# Mice Inherit Specific Memories, Because Epigenetics?

By Virginia Hughes

Two weeks ago I wrote about some tantalizing research coming out of the Society for Neuroscience meeting in San Diego. Brian Dias, a postdoctoral fellow in Kerry Ressler's lab at Emory University, had reported that mice inherit specific smell memories from their fathers — even when the offspring have never experienced that smell before, and even when they've never met their father. What's more, *their* children are born with the same specific memory.

This was a big, surprising claim, causing many genetics experts to do a double-take, as I discovered from a subsequent flurry of Tweets. "Crazy Lamarkian shit," quipped Laura Hercher (@laurahercher), referring to Lamarckian inheritance, the largely discredited theory that says an organism can pass down learned behaviors or traits to its offspring. "My instinct is deep skepticism, but will have to wait for paper to come out," wrote Kevin Mitchell(@WiringTheBrain). "If true, would be revolutionary."

The paper is out today in *Nature Neuroscience*, showing what I reported before as well as the beginnings of an epigenetic explanation. (Epigenetics usually refers to chemical changes that affect gene expression without altering the DNA code).

Having the data in hand allowed me to fill in the backstory of the research, as well as gather more informed reactions from experts in neuroscience and in genetics. I've gone into a lot of detail below, but here's the bottom line: The behavioral results are surprising, solid, and will certainly inspire further studies by many other research groups. The epigenetic data seems gauzy by comparison, with some experts saying it's thin-but-useful and others finding it full of holes.

## SO WHAT IS THE SURPRISING PART, AGAIN?

If you've followed science news over the past decade then you've probably heard about epigenetics, a field that's caught fire in the minds of scientists and the public, and understandably so. Epigenetic studies have shown that changes in an organism's external environment — its life experiences and even its choices, if you want to get hyperbolic — can influence the expression of its otherwise inflexible DNA code. Epigenetics, in other words, is enticing because it offers a resolution to the tedious, perennial debates of nurture versus nature.

But some scientists dispute the notion that epigenetic changes have much influence on behavior (see this *Nature*feature for a great overview of the debate). Even more controversial is the idea that epigenetic changes can be passed down from one generation to the next, effectively giving parents a way to prime their children for a specific environment. The key question isn't whether this so-called 'transgenerational epigenetic inheritance' happens — it does — but rather how it happens (and how frequently, and in what contexts and species).

Source: http://phenomena.nationalgeographic.com/2013/12/01/mice-inherit-specific-memories-because-epigenetics/

That's what Dias and Ressler wanted to investigate. Trouble is, environmental influences such as stress are notoriously difficult to measure. So the researchers focused on the mouse olfactory system, the oft-studied and well-mapped brain circuits that process smell. "We thought it would give us a molecular foothold into how transgenerational inheritance might occur," Dias says.

The researchers made mice afraid of a fruity odor, called acetophenone, by pairing it with a mild shock to the foot. In a study published a few years ago, Ressler had shown that this type of fear learning is specific: Mice trained to fear one particular smell show an increased startle to that odor but not others. What's more, this fear learning changes the organization of neurons in the animal's nose, leading to more cells that are sensitive to that particular smell.

Ten days after this fear training, Dias allowed the animals to mate. And that's where the crazy begins. The offspring (known as the F1 generation) show an increased startle to the fruity smell even when they have never encountered the smell before, and thus have no obvious reason to be sensitive to it. And their reaction is specific: They do not startle to another odor called propanol. Craziest of all,*their* offspring (the F2 generation) show the same increased sensitivity to acetophenone.

The scientists then looked at the F1 and F2 animals' brains. When the grandparent generation is trained to fear acetophenone, the F1 and F2 generations' noses end up with more "M71 neurons," which contain a receptor that detects acetophenone. Their brains also have larger "M71 glomeruli," a region of the olfactory bulb that responds to this smell.

"When Brian came in with the first set of data, we both just couldn't believe it," Ressler recalls. "I was like, 'Well, it must just be random, let's do it again.' And then it just kept working. We do a lot of behavior [experiments], but being able to see structural change that correlates with behavior is really pretty astounding."

Still, those experiments couldn't rule out some kind of social, rather than biological transmission. Perhaps fathers exposed to the fear training treated their children differently. Or maybe mothers, sensing something odd in their mate's behavior, treated their children differently.

To control for these possibilities, the researchers performed an in vitro fertilization (IVF) experiment in which they trained male animals to fear acetophenone and then 10 days later harvested the animals' sperm. They sent the sperm to another lab across campus where it was used to artificially inseminate female mice. Then the researchers looked at the brains of the offspring. They had larger M71 glomeruli, just as before. (The researchers couldn't perform behavioral tests on these animals because of laboratory regulations about animal quarantine.)

"For me it clicked when we did the IVF," Dias says. "When the brain anatomy persisted, that to me emphasized that it's not really a social transmission. It's inherited."

Other researchers also seem convinced. "It is high time public health researchers took human transgenerational responses seriously," says Marcus Pembrey, emeritus professor of paediatric genetics at University College London, who has been championing the idea of epigenetic inheritance for over a decade. "I suspect we will not understand the rise in neuropsychiatric disorders or obesity, diabetes and metabolic disruptions generally without taking a multigenerational approach," he says.

In an interesting historical aside, Pembrey also notes that the new study echoes an experiment that Ivan Pavlov did* 90 years ago, in which he trained mice to associate food with the sound of a bell. Pavlov "reported that successive generations took fewer and fewer training sessions before they would search for food on hearing a bell even when food was absent," Pembrey says. Nevertheless, the idea that experience could be biologically inherited fell out of favor in the 20th century. "If alive today, Pavlov would have been delighted by the Dias and Ressler paper, first as a vindication of his own experiment and results, and second by the amazing

experimental tools available to the modern scientist."

Neuroscientists, too, are enthusiastic about what these results might mean for understanding the brain.

"To my knowledge this is the first example, in any animal, of epigenetic transmission of a simple memory for a specific perceptual stimulus," says Tomás Ryan, a research fellow at MIT who studies how memories form in the brain. "The broader implications for the neuroscience of memory and to evolutionary biology in general could be paradigm shifting and unprecedented."

There are still some unanswered questions, Ryan notes. For example, the researchers didn't do a control experiment where the F0 animals are exposed to the fruity odor *without* the shock. So it's unclear whether the "memory" they're transmitting to their offspring is a fear memory, per se, or rather an increased sensitivity to an odor. This is an important distinction, because the brain uses many brain circuits outside of the olfactory bulb to encode fear memories. It's difficult to imagine how that kind of complicated brain imprint might get passed down to the next generation.

Ressler and Dias agree, and for that reason were careful not to refer to the transmitted information as a fear memory. "I don't know if it's a memory," Dias says. "It's a sensitivity, for now."

## WHAT'S THAT GOT TO DO WITH EPIGENETICS?

So let's call it a sensitivity. How could a smell sensitivity, formed in an adult animal's olfactory bulb in its brain, possibly be transmitted to its gonads and passed on to future generations?

The researchers are nowhere near being able to answer that question, but they have some data that points to epigenetics.

There are several types of epigenetic modifications. One of the best understood is DNA methylation. There are millions of spots along the mouse genome (and the human genome), called CpG sites, where methyl groups can attach and affect the expression of nearby genes. Typically, methylation dials down gene expression.

Dias and Ressler sent sperm samples of mice that had been fear-conditioned to either acetophenone or propanol to a private company, called Active Motif, which specializes in methylation analyses. The company's researchers (who were blinded to which samples were which) mapped out the sperm methylation patterns near two olfactory genes: Olfr151, which codes for the M71 receptor that's sensitive to acetophenone, and Olfr6, which codes for another odor receptor that is not sensitive to either odor.

It turns out that Olfr151, but not the other gene, is significantly less methylated in sperm from animals trained to fear acetophenone than in sperm from those trained to fear propanol. Because less methylation usually means a boost in gene expression, this could plausibly explain why these animals have more M71 receptors in their brains, the researchers say.

What's more, the same under-methylation shows up in the sperm of F1 animals whose fathers had been trained to fear acetophenone.

"It's a very precise signal," Ressler says. "The convergence of this data, we think, shows that this is a really profound and robust phenomenon."

Others, though, find a number of flaws in this epigenetic explanation.

Timothy Bestor, professor of genetics and development at Columbia University, points out that methylated CpG sites only affect gene expression when they are located in the so-called gene promoter, a region about 500 bases upstream of the gene. But the Olfr151 gene doesn't have any CpG sites in its promoter.

That means the differences in methylation reported in the paper must have occurred within the body of the gene itself. "And methylation in the gene body is common to all genes whether they're expressed or not," Bestor says. "I don't see any way by which that gene could be directly regulated by methylation."

But what would explain the methylation differences between the trained animals and controls? They're pretty subtle, he says, and "could easily be a statistical fluke."

Bestor was skeptical from the outset, based on the mechanics of the reproductive system alone. "There's a real problem in how the signal could reach the germ cells," he says.

For one thing, the seminiferous tubules, where sperm is made inside of the testes, don't have any nerves. "So there's no way the central nervous system could affect germ cell development." What's more, he says it's not likely that acetophenone would be able to cross the blood-testis barrier, the sheet of cells that separates the seminiferous tubules from the blood.

By this point in my conversation with Bestor, I was starting to feel a bit defensive on behalf of epigenetics and all of its wonder. "Are you saying you think epigenetic inheritance is a bunch of bologna?" I asked helplessly.

"No," he said, laughing. "It's just not as dynamic as people think."

## WHAT'S NEXT?

A good next step in resolving these pesky mechanistic questions would be to use chromatography to see whether odorant molecules like acetophenone actually get into the animals' bloodstream, Dias says. "The technology is surely there, and I think we are going to go down those routes."

First, though, Dias and Ressler are working on another behavioral experiment. They want to know: If the F0 mice un-learn the fear of acetophenone (which can be done by repeated exposures to the smell without a shock) and then reproduce, will their children still have an increased sensitivity to it?

"We have no idea yet," says Ressler, a practicing psychiatrist who has long been interested in the effects of post-traumatic stress disorder (PTSD). "But we think this would have tremendous implications for the treatment of adults [with PTSD] before they have children."

It will take a lot more work before scientists come close to understanding how these data relate to human anxiety disorders. So what, after all of these words, should we take away from this study now?

Hell if I know. Here's the most rational and conservative appraisal I can muster: Our bodies are constantly adapting to a changing world. We have many ways of helping our children make that unpredictable world slightly more predictable, and some of those ways seem to be hidden in our genome.

Anne Ferguson-Smith, a geneticist at the University of Cambridge, put it more succinctly. The study, she says, "potentially adds to the growing list of compelling models telling us that something is going on that facilitates transmission of environmentally induced traits."

Scientists, I have to assume, will be furiously working on what that *something* is for many decades to come. And I'll be following along, or trying to, with awe.

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*Update, 12/1/13, 2:35pm: It seems that that Pavlov experiment may have been retracted in 1927, though I don't know anything about that beyond what is stated here.

*Style note: A few paragraphs of this post were adapted from my earlier post on this research,* published November 15.