Tracking Insulin to the Mind

Although the idea is controversial, recent evidence suggests that insulin may be needed for normal brain functions—including learning and memory

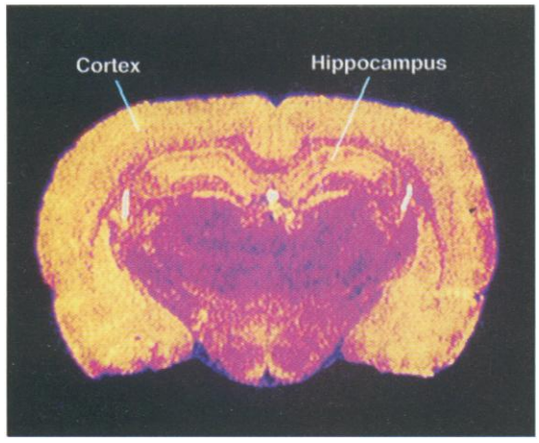
When one thinks of the hormone insulin, what comes to mind is not ... the mind. Insulin has long been known as the signal that tells every muscle, liver, and fat cell to pull the sugar glucose in from the blood so it can be used to generate the energy the body needs to survive. But the hormone is supposed to hold no sway over the brain—or so the endocrinology textbooks say. Now, growing, although controversial, evidence is beginning to contradict this dictum, suggesting not only that insulin is vital in the brain but that the hormone may influence the brain's most precious functions: learning and memory.

Several lines of work in both lab animals and humans suggest that when neurons in cognitive brain areas such as the hippocampus and cerebral cortex don't get enough insulin or can't respond to it properly, everything from very mild memory loss to Alzheimer's disease can result. "Insulin is active in the brain in more significant ways than people have assumed," says behavioral neuroscientist Claude Messier of the University of Ottawa in Ontario, Canada, whose own work is contributing to that conclusion. "It's a hot topic," adds Mony de Leon of New York University (NYU) School of Medicine, who is one of the researchers newly attracted to the field. Exploring insulin's role in cognition, experts say, might one day point the way to drugs that could reduce memory loss in Alzheimer's disease and normal aging.

Other researchers aren't so sure. "There simply isn't enough information to say that insulin improves memory," says psychologist Paul Gold at the University of Virginia, Charlottesville. One major problem with the insulin hypothesis is that even its proponents can't agree on how the hormone might influence cognition.

Some experts suggest that insulin works in the brain much as it works elsewhere in the body—by chaperoning glucose into brain neurons, thereby helping them maintain their energy production. In that case, memory loss might result when brain cells lack insulin or become resistant to it, starving them of glucose—a condition that would amount to diabetes of the brain. But there are also hints that insulin has other beneficial roles, such as spurring neuronal growth and inhibiting the formation of brain lesions called neurofibrillary tangles that characterize Alzheimer's disease.

Early inklings that insulin might play a role in cognition came in the mid-1980s when a team led by diabetes expert Jesse Roth and neuroscientist Candace Pert, who then were both at the National Institutes of Health, discovered that parts of the rat brain important to learning and memory, including the hippocampus and parts of the cerebral cortex, are densely peppered with the receptors through which insulin exerts its effects on cells. Nobody knows just what the receptors are doing there. But neuroscientist Siegfried Hoyer at the University of Heidelberg in Germany began contemplating the heretical notion that they could help neurons metabolize glucose. At the time, virtually all experts believed that this does not require insulin, primarily because no one had found glucose-transporting molecules that respond to insulin in neurons.



J. M. HILL, M. LESNIAK / NIH

Lighting up. Staining with radioactive insulin shows that the rat brain is well supplied with insulin receptors. White indicates the greatest receptor density and purple the least, with yellow in between.

Early links to insulin

But Hoyer's team soon found a hint that defective glucose metabolism could contribute to Alzheimer's disease. They showed that patients with early-stage disease have much more unmetabolized glucose in their cerebral blood than controls have. Because the brains of the patients showed no corresponding decrease in oxygen consumption, Hoyer concluded that they were keeping up their metabolic rates abnormally, by oxidizing chemicals other than glucose. Indeed, he suggested that the neurons, like starving people, might be devouring parts of themselves and thus contributing to the cell damage and death that occurs in Alzheimer's disease.

Hoyer also reasoned that a defect in the ability of the patients' brain cells to respond to insulin might be what was keeping the glucose levels high in the blood coming from their brains, just as patients with type II diabetes have high levels of blood glucose because their liver, muscle, and fat cells are resistant to insulin. To test the idea, he decided to study the effect of disarming the insulin receptor in the brains of rats, making them insensitive to insulin.

When his team injected streptozotocin, a chemical that damages the insulin receptor, into the brains of 18 rats, the researchers found that it seriously impaired the rats' ability to remember a compartment in which they had received an electric shock. And as yet unpublished work by the Heidelberg group now demonstrates that the memory loss that results from impaired insulin signaling in rats is progressive, like the cognitive decline seen in Alzheimer's patients. Concludes Hoyer: "We believe that some cases of Alzheimer's disease are like diabetes mellitus."

By the early 1990s, other lines of research also began suggesting a role for insulin—or at least glucose metabolism—in memory. Glucose had been shown to enhance memory in rats, and Gold and his colleagues found that temporary and modest increases in blood levels of glucose can improve memory in people as well, including both Alzheimer's patients and normal elderly adults. Because glucose injections into the brains of rats enhanced their memory, Gold concluded that glucose exerts its effects by acting directly on neurons. "Insulin cannot explain much of what we know about glucose enhancement of memory," he maintains. But neuroscientist Suzanne Craft of the Seattle Veterans Administration Medical Center and the University of Washington and her colleagues thought that insulin might be behind effects such as those Gold saw.

She and her colleagues set out to separate the effects of insulin from those of glucose alone in Alzheimer's patients. In an initial experiment, the researchers found that both insulin and glucose infusions produced strik-

ing improvements in verbal memory in both early-stage Alzheimer's patients and controls. For example, the patients' scores went from "borderline" dementia to "low average." But because glucose infusions normally produce a rise in insulin, Craft and her colleagues repeated the experiment in another group of Alzheimer's patients, this time raising blood glucose to a level that previously improved memory while preventing an insulin rise. And they saw no memory improvement. Together, the two sets of experiments show that insulin does indeed mediate the cognitive enhancements originally seen with glucose, Craft reported at the 1996 meeting of the Society for Neuroscience.

More recently, her team has found hints that something has gone wrong with the hormone in the brains of people with Alzheimer's. In the January 1998 issue of *Neurology*, her team reports finding both significantly higher plasma insulin levels and lower insulin levels in the cerebrospinal fluid (CSF) of Alzheimer's patients as compared to controls. The researchers also found a correlation between the ratio of CSF insulin to plasma insulin and severity of dementia in the 25 patients they studied, with the more severely afflicted patients displaying the lowest ratios.

The imbalance might result because insulin isn't working effectively in the brains of these patients, Craft says. The pancreas might then churn out more insulin to compensate, which in turn might cause cells at the blood-brain barrier to produce fewer insulin transporter molecules, reducing the amount of the hormone that slips into the brain. Alternatively, abnormally fast breakdown of insulin in the brain could produce a deficit of CSF insulin and then send a signal to the periphery to rev up insulin production.

Hoyer's team has also found hints of some kind of insulin defect in the brains of Alzheimer's patients. In a study to appear in the *Journal of Neurotransmission*, they found unusually high numbers of insulin receptors in the cortical areas of brains from 17 patients who died of Alzheimer's disease. At the same time, these receptors seemed unable to convey the insulin signal properly, because an enzyme that comprises part of them was less active than normal. The scientists interpret the proliferation of receptors as the brain's attempt to compensate for a lack of insulin—a deficit that, they speculate, is compounded by a defect in the receptor itself.

Hoyer and others don't propose that insulin resistance is the primary cause of Alzheimer's disease, but they believe it could be one of several contributing factors, which include the accumulation of the small protein β amyloid into so-called plaques, a hallmark feature of the disease. Exactly how various factors might interact to produce dementia is not yet clear. However, some experts theorize that milder forms of insulin resistance, or perhaps insulin resistance in the absence of other factors linked to dementia, could lead to lesser memory deficits such as those that appear in normal aging and with type II diabetes, a disease that is often accompanied by memory problems.

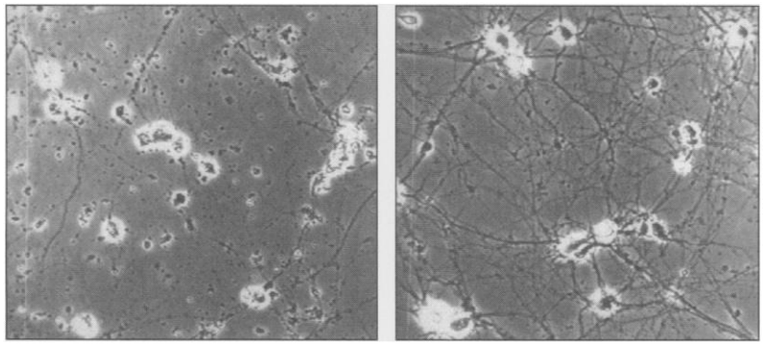
Unanswered questions

Even if other experiments confirm that problems in insulin signaling can create cognitive deficits and contribute to dementia, researchers will still need to explain how. Hoyer, for example, has some evidence to support his idea that the insulin signaling problems could create a memory-sapping energy deficit by impairing the ability of neurons to take up and metabolize glucose. He has shown, for instance, that treatment with streptozotocin—the drug that inactivates the insulin receptor—interferes with glucose metabolism in rat brains. Other researchers suggest that inadequate glucose metabolism might also create a deficit of the memory-enhancing neurotransmitter acetylcholine, which requires acetyl-CoA, a product of glucose breakdown, for its synthesis.

However, so far no one has shown conclusively that insulin promotes glucose uptake by neurons. Indeed, only now have researchers found insulin-sensitive glucose transporters in the mature mammalian brain. But even biochemist Ian Simpson, who, with his colleagues at the National Institute of Diabetes and Digestive and Kidney Diseases, demonstrated that such transporters exist in adult rodent brains, won't speculate about their role, which he calls simply "intriguing."

There is, however, evidence that insulin benefits neurons in other ways. Last August, for example, Ming Hong and Virginia Lee at the University of Pennsylvania School of Medicine in Philadelphia showed in neuronal cell cultures that insulin inhibits a key event in the formation of one of the pathological hallmarks of Alzheimer's disease, the neurofibrillary tangles. Researchers have previously linked the formation of the tangles to the addition of excess phosphate groups to a protein called tau, the principal tangle protein. Lee's team has now shown that insulin prevents this hyperphosphorylation, apparently by dampening the activity of one of the key tau-phosphorylating enzymes, glycogen synthase kinase–3.

In addition, a pair of papers published last December in *Molecular Brain Research* by neurobiologist William Wallace of the National Institute on Aging in Baltimore and his colleagues suggests that insulin may also act as a neuronal growth factor. The researchers were investigating how amyloid precursor protein (APP), β amyloid's parent molecule, affects certain cultured rat cells. The Wallace team found not only that APP treatment caused these cells to send out neuronlike extensions but that APP promotes this growth by activating the same molecular signaling pathway that insulin does. The finding suggests that insulin too may promote neuron outgrowth—and may thus help maintain neuron health. "In addition to or instead of insulin's effect on glucose and metabolism, insulin may be acting along this pathway to promote growth," Wallace says.



J. J. LUO, J. W. KUSIAK, W. C. WALLACE

Growth promoter. Working through an insulin signaling pathway, amyloid precursor protein causes neurons at right to send out more projections.

But before the larger community of neuroscientists is convinced that insulin is doing anything to affect cognition in the adult mammalian brain, researchers face two challenges. They will have to identify the molecular ripples insulin sends out when it contacts neurons in living animals. And they will then have to pinpoint problems along those insulin-sensitive molecular pathways in the brains of animals and humans with signs of memory loss. "There's evidence suggesting that insulin ought to play a role in cognitive function, but it doesn't add up to a complete story," cautions NYU's de Leon.

If the gaps in this story are filled in, experts might design drugs that augment specific effects of insulin in order to counteract memory loss. They could also test their hunch that insulin resistance is contributing to the problem by trying to correct it with the next wave of diabetes drugs, such as the recently approved compound Rezulin, and seeing if memory improvements follow. Craft is optimistic: "In my mind, one of the best predictors of how you age cognitively is your glucoregulatory status," she says.

But the jury is still out. Says Ottawa's Messier: "A key regulating hormone in our

body—that is, insulin—seems to have a profound effect on our brain, but we don't know what it's doing."

–Ingrid Wickelgren

Additional Reading

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PHYSICS

From a Turbulent Maelstrom, Order

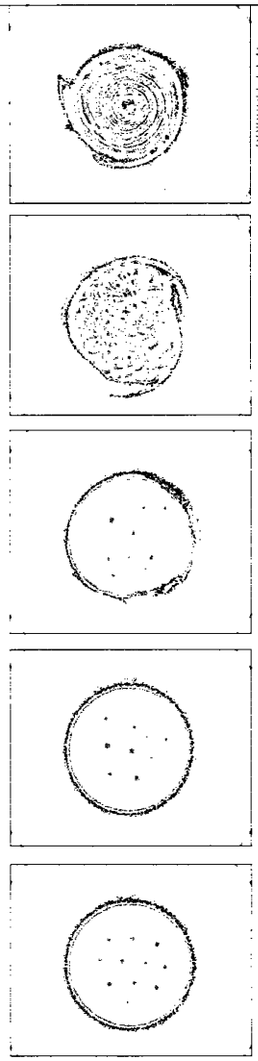
Daniel Dubin and Dezhe Jin didn't set out to introduce a Zen koan—a paradoxical statement that stimulates the intuition—into physics. But the team at the University of California, San Diego (UCSD), is offering a notion that sounds very like a Zen paradox to explain a bizarre phenomenon seen 4 years ago in turbulent gases of electrons. The vortices that develop in these fluids arrange themselves in neat, long-lasting "crystals," looking like a phalanx of tornadoes marching in perfect formation. Dubin and Jin have now shown that this kind of order can be the natural consequence of an increase in disorder.

Theory as well as common sense had rebelled at the finding, because stable patterns should be anathema to the high entropy, or randomness, of turbulent flows. But in a flash of insight, the team realized that each large vortex in these electron fluids acts as a Mixmaster, stirring and randomizing the background flow. That entropy increase opens the way for the vortices to gel into predictable, orderly, crystalline patterns. "The moral," says Dubin with the cryptic laugh of a Zen master, "is that entropy is maximized except where it isn't."

Other physicists are suitably bemused. "It's very hard to get your head around," says Michael Brown, a physicist at Swarthmore College in Pennsylvania, of the theory. But the theory, which is accepted for publication at *Physical Review Letters*, accurately predicts not only the crystals but also the distribution of vorticity or "swirliness" seen in the random sloshing of the background. "It is very much in quantitative agreement with the experiment," says UCSD's Fred Driscoll, whose own group discovered the crystals (*Science*, 9 December 1994, p. 1638). The new understanding should help the teams search for the behavior in other laboratory systems and ultimately in nature.

In the original experiments, Driscoll and his colleagues, including Kevin Fine, Ann Cass, and others, caged about a billion electrons at a time in a vacuum chamber using strong magnetic fields. Electrons trapped in this way bounce back and forth so rapidly between charged plates capping the field lines that they smooth out any structures that might form in that direction. The experimenters focus on two-dimensional (2D) patterns that develop across the field lines, like the eddies in a spinning bucket of water or the swirls in a suspended soap film. The big difference between the electrons and other turbulent fluids is that Driscoll's magnetically caged plasma has almost no viscosity or friction with the walls, so it offers a purer picture of turbulence and any structures that may emerge from it.

Driscoll generates turbulent initial states by injecting pulses of electrons from filaments mounted on one of the end caps. He then "photographs" the plasma after various time delays by dumping it onto a phosphorescent screen at the other end, which glows brighter where the electrons are concentrated. These snapshots showed that one or several strong vortices grew as the smaller vortices present in the stormy initial state merged. The vortices, embedded in weaker background eddies, eventually stopped rattling around and "chilled" into crystalline patterns.



DRISCOLL LAB

Orderly tornadoes. Vortices in a turbulent sea of electrons "crystallize" into a regular pattern.

Dubin and Jin tried to understand the crystals by drawing on a venerable tradition dating back to work by David Montgomery, now at Dartmouth College in Hanover, New Hampshire, and others in the 1970s. The pair treated the 2D plasma vortices and eddies statistically. They analyzed the ways in which vorticity could be distributed in the fluid to find the most likely patterns, regardless of how they came about. It's essentially the same approach that says roughly equal numbers of heads and tails will come up when you toss 100 pennies, no matter how individual pennies spin and fall. Because the most likely patterns are also the most disordered ones, theorists call this the maximum-entropy approach.

If the large vortices break up over time, maximum entropy theory would predict a vorticity distribution something like sand thrown randomly into a pile, with a single, broad peak. But the two theorists recognized, says Dubin, "that the vorticity in the strong vortices is trapped and cannot be mixed. Those vortices are so strong that nothing can get to them." These persistent, large vortices stir up the background, increasing its entropy and losing energy. And because the total entropy increases, the vortices can settle into an orderly pattern—a crystal.

Bizarre as the outcome may seem, it's not the first time physicists have recognized that entropy can create paradoxical patches of order (*Science*, 22 March, p. 1849). And the theory accurately predicts the details of the vortex crystals Driscoll's group observed. "The amazing thing to me, as an experimentalist, is that the theory actually works," says Driscoll.

It may also work in systems far from the frictionless gas of electrons. For example, the energy of vortices in a large and nearly 2D system like the film of atmosphere on Jupiter might overwhelm viscosity, allowing such strange effects to emerge. They could only do so, however, if crystalline patterns can take shape from vortices that can spin in both directions, unlike those in the electron gas, which are forced to spin in the same direction by the interaction of the electrons and the magnetic field lines.

So far Dubin and Jin's theory doesn't say whether that is possible. But both the theorists and the experimentalists are hoping to find out. Adding the electrons' positively charged, antimatter counterparts—positrons—to the laboratory systems would produce both left- and right-handed vortices. To paraphrase a famous koan, that should reveal whether the crystals amount to more than the sound of one hand clapping.

–James Glanz