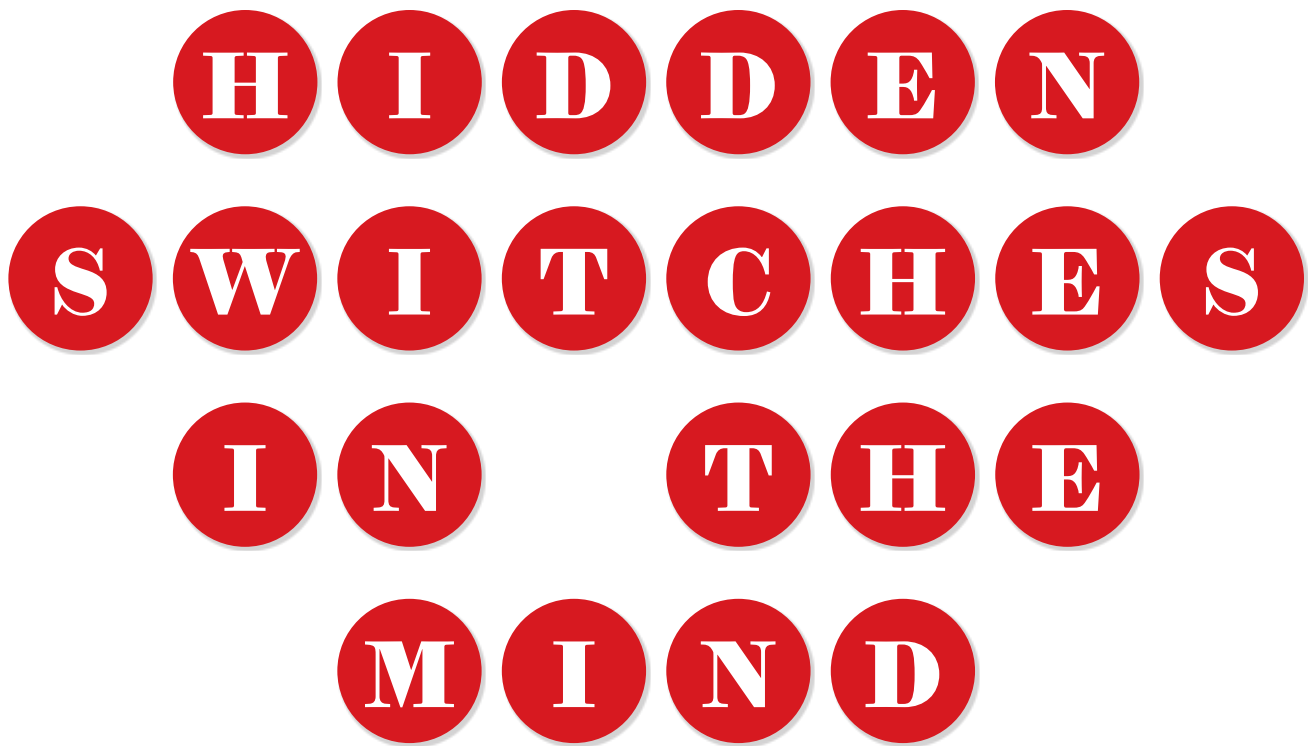




NEUROSCIENCE



Experience may contribute to mental illness in a surprising way: by causing “epigenetic” changes—ones that turn genes on or off without altering the genes themselves

By Eric J. Nestler

Eric J. Nestler is Nash Family Professor of Neuroscience and director of the Friedman Brain Institute at the Mount Sinai Medical Center in New York City. His laboratory focuses on uncovering the molecular mechanisms of drug addiction and depression.



MATT IS A HISTORY TEACHER. HIS TWIN BROTHER, GREG, IS A DRUG ADDICT. (Their names have been changed to protect their anonymity.) Growing up in the Boston area, both boys did well in high school: they were strong students in the classroom and decent athletes on the field, and they got along with their peers. Like many young people, the brothers snuck the occasional beer or cigarette and experimented with marijuana. Then, in college, they tried cocaine. For Greg, the experience derailed his life.

At first, he was able to function normally—attending classes and maintaining connections with friends. But soon the drug became all-important. Greg dropped out of school and took on a series of menial jobs in retail and fast-food joints. He rarely held a position for more than a month or two, generally getting fired for missing too much work or for arguing with customers and co-workers. His behavior became increasingly erratic—sometimes violent—and he was arrested repeatedly for stealing to support his habit. Multiple efforts at treatment failed, and by the time the courts sent Greg, then 33 years old, to a psychiatric hospital for evaluation, he was destitute and homeless: disowned by his family and a prisoner of his addiction.

What made Greg so susceptible to the siren song of cocaine—to the point that the drug essentially destroyed his life? And how did his identical twin, who shares the exact same genes, escape a similar fate? How can exposure to a drug set up some individuals for a lifelong addiction, while others can move past their youthful indiscretions and go on to lead productive lives?

These questions are not new, but neuroscientists are beginning to take a fresh approach to finding the answers, borrowing from discoveries first made in other fields. Over the past 10 years biologists studying embryonic development and cancer have uncovered an extensive array of molecular mechanisms that allow the environment to alter how genes behave without changing the information they contain. Instead of mutating genes, these “epigenetic” modifications mark them in ways that can alter how active they are—in some cases for a lifetime.

Now my laboratory and others in the field are finding signs that epigenetic changes caused by drug use or chronic stress can change the way the brain responds to experience: priming an in-

dividual to react with resilience or to succumb to addiction, depression or a range of other psychiatric disorders. Although we are still at the earliest stages of understanding this powerful molecular interplay between genes and the environment, we are hopeful that what we learn may lead to improved treatments for these devastating conditions—and may even offer new insights into how mental illness can pass from generation to generation.

BEYOND GENES

OUR EFFORTS to untangle the epigenetic influences on mental illness are helping to fill in blanks left by decades of earlier research into the genetic roots of addiction, depression, autism, schizophrenia and other psychiatric disorders. Like most common medical conditions, these neurological afflictions are highly heritable: roughly half the risk for addiction or depression is genetic—which is greater than the genetic risk for high blood pressure or most cancers. But genes are not everything. As we saw with Greg and Matt, even having identical genes is no guarantee that two individuals will contract the same illness. Instead psychiatric disorders are precipitated in genetically susceptible individuals by environmental inputs—exposure to drugs or stress, say—and can even be influenced by random molecular events that occur during development. No two people will have exactly the same experiences or developmental history.

So the question becomes: By what mechanisms can such inputs lead to mental illness? At one level, the answer is obvious: nature and nurture come together in the nerve cells in the brain. These cells process everything we experience—whether it is watching a movie, getting a hug, snorting cocaine or thinking about what is for dinner—and then share information with

IN BRIEF

New findings suggest that experiences can contribute to mental illness by adding or removing “epigenetic” marks on chromosomes. These tags are particular chemicals that can influence gene activity without changing the information encoded in the genes.

Studies in mice demonstrate a role for long-lasting epigenetic modifications in such disorders as addiction and depression.

Epigenetic changes can also affect maternal behaviors in ways that reproduce the same behaviors in

their offspring, even though the changes are not passed down through the germ line.

Researchers hope the new findings will lead to better treatments, although the path to those treatments is not yet obvious.

one another by releasing and recognizing chemicals called neurotransmitters. Neurotransmitters can activate or inhibit individual nerve cells and switch a range of responsive genes on or off. Which genes a particular neurotransmitter affects will help to determine how a nerve cell will respond to an experience and ultimately shapes the way an individual behaves.

Many of these effects last only briefly. For example, exposure to cocaine will activate the reward center in the brain, leading to transient euphoria. That feeling soon fades, and the system resets itself. Still mysterious is how drugs, stress or other experiences can engender longer-term effects, causing an individual to succumb to depression or addiction. That, many neuroscientists are starting to think, is where epigenetics comes in.

MAKING MARKS

TO UNDERSTAND why epigenetics has attracted our attention, it helps to know a little something about how gene activity is regulated. A gene, in simplified terms, is a stretch of DNA that typically specifies the makeup of a protein; proteins carry out most processes in cells and thus control cell behavior. This DNA is not tossed haphazardly into the cell's nucleus. Rather it is wrapped around clusters of proteins called histones—like thread on a spool—and then further bundled into the structures we call chromosomes. The combination of protein and DNA in chromosomes is known as chromatin.

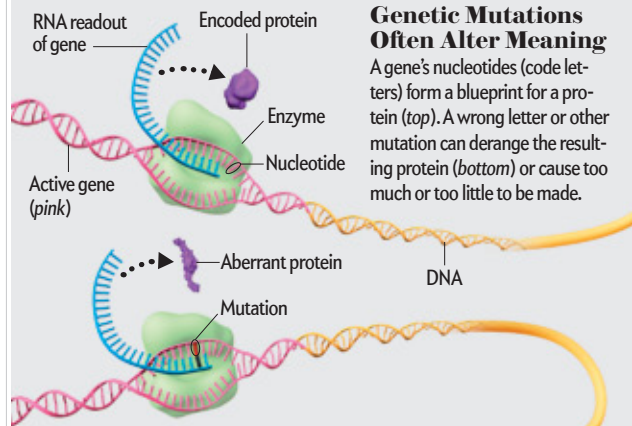
This packaging of DNA does more than keep the nucleus tidy. It also helps to regulate the behavior of the resident genes. Tighter packing tends to keep genes in an inactive state, preventing access by the machinery that turns genes on. In a nerve cell, for example, genes that encode liver enzymes are tucked away in densely packaged chromosomal regions. When a gene is needed, however, the section of DNA on which it resides unfurls somewhat, making the gene accessible to cellular machinery that transcribes the DNA into a strand of RNA. In most cases, that RNA will then serve as a template for producing the encoded protein. Stimulating a neuron, for example, might prompt that cell to boost the transcription of genes coding for certain neurotransmitters, leading to increased synthesis of those messaging molecules.

Whether a segment of chromatin is relaxed (primed for activation) or condensed (shut down either permanently or temporarily) is influenced by epigenetic marks: chemical tags that are attached to the resident histones or to the DNA itself. These tags can take various forms and together create a kind of code that indicates how tightly packed the chromatin should be and whether the underlying genes should be transcribed [see box at right]. An individual gene may be more—or less—active, depending on how its chromatin is marked.

Epigenetic modifications are made by a variety of enzymes, some of which add the chemical tags and some of which remove them. C. David Allis of the Rockefeller University, a leader in the field, has dubbed these enzymes the “writers” and “erasers” of the epigenetic code. For example, an enzyme known as a histone acetyltransferase, which attaches an acetyl group to a histone protein, is a writer, and a histone deacetylase, which removes this mark, is an eraser. The marks then attract other proteins that act as “readers.” Readers bind to specific epigenetic tags and can loosen or condense the surrounding chromatin by recruiting other regulatory proteins that stimulate or repress

Genetics vs. Epigenetics

Many new insights into mental illness have come from studying epigenetic modifications of genes, which differ from genetic mutations (below). Both kinds of alterations can disturb the functioning of the brain and other tissues.

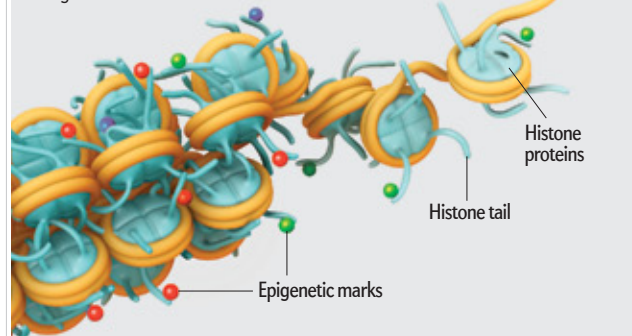


Genetic Mutations Often Alter Meaning

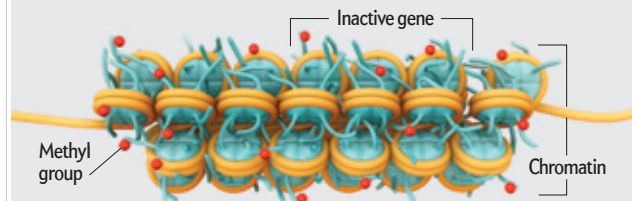
A gene's nucleotides (code letters) form a blueprint for a protein (top). A wrong letter or other mutation can derange the resulting protein (bottom) or cause too much or too little to be made.

Epigenetic Changes Alter Activity

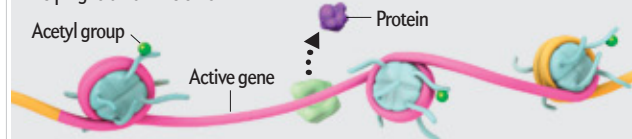
Chemical tags known as epigenetic marks sit atop genes, either on the DNA itself or on the histone proteins around which DNA is wrapped (below). Changes in the mix of these marks can alter a gene's behavior, turning the gene off, so that protein synthesis is inhibited, or turning it on—all without changing the information the gene contains.



Gene off: Some epigenetic marks inhibit genes by inducing tight folding of chromatin (DNA complexed with histones and other proteins) and thus keeping genes from being read; methyl groups sometimes play that role.



Gene on: Other marks, such as acetyl groups, tend to spur gene activity by helping to unfurl the chromatin.

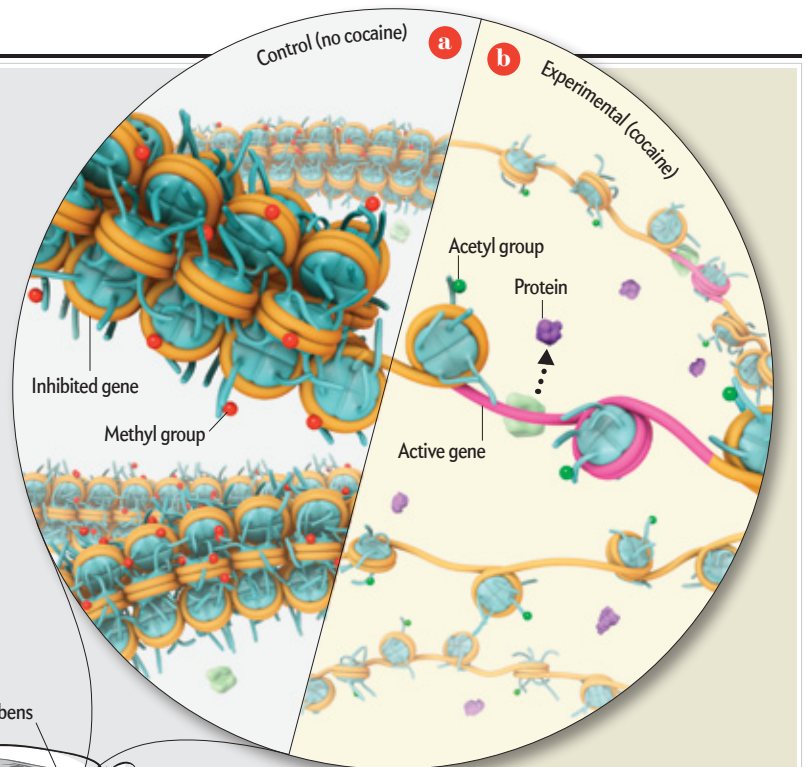


Epigenetics of Addiction

Studies of mice have shown that chronic cocaine exposure shifts the balance of epigenetic marks on genes in the brain's reward center. This shift renders the animals more sensitive to the drug's effects and more prone to becoming addicted.

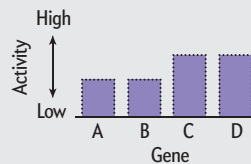
What Changes Exactly?

Even a single dose of cocaine can alter the epigenetic landscape of genes in the nucleus accumbens, a part of the reward center. In the absence of drugs (a), methyl marks predominate, keeping the affected chromatin tightly wound and its genes quiet. Cocaine causes acetyl groups to predominate and chromatin to loosen (b). Then many genes encoding proteins involved in the pleasurable response to the drug become active.

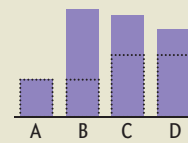


Nucleus accumbens

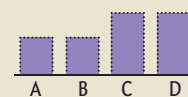
No cocaine



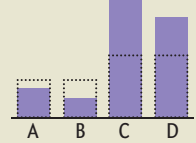
Initial cocaine exposure



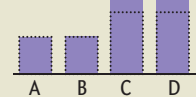
One drug-free week later: Gene activity returns to normal



Chronic exposure



One drug-free week later: Some genes remain overactive



Lasting Effects

The initial exposure to cocaine transiently elevates the activity of many genes (represented schematically by changes in B, C and D, near right), but activity soon returns to baseline. Chronic exposure, however, has more complex effects: it renders some genes less sensitive to the drug (B, far right), while boosting the activity of others even higher than before (C and D). Some of those genes remain overactive for an abnormally long time.

transcription of the resident genes. Histones that are highly acetylated, for instance, attract readers that tend to open up the chromatin and other proteins that promote gene activation. Histones carrying an abundance of methyl groups, in contrast, attract readers that can either suppress or stimulate transcription, depending on the exact location of the methyl marks.

The environment can influence gene activity by regulating the behavior of epigenetic writers and erasers—and thus the tagging, and restructuring, of chromatin. Sometimes the tags persist for just a short time, say, to allow a nerve cell to respond rapidly to intense stimulation by producing a sustained wave of neurotransmitter release. Often the tags stay put for months or years—or even for the life of the organism: strengthening or weakening the neural connections involved in laying down memories, for example.

The addition and removal of acetyl and methyl groups—and other marks—can thus help the brain to respond and adapt to environmental challenges and experience. My lab and others are now finding in animal studies, however, that these beneficial epigenetic processes can go awry in conditions such as addiction and depression, where alteration of the normal array of modifications may serve to activate cravings, induce feelings of defeat or otherwise predispose an animal to a lifetime of maladaptive behavior. Examination of human brain tissue, retrieved post-mortem, suggests that the same may be true in people.

PRIMED FOR ADDICTION

THE FINDINGS related to addiction build on past insights into how drugs of abuse usurp the brain's natural reward center. Many studies, for instance, have identified sweeping changes in the ac-

tivation of genes in response to cocaine, opiates or other addictive substances. Some of these changes in gene “expression” were shown to persist even after months of abstinence, although researchers have been hard-pressed to explain the mechanism underlying the persistence. Given the long-lasting effects that epigenetic changes can have, about 10 years ago my lab set out to examine whether cocaine could alter the activity of genes in the brain’s reward center by changing their epigenetic tagging. Cocaine is a powerful drug that is as addictive in animals as it is in people. Hence, its long-term influences can be readily studied in a lab setting.

A single dose of cocaine induces robust and widespread changes in gene expression, as measured by concentrations of messenger RNA—a direct readout of gene activation. One hour after mice receive their first injection of cocaine, nearly 100 genes get newly switched on. Even more interesting is what happens when animals are chronically exposed to the drug. A handful of the genes turned on by acute exposure to cocaine fall silent if it is given every day. These genes become “desensitized” to the drug.

A much larger number of genes, however, do just the opposite: although they become transiently active in response to the initial exposure to cocaine, chronic exposure to the drug boosts their activity levels even higher—in some cases for weeks after an animal’s last injection. What is more, these genes remain highly sensitive to cocaine even after the animal has had no exposure to the drug for some time. Chronic use of cocaine thus primes these genes for future activation—in essence, allowing them to “remember” the rewarding effects of the drug. This priming also sets up the animal for relapse, paving the way to addiction. The heightened sensitivity, it turns out, stems from epigenetic modifications of the genes.

Using powerful techniques for cataloguing the epigenetic marks across the entire mouse genome, we have been able to demonstrate that chronic cocaine administration selectively reconfigures the collection of acetyl and methyl tags on hundreds of genes within the brain’s reward center. Collectively, these changes tend to loosen the chromatin structure, rendering these genes more prone to activation by subsequent exposure to cocaine. Again, many of these changes are transient—lasting only a few hours after the animal receives the drug. Some last much longer, however: we have recorded changes that persist for at least a month, and we are beginning to look at even longer periods.

We are also starting to get a handle on the mechanisms that underlie these persistent changes. In our lab, we find that chronic cocaine administration dampens the activity of certain erasers that remove acetyl groups, as well as of particular writers that add inhibitory methyl groups. Chromatin that is more highly acetylated—or less methylated—remains in a more open, relaxed state, making its resident genes more amenable to activation. Chronic cocaine exposure also manipulates the activity of other writers and erasers in the brain’s reward center, leaving in its wake an array of epigenetic marks that favor gene activation. In support of this observation, we find that when we artificially tweak the activities of these writers and erasers to mimic the effects of chronic drug use, without actually administering the abused drug, we cause animals to be more sensitive to the pleasurable effects of cocaine—one of the hallmarks of addiction.

The changes in writer and eraser activity following chronic

cocaine use are also long-lasting, which may account for the long-term changes in the activities of the marked genes—and the way the animal will respond to a range of future experiences. Because the brain’s reward center reacts to such a wide variety of stimuli—including food and sex—manipulating the activity of neurons in this center can fundamentally alter the way an animal behaves.

MARKED FOR DEPRESSION

NEURAL ADAPTATIONS that affect long-term behavior also underlie one of the most chronic, debilitating and common psychiatric conditions: depression. Like addiction, aspects of this disorder can be readily studied in animals. In my laboratory, we work with mice that have been subjected to chronic social defeat. Mild-mannered male mice are paired off with more aggressive animals. After 10 days of being bullied, the docile mice display many of the signs of human depression: they no longer enjoy pleasurable activities (sex, eating sweets), and they become more anxious and withdrawn and less adventurous; they can even overeat to the point of becoming obese. Some of these changes last for months and can be reversed by chronic administration of the same antidepressants used to treat depression in humans.

Looking more closely at the mice’s DNA, we saw changes in epigenetic modification across some 2,000 genes in the brain’s reward center. For 1,200 of these genes, we measured an increase in a particular epigenetic mark—a form of histone methylation that represses gene activity. So it seems that depression may shut down genes important to activating the part of the brain that allows an animal to feel good, creating a sort of “molecular scar.” Many of these stress-induced changes, we found, could be reversed by treating the mice for one month with imipramine, a widely prescribed antidepressant. Similar epigenetic changes have been detected in human brain samples obtained from individuals who were depressed at their time of death.

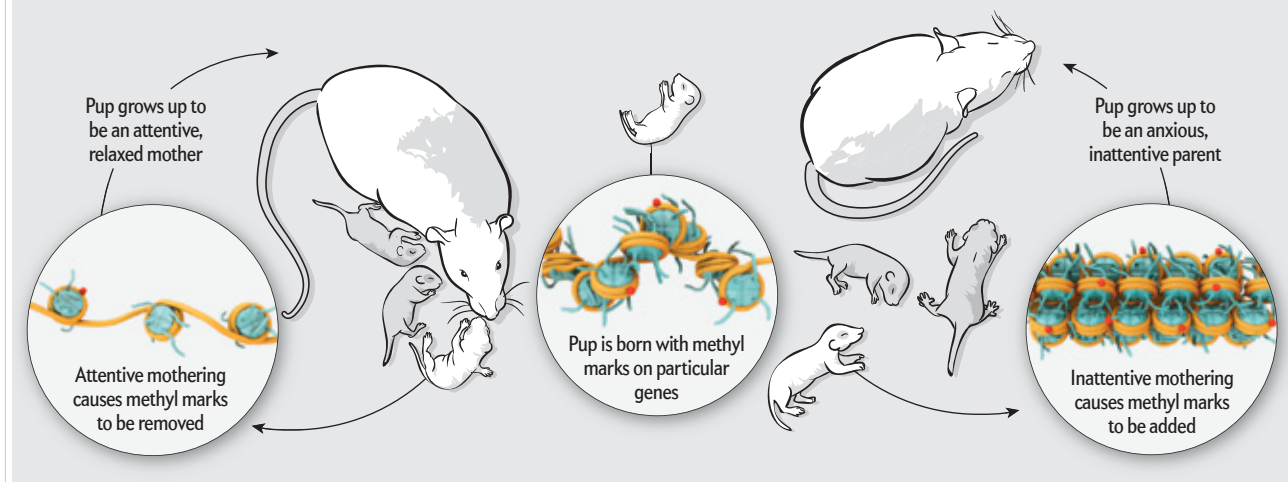
Although depression is a common problem in the human population, not all people are equally vulnerable. And we found the same is true for mice. Roughly one third of the males that receive a daily “dose” of social defeat appear to be resistant to depression: despite being subjected to the same relentless stress, they show none of the withdrawal or listlessness displayed by their susceptible peers. This resiliency reaches down to the level of their genes. Many of the stress-induced epigenetic changes we see in susceptible mice do not occur in the resilient mice. Instead these animals show epigenetic modification of an additional set of genes in the reward center that are not similarly modified in the mice that become depressed. The findings suggest that this alternative pattern of modification is protective and that resiliency is more than just an absence of vulnerability; it involves an active epigenetic program that can be called on to combat the effects of chronic stress.

We also found that the protective genes that are epigenetically modified in resilient mice include many of the same ones whose activity is restored to normal in depressed mice treated with imipramine. A subset of these genes are known to boost the activity of the brain’s reward center and, hence, to ward off depression. These observations raise the possibility that, in people, antidepressants may work in part by activating some of the same protective epigenetic programs that function in individuals less prone to depression. If so, in addition to searching for

My Mother, Myself

Studies in rats have shown that epigenetics can influence maternal behavior and that this effect can be passed from one generation to the next by acting on the pup's brain alone, without altering germ cells. When pups are born, genes involved in regulating the animals' responses to stress are decorated with inhibitory methyl marks, which enhance sensitivity to stress. If the pups are

raised by a relaxed and nurturing mother, many of their methyl groups will melt away, leaving the animals calmer. When these pups mature, they, too, will be easygoing, attentive parents. If the pups, however, are raised by a fearful, passive mother, their genes will gain methyl marks. They grow up to be nervous and neglectful caretakers.



drugs that block the bad effects of chronic stress, we should also be able to identify drugs that boost the brain's natural mechanisms of resilience.

A MOTHER'S LEGACY

THE EFFECTS I have discussed so far have been seen to persist for a month—the longest time period we have examined. But epigenetic modifications can promote behavioral changes that last a lifetime, as has been demonstrated by Michael Meaney of McGill University and his colleagues. Meaney has examined the effects of maternal care on epigenetic modification—and on the subsequent behavior of the offspring.

The researchers observed that some rat mothers display high levels of nurturing behavior, licking and grooming their pups. Others are less diligent. The offspring of more active mothers are less anxious and produce less stress hormone when disturbed than pups cared for by more passive mothers. What is more, females raised by nurturing mothers become nurturing mothers themselves.

Meaney's group went on to show that the effects of maternal behavior are mediated, at least in part, through epigenetic mechanisms. Pups raised by passive mothers show more DNA methylation than aggressively groomed pups in the regulatory sequences of a gene encoding the glucocorticoid receptor—a protein, present in most cells in the body, that mediates an animal's response to the stress hormone cortisol. This excessive methylation—detected in the hippocampus, a brain region involved in learning and memory—causes nerve cells to make less of the receptor. Because activation of the glucocorticoid receptor in the hippocampus actually signals the body to slow production of cortisol, the

epigenetic reduction in receptor number exacerbated the stress response in the animals, making them more anxious and fearful—traits that persisted throughout their lifetime. The effects at the glucocorticoid receptor may be just part of the story. Frances Champagne of Columbia University and her colleagues have found similar epigenetic differences at the gene encoding the estrogen receptor in pups raised by active and passive mothers. It is likely, then, that epigenetic marking of many other genes will turn out to be involved in programming responses to, and thus inheritance of, something as complex as maternal behavior.

In this situation, it seems, epigenetic changes produced in a gene in one generation can, in effect, be handed down to the next generation, even though the changes are not passed through the germ line. A mother's behavior changes the epigenetic regulation of genes in a pup's brain, and then the pup displays the same behavior, which alters the epigenetic markings and behavior in its pup, and so on.

EPIGENETIC CURE

A KEY CHALLENGE in the coming decades will be exploiting what we are learning about epigenetic modifications and behavior to develop improved methods for treating various psychiatric disorders. Our lab and others, for example, have found that drugs that keep histones coated with acetyl groups—by inhibiting the enzymes that erase those marks—have potent antidepressant effects. Furthermore, although passive mothering is associated with changes in DNA methylation, Meaney has found that the same drugs can promote nurturing behavior (because enhanced acetylation can counter the repressive effects of too much methylation).

Although these results are promising, the inhibitors cur-

rently on the market are not likely to be useful for combating mental illness. The acetyl erasers—histone deacetylases—regulate epigenetic markings in cells throughout the brain and all over the body, so drugs that disable them indiscriminately have serious side effects and can be toxic. One alternative would be to generate medicines able to selectively inhibit the forms of histone deacetylases that are enriched in areas of the brain most affected in specific psychiatric conditions—the reward center, for example. Another option would be to identify novel proteins involved in epigenetic modification in the brain. In the end, though, the most fruitful approach might be to determine which genes are the subjects of epigenetic modification in depression or addiction: the genes for specific neurotransmitter receptors or signaling proteins, say, that are involved in neural activation. We can then focus our efforts on designing drugs that target the activity of those particular genes—or the protein products of the genes—directly.

PASSING IT ON

ONE INTRIGUING QUESTION that remains to be settled is: To what degree are the epigenetic changes that accompany neuropsychiatric conditions heritable? In Meaney's experiments, rats "inherit" certain behavior patterns—and the accompanying epigenetic profiles—from their mothers. But those changes, which are directly influenced by behavior, occur in the brain. They are not conveyed by marks on genes in the germ cells that form a new embryo. A more provocative question is: Can such experiences cause epigenetic changes in sperm and egg cells, which can then be passed directly to an individual's progeny?

It is certainly not farfetched to think that chronic stress or a drug of abuse could alter the activity of genes in sperm or eggs; after all, stress hormones and drugs are not confined to the brain but flood the entire body, including the testes and ovaries. What is hard to understand, however, is how such a change in sex cells could be maintained across generations. Acquired epigenetic modifications are erased during the type of cell division that gives rise to sperm and eggs. Also, how would the alterations, if present in an embryo, wind up influencing the activity of genes in only select parts of the brain or in the endocrine organs of an adult?

Nevertheless, intriguing work hints that some epigenetic modifications may be heritable. Several groups have found that chronically stressed rodents give birth to offspring that are particularly sensitive to stress. For example, Isabelle Mansuy of the University of Zurich and her colleagues subjected mouse pups to maternal separation during their first two weeks of life and found that, in adulthood, the male offspring exhibit signs of depression. When these males are bred with normal female mice, the resulting offspring also show similar depressionlike behaviors as adults, even though they were not subjected to stress during their upbringing. This transmission of vulnerability to stress correlates with altered levels of DNA methylation of several specific genes in both sperm and brain.

We performed a similar study in our lab. Using our model of social defeat, we subjected male mice to chronic stress. We then waited one month, let these males mate and discovered that their offspring showed a profound increase in their susceptibility to depression. Then we took the experiment one step further. If the epigenetic modifications that make mice susceptible

to depression were truly heritable, then the changes should reach the animals' sex cells. So we took sperm from our bullied males and used it to fertilize eggs from a normal female. The offspring of this artificial union, we discovered, were almost completely normal: they showed only slight indications of the withdrawn behavior and anxiety evinced by their fathers.

This experiment is not definitive, because epigenetic marks might somehow be stripped from sperm during the in vitro fertilization process. The results, however, suggest that the females that had physically mated with intimidated males treated their pups differently than females that mated with normal males—or that never met the fathers of their pups. Consequently, the offspring's depression may have stemmed from an early behavioral experience and not from a direct epigenetic inheritance carried through sperm or eggs.

That is not to say that such transgenerational transmission is impossible. Currently, though, we have no definitive evidence to indicate that it occurs. To address that question, we must develop experimental tools that will enable us to identify the relevant epigenetic modifications in germ cells—and to establish that these modifications are both necessary and sufficient to induce the transmission of traits observed.

Eighteenth-century biologist Jean-Baptiste Lamarck was known for his theory of inheritance of acquired characteristics. According to this idea, traits that organisms pick up over a lifetime—a well-exercised musculature, for example—can be passed on to their offspring. Of course, we now know that an individual's genes play the dominant role in determining physiology and function. At the same time, scientists are increasingly coming to appreciate that exposure to the environment and to different experiences (including random occurrences) throughout development and adulthood can modify the activity of our genes and, hence, the ways these traits manifest themselves. And we know now that epigenetic mechanisms mediate this interplay between nature and nurture. We still have much more work to do to fully understand how, and to what extent, epigenetics influences our behavioral traits and susceptibility to mental illness and whether such vulnerabilities can be passed to future generations. No doubt Lamarck and his critics would have delighted in debating the possibilities. ■

MORE TO EXPLORE

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SCIENTIFIC AMERICAN ONLINE

Listen to an interview with the author and learn more about epigenetics at ScientificAmerican.com/dec2011/epigenetics