# Behavioral Epigenetics: How Nurture Shapes Nature

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**Experience and social environment have a role—probably a key role—in development.**



Identical twins have the same genes. Yet as individuals, they can be quite unalike in behavior, in personality, in health, and even in appearance, and they tend to grow more different as they age. How can genes that seem to be identical produce such different effects?

A big part of the answer, scientists now think, is epigenetics—how nurture shapes nature. Epigenetic mechanisms are molecular events that govern the way the environment regulates the genomes of organisms. Epigenetic processes lead to individual differences in appearance, physiology, cognition, and behavior—the group of traits known as the *phenotype*. Scientists are at the very earliest stages of investigating them. The goal is to pry open one of nature's most challenging black boxes: explaining how life experiences are transmuted into persistent changes in body function and behavior.

In its brief history, epigenetics research has concentrated mostly on the early development of organisms. One strain of these investigations is development of behavior, and this line of research now has its own name: *Behavioral epigenetics* refers to the study of how signals from the environment trigger molecular biological changes that modify what goes on in brain cells. Here, the term *environment* encompasses pretty much everything that happens in every stage of life: social experience; nutrition; hormones; and toxicological exposures that occur prenatally, postnatally, and in adulthood. If research on epigenetics is in its infancy, research on behavioral epigenetics is in embryo.

Despite its embryonic state, behavioral epigenetics is already a vast topic, rife with complexities that grow more intricate every day. Discoveries seem to lead not to illumination but to more questions, and we have space here to touch on barely a few. Yet behavioral epigenetics has been held out as promising to elucidate, and perhaps even solve, immense medical troubles, such as mental retardation, autism, schizophrenia, and neurodegenerative disorders, and even social challenges, such as aging, addiction, suicide, child abuse, and child neglect.

## Learning and remembering

The basis of all behavior is learning and memory. Epigenetic modifications to a number of genes have now been shown to figure in learning and remembering.

J. David Sweatt, director of the McKnight Brain Institute at the University of Alabama at Birmingham, notes a striking parallel between developmental processes and the mechanisms of memory—changes driven by experience—in the adult nervous system. "It's not just that development and behavioral memory are rough analogs of each other, but rather that they are molecular homologues of each other," he says. The two most studied epigenetic processes—regulation of the structure of three-dimensional DNA and its associated proteins, plus chemical adjustments to DNA through mechanisms like histone modification—are essential both in development and in long-term memory formation. "It's as if evolution has been efficient in the set of molecular mechanisms that cells use to trigger persisting changes. It uses those mechanisms in development when it's patterning the organism, when it's turning an embryonic stem cell into a neuron or a liver cell," he says. "Then in the adult nervous system it has coopted some of those same mechanisms to trigger experience-dependent, persisting change in the function of neurons in the nervous system."

Several studies have established that both DNA methylation and histone modifications are essential for learning and remembering. Some examples are based on fear conditioning, in which mice learn to show fear of a particular location where they have been subjected to electric shocks.

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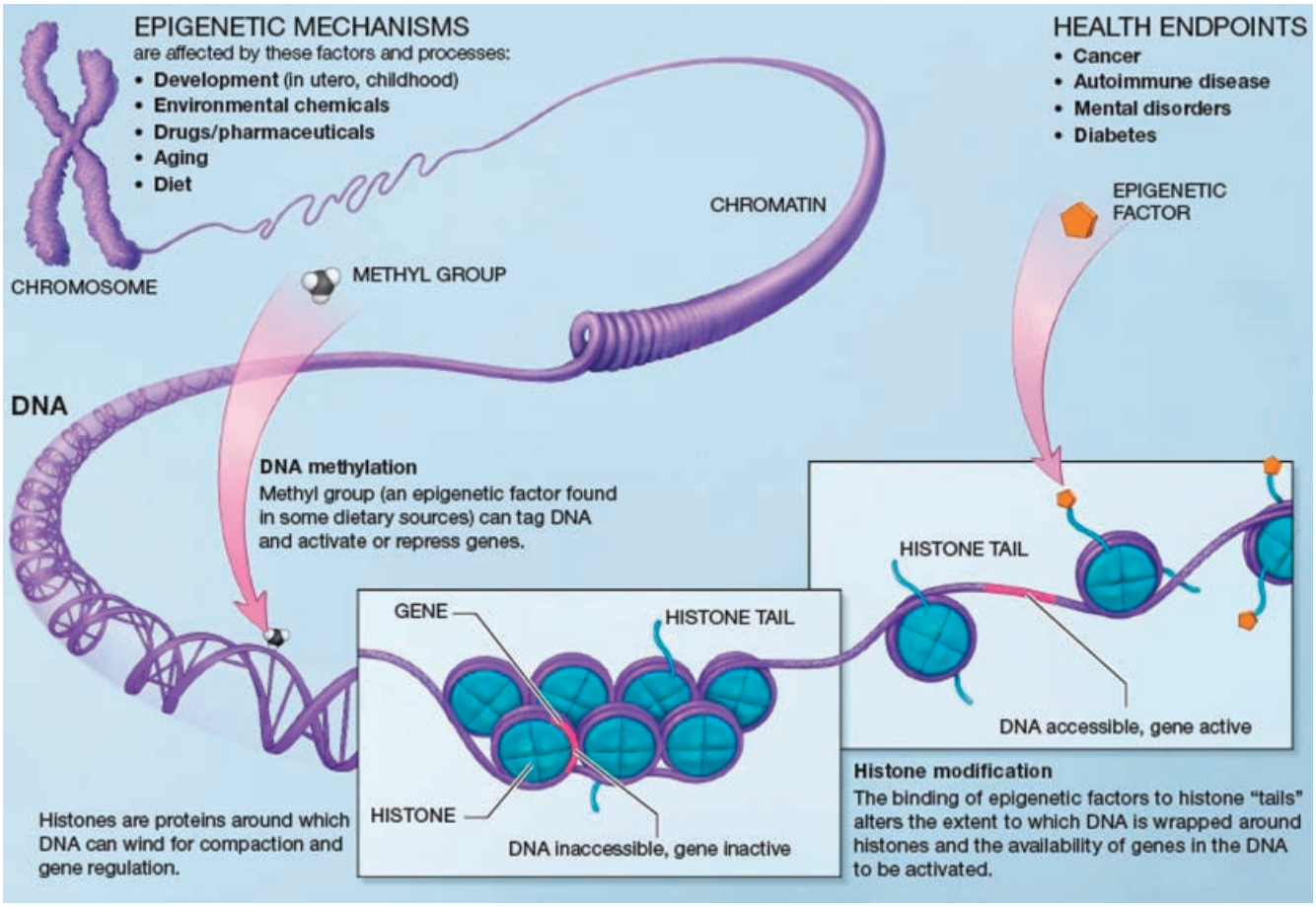


***Epigenetics may function in important ways during early development and in response to a variety of environmental triggers. Some of the mechanisms thought to be involved are DNA methylation, DNA packaging by histones, and histone modifications. Illustration: National Institutes of Health.***

After this conditioning, DNA methyltransferase, the enzyme that attaches a methyl group to DNA, increases in the hippocampus, the brain region where memories are forged. Inhibiting the enzyme prevents memories from forming. Forming memories of and remembering this contextual fear also boosts acetylation of histones in the hippocampus. Blocking histone acetylation therefore interferes with the behavior usually associated with the fear, but blocking deacetylation reverses these effects and also strengthens the formation of the fear memories.

It used to be—and still is, to some extent—that researchers believed that once epigenetic marks—particularly DNA methylation—were made, they were immutable except in special cases like cancer. The central dogma dictated that that the marks were laid down when cell fate was determined, and that those marks were unchangeable for the remainder of an animal's lifetime.

Now the take-home message from Sweatt's lab and those of other behavioral epigenetics pioneers of the mammal nervous system, such as Michael Meaney of Douglas Hospital and Moshe Szyf of McGill, both in Montreal, and Eric Nestler at Mount Sinai in New York City, is just the opposite. Recent work from labs investigating this new subfield of behavioral epigenetics has shown, Sweatt says, that there is dynamic regulation of epigenetic marks in nondividing cells in the mature nervous system. At least a subset of genes undergo active demethylation and remethylation, which is driven by the environment or by experience. This dynamism, he says, can lead to either transient or persistent functional changes in the nervous system.

Sweatt's recent work has concerned the potential role of DNA methylation in regulating long-term memory storage in the cortex. He and his colleagues have reported that putting DNA methytransferase inhibitors into an animal's anterior cingulate cortex a month after it has learned something partly erases that memory, diminishing it by half. The role of DNA methylation in long-term memory storage is at the moment a wide-open question and a focus in his lab, Sweatt says.

## The influence of mom and dad

The epigenetics of parental care got its start some two decades ago when Michael Meaney and his colleagues

showed that rats' mothering styles influenced their pups' response to stress as adults as a result of effects on the glucocorticoid receptor in the hippocampus. Offspring of nurturing mothers tended to be less anxious than those of more lackadaisical mothers. The Montreal researchers showed how early experience could shape an adult animal's behavior and even disease susceptibility, and they attributed these findings to gene changes wrought by epigenetic events.

Environmental chemicals can also affect parenting and offspring behavior. Many studies have been done on the ubiquitous endocrine disrupter bisphenol A, which alters DNA methylation. It has a great many effects in rats and mice treated during gestation, both on recipients and on their offspring. Learning, memory, and behavior, including maternal behavior, seem particularly affected. For example: Treated moms do less licking and grooming of their pups, and the pups tend to explore less and behave more anxiously, avoiding new places.

Researchers in Frances Champagne's lab at Columbia University in New York City are comparing social enrichment with social isolation or social impoverishment in rodents soon after their births, examining how those different environments change genes that govern social and reproductive behavior. Champagne's is among several labs to show that social experiences—in particular, social experiences that are relevant to mammalian development—can induce epigenetic changes. These researchers are studying not just extremes of maternal care, but also how natural variation in mothering styles can induce significant differences in epigenetic profiles.

Their latest work defines the outcomes of communal rearing in mice. Communal rearing, which comes naturally to mice but is not found often in the lab, induces multiple changes in both the brain and behavior that persist across generations, even in those offspring who were not reared in a communal nest.

There has been a big increase in research on fathers' experiences and how those are transmitted to offspring, Champagne says. Paternal effects may be particularly helpful in sorting out confounding factors in epigenetic studies, because what fathers transmit to offspring biology is only through sperm and whatever epigenetic marks they retain. There is no cytoplasm, no mitochondria, no uterus, and no messy maternal behavior to complicate interpretation. "It's a way of actually seeing whether there's some sort of germ cell epigenetic change," she says.

Subjecting male lab animals to endocrine-disrupting chemicals and other toxins has produced behavioral effects in their offspring, even when the exposure takes place well before mating. When male mice and rats are exposed to alcohol before mating, their offspring do less well at discrimination on spatial tasks, and they are more aggressive, take more risks, and display more anxiety-like behavior than offspring of unexposed animals. Males exposed to cocaine have offspring with smaller brains and deficits in attention and working memory. Even males exposed to toxins during their own embryonic development transmit detrimental effects to their offspring. In all of the examples mentioned here, epigenetic changes, especially those in DNA methylation, have been observed.

## Imprinting

Horses and donkeys are both equids, but their evolution diverged millions of years ago. Still, they are closely enough related that they can interbreed. But the hybrids they produce look different from each other depending on whether the mother is a horse or a donkey. If she is a horse, her baby is a mule and has very long ears. If she is a donkey, her baby is a hinny. Hinnies are rare, but they are generally smaller than mules, with shorter ears.

People have known for thousands of years that horse–donkey hybrids differ depending on which species is the mother and which the father. The process thought to be responsible for these differences—*genomic imprinting*—has been known for only a few decades.

***Hinnies are rare horse–donkey hybrids born to donkey mothers. The differences between mules and hinnies result from* genomic imprinting*, the silencing of genes from one or the other parent. Photo credit: Sage Ross ("Ragesoss"), wikimedia.***

Genomic imprinting is an epigenetic mechanism, one of the forms of biological inheritance that operate outside the traditional Mendelian mode. Imprinting is a particularly useful model for investigating epigenetic gene regulation and is a major source of epigenetic regulation in the brain.

With genomic imprinting, DNA methylation silences some genes or gene clusters—in egg, sperm, or zygote—depending on which parent they came from. For an imprinted gene, the allele from one parent or the other is shut down and makes no product. The other allele is expressed and produces characteristic outcomes in the offspring. Thus, mom's and dad's chromosomes are not functionally the same.

Imprinting is required for normal development, although if the functioning imprinted gene is defective, as sometimes happens, the outcome can also be fatal, or at least debilitating. Some 30 serious disorders are attributed to disrupted imprinting. Some are rare, but more common afflictions, such as cancers and autism, have also been linked to genomic imprinting.

Flowering plants make use of genomic imprinting, but among animals, only placental mammals have discovered it. There are similarities between imprinting in plants and mammals, and even similarities in hypotheses about why imprinting originated and is maintained, but we'll focus here on mammals. Much about the evolution of imprinting is murky, but it seems clear that imprinting evolved at different sites in the genome at different stages of mammalian evolution and that once imprinting has evolved at a site, the site remains imprinted.

Most known imprinted genes are involved in placental and embryonic development and fetal growth, which bolsters the leading notion about why genomic imprinting came to be: It is an evolutionary battle of the sexes, usually referred to as the *conflict hypothesis*. It is in mom's interest to conserve resources and distribute them equally among her offspring, who may not all have the same father, by putting the brakes on genes that promote strong growth in any single embryo. It is in dad's interest for his particular offspring to flourish and monopolize mom's resources by shutting down genes that hinder growth.

As of one year ago, about 100 imprinted genes had been identified, and many of these were active in the brain. Researchers believed that paternal genes were preferentially silenced in the cortex and that maternal genes were silenced in the hypothalamus. In August 2010, Harvard researchers rewrote that scenario. They reported finding more than 1300 imprinted genes in the mouse brain. "Imprinting has been mainly thought of in the context of development. There were reports of potential importance for brain function, but I think our work enabled this importance to emerge," says senior author Catherine Dulac.

Sweatt, who was not involved in the study, says it "fundamentally changes the way you have to think about the role of epigenetic mechanisms in controlling nervous system function. It's much more widespread than anyone had imagined." But he notes that it is still an open question what the functional roles of those various imprinting mechanisms are.

There are, of course, complexities, Christopher Gregg, the paper's first author, points out. Those 100 previously identified imprinted genes are what he calls "canonical," whereby all copies of the imprinted allele from one sex are silenced. That estimate probably remains correct, Gregg says. What the study revealed is that most imprinted genes do not display that all-or-none pattern. In some, one parent's copy was silenced in some tissues but worked fine in others, whereas the other parent's copy did the opposite. Nearly 350 imprinted genes were turned off only in one sex or in the other. Different genes were also imprinted in embryos and adults. In embryo brains, about 60 percent of the silenced genes belonged to the father. In adults, the ratio was reversed and more prominent: 70 percent of the maternal imprinted genes were shut down, and the effect was especially pronounced in the cortex and the hypothalamus.

One particularly dramatic example of imprinting's effects was published in January 2011. Among the imprinted genes that affect fetal growth, metabolism, and fat storage is growth factor receptor-bound protein 10 (Grb10). In mice, dad's copy normally works in the brain, and mom's works in the rest of the body. Just as the conflict hypothesis predicts, silencing the maternal Grb10 allele leads to bigger, heavier babies, but silencing the paternal allele had an unforeseen effect on behavior: It made the mice more dominant. When two mice who do not know each other meet experimentally in a narrow glass tube, one usually backs off. Mice with a silenced dad gene never did. They routinely dominated the wild-type mice, grooming them frequently and so aggressively that they pulled out whiskers and inflicted other injuries. Sex was irrelevant for this behavior; social dominance occurred whether the altered mouse was male or female.

Why this gene evolved to promote entirely different effects in different tissues is unknown. The researchers speculate that the apparent function of the unsilenced dad copy, keeping a lid on aggressive behavior and avoiding conflict, "can be viewed as a risk-averse phenotype aimed at maximizing reproductive success by avoiding the potentially detrimental consequences of challenging for social status." The gene, they say, is likely conserved in humans.

## Epigenetics through the generations

How far in time can parental epigenetic effects extend? In single cells and plants, quite a number of generations, it appears. In animals, the evidence to date suggests that behavioral effects appear in offspring, the F1 generation, and even in grandoffpsring, F2s, and then seem to peter out. In broad evolutionary terms, the social experiences of one generation being transmitted reliably across multiple generations would be disadvantageous, Champagne points out.

**Technical explanations**

*Epigenetics* is a catchall and imprecise term for a jumbled collection of biological processes that operate "above the genes." It is usually said that these mechanisms modify gene action without changing gene sequences, and it is often said that they turn genes on and off or up and down.

Purists insist that these mechanisms must be heritable during somatic cell division or in the germline. And yet a process that is probably not heritable, posttranslational histone modification, is always on the list of epigenetic mechanisms and has been researched heavily. Histones are proteins that resemble spools for winding up DNA tightly enough to stuff into the cell nucleus. In the epigenetic mode, histone proteins are chemically modified—for instance, by adding or subtracting a methyl group, acetyl group, or other chemical tags. Histone methylation characteristically tightens up DNA, restricting access to genes. Histone acetylation unravels DNA, making genes more accessible.

The other chief focus of epigenetic attention has been DNA methylation, the tagging of specific points on the DNA molecule with a methyl group. DNA methylation, which usually (but not always) silences genes, is also the chief focus of this article.

Noncoding RNA is usually on the list of epigenetic mechanisms, but much less is known about its epigenetic features. Some authorities also include prions, because they change a protein's shape while retaining its original composition. Noncoding RNA, along with DNA methylation and histone modification, were discussed in a previous article on epigenetics basics, "Epigenetics and development," which appeared in *BioScience* in October 2009.

Circumstances change, and the social experiences might be irrelevant, or even harmful, several generations later.

Few human studies have been done, but one in which carefully kept nineteenth and early twentieth century parish records from northern Sweden were examined showed that risk of diabetes, cardiovascular disease, and early death is linked in men (but not women) with grandfathers who had plenty of food available just before puberty.

Champagne points to mouse studies showing that exposure to endocrine-disrupting chemicals seems to have effects that persist to the F5 generation, "which does suggest that there must be some sort of germ line incorporation of the epigenetic change," she says. Doing multigenerational studies in complex organisms is difficult, she points out, and mice are complex organisms. "A lot of people are trying now to incorporate it into their studies so we can see what is transmitted over generations and how many generations this can persist."

Sweat adds, "It's reasonable to think that these transgenerational effects will have a half life, so to speak, that they probably last for two, three, four generations and then are subject to reversing back to the original baseline state. But the question has not really been addressed, at least in terms of mammalian systems." Technical advances mean that researchers can now measure epigenetic marks like DNA methylation directly, he points out. It is becoming possible to ask whether a molecular mark is short-lived or really is heritable in an infinitely repeating sense. "People just really haven't done the experiments in mammalian systems to try to figure that out."

## Toward the future of epigenetics

Sweatt believes that understanding the role of epigenetic mechanisms in regulating fundamental cell biology will be transformative. He argues, "It's somewhat like the advent of molecular biology in the biological sciences in the '70s. These epigenetic molecular mechanisms are just going to permeate all aspects of functional cell biology by the time this is all figured out." Like other researchers, however, he emphasizes that realizing the enormous promise of epigenetics will take an enormous amount of work and lots of time. Meanwhile, pharmaceutical researchers have voted with their feet. Their journals are home to a growing number of articles on the prospects for exploiting epigenetic mechanisms for drug therapy.

The hurdles left to epigenetics research are also enormous. Champagne emphasizes the degree of control needed in a lab animal facility in order to do epigenetic studies over several generations. She also points out that human studies are limited, because access to tissue is limited. Getting brain tissue from living humans is not an option, and it is not clear that blood will ever be an acceptable surrogate tissue, especially for behavioral epigenetics. "We need to do a lot more work on that," she says.

Perhaps even more challenging than lab obstacles is the task of managing and sharing the many terabytes of data that epigenomics research generates. Members of the National Institutes of Health Roadmap Epigenomics Mapping Consortium lamented last year: "The sheer volume and complexity of consortium-generated data has pushed the limits of existing analytical and visualization tools."

But if at some future date, epigenetic reprogramming of cells, especially neurons, seems likely to potentially fix (or even prevent) mental disorders, aging, and social ills such as addiction and suicide, how long will it be before we try our hand at such epigenetic improvements?

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