

Bench Report

Notes in the Margins

During brain development, the chemical marks that stud the length of DNA may be just as important as the genes themselves.

WHEN RESEARCHERS IN Terrence Sejnowski's laboratory added a new drug to the cocktail of compounds they were giving young mice, they didn't expect to see much effect on the rodents' brain cells. The mice were already receiving ketamine, a drug that altered brain cell development and induced neurological and behavioral signs of schizophrenia, helping the team study the roots of the disease.

When the mice received the additional drug, 5-azacytidine, in conjunction with ketamine, the effect was profound. The brain cell alterations and behavior changes produced by ketamine seemed to stop entirely.

"All of a sudden, this experiment became really exciting," says Sejnowski, an HHMI investigator at the Salk Institute for Biological Studies.

5-azacytidine is a methylation inhibitor; it halts the addition of methyl chemical groups to DNA, and changes how mice learn. "We hadn't anticipated any role of methylation in the underlying pathophysiology of

schizophrenia or ketamine's effects," he says.

Luckily for him, a methylation expert was just down the hall. Joe Ecker, an HHMI-GBMF investigator at Salk, was an expert on methyl groups in plants and had begun to shift his attention toward mammalian biology. The timing for a collaboration was perfect.

Methylation—adding a methyl chemical group to the DNA of a plant or animal—doesn't change the information held in a gene. Instead, it influences which genes are expressed in a cell, and at what level. When a methyl group is tacked onto a gene, it keeps other regulatory molecules from binding to the gene and reading its code, so the gene's protein product is less likely to be made. Ecker, whose early research focused on methylation in the plant *Arabidopsis*, had developed a method to map the location of all methyl groups in a cell's DNA and had recent success in applying this method to human stem cells.

So Ecker, Sejnowski, and members of their labs, including staff scientist Margarita Behrens, teamed up to study whether methylation might be involved in learning and memory, or in brain development in mammals. Using Ecker's mapping methods on brain cells, they got another surprise. In mammals,

most methylation is known to occur at DNA sites where there is a particular combination of nucleotides—a cytosine followed by a guanine. This is called CG methylation. But Ecker's mapping methods, applied to the brain cells of mice, told a different story: the scientists found unusual methylation levels at other nucleotide combinations—that is, non-CG methylation.

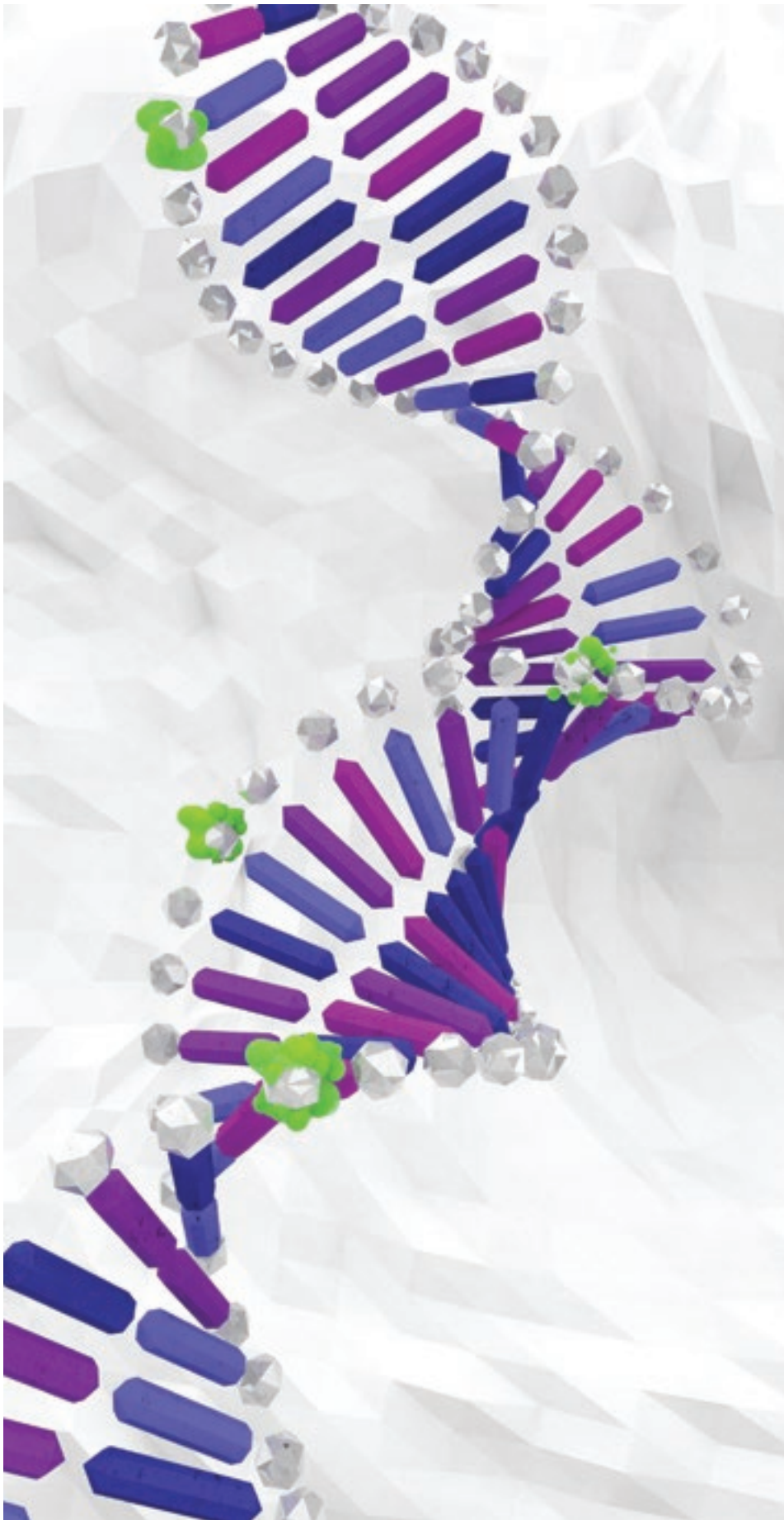
In humans, non-CG methylation had previously been detected only in stem cells—by Ecker's group. Moreover, researchers' prior efforts to map brain methylation made the erroneous assumption that all human DNA methylation occurred at CG sites. Because Ecker's method highlighted all methylation marks, it showed what others had missed.


Non-CG methylation marks, the team discovered, appear as the brain develops. "It was particularly striking that there are none of these marks in the fetal brain," says Ecker. "But as brain development progresses, there is a rapid increase in non-CG methylation."

The scientists mapped the patterns of non-CG methyl marks in several types of brain cells, throughout development, in a study reported August 9, 2013, in *Science*. In both humans and mice, non-CG methylation in brain cells increased between birth and adolescence. Moreover, the exact location of methylation in the genome was remarkably similar among humans, and even similar to the pattern seen in mice, Ecker says, suggesting that the marks affect fundamental processes in brain development or function.

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—JOE ECKER



 Watch an animation showing how methylation stops gene expression at www.hhmi.org/bulletin/winter2014.

“The increase in non-CG methylation occurs during a time in development when many new cellular connections are being made in the brain, and this is the exact period when things are known to go wrong in various diseases,” he adds.

“This is so exciting because for a disease like schizophrenia, the genetic component is only around 50 percent heritable [based on twin studies], and nobody knows what explains the other 50 percent,” says Sejnowski. “These findings open the door for a whole new area to delve into.”

Methylation marks, which can be influenced by genetics and are passed between generations in mice, may also be influenced by environment. Methylation patterns can be altered by diet and chemical exposure, and may be influenced by other factors such as drugs. Further research could reveal a role in disease development, including schizophrenia.

To speed research in this area, Ecker, Sejnowski, and Behrens are sharing their maps, creating a public database that contains the full methylation patterns—or methylomes—of the individuals they’ve studied so far.

“We’ve just laid the foundation,” says Sejnowski. “We’re only beginning to understand all these mechanisms that are constantly at work in our brains while we’re running around planning things, learning, remembering, and making decisions.”

—Sarah C.P. Williams