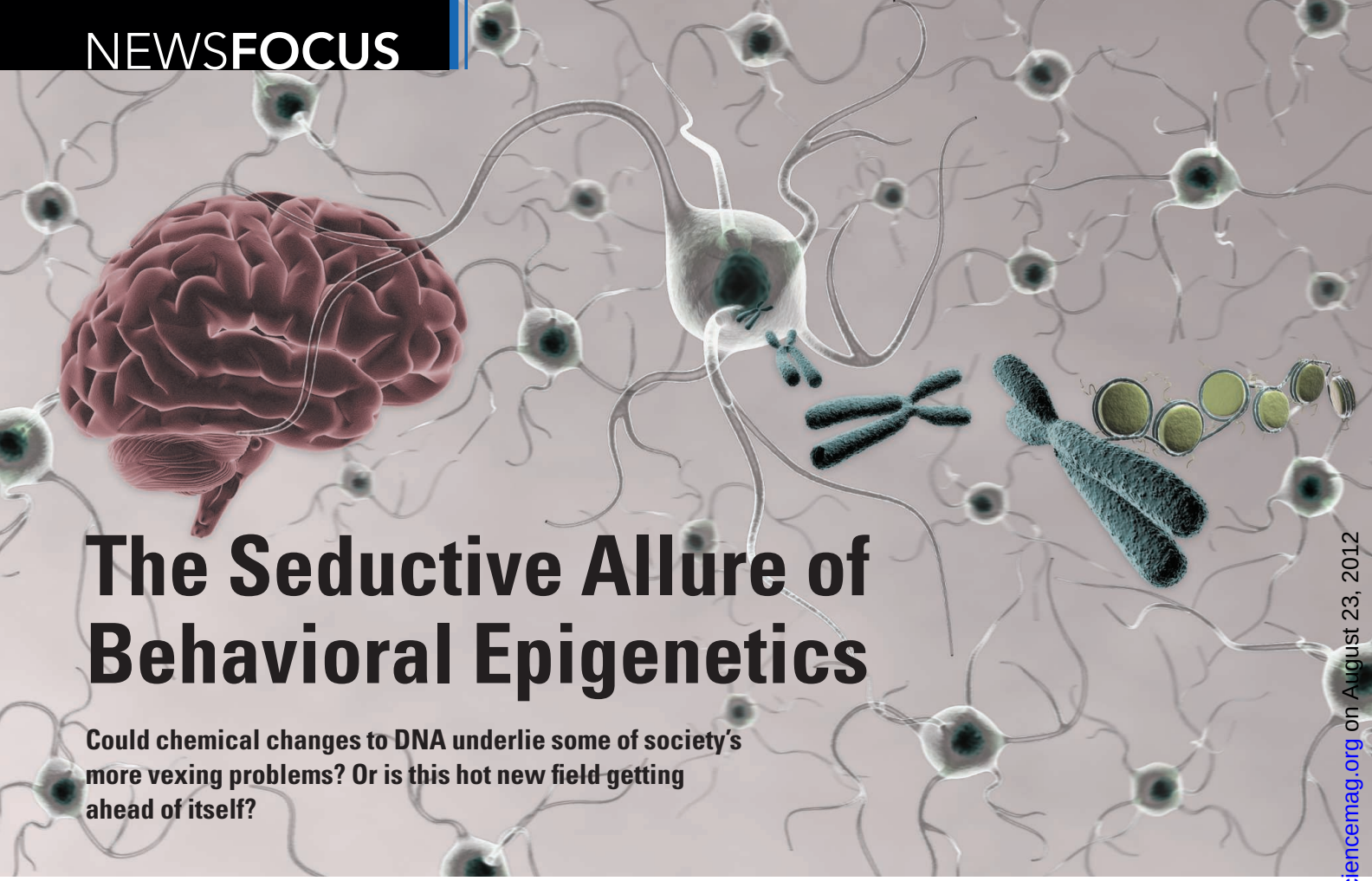

The Seductive Allure of Behavioral Epigenetics

Could chemical changes to DNA underlie some of society's more vexing problems? Or is this hot new field getting ahead of itself?

MICHAEL MEANEY AND MOSHE SZYF WORK in the same Canadian city, but it took a chance meeting at a Spanish pub more than 15 years ago to jump-start a collaboration that helped create a new discipline. Meaney, a neuroscientist at the Douglas Mental Health University Institute in Montreal, studies how early life experiences shape behavior later in life. Across town at McGill University, Szyf is a leading expert on chemical alterations to DNA that affect gene activity. Sometime in the mid-1990s, both men attended the same meeting in Madrid and ended up at a bar talking and drinking beer. "A lot of it," Szyf recalls.

Meaney told Szyf about his findings that rat pups raised by inattentive mothers tend to be more anxious as adults than pups raised by more nurturing mothers. He also described how the activity of stress-related genes was altered in the undernurtured pups. At some point in the conversation, Szyf had a flash of insight: This difference must be due to DNA methylation—the chemical alteration he had been studying in stem cells and tumor cells.

The idea cut against the conventional thinking in both fields. In neuroscience, the prevailing wisdom held that long-term changes in behavior result from physical changes in neural circuits—such as when neurons build new synapses and become more sensitive to messages from their neighbors. And most scientists who studied DNA methylation thought the process was restricted to embryonic development or cancer cells.

Back in Montreal, the pair eventually began collaborating, and Szyf's hunch turned out to be right. Their research, along with work from a handful of other labs, has sparked an explosion of interest in so-called epigenetic mechanisms of gene regulation in the brain. Long familiar to developmental and cancer biologists, these molecular mechanisms alter the activity of genes without changing their DNA sequence (*Science*, 10 August 2001, p. 1064). And they have recently become a white-hot topic in neuroscience. Meaney and Szyf's work suggests that epigenetics could explain how early life experiences can leave an indelible mark on the brain and influence both behavior and physical health later in life. These effects may even carry over to subsequent generations. Meanwhile, other researchers have implicated epigenetics in drug addiction. Still others have described important roles in cognition (see sidebar, p. 27).

Some researchers speculate that if these rodent findings extend to humans, epigenetics could turn out to be at the heart of some of the most vexing problems in society. These ills include the long-term health problems of people raised in lower socioeconomic environments, the vicious cycle in which abused children grow up to be abusive parents, and the struggles of drug addicts trying to kick the habit.

Tempting as such speculation may be, others worry that the young but fast-growing field of behavioral epigenetics is getting ahead of itself. They point out that so far there's very little evidence in humans that epigenetics connects early life experience to behavioral or health problems later in life. Moreover, several experimental obstacles will make finding proof exceedingly difficult. "I think there's been a lot of putting the cart before the horse," says Gregory Miller (no relation), a psychologist at the University of British Columbia (UBC), Vancouver.

Online
sciencemag.org
Podcast interview with author Greg Miller.

The importance of a loving mother

In 2004, Szyf and Meaney published a paper in *Nature Neuroscience* that helped launch the behavioral epigenetics revolution. It remains one of the most cited papers that journal has ever published. The paper built on more than a decade of research in Meaney's

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lab on rodent mothering styles.

Rat moms vary naturally in their nurturing tendencies. Some lick and groom their pups extensively and arch their backs to make it easier for their young to nurse. Others spend far less time doting on their pups in this way.

Meaney had found that the type of mothering a rat receives as a pup calibrates how its brain responds to stress throughout its life. Rats raised by less-nurturing mothers are more sensitive to stress when they grow up. When confined to a Plexiglas tube that restricts their movement, for example, they exhibit a greater surge in corticosterone, a hormone pumped out by the adrenal glands in times of stress. The likely cause is reduced numbers of a receptor for steroid hormones in the brain. This so-called glucocorticoid receptor is part of a negative feedback loop that dials down the volume on communication between the brain and adrenal glands, thereby reducing reactivity to stress.

The *Nature Neuroscience* paper linked this reduction in glucocorticoid receptors to DNA methylation. Rats raised by less-nurturing moms tended to have more methyl groups attached to the promoter region, the "on" switch, of the glucocorticoid receptor gene. These methyl groups block access by the transcription factors that turn the gene on. As a result, fewer receptors are produced. Subsequent experiments showed that enzymes that reverse DNA methylation of the glucocorticoid receptor gene also reverse the effects of unenthusiastic mothering on the offspring's hormonal and behavioral responses to stress.

Several of Meaney's students have carried on with this work and extended it in new directions. Frances Champagne, a co-author of the 2004 paper, went on to show that female rats raised by nurturing mothers are more nurturing mothers themselves. She also found that pups raised by less-nurturing moms exhibit greater methylation—and reduced expression—of the gene for a particular estrogen receptor in the hypothalamus, a brain region involved with reproductive behavior. This receptor amplifies signaling by oxytocin, a hormone that promotes mother-infant bonding.

Now at Columbia University, Champagne has been investigating the long-term effects of other kinds of social experiences early in life. She has found that mice raised communally by multiple mothers (as rodents raise their young in the wild) are better socially adjusted as adults: They are less likely to pick a fight with a stranger put into their cage, for example. Communally raised mice also spend more time fussing over their own offspring. Fussed-over daughters in turn tend to grow up to be nurturing mothers. These changes correlate with a higher density of oxytocin receptors in some brain regions, the researchers reported last September in *Frontiers in Behavioral Neuroscience*. Champagne's lab is now investigating whether that higher density could be due to epigenetic modifications. "What's exciting to me is that the social world, which can be perceived as being this ethereal thing that may not have a biological basis, can affect these mechanisms," she says.

Adversity takes its toll

Whereas a nurturing environment can predispose a rodent to be calmer in adulthood and raise a nurturing family of its own, an adverse environment can have the opposite effect. There's evidence that this effect, too, may involve epigenetic changes. Last year, researchers led by Tania Roth and J. David Sweatt of the University of Alabama, Birmingham, helped show this by building on earlier work showing that rat mothers denied access to the materials needed to make a proper nest become anxious and spend less time nurturing their young. Pups raised by these stressed-out rat moms exhibited increased methylation of the gene for BDNF, a neural growth factor, in the brain's prefrontal cortex, they reported in the 1 May 2009 issue of *Biological Psychiatry*. In addition, this methylation pattern, which would tend to reduce the amount of BDNF produced, was passed on to the subsequent generation.

Exactly what low BDNF levels mean for a mouse isn't entirely known. However, levels of this growth factor are reduced in mouse models of depression and anxiety, at least in certain brain regions, and restoring it can mimic the effect of antidepressant drugs. In 2006, Eric Nestler, currently at Mount Sinai Medical Center in New York City, and colleagues reported in *Nature Neuroscience* that the *Bdnf* gene is down-regulated in the hippocampus of adult mice exposed to social stress—in the form of chronic bullying by a bigger mouse. In the same paper, Nestler's team linked this reduction in *Bdnf* activity to epigenetic modifications involving histones, tiny protein spools that keep DNA wrapped up. Chronic stress triggered an increase in a type of histone methylation that suppresses gene activity by keeping the DNA containing the *Bdnf* gene tightly wound. Antidepressant drugs, on the other hand, boosted histone acetylation, which helps unwind DNA from histones and promote *Bdnf* activity. Such findings hint that epigenetic modifications could be an important link between adverse life experiences and the risk of psychiatric disorders such as depression and anxiety, Nestler says.

Different upbringings. Being raised by a nurturing (*top left*) or a lackadaisical (*top right*) mother can cause epigenetic differences that affect a rat pup's behavior later in life. Whether similar differences occur in people raised in wealthy (*bottom left*) or impoverished (*bottom right*) neighborhoods remains an open question.

CREDIT (CLOCKWISE FROM LEFT): MICHAEL MEANEY LAB; TIME & LIFE PICTURES/GETTY IMAGES; PHOTOS.COM; ISTOCKPHOTO

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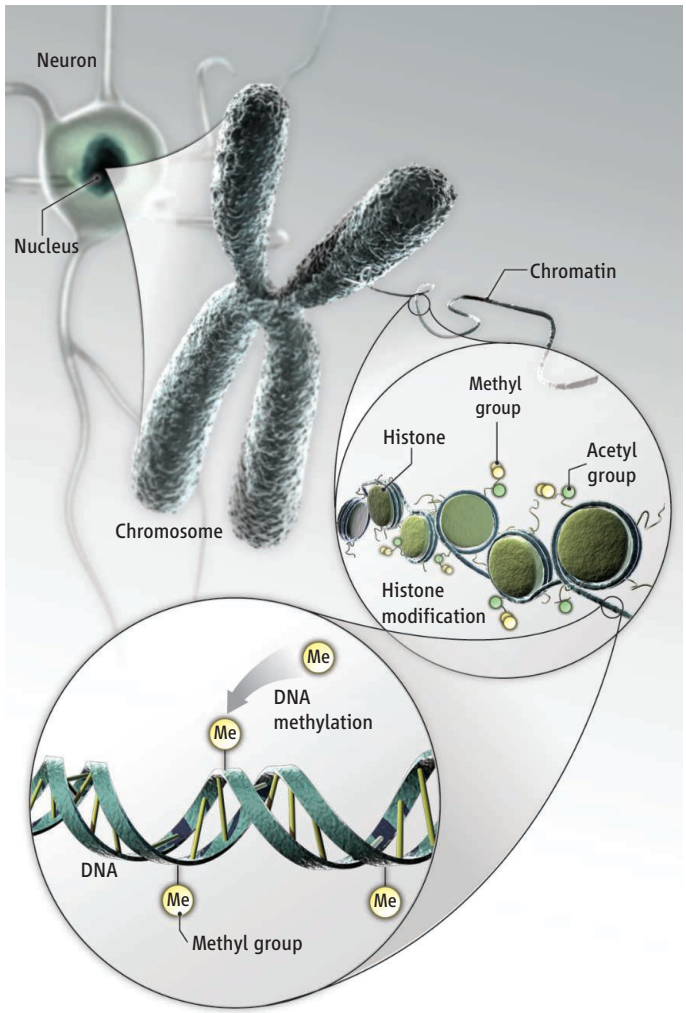
Another line of work in Nestler's lab suggests that epigenetic mechanisms could also play an important role in drug addiction. His team has now documented several epigenetic changes provoked by cocaine administration in rodents. The drug increases acetylation and decreases methylation of histones—both of which tend to promote gene activity—in the brain's reward circuitry. "Cocaine produces changes in the genome that make the brain more sensitive to the next dose of cocaine," Nestler says.

For example, in the 8 January issue of *Science* (p. 213), Nestler and colleagues reported that repeated cocaine administration suppresses methylation of a particular histone in one reward region, the nucleus accumbens. Suppressing this type of histone methylation in mice that had never received cocaine triggered the growth of extra dendritic spines—tiny extensions on neurons that tend to sensitize them—in the nucleus accumbens. Suppressing methylation also increased the animals' preference for cocaine once they finally tried it. In contrast, enhancing histone methylation reduced cocaine-seeking behavior. Nestler cautions that any treatments for human drug addicts are a long way off, but he says a better understanding of the genes altered by such epigenetic changes could eventually point to new treatments to break the hold of addiction.



Epigenetic breakdown. Several epigenetic mechanisms alter gene activity in neurons, with potentially important effects on brain function and behavior. Histone acetylation tends to promote gene activity, whereas histone methylation and DNA methylation tend to inhibit it.

The human story

If the rodent research on epigenetics translates to humans, the implications could be far-reaching. The effects of adverse environments early in life are well documented and notoriously hard to shake. Childhood abuse, for example, elevates the lifelong risk of depression, anxiety, and suicide. Growing up in an impoverished environment also takes a lasting toll, affecting physical health as well as behavior. Could epigenetics be part of the reason?

So far, the evidence in humans is scant. A major reason, and a huge obstacle to the field in general, is that human brain tissue is hard to come by. In one of the few studies to date, Meaney, Szyf, and postdoctoral fellow Patrick McGowan examined postmortem brains from 24 people who had committed suicide, half of whom had been abused as children. From their rodent work, the researchers hypothesized that those who had been abused might have more methyl groups on the glucocorticoid receptor gene than did those who had not been abused. That's indeed what they found, the researchers reported in the March 2009 issue of *Nature Neuroscience*.

Lacking access to brain tissue, researchers have looked elsewhere in the body for signs of epigenetic alterations. In the March-April 2008 issue of *Epigenetics*, a team led by Tim Oberlander at UBC reported increased methylation of the glucocorticoid receptor gene in cells isolated from umbilical cord blood in 33 infants born to women who suffered symptoms of depression during their pregnancy. Those infants also had higher cortisol concentrations in their saliva when tested 3 months later, suggesting an increased susceptibility to stress.

"Those data are very consistent with the rodent work, although there's some ambiguity about what's actually going on," says UBC's Miller, who was not involved with the study. One issue, he and others point out, is that cord blood contains a mishmash of different cell types. Methylation patterns can vary significantly from one cell type to another, and the proportion of cell types in cord blood can vary significantly from one individual to another. As a result, Miller says, the findings in the *Epigenetics* paper could be accounted for by a difference in the combination of cell types sampled rather than a difference in methylation in any particular cell type. "That's a real problem for that study and others like it," he says.

In Vancouver, Miller and UBC colleagues Edith Chen and Michael Kobor head an ongoing study of gene expression in people raised in different socioeconomic conditions. Their work focuses primarily on white blood cells. Miller says he expected to find increased methylation of the glucocorticoid receptor gene studied by Meaney's group, reasoning that the lower socioeconomic environment of his subjects might be roughly analogous to the un-nurturing environment of Meaney's rat pups. So far they haven't found it. "We're not seeing anything in the way of DNA methylation in the glucocorticoid receptor [gene]," he says. One possible explanation, Miller says, is that blood and brain cells don't necessarily undergo the same epigenetic changes in response to a given life experience.

Miller also suspected that he and his colleagues would find epigenetic alterations in genes related to physical health. Because of its long-lasting effects on gene activity, DNA methylation seemed like a potentially attractive explanation for why people who grow up in poor households have an increased lifetime risk of health problems. (A 2006 study of graduates of Johns Hopkins University School of Medicine, for example, showed that even in this well-educated, affluent population, those who'd grown up in poor house-

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holds decades earlier had 2.4 times the risk of heart disease compared to those who'd grown up in wealthier households.)

Socioeconomic status early in life does appear to alter gene expression: In a 25 August 2009 paper in the *Proceedings of the National Academy of Sciences* (*PNAS*), Miller and colleagues reported disparities in the activity of more than 100 genes related to immune system function in the white blood cells of men who lived in lower socioeconomic environments before the age of 5. The net result of these changes in gene activity would tend to increase inflammatory immune responses, a potential contributing factor to the documented increases for infectious and cardiovascular diseases related to poverty.

But epigenetics does not seem to be the cause of these changes. "We spent the last few years trying to see if we could find evidence of epigenetic alterations in the immune system that are related to early life experience," Miller says. "This work is still ongoing, so I think it would be premature to conclude anything definitively, but we've had less success than we'd hoped and imagined."

Others also feel that too much emphasis is being put on epigenetics as the link between environment and genes. "The big-picture story is that clearly social interactions can regulate gene expression, but they do so in different ways in different tissues," says Steve Cole, a genomics researcher at the University of California (UC), Los Angeles, who collaborated on the *PNAS* study. Cole notes that epigenetics represents only one class of potential mechanisms for altering gene activity. He argues that changes in the activity of transcription factors can cause long-term changes in gene expression without help from DNA methylation or other epigenetic mechanisms.

The excitement over the Meaney findings has led many researchers to look for epigenetic alterations, but Cole says he knows of several others who are coming up empty-handed: "Lots of people have spent lots of time and money and are now a little grumpy about this."

At UC Berkeley, one of Meaney's former students, Darlene Francis, says she has mixed feelings too. "These phenomena are really exciting from a public-health perspective," says Francis, a neuroscientist and public-health researcher. Epigenetics provides a potential explanation for how social conditions can affect biology in ways that can contribute to poor health, Francis says: "This allows folks who are convinced that social forces are huge contributors to risk and vulnerability to make more effective arguments." At the same time, Francis says too many researchers are embarking on "undirected" searches for epigenetic alterations in human populations without a solid rationale. "What some people take away [from the rodent work] is that methylation is now the cause and solution to a lot of life's problems," she says. "I get frustrated with the overextrapolation of the animal findings, and some of it is my work so it's ironic," Francis says. "I don't know when I became the sensible one."

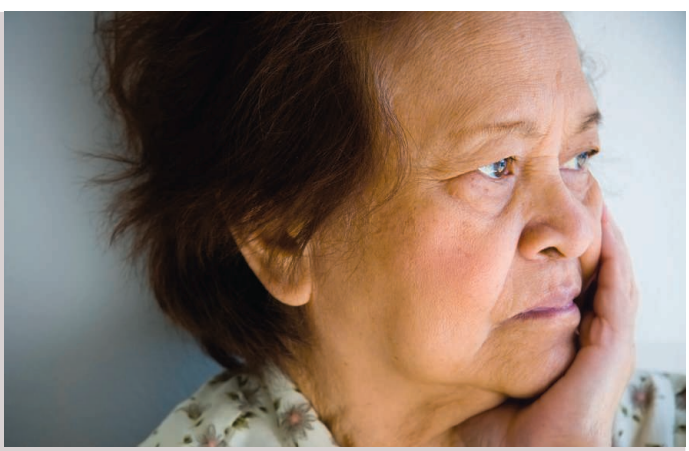
–GREG MILLER

A Role for Epigenetics in Cognition

The push to show that epigenetics can translate early life experiences into lasting changes in behavior (see main text, p. 24) has been accompanied by a parallel surge of interest in how chemical modifications to DNA can affect cognition. This work sprang from research in the late 1990s showing that abnormalities in DNA methylation are involved in developmental disorders that cause intellectual impairment, including Angelman syndrome and Rett syndrome, says J. David Sweatt, a neurobiologist at the University of Alabama, Birmingham. Sweatt's lab and others have since found evidence that epigenetic mechanisms play important roles in learning and memory in adult rodents. One recent study even suggests that these mechanisms may help explain why memory declines with age.

Sweatt's team recently discovered that training mice to associate a certain location with a mild electric shock reduced DNA methylation in the hippocampus, a brain region crucial for memory formation. More specifically, the *Bdnf* gene was demethylated, boosting its activity, they reported 15 October 2008 in *The Journal of Neuroscience*. This gene encodes a growth factor that promotes new synaptic connections between neurons so that a memory is retained. Injecting a drug that inhibits demethylation of DNA into the hippocampus prevented the increase in *Bdnf* activity after learning and weakened rodents' memory of the place where they had received a shock. The work is one of several clues that DNA methylation affects the formation and maintenance of memories.

Other work has focused on histone acetylation, a chemical modification that unwinds DNA from protein spools called histones, thereby enabling gene activity. One of the most intriguing studies to date was led by Li-Huei Tsai and André Fischer at the Massachusetts Institute of Technology in Cambridge. In the 10 May 2007 issue of *Nature*, they reported that a drug that promotes histone acetylation improved learning and memory in a mouse model of Alzheimer's disease. When injected into the hippocampus, the drug even seemed to restore a forgotten memory of a location where the rodents had previously received a shock.



Maintaining memories. Epigenetic mechanisms in the brain seem to play important roles in memory and may be an attractive target for drugs to stave off memory loss in old age.

More recently, Fischer, who is now at the European Neuroscience Institute in Göttingen, Germany, has been investigating alterations in histone acetylation that occur naturally with age. In the 7 May issue of *Science* (p. 753), he and colleagues reported that adult mice, compared with juveniles, exhibit reduced histone acetylation and diminished activation of genes in the hippocampus that were related to learning and memory. As in the Alzheimer's mice, drugs that boosted histone acetylation improved the older mice's performance on tests of rodent cognition.

Biotech and pharmaceutical companies are already exploring these drugs, called histone deacetylase inhibitors, for treating Alzheimer's disease, says Ottavio Arancio, a neuroscientist at Columbia University. Some of these drugs are already approved for treating cancer. However, because the drugs alter the activity of multiple genes, Arancio cautions that more work is needed to determine whether they can aid memory without causing serious side effects. **–G.M.**

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