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IN THEIR NURTURE

Can epigenetics underlie the enduring effects of a mother's love? **Lizzie Buchen** investigates the criticisms of a landmark study and the controversial field to which it gave birth.

After dropping a pair of male and female adult rats into a rectangular Plexiglas container, Frances Champagne can expect one of a few scenarios to ensue. The male will definitely try to mate with the female — but the female is less predictable. She might approach him, appraise his scents and arch her back to allow him to mount her. Should a second male enter the cage after she's mated with the first, she may be similarly hospitable.

Some females play it coy, however, evading the male, requiring more courtship and, if mating does occur, avoiding another go. A number of factors can influence what the female does, but to Champagne, a behavioural scientist at Columbia University in New York, one is particularly beguiling: how often the female rat's mother licked and groomed her during her first week of life[1]. Doting mothers have prudish daughters, whereas the daughters of inattentive rats cavort around like mini Mae Wests. At the heart of these differences lies the sex hormone oestrogen, which drives female sexual behaviour. Champagne says that neglected rats might respond to it more strongly than those raised by attentive mums.

The phenomenon is just one example of how experiences early in life can shape behaviour, and it may apply to humans. It is known, for example, that children who grow up in poverty are at greater risk as adults for problems such as drug addiction and depression than those with more comfortable upbringings, regardless of their socioeconomic situation later in life. But what is it about early experiences that has such a lasting effect? For Champagne and many of her colleagues, the answer has been apparent for nearly a decade. Life experiences alter DNA; not necessarily its sequence but rather its form and structure, including the chemicals that decorate it and how tightly it winds and packs around proteins inside the cell. These changes, often referred to as epigenetic modifications, make genes easier or more difficult for the cell's protein-making machinery to read (see 'The marking of a genome').

The most enduring epigenetic change is thought to be the attachment of methyl groups to specific nucleotides in DNA, which can completely silence the expression of nearby genes. Champagne says that her neglected rats might have less methylation near the oestrogen receptor gene. And such differences occur specifically in regions of the hypothalamus known to be involved in sexual behaviour. Less methylation leads to increased expression of the oestrogen receptor throughout life, she reasons, making the adult daughters more responsive to the hormone's influence when sizing up suitors.

The idea that epigenetics could explain the lasting effects of something as short-lived but profound as a mother's affection has breathed life into the behavioural sciences, providing a molecular middle ground in the centuries-old debate over nature versus nurture. Epigenetic changes could be the conduit through which environment elicits life-long biological change. Many behavioural scientists have latched onto the idea, searching for epigenetic explanations for a number of differences in behaviour, including homosexuality, intelligence and conditions such as autism and schizophrenia. Although experience has been connected to altered methylation for only a handful of genes, epigenetics has become one of the hottest areas of behavioural science. But it is also one of the most hotly contested.

A struggle for acceptance

Behavioural epigeneticists have run up against deep resistance to their ideas — generally from molecular biologists and biochemists, who have been studying DNA methylation since the 1960s. When methyl groups coat DNA early in embryonic development, the affected genes are turned off for the life of the animal; for example, this mechanism permanently shuts down one of the two X chromosomes in female mammalian cells. Many find the idea that DNA methylation could be influenced by maternal care hard to swallow. Behavioural epigenetics "is a field that has a lot of deep problems", says Timothy Bestor, a geneticist also at Columbia University who studies DNA methylation in sex cells. The evidence supporting it is weak and grossly over-interpreted, according to Bestor and other critics, and the mechanism by which it works remains unclear. To prove the field's worth to the hardcore molecular biologists, the behaviourists will have their work cut out for them.

The debate has been raging since the early 2000s, when Champagne was in graduate school at McGill University in Montreal,

Canada. Her adviser, Michael Meaney, a behavioural scientist, was trying to explain why rats raised by attentive mothers were, as adults, able to deal with stress better than rats raised by more negligent mothers. Meaney's group found that levels of the glucocorticoid receptor, a protein that regulates the reaction to stress hormones, were different in these two groups, but the group puzzled over how the difference came about. Serendipitously, Meaney met Moshe Szyf, a molecular biologist at McGill who was studying DNA methylation in cancer. His research had shown that DNA methylation might act as an on–off switch for cancer-causing genes[2]. As the two discussed Meaney's stressed-out rats, they wondered whether the same mechanism might be at play. They collaborated, and found different methylation patterns between groomed and ungroomed pups. Their work suggested that a mother rat's preening could remove methyl groups from her pups' DNA. This alteration, they argued, made the gene for the glucocorticoid receptor more accessible to protein-making machinery.

Szyf was excited, but surprised. DNA methylation was thought to be stable. For this reason, says Szyf, the paper struggled through the review process, and was rejected by both *Nature* and *Science*. "The main review was, 'We never heard that DNA methylation changes after birth'," he says. "Something that doesn't fit with their dogma has to be wrong."

After two-and-a-half years, the paper found an outlet in *Nature Neuroscience*[3] in 2004. And among behavioural neuroscience researchers, Meaney says, it caused a stir. "They understood immediately that epigenetic mechanisms were a great candidate that could explain the enduring effects of the early environment," he says. A deluge of research projects ensued, and are beginning to bear fruit. In December, Dietmar Spengler at the Max Planck Institute of Psychiatry in Munich, Germany, and his colleagues showed that separating mouse pups from their mothers for short periods of time reduced the methylation near the arginine vasopressin gene, possibly leading to a depression-like condition[4]. In May, David Sweatt at the University of Alabama at Birmingham showed that stress in early life changed the methylation status of the rat *Bdnf* genes, which encodes a growth factor involved in brain development and plasticity[5].

The work is starting to extend to humans as well. In 2009, Meaney and his collaborators compared the brains of people who had committed suicide and who were severely abused as children with those who were not. His data suggested that those who had been subject to abuse showed methylation changes in stress-related genes similar to those found in rats raised by inattentive mothers[6]. That same year, epigeneticist Jessica Connelly at Duke University in Durham, North Carolina, and her colleagues found methylation differences in the gene encoding the receptor for oxytocin — a hormone believed to influence social behaviour — in people with autism[7]. Now at the University of Virginia in Charlottesville, Connelly is pursuing this link further in humans and in prairie voles, which form close social bonds, so can potentially be used to help study human social behaviour. And Champagne, who started her own lab in 2006, is teaming up with researchers at the Columbia Center for Children's Environmental Health to see if pollution in northern Harlem and the South Bronx in New York is leading to epigenetic changes in children, making them more prone to conditions such as asthma and obesity.

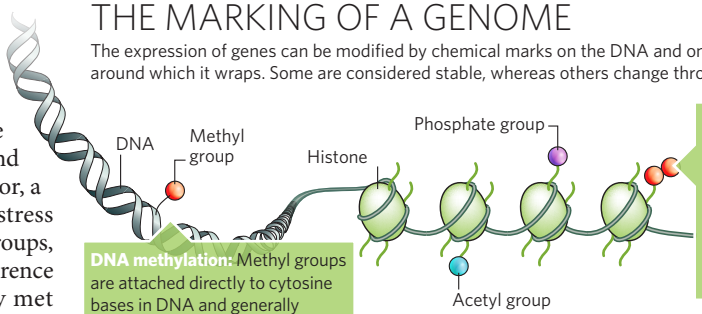
> **"No one can take methylated DNA, mix it with enzymes *in vitro* and demethylate it."**

Meany's 2004 study[3] eventually became one of *Nature Neuroscience*'s most cited papers. But the criticisms have not stopped.

Molecular biologists' main problem with the behavioural neuroscientists' data is that they are highly correlative, and the underlying mechanisms are still largely unknown. Scientists who cut their teeth on *in vitro* systems — with their exacting control of variables and unambiguous data — cannot wrap their heads around messy data and tenuous links. "What's really important to understand is the enormous gap in mechanistic knowledge between people who work in simple systems, such as epigenetic inheritance in yeast, where people have spent years to go over mechanistic details and really understand how it works, to someone who looks at the enormous complexity of the brain," says Catherine Dulac, a molecular biologist at Harvard University in Cambridge, Massachusetts.

THE MARKING OF A GENOME

The expression of genes can be modified by chemical marks on the DNA and on the proteins around which it wraps. Some are considered stable, whereas others change throughout life.



DNA methylation: Methyl groups are attached directly to cytosine bases in DNA and generally silence nearby genes. Removal of these marks occurs during...

Histone modification: The histone proteins around which DNA wraps can be methylated, phosphorylated, acetylated and more. These marks influence the binding of other proteins, chromatin structure and gene expression. They can generally be remodelled throughout life.

...fertilization
Just after embryo formation, methyl groups are stripped from the paternal DNA and methylation is reset for development.

...fetal development
Genome-wide demethylation occurs again during the development of primordial sex cells (sperm or eggs) in the fetus.

...perinatal development?
Before and after birth, diet and stress may contribute to further demethylation of genes, which may influence behaviour.

The mechanics of upbringing

One of the biggest bones of contention with the work of Meaney and Champagne is that there is no proven mechanism for actively removing methyl groups from DNA. A number of groups have proposed and published evidence of a 'demethylase' that would do this. Szyf's group, for example, published results in 1999 showing that a protein called MBD2 could rapidly remove methyl groups from DNA[8]. But critics argue that these have not stood the test of reproducibility.

Adrian Bird, who studies DNA methylation at the University of Edinburgh, UK, calls it one of many "false alarms". He showed in 2001 that mice lacking the *Mbd2* gene have normal patterns of DNA methylation, suggesting that it does not have a demethylase role[9]. The crucial missing piece, he says, is a pure biochemical demonstration of enzymatic activity. "No one can take a piece of methylated DNA, mix it with some enzyme *in vitro* and demethylate it. It doesn't mean it can't happen, but show me

the incontrovertible evidence."

Another issue is that the methylation changes documented by that Meaney, Szyf, Champagne and others seem trivially tiny, appearing on only a handful of nucleotides in a small region of a gene, for example. And those changes only occur in a fraction of the total cells. To epigeneticists who study robust changes in methylation such as those that occur in embryos and zygotes, this looks like noise. It is also unclear whether these changes are actually happening in neurons. They could, for example, be happening in glia, cells that mainly provide support and protection for neurons. "Quite often one sees statistically significant differences in DNA methylation but they're very small," says Bird. "The question is, are they biologically significant in addition to statistically significant?"

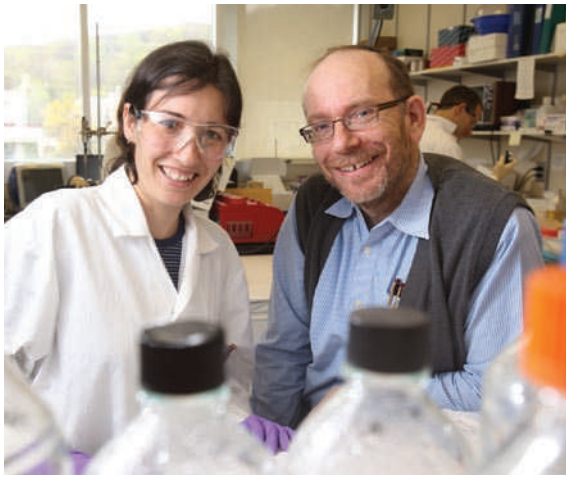
Proponents are unfazed by the wall of scepticism. Szyf insists that his group has shown that small changes make a difference. In their human study, he says, "we only got a few cytosines methylated, and that made us worried". So they engineered a version of the DNA region they were studying — near a glucocorticoid receptor gene — that had only those specific sites methylated. It shut down gene expression for cells in a dish[6].

Bestor, still unconvinced, responds that because the researchers only provide percentages of methylation in the brain, it is hard to tell whether any given cell would actually have all the sites methylated.

As for the demethylase, Champagne says one has to exist. "There's a chemical reaction. DNA methyl groups are coming off. So there has to be an enzyme to do it." Plant cells have a well characterized demethylase, and many suspect that one will turn up in animal cells. Szyf, moreover, stands by the *Mbd2* findings that Bird could not corroborate.

As for the size of the changes, Champagne understands why many molecular biologists are wary. "If you're used to seeing effect sizes in a cell-culture dish, the kinds of effect sizes reported in behavioural models might mean nothing to you," she says. "If you saw that effect in a dish, it'd be error." But she points out that small changes can have big effects in the nervous system. "With behaviour, it's so dependent on where the changes are happening in the brain, what part of the circuits are affected," says Champagne.

She acknowledges, however, that there are still many unknowns. She also appreciates that scepticism is healthy for the field, which even she thinks may be getting too hot, too fast. Critics, she says, "keep everyone honest. The enthusiasm in the field is obviously great, but I think people's expectations of what this means need to chill out a little bit. There are a few things we need to work out".



Frances Champagne (left), postdoc Nada Borghol and Moshe Szyf study epigenetics in rodents and humans.

O. EGAN

Szyf says, however, that the responses to behavioural epigenetics reflect a difference in ethos in fields that need to come together. "The psychiatry field is glad to have this mechanism they were missing," he says. "It was the thing that bothered them and now it's like, 'Oh wow, this makes so much sense.' But then the epigeneticists say, 'Oh come on, that's just magic.'" Szyf says that almost all of the papers that cite his and Meaney's 2004 study are from the behavioural sciences — not genetics or molecular biology.

> "People's expectations of what this means need to chill out a little bit. There are a few things we need to work out."

Some molecular biologists are warming to the idea, however. "We're starting to see this in more than one gene, more than one neuronal region," says Huda Zoghbi, a geneticist at Baylor College of Medicine in Houston, Texas, who studies the role of DNA methylation in a form of mental retardation. "There are a few issues, but I think it's intriguing and we really have to take stock in it, and start thinking about how this is happening."

Determining exactly how it is happening remains the challenge. Even if researchers can work out how methyl marks are removed from a gene, they have to show the mechanism by which a life experience such as maternal care would cause that change. Meaney has proposed that mother rats' licks increase levels of the neurotransmitter serotonin, and that this increase could result in methylation changes, but no experiments have provided evidence that would explain how this link would work.

The behavioural epigeneticists know they have work to do to answer the criticisms and they are setting about doing it. Szyf's lab is using a technique that can separate neurons from glia and other cell types so he can work out where the methylation is occurring, and Meaney says experiments are in progress to understand the series of events by which the environment might be altering methylation. Champagne is working with mice in hopes of doing more genetic experiments, and says that behavioural scientists are increasingly collaborating with classically trained molecular biologists. She has recently hired a postdoc trained in epigenetics. "The question always becomes, how do you transduce a social experience to the level of DNA methylation? Right now it's very speculative. We don't know. To really study that, you have to go back to a dish, ultimately."

Until these sorts of hard data arrive, many molecular biologists say that they will stay in the sceptics' corner. "It's an interesting possibility, but I do think people have jumped the gun and seen more positive results than are really out there," says Bird. "I'm perfectly happy to believe that it's very important, but I'm also happy to believe that it's irrelevant." ■

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