNA which produces protein. The second region of DNA does not participate in the production of protein. Instead, it contains regulatory elements that determine the level of activity of this part of the gene. That, we consider to be the most common place because we know the offspring which don't have glucocorticoid receptor gene, they just don't make as much of it. So we figured these regulatory regions are probably primary regulatory actions. So what Jonathan's lab work did was to log and describe that part of the gene which had a regulatory effect. What we found was, in fact, there were many sequences of DNA, each of which were perfectly capable of turning on the glucocorticoid receptor gene. Our challenge then was to find which of these regulatory areas, which of these so called promoters were active within hippocampus neurones and was regulated by the behaviour of the mother. And so we found this guy right here. This is actually called '1 (7) promoter' and it is one span of DNA which is critical in determining how much glucocorticoid receptor you produce in hippocampus brain cells.

What we then found was that in fact this region of DNA right here is more active in turning on the glucocorticoid receptor gene if the animal was reared by a high licking mother compared to a low licking mother. So now we've got a particular region that we know determines the antipathy of the glucocorticoid receptor gene and it's regulated by maternal care. We then found when we sequenced this region of the glucocorticoid is that it contains a region which can physically directly interact with the NGFIA. Ok, think about this. This is a little bit for us, well, chemistry geeks because here you've got protein signalling known as being activated by maternal behaviour and here you've got a region of the DNA which can directly interact with that maternal signal. So now what we have is the prospect of being able to define precisely how a social signal is interactive with DNA. Of course, as I mentioned to you earlier what we have previously shown is that , yeah, mother's licking turns on NGFIA, these pups produced by licking mothers produce higher NGFIA and what we also showed is that if you take hippocampus brain cells of pups born and reared by high or low licking mothers is that you can actually find there is more NGFIA physically interacting with this regulatory sequence in pups reared by high rather than low licking mums. There is more of it directly signalling from the DNA. And what that does of course when this signals when it signals this particular promoter is it turns it on and it causes pups to produce more glucocorticoid receptor.

So, in a nutshell then, what happens is the mother's licking causes an increase in NGFIA that binds to this regulator 1(7) promoter, turns on the glucocorticoid receptor and so the pup is making more of this glucocorticoid receptor gene. That is interesting but it's only part of the problem. In fact, the main challenge here is really not to understand how the pup in early infancy glucocorticoid receptor ……The really challenge is to understand how it is that two, four, six months later when the pup is clearly removed from its mother it is still making glucocorticoid receptor. So part of the problem is underscored here by well, if you look at the brain of infant rats the imagined NGFIA is indeed regulated by the mother's licking but in adulthood that effect is abolished. There is no longer a difference in that NGFIA**.** So that can't explain why they are making more of these glucocorticoid receptors. So what we suggested was this could be the increased reaction of NGFIA with the DNA during early live, in the first weeks of life when these animals were developing.

This somehow posits a structural change in the DNA and this structural change later continues and mediates this particular form. And the structural changes we talked about and considered was the possibility that it might lead to a difference in DNA methylation and so what we actively did then was to look at the methylation of this critical region of the DNA that regulates the glucocorticoid receptor. Now, here's that particular region. This is the region that physically interacts with NGFIA and what you'll note, if you look closely enough, is there are actually two cytosines, two of these seeds lying within the region which can physically interact with NGFIA. Cytosines can be methylated. So what we did was to simply take this sequence and ask the question whether or not the mother's behaviour might determine whether either this cytosine or this one here might be methylated or not and the answer was, yes, it does. So you've got two cytosines. This is just the same sequence which binds NGFIA. That's the part of the promoter which physically interacts with this transcription factor. Two cytosines. One on the right which we refer to three pronged and one on the left which we…..So we are actually doing studies where we characterise the methylation state of these individual cytosines. We find that this particular cytosine sitting right here it generally tends to be methylated. It carries that methyl marker regardless of whether the animal was raised by a high or low licking mother. Over here, this particular cytosine in contrast is heavily methylated; it carries the methyl marker here. It was raised by a low licking mother but it very rarely carries that methyl marker if the animal was raised by a high licking mother. The mother's behaviour is statistically associated with the temperance and presence in rats of the methyl group of this particular cytosine.

Ok, the second thing we can do is go back to our……Showing in fact, this difference I showed you right here is entirely driven by the mother's behaviour towards……Now the next question you can ask is so bloody what? We've got one signal cytosine nucleotide and it either carries or does not carry this methyl marker. Does that really change anything? Is that really meaningful? So we did a series of studies in which we asked the question: 'if the methyl marker is present on this single cytosine, as so commonly is on animals raised by low licking mothers does that alter the ability of NGFIA to physically interact with the site? In contrast, in animals who were raised by high licking mothers who don't carry the methyl marker, is NGFIA better able to interact with DNA at that site? The answer is yes, that's true. So, in a sense what you are doing by adding a single methyl group onto that site is you are decreasing the ability of NGFIA to physically bind to the DNA and to turn on the glucocorticoid receptor. Fair enough? That's what the mother's licking behaviour is doing and in fact, what you can then do is to understand is why it is that despite the fact that as adults these animals produce the same amount of NGFIA they produce different amounts of glucocorticoid receptor. And the explanation is as follows.

If the animal carries the methyl marker at this particular site, even though it makes scads of NGFIA, the NGFIA cannot physically interact with that site and therefore cannot turn on the glucocorticoid receptor and thus this is apparent in animals raised by low licking mothers. In contrast, if the animal was reared by a high licking mother, it does not carry the methyl marker, NGFIA can now interact with the site and turn on the glucocorticoid receptor. The behaviour of the mother is altering the ability of this signal to turn on this particular function and that, for example, something we have been giving considerable attention and there is a great deal of evidence now for exactly that model. We can now go back experimentally and reverse this difference in methylation. It completely reverses the differences in the glucocorticoid receptor in production and the way in which these animals respond to stress. And that is what is going on in these slides which I am going to spare you from.

I'm going to take you through just two quick messages as I think I'm starting to get a little …….here.

The first question is how does this happen? How in fact does maternal care alter the methylation of this particular cytosine? Now, I took you through this particular schema and we really need to go into it in order to see it. What I'm telling you're here is maternal care creates a whole series of signals occurring outside as well as inside the hypothalamus cells and the critical signal for the activation of glucocorticoid receptor is the ability of NGFIA to physically interact with this glucocorticoid receptor site. What I'm going to tell you is, in fact, is the research strongly suggests is it is exactly the increased interaction of NGFIA with the genome in early life that produces the difference in DNA methylation. The way that seems to happen was a bit of a surprise to us.

What you are looking at here, remember I told you the difference on the chromosome is one single cytosine is the nucleotide which sits right here within the region of DNA and binds NGFIA. So what we simply did with this study was to take animals of different ages and ask: 'when does the difference in that methylation occur? Well, it isn't occurring before they are born because at that particular point in time it doesn't matter if you take hippocampus cells from animals born to high licking and lower licking mothers or even just from higher licking mothers; they do not contain the methyl mark. Shortly after birth the methyl mark is attached to cytosine and that occurs in the offspring that were born to both high and low licking mothers. In the offspring of low licking mothers the methylation marker is attached and remains fixed to that cytosine. In offspring born to high licking mothers, the methyl marker has been actively removed. It has been removed through the binding of NGFIA. It is the binding of NGFIA to this site which causes the methyl mark to be actively removed.

So, in essence what you are looking at here is a story through which the mother's behaviour is shaping the chemical signals that are superimposed on the DNA and in doing so giving rise to the change in the gene's……and the activity of the glucocorticoid receptors, as well as changes of course, in adult responses to stress.

So the second part I'm going to talk to you about is the question, very simply, as to whether or not a similar process might be possible for human subjects. The way we tested this again, working in collaboration this time with staff at…….MGill. And what we were doing here was to say. 'ok, look, we know we are not going to be able to do studies as definitively with humans as we can with a rat' [laughter] 'but perhaps what we can do is to come up with some approximations'.

So lets start working, at least, with the same brain tissue. This is hippocampal brain tissue that comes from what is known as the Quebec Suicide Brain Bank. Tragically, this brain banks is formed by donations through individuals who have died from suicide or have died through involuntarily but no less secondary death. So you know have access to tissues that are biochemically intact which you can start to study. I want you to assume that since some of these individuals died involuntarily and others through their own doings that there might be some difference in terms of underlying psycho pathology here and that, in fact, is actually buttressed by the activities of…….whose digging constructs what's know as forensic genotype. Essentially, what they do is they interview family members of the victims and through the interviews with family members and friends they're able to judge not only the history of psycho pathology of the individual also characterise their developmental history as well. The second thing you can do is you can say, 'I'm going to look at the same region of the DNA that I studied in the rat' because of the work of……and people don't do that often unless they [1:04:48]. They characterise the same region of the genome in humans that we have previously characterised in the rat. So what we are doing then is to excise hippocampus tissue from suicide victims controlled and we

are looking at the comparable region of the genome; we are looking at the glucocorticoid receptor. We are interested in that region of the DNA that regulates the activity glucocorticoid receptor and in a human the promoter of it is comparable to this '1(7) promoter' that activates the glucocorticoid receptor in the brain cells and is known as this one path promoter right here. And it also contains a sequence which combines NGFIA so it operates very similarly to the way it actually does in a rat. So the

So the first thing we did when we looked at these brains is to characterise possible differences in glucocorticoid receptor levels. What we found was that in hippocampus that came from controls, the produced more of this glucocorticoid receptor gene than any of the individuals who had died from suicide. We also found that this one act promoter was more active in hippocampus cells in the controls versus suicide,as you would expect. So in the regulatory region there is more active energy which is producing more glucocorticoid receptors and that difference was apparent individuals who had completed suicide versus controls. Then because of…….forensic genotype we were then able to take this suicide group and to break them into two cell populations. One suicide group were positive for a history of abuse and child neglect, that contained and including severe emotional neglect , physical and sexual abuse. The other group was negative of this developmental history. What you can see from this graph is that yes, indeed, it was only those individuals which were positive for abuse that showed differences in the glucocorticoid receptor as well as differences in…….the one path promoter activity. What we then did was to look at the methylation of this region and we found again that those individuals who were positive for a history of abuse had an increase in the methylation signal comparable to what we had seen in offspring reared by low licking mothers.

You will note that neither in terms of methylation nor in terms of glucocorticoid receptor was there any difference between suicides negative for abuse and controls. We then went onto show that the difference in methylation occurs at a very particular site and then indeed, as you go back back this is associated with a blockade in NGFIA to drive the glucocorticoid receptor. So the history of childhood adversity in these circumstances was associated with change in the methylation status of this gene and a difference in the activity of the gene itself. So, again this is certainly not most convincing, by any means that the cognitive processes are occurring, nor is it illuminating the mechanism by which human parenting directly intervenes but it is a story which is largely consistent with what we are seeing in the lab. Of course, one of the major features from all of this which we are coming through is these basic fundamental biological features of parental interactions and activity of genes seem to have a theme which runs through more than one species.

Ok, I think at that point I will leave it here and take any questions. Thank you very much.

[Applause]