Is Hippocampal Cell Death a Myth?

Results of new neuronal counting methods are challenging the view that the "benign" memory loss of normal old age is due to loss of neurons in the hippocampus

A man in his late sixties wonders if he's losing his mind. He misplaces keys, glasses, and other necessities with alarming frequency, and he forgets appointments much more than he used to. Yet he shows no signs of dementia, such as having no memory of a meeting that occurred just minutes ago. The man is merely experiencing something called "benign forgetfulness," as about one third of people over 65 do.

What accounts for this forgetfulness? No one knows for sure, but studies done in several labs over the past 2 decades suggested it could be due to the death of nerve cells in the hippocampus, a brain structure known to be critical to learning and memory. Now, though, powerful new methods of counting neurons appear to have contradicted much of this work.

The accumulating evidence, much of it from Mark West at the University of Aarhus in Denmark and his colleagues, implies that neither rats nor humans lose neurons as they age in the part of the hippocampus—known as the CA region—traditionally linked with memory impairment. "The failure to see neuron loss in the hippocampus is really quite amazing," says Michael Meaney, a neuroscientist at Canada's McGill University.

If the Aarhus team is correct, and at least two other labs are finding similar results, neurobiologists would have to revise their theories about what causes benign memory impairment. "When we talk about normal age-related decline in memory, we assume a loss of cells. [West's] study is calling that into question," says Fred Gage, a neurobiologist at the Salk Institute in San Diego. That might be encouraging news for people like the forgetful older man, because it may mean that treating age-related memory loss will be possible. Replacing missing neurons is a tall order, but if memory loss is due to a change in cell function, drugs might reverse it.

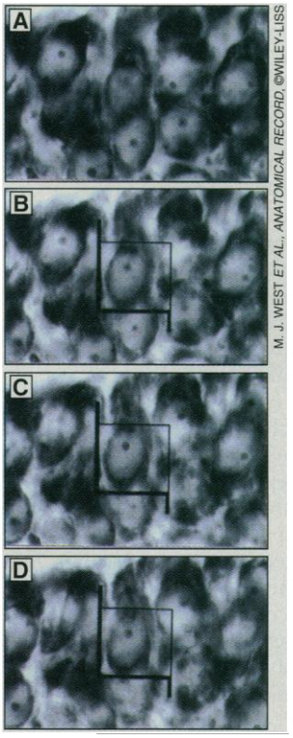
Still, many neurobiologists are taking a wait-and-see attitude about the new findings because of the large number of studies suggesting that hippocampal neurons are lost during normal aging as well as in people with Alzheimer's disease, who suffer severe, progressive memory loss. In the mid-1970s, for example, Philip Landfield's team, then at Bowman Gray School of Medicine in Winston-Salem, North Carolina, used well-established techniques for determining brain-cell density to compare the hippocampi of young rats with those of old rats, and found a 20% to 30% percent drop in cell density in the CA region over the 25-month rodent lifetime. Most of the cells lost were pyramidal neurons, pyramid-shaped cells that sport critical long-distance connections to other parts of the hippocampus and to other brain regions. Other researchers quickly replicated these findings, and by the end of the 1980s, both the human and rat data pointed to a gradual loss of pyramidal neurons in the hippocampus with age—to the tune of 3% to 9% per decade in humans.

Then in 1990, Meaney's team correlated such losses with cognitive impairment. They found that aged rats who were cognitively impaired—as measured by their poor performance in the Morris water maze, a standard test in which the animals have to remember the location of a camouflaged, underwater platform—lost half their cells in CA1 and CA3, while unimpaired rats of the same age lost just one fifth of their cells.

But even then neurobiologists recognized that the cell density studies were imperfect. Because researchers usually obtain density counts in only a few sections of hippocampal tissue, there's the possibility that the areas studied are not representative of the whole brain region. In addition, the processing needed to ready brain tissue for the density counts could skew the results. "Density measures can be affected by a number of factors, and one of them is aged brains may shrink less when you process them than young brains do," says neuroscientist Peter Rapp of the State University of New York, Stony Brook, one of the researchers finding results confirming those of West. Thus, neuronal densities could appear lower in older brains than in younger ones, even if their total cell counts are the same.

Aware of these problems, West wanted to find a more accurate way of counting neurons. "We have never felt that the density data by themselves were very valuable," he says. So West teamed up with Hans Gundersen, also at Aarhus, who in 1984 had devised a new way of counting kidney cells. Together they adapted it to the nervous system.

In this method, dubbed the "optical fractionator" technique, researchers cut tissue slices all the way through the hippocampus, as one might slice a loaf of bread. They then pick out slices at even intervals—say, every tenth slice—and examine particular regions under a microscope, counting all the neurons in each when they first come into the plane of focus. The focusing trick ensures that each cell is counted only once. Because the researchers know what fraction of the hippocampus they've examined, they can calculate the total number of neurons in the brain structure. "The neat aspect of our technique is that we can estimate the total number of cells and not packing density," says West.



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Tuning in. In optical fractionation, a neuron is counted only when its nucleus comes into sharp focus, as in "C," which helps ensure it's counted just once.

After perfecting his method in the early 1990s, West applied it to brain autopsy tissue from humans aged 13 to 85 who had not died of any neurodegenerative disease. To his surprise, he saw almost no signs of neuronal loss with age in any of the CA regions, but big losses in two other hippocampal regions, the subiculum and hilus. Researchers are not sure what to make of those changes because the subiculum and hilus are not known to play a major role in memory, but the lack of change in the memory-related CA regions, confirmed in later studies, bucks the conventional wisdom that the pyramidal cells there dwindle with age.

An even more recent study, which appears in the January/February issue of *Neurobiology of Aging*, also challenges the link between hippocampal cell counts and memory. West, Thøger Rasmussen, and their colleagues tested the memories of 52 old rats using the Morris water maze and then counted hippocampal neurons in the five worst and five best performers, as well as in five young control rats. Rats in all three

groups had similar neuronal counts. The Aarhus neurobiologist believes his results show that cell loss does not accompany aging—even when there's obvious memory impairments—in rats. However, he's still concerned about the discrepancies between his human data, which do show some loss of hippocampal neurons, although not in the CA regions, and his results in rats, where there was no loss at all.

Still, other neurobiologists are enthused about the new neuronal counting method. "West's work is the definitive work in counting cells in the brain as a function of age or disease," says Paul Coleman, a neurobiologist at the University of Rochester Medical School in New York. But some experts aren't convinced that the older methods were flawed enough to produce consistently erroneous results. "I can't explain why there are discrepancies—Mark does excellent work—but I'm very confident in our data," says Landfield, now at the University of Kentucky College of Medicine in Lexington. The large gaps between cells and very obvious decreases in packing density in hippocampal tissue from older brains his team found cannot, he maintains, be explained by changes in the volume of the hippocampus. He adds that his team has recently completed a study using techniques similar to West's and still finds a decrease in neuron number with age.

Others, such as Stony Brook's Rapp, point out that West's behavioral tests failed to rule out reasons for a rat's poor performance in the water maze—such as age-related blindness or decreased motor skills—that have nothing to do with memory loss. If other factors were responsible, then a failure to find cell loss in the hippocampus would be less surprising. West agrees he couldn't test for everything, but insists his methods were good enough to pinpoint rats that had at least some types of cognitive impairment.

Everyone agrees that the only way to resolve the issue of what happens in the hippocampus during aging is with more data, and some of that may not be long in coming. Rapp and psychologist Michela Gallagher at the University of North Carolina, Chapel Hill, say they have new rat data corroborating West's results. "I think we're going to shortly arrive at a fairly convincing demonstration that hippocampal lesions don't exist [in normal aging]," predicts Gallagher.

If so, something else—perhaps a loss of connections between neurons or functional changes such as a disruption of cells' ability to communicate chemically with each other—could underlie a fading memory. In that case, the search would be on for treatments that could spell clearer skies for people under the forgetful cloud of old age.

–Ingrid Wickelgren

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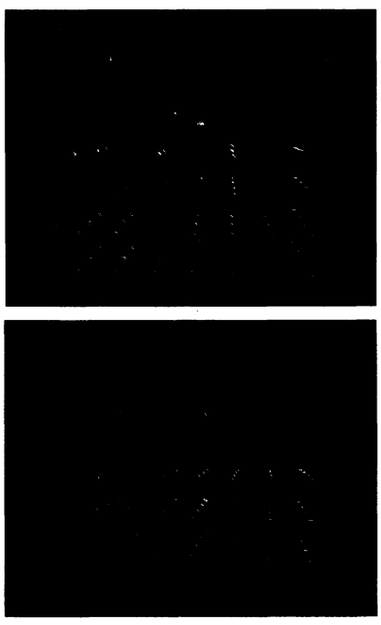
MICROELECTRONICS

Isotope Switch Toughens Transistors

A favorite bugaboo of English teachers is the dangling modifier, a description unattached to any subject. When it comes to integrated circuits (ICs), though, the most dreaded hang-up of all is the dangling bond. Instead of hampering the flow of narrative, dangling bonds—unoccupied chemical binding sites in, say, silicon atoms—clutch at free electrons and resist the flow of current, slowing transistors and damaging them with the heat that's generated. Chipmakers try to delay the damage by diffusing hydrogen into nearly finished wafers to cap off the bonds. But as circuits get smaller and the electric fields that drive them get larger, that remedy falls short, as energetic "hot" electrons strip away the protective hydrogen.

That's why some semiconductor researchers are reaching for superlatives to describe the discovery that a simple isotope switch—deuterium for hydrogen—can improve transistor lifetimes by factors of 10 to 50, according to a paper just accepted at *Applied Physics Letters*. Twice as heavy as hydrogen but virtually identical chemically, deuterium is much harder to separate from bonds on a wafer's silicon substrate, say the authors, Joseph Lyding and Karl Hess of the Beckman Institute at the University of Illinois, Urbana-Champaign, and Isik Kizilyalli at AT&T Bell Laboratories in Orlando, Florida.



LYDING *ET AL.*

Holding the line. In simulations, deuterium atoms *(purple)* cluster at a silicon-oxide boundary, while hydrogens *(white)* stray.

The finding "has huge implications worldwide," says Dan DiMaria of IBM's Thomas J. Watson Research Center in Yorktown Heights, New York. It could be "extremely important," he says, for understanding chip failure. And while some researchers, including DiMaria, doubt that the technique will quickly penetrate chip manufacturing because of the large investment in existing protective strategies, others suggest that it could soon yield more durable circuits that could be driven harder.

The bonds in question lie at the interface between a wafer's silicon base, or substrate, and the insulating layer of silicon dioxide grown over it. Current flows along this interface when the circuit—a transistor, for example—is open. The problem, says Hess, is that the oxide "does not fit perfectly on top of the silicon," having a slightly larger spacing between the atoms in its lattice. This mismatch leaves dangling silicon bonds that can snap up electrons and impede current flow.

For decades, says Hess, chipmakers have battled this problem by bathing chips in hydrogen gas during a final, high-temperature "annealing" step. The hydrogen atoms slip through the IC's layers and terminate the dangling bonds—at least until hot electrons strip away the hydrogen. Other measures, such as incorporating nitrogen into the oxide lattice, which may help confine the hydrogen, or designing a transistor's current drain to reduce the maximum electric fields, can help. But they can be expensive and cut into performance.

That's where matters stood until last October, says Lyding, when he was "shooting the breeze" in Hess's office about some basic experiments he had done showing that deuterium is much harder to knock loose from a bare silicon surface than hydrogen is. When Hess saw the work's implications for ICs—his own specialty—he "jumped out of his chair," says Lyding. They gave the deuterium treatment to some chips obtained from AT&T, then began collaborating with Kizilyalli, who showed the large improvement in lifetime in "accelerated" tests that drive the chips harder than spec. At least part of the explanation is simple, says Hess: "If you played pool and had a smaller [cue] ball, it would not transfer as much energy to the bigger balls"—the larger deuterium atoms.

"I didn't realize [the effect] would be so major," says Kizilyalli. Because deuterium is inexpensive and the treatment is simple, "it will be readily adopted by industry," says Jeffrey Bude of AT&T Bell Laboratories in Murray Hill, New Jersey.

Others aren't so sure. "This kind of data really needs to be verified" by other groups, says Leo Yau of Intel Corp. in Beaverton, Oregon, and industry will want to know whether the technique is compatible with existing processes. Still, the dread of the dangling bond is so great that even Yau admits, "If it works, people will use it."

–James Glanz