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By cloning mouse neurons, scientists find brain cells with 100+ unique mutations

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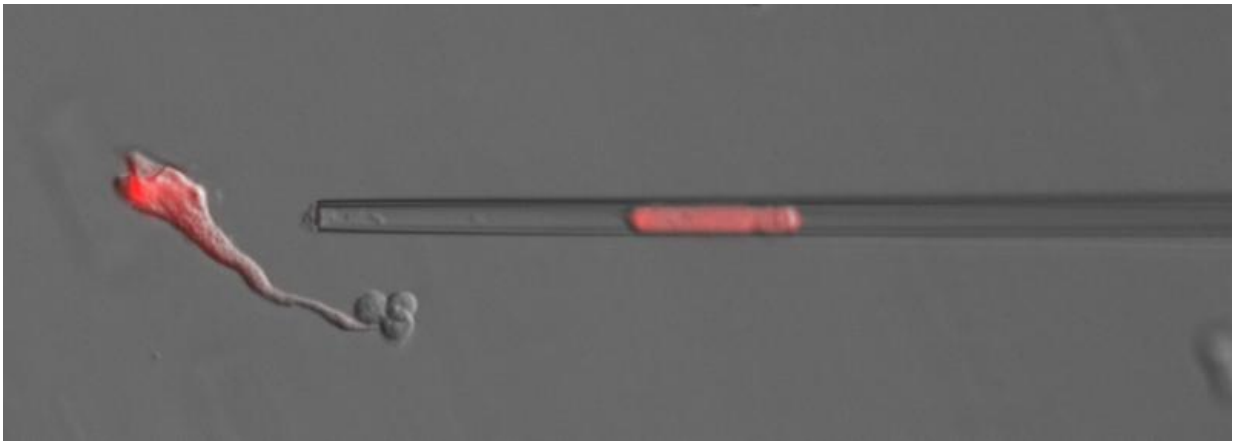

TSRI Research Assistant Alberto Rodriguez uses a tiny straw-like micropipette to pick up red fluorescent neurons and transfer their genomes into an egg.

In a new study published today in the journal *Neuron*, scientists from The Scripps Research Institute (TSRI) are the first to sequence the complete genomes of individual neurons and to produce live mice carrying neuronal genomes in all of their cells.

Use of the technique revealed surprising insights into these cells' genomes—including the findings that each neuron contained an average of more than 100 mutations and that these neurons accumulated more mutations in genes they used frequently.

"Neuronal genomes have remained a mystery for a long time," said TSRI Associate Professor Kristin Baldwin, senior author of the new study and member of the Dorris Neuroscience Center at TSRI. "The findings in this study, and the extensive validation of genome sequencing-based mutation discovery that this method permits, open the door to additional studies of brain mutations in aging and disease, which may help us understand or treat cognitive decline in aging, neurodegeneration and neurodevelopmental diseases such as autism."

Unique Mutations

Our individual genomes are inherited from our parents and make us unique in our behavior, appearance and susceptibility to disease. While new mutations in genomes of individual cells are known to cause cancer, only recently have researchers begun to appreciate how different the genomes within normal cells of the body may be. Several lines of research have suggested cells in the brain may be particularly unique—and prone to accumulating new mutations of various sorts, including "jumping" genes called transposons.

Many of these mutations may not be harmful—but collecting too many mutations, or having them build up in genes needed for a cell's function, might lead to loss of neurons or incorrect brain wiring, which are suspected causes of diseases such as Alzheimer's and autism.

"We need to know more about mutations in the brain and how they might impact cell function," said TSRI Research Associate Jennifer Hazen, co-first author of the new study with Gregory Faust of the University of Virginia School of Medicine.

However, studying mutations in single neurons has presented a challenge: A single cell doesn't contain enough genetic material for analysis, yet these mutations only exist in single cells. Unfortunately,

current single-cell analysis approaches introduce new DNA errors and also destroy the only copy of the cell's DNA in the process, making it impossible to go back and check to see if the mutations were really there. Scientists can't generate copies of neurons because, unlike other cell types, neurons don't divide in cell culture.

"There has been no easy way to get more copies of a neuron," explained TSRI Research Assistant William Ferguson, a co-author of the paper.

Copies Through Cloning

The new study helps solve this problem. The team took a mouse neuron's nucleus, which houses its DNA, and inserted it into an egg cell, which then divided and copied the mutation. The cloned cells then developed into thousands, or even millions, of stem cells with enough DNA for genomic analysis. The researchers repeated the process to create several lines of cloned neurons.

"We worked to get the egg itself to copy the genomes of brain cells using cloning," said Baldwin.

"We're tricking the neuron into thinking it's not a neuron," added Hazen. "This gives us a renewable source of copies of these genomes."

To confirm that the cloned cells were indeed neurons, rather than other brain cells, the researchers tagged the cells with bright fluorescent markers. "When you see the marker, it's a sigh of relief—it worked," said TSRI Research Assistant Alberto Rios Rodriguez, a co-author of the study. Genomic analysis of the cloned cells provided further evidence that the neuron's unique mutations were indeed being passed along.

For the first time, the team was even able to make cloned stem cell lines neurons from mice older than eight weeks. This allowed the researchers

to see mutations that build up over time. Even more strikingly, several of these stem cell lines could be grown into fertile adult mice which were clones of a single mouse neuron and carried the neuronal mutations in every cell on top of the rest of the DNA from the original mouse.

Sergey Kupriyanov, director of the Mouse Genetics Core at TSRI and co-author of the study, called the project "technically challenging." The researchers discovered that not every mutated neuron could be developed into a stem cell line, although more research is needed to explain why.

The stem cell lines that did develop, however, provided some surprising insights into the brain.

Implications for Human Neurons

The researchers found that neurons accumulate more mutations in the genes they use, which contrasts with other cell types that seem to protect their commonly used genes.

"Even more surprisingly," said Baldwin. "We found that every neuron we looked at was unique—carrying more than 100 DNA changes or mutations that were not present in other cells."

The researchers aren't sure why this diversity is so common—there's no evidence that neurons rearrange their DNA like blood cells do—but Baldwin said that if this phenomenon holds true in humans, our brains could hold 100 billion unique genomes.

Next, the researchers plan to use their technique to study neuronal genomes of very old mice and those with neurologic diseases. They hope this work will lead to new insights and therapeutic strategies for treating brain aging and neurologic diseases caused by neuronal mutations.

More information: "The Complete Genome Sequences, Unique Mutational Spectra, and Developmental Potency of Adult Neurons Revealed by Cloning" *Neuron*, March 3, 2016. DOI: 10.1016/j.neuron.2016.02.004

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