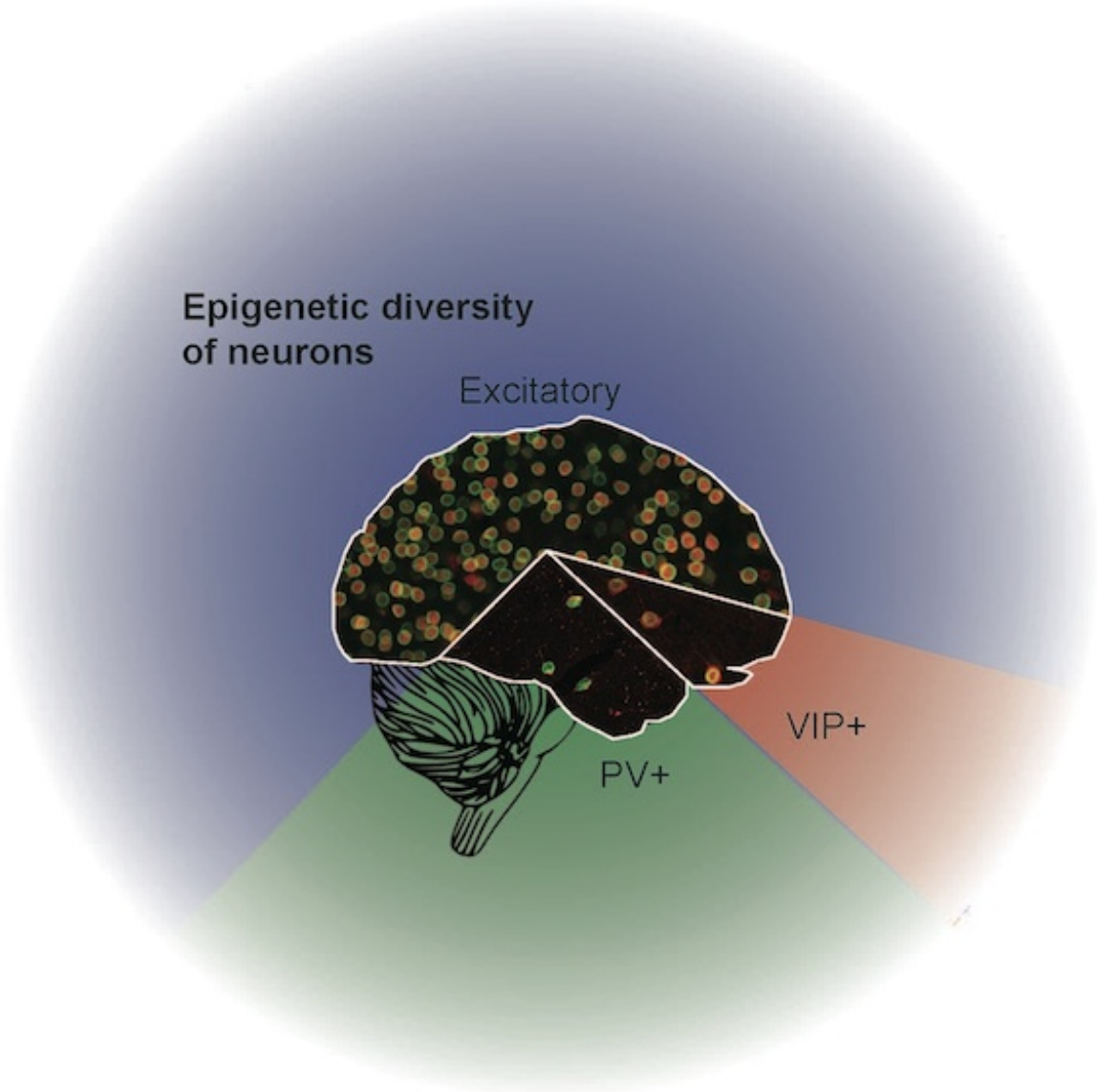
# Researchers survey the epigenetic diversity of neurons

June 25 2015



Credit: Eran Mukamel, Salk Institute

Howard Hughes Medical Institute (HHMI) scientists have profiled key features of the genetic material inside three types of brain cells and found vast differences in the patterns of chemical modifications that affect how the genes in each type of neuron are regulated. The analysis was made possible by a new method of collecting and purifying the nuclei of specific kinds of cells. Doing this type of study on cells in brain tissue has been challenging because the cells are densely packed and intimately intertwined.

A team of scientists led by Jeremy Nathans, an HHMI investigator at Johns Hopkins University, and Joseph Ecker, an HHMI investigator at the Salk Institute for Biological Studies, published the findings June 18, 2015, in the journal *Neuron*. Nathans and Ecker collaborated on the studies with researchers in the labs of HHMI investigator Terrence Sejnowski at Salk and Sean Eddy, a group leader at HHMI's Janelia Research Campus. The researchers say the new method for obtaining cell-type specific nuclei, an adaptation of technology previously used in plants, will enable a wide range of studies in mammalian tissues.

Nathans is a neuroscientist who studies the how cells in the retina—the light-absorbing structure at the back of the eye, which is considered part of the brain—assume their correct identities, and how those cells respond to injury and disease. In 2010, he and Alisa Mo, an MD/PhD student in his lab, learned of a new methods scientists were using to separate genetic material according to cell type. Rather than disentangling entire cells, HHMI investigator Steven Henikoff and postdoctoral researcher Roger Deal at the Fred Hutchinson Cancer Research Center had simplified the problem by isolating only the nuclei of the cells they were interested in. The bulk of a cell's DNA is

contained in its nucleus, as well as enough RNA for analyses of gene activity. Henikoff and Deal had developed the method, which they called INTACT, for plants, but Nathans and Mo thought it could be adapted for use in mammals.

Henikoff's team genetically manipulated the cells in their plants so that the cells they wanted to study —and only those cells—produced a particular protein that protruded from the nucleus. They could then break up the plant cells and fish out the nuclei they were interested in with an antibody that stuck specifically to the nuclear protein they had introduced.

"It's a very clever strategy," Nathans says. "We got to thinking about this in the context of the brain, where it's not so easy to isolate intact cells."

Gilbert Henry, a research specialist in Eddy's lab, was already engineering INTACT to work in fruit flies, and offered insight that helped Mo develop a similar system in mice. Mo engineered mice that carried a gene for a tagged nuclear membrane protein Henry had designed, SUN-1, which would be turned on only in cells that made another protein, Cre. Taking advantage of mice that produce Cre in specific cell types, they could breed animals in which their nuclear tag was limited to the cells they wanted to study. Then they could use an antibody that clung to a tag on the SUN-1 protein to retrieve nuclei of those particular cells.

"We built this to be potentially expressable in any cell," Nathans says. "It just needs the Cre reaction to turn it on, and there are many mouse lines available that express Cre in different cell types."

For Nathans and Ecker, the first application of the technology was to map out epigenetic modifications in various cell types. Epigenetic modifications are chemical changes to DNA that affect how genes are

regulated. They are widespread and important in shaping cells' identities and function.

Ecker studies epigenetic processes in plants and animals, and is a leader in studies of methylation, in which small chemical tags called methyl groups are attached to specific sites in the genome. In 2013, he and collaborators mapped how methylation patterns in brain tissue change during development. "Now what we want to do is begin to profile different subtypes of neurons to begin to look at diversity," he says.

For their experiments, the team isolated the nuclei of three types of neurons: excitatory neurons and two kinds of inhibitory neurons, PV interneurons and VIP interneurons. "These neurons play very distinct functional roles," Mo says. "Excitatory neurons give rise to the output of the brain, whereas inhibitory neurons are modulators of the output." Importantly, individual classes of inhibitory neurons are quite sparse in the brain, so any unique features of those cells would likely have been lost in earlier studies that looked at the tissue as a whole.

The team mapped methylation sites for each cell type. They also looked at how accessible different regions of DNA were inside each cell type. DNA packaging bundles up chromosomes, packing most of the DNA away but leaving some regions accessible to the machinery that switches genes on and initiates protein production, so accessibility has a profound effect on gene activity. Additionally, the scientists analyzed nuclear RNA, a measure of the activity of specific genes.

The experiments generated massive amounts of data. Eran A. Mukamel, a computational biologist in HHMI investigator Sejnowski's lab, applied sophisticated analyses to make sense of it all.

The variation in the epigenetic landscapes of the three cell types was staggering. The scientists identified more than 200,000 sites where

chromatin accessibility and DNA methylation signatures differed between the cells.

"We weren't sure what to expect. This was an exploratory, discovery-level project," Ecker says. One surprise, he says, is that the two types of inhibitory neurons in their study differed from one another as much as they differed from the excitatory neurons. "Although the two types of inhibitory neurons are very similar to each other in terms of morphology, synaptic connectivity, and the kinds of small molecules they use for signaling, they're very different in terms of the methylation pattern. That means that there's a lot of additional information here, outside of where genes are expressed," he says.

One important level of information discovered in their data was a record of past gene expression. "We could see a particular methylation signature of what likely happened previously," Ecker says.

Many hints toward future lines of research emerged from the data, and Nathans and Ecker both plan to continue using the INTACT system in their own research. Meanwhile, they expect other scientists to adopt the technology for their own experiments. The method is straightforward and the genetically engineered mice are freely available, they say. Mo says there are many opportunities to explore how disease and development alter epigenetic patterns in specific types of neurons, but points out that the system can also be applied outside of the brain. "We developed a broad approach that can be applied to many different systems. It's not limited to any type of organ or disease model," she says.

**More information:** "Epigenomic Signatures of Neuronal Diversity in the Mammalian Brain." DOI: dx.doi.org/10.1016/j.neuron.2015.05.018