

Building a Brainier Mouse

by Joe Z. Tsien

When I decided to become a scientist, never in my wildest dreams did I imagine that my work would provide fodder for CBS's *Late Show with David Letterman*. But last September, after my colleagues and I announced that we had doctored the genes of some mice to enhance their learning and memory skills, I turned on my television to find that my creations were the topic of one of Letterman's infamous Top Ten Lists. As I watched, the comedian counted down his roster of the Top Ten Term Paper Topics Written by Genius Mice. (My personal favorites are "Our Pearl Harbor: The Day Glue Traps Were Invented" and "Outsmarting the Mousetrap: Just Take the Cheese Off Really, Really Fast.")

My furry research subjects had become overnight celebrities. I received mail by the bagful and was forwarded dozens of jokes in which "smart" mice outwitted duller humans and their feeble traps. It seemed that the idea of a more intelligent mouse was something that everyone could identify with and find humorous.

But my co-workers and I did not set out merely to challenge the inventiveness of mousetrap manufacturers. Our research was part of a decades-long line of inquiry into exactly what happens in the brain during learning and what memories are made of. By generating the smart mice—a strain that we dubbed *Doogie* after the boy genius on the TV show *Doogie Howser, M.D.*—we validated a 50-year-old theory about the mechanisms of learning and memory and illustrated the central role of a particular molecule in the process of memory formation. That molecule could one day serve as a possible target for drugs to treat brain disorders such as Alzheimer's disease or even, perhaps, to boost learning and memory capacity in normal people.

Understanding the molecular basis of learning and memory is so important because what we learn and what we remem-

By genetically engineering a smarter than average mouse, scientists have assembled some of the central molecular components of learning and memory

ber determine largely who we are. Memory, not merely facial and physical appearance, defines an individual, as everyone who has known someone with Alzheimer's disease understands all too well. Furthermore, learning and memory extend beyond the individual and transmit our culture and civilization over generations. They are major forces in driving behavioral, cultural and social evolution.

The ABCs of Learning and Memory

The human brain has approximately 100 billion nerve cells, or neurons, that are linked in networks to give rise to a variety of mental and cognitive attributes, such as memory, intelligence, emotion and personality. The foundations for understanding the molecular and genetic mechanisms of learning and memory were laid in 1949, when Canadian psychologist Donald O. Hebb came up with a simple yet profound idea to explain how memory is represented and stored in the brain. In what is now known as Hebb's learning rule, he proposed that a memory is produced when two connected neurons are active simultaneously in a way that somehow strengthens the synapse, the site where the two nerve cells touch each other. At a synapse, information in the form of chemicals called neurotransmitters flows from the so-called presynaptic cell to one dubbed the postsynaptic cell.

In 1973 Timothy V. P. Bliss and Terje Lømo, working in Per Andersen's laboratory at the University of Oslo, discovered an experimental model with the hallmark features of Hebb's theory. They found that nerve cells in a sea horse-shaped region of the brain, appropriately called the hippocampus (from the Greek for "horse-headed sea monster"), become more tightly linked when stimulated by a series of high-frequency electrical pulses. The increase in synaptic strength—a phenomenon known as long-term potentiation (LTP)—can last for hours,

days or even weeks. The fact that LTP is found in the hippocampus is particularly fascinating because the hippocampus is a crucial brain structure for memory formation in both humans and animals.

Later studies by Mark F. Bear of the Howard Hughes Medical Institute at Brown University and other scientists showed that applying a low-frequency stimulation to the same hippocampal pathway produces a long-lasting *decrease* in the strength of the connections there. The reduction is also long-lasting and is known as long-term depression (LTD), although it apparently has nothing to do with clinical depression.

The strengthening and weakening of synaptic connections through LTP- and LTD-like processes have become the leading candidate mechanisms for storing and erasing learned information in the brain. We now know that LTP and LTD come in many different forms. The phenomena also occur in many brain regions besides the hippocampus, including the neocortex—the "gray matter"—and the amygdala, a structure involved in emotion.

What is the molecular machinery controlling these forms of synaptic changes, or plasticity? Studies in the 1980s and 1990s by Graham L. Collingridge of the University of Bristol in England, Roger A. Nicoll of the University of California at San Francisco, Robert C. Malenka of Stanford University, Gary S. Lynch of the University of California at Irvine and other researchers have found that the changes depend on a single type of molecule. The researchers demonstrated that the induction of the major forms of LTP and LTD requires the activation of so-called NMDA receptors, which sit on the cell membranes of postsynaptic neurons.

NMDA receptors are really minuscule pores that most scientists think are made up of four protein subunits that control the entry of calcium ions into neurons. (The name of the receptors derives from *N*-methyl-D-aspartate, an artificial chemical



JANA BRENNING (digital illustration); PHOTODISC (mazes); CORBIS (lamp); PETER MURPHY (mouse)

THE BASICS

A MOUSE NAMED DOOGIE

The author reviews the qualities of “smart” mice—and their implications for people

How are *Doogie* mice different from other mice? They have been genetically engineered to make more than the usual amount of a key subunit of a protein called the *N*-methyl-D-aspartate (NMDA) receptor.

What does the NMDA receptor do? It helps to strengthen the connection between two neurons that happen to be active at the same time. Scientists theorize that such strengthening is the basis for learning and memory.

How smart are *Doogie* mice? They will never do differential equations or play the stock market, but they are better than normal mice at distinguishing between objects they have seen before and at recalling how to find a platform in a tank of murky water, for instance.

How does their genetic alteration make them smarter? The NMDA receptors of *Doogie* mice stay open nearly twice as long as those of normal mice. The extra time somehow helps them form a new memory more effectively.

Could the same technique be used to enhance people’s ability to learn and remember? Theoretically, the possibility exists. But learning and memory in humans are much more complex than recognizing objects or remembering a water maze. Besides the scientific and technical barriers, the safety and ethical issues surrounding human genetic engineering would also need to be addressed. It is much more likely that pharmaceutical companies will first attempt to develop drugs that interact with the NMDA receptor to boost memory ability in people with memory deficits.

that happens to bind to them.) They are perfect candidates for implementing the synaptic changes of Hebb’s learning rule because they require two separate signals to open—the binding of the neurotransmitter glutamate and an electrical change

of the University of Edinburgh and his colleagues have observed that rats whose brains have been infused with drugs that block the NMDA receptor cannot learn how to negotiate a test called a Morris water maze as well as other rats. The finding is largely consistent with the prediction for the role of



The idea of a more intelligent mouse was something that everyone could identify with and find humorous.

called membrane depolarization. Accordingly, they are the ideal molecular switches to function as “coincidence detectors” to help the brain associate two events.

Although LTP and LTD had been shown to depend on NMDA receptors, linking LTP- and LTD-like processes to learning and memory turned out to be much more difficult than scientists originally thought. Richard G. M. Morris

LTP in learning and memory. The drugs often produce sensory-motor and behavioral disturbances, however, indicating the delicate line between drug efficacy and toxicity.

Four years ago, while I was working in Susumu Tonegawa’s laboratory at the Massachusetts Institute of Technology, I went one step further and developed a new genetic technique to study the NMDA receptor in learning and memo-

ry. The technique was a refinement of the method for creating so-called knockout mice—mice in which one gene has been selectively inactivated, or “knocked out.” Traditional knockout mice lack a particular gene in every cell and tissue. By studying the health and behavior of such animals, scientists can deduce the function of the gene.

But many types of knockout mice die at or before birth because the genes they lack are required for normal development. The genes encoding the various subunits of the NMDA receptors turned out to be similarly essential: regular NMDA-receptor knockout mice died as pups. So I devised a way to delete a subunit of the NMDA receptor in only a specific region of the brain.

Scoring a Knockout

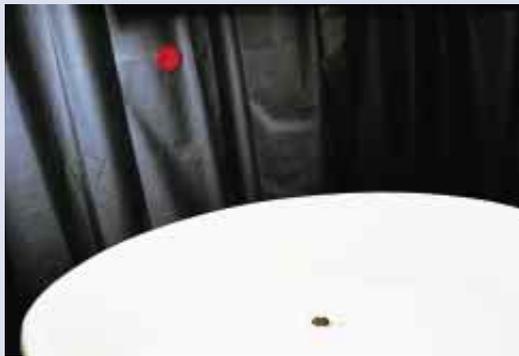
Using the new technique, I engineered mice that lacked a critical part of the NMDA receptor termed the NR1 subunit in a part of their hippocampus known as the CA1 region. It was fortunate that we knocked out the gene in the CA1 region because that is where most LTP and LTD studies have been conducted and because people with brain damage to that area have memory deficits. In collaboration with Matthew A. Wilson, Patricio T. Huerta, Thomas J. McHugh and Kenneth I. Blum of M.I.T., I found that the knockout mice have lost the capacity to change the strength of the neuronal connections in the CA1 regions of their brains. These mice exhibit abnormal spatial representation and have poor spatial memory: they cannot remember their way around a water maze. More recent studies in my own laboratory at Princeton University have revealed that the mice also show impairments in several other, nonspatial memory tasks.

Although these experiments supported the hypothesis that the NMDA receptors are crucial for memory, they were not fully conclusive. The drugs used to block the receptors could have exerted their effects through other molecules in addition to NMDA receptors, for example. And the memory deficits of the knockout mice might have been caused by another, unexpected abnormality independent of the LTP/LTD deficits.

To address these concerns, a couple of years ago I decided to try to increase the function of NMDA receptors in

TESTING DOOGIE

Putting the Smart Mouse through Its Paces



In the initial tests of *Doogie* mice, we found that they were more likely than normal mice to recognize a familiar object over a novel one, such as the red toy in the photograph above. But that test, which is called an object-recognition task, assesses only one type of memory.

To further evaluate whether *Doogie* mice have enhanced learning and memory abilities, we used a more complex laboratory test called the Morris water maze. In this test we put a mouse into a circular pool that was 1.2 meters in diameter and filled with murky water. We placed into the pool a nearly invisible, clear Plexiglas platform that was almost—but not quite—as tall as the water was deep, so that it was just hidden beneath the surface. We surrounded the pool with a black shower curtain that had certain landmarks on it, such as the red dot in the top photograph at the left. Mice do not like to get wet, so in these tests they generally swim around until they find the platform, where they can pull themselves almost out of the water and rest.

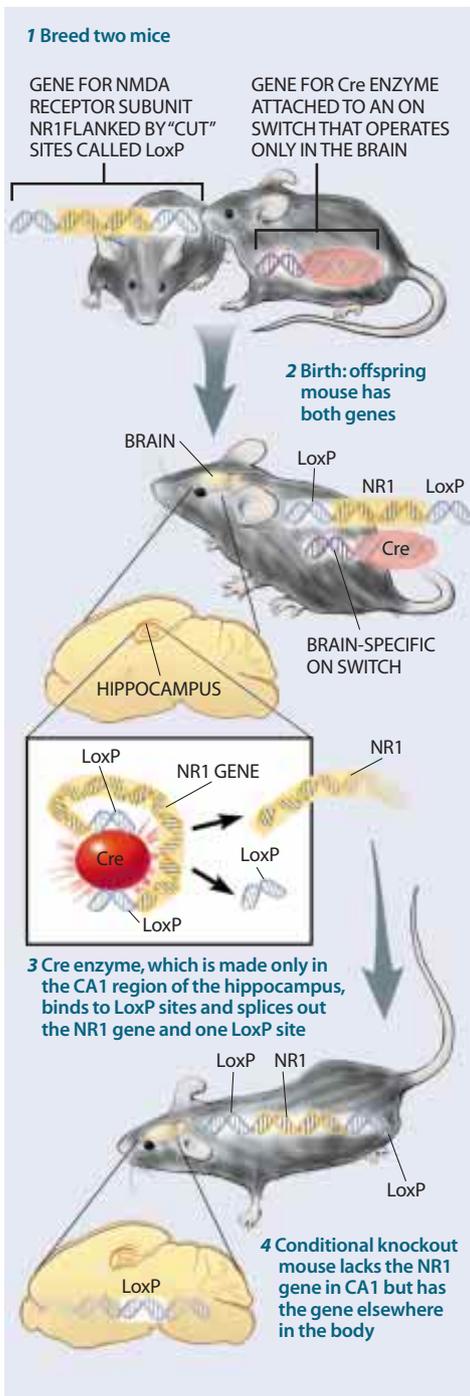
We found that the *Doogie* mice located the submerged platform faster than normal mice, so we took the test a step further: we removed the platform to see if the animals would remember where the platform had been in relation to landmarks such as the red dot. When we put them back into the pool, *Doogie* mice spent more time than normal mice in the quarter of the pool where the platform had been, indicating that they remembered where it should be. What did they get as a reward? A towelling off and a stint under the heat lamp.

—J.Z.T.



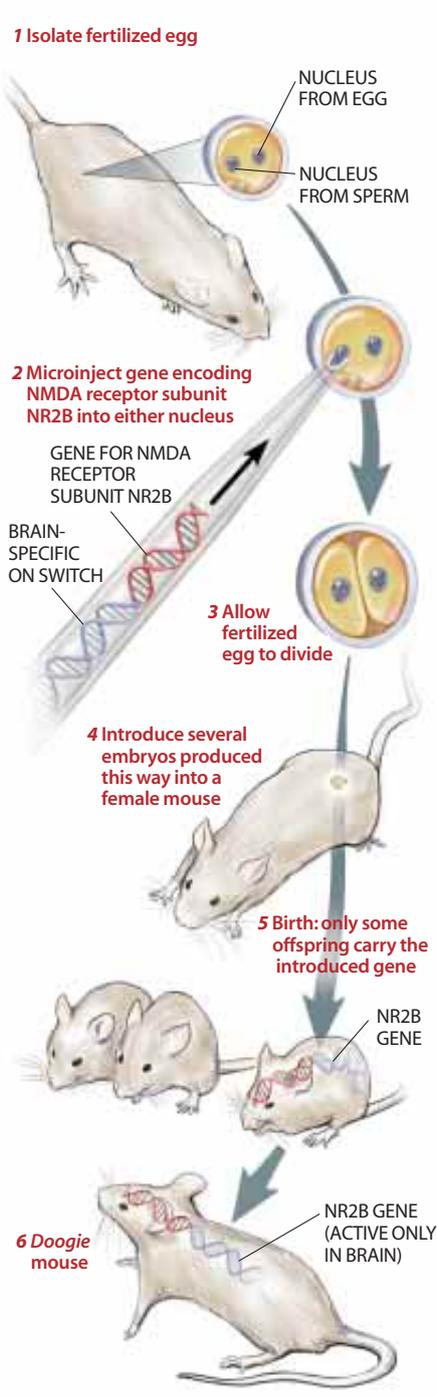
HOW TO MAKE A DUMB MOUSE

Remove part of a key receptor from its brain



HOW TO MAKE A SMART MOUSE

Add an extra copy of part of a key receptor to its brain



diverse as birds, rodents and primates remain open longer in younger individuals than in adults. Some researchers, including my colleagues and me, have speculated that the difference might account for the fact that young animals are usually able to learn more readily—and remember what they have learned longer—than their older counterparts.

As individuals mature, they begin to switch from making NMDA receptors that contain NR2B subunits to those that include NR2A subunits. Laboratory studies have shown that receptors with NR2B subunits stay open longer than those with NR2A. I reasoned that the age-related switch could explain why adults can find it harder to learn new information.

So I took a copy of the gene that directs the production of NR2B and linked it to a special piece of DNA that served as an on switch to specifically increase the gene's ability to make the protein in the adult brain. I injected this gene into fertilized mouse eggs, where it was incorporated into the chromosomes and produced genetically modified mice carrying the extra copy of the NR2B gene.

Working in collaboration with Guosong Liu of M.I.T. and Min Zhuo of Washington University, my colleagues and I found that NMDA receptors from the genetically engineered mice could remain open for roughly 230 milliseconds, almost twice as long as those of normal mice. We also determined that neurons in the hippocampi of the adult mice were capable of making stronger synaptic connections than those of normal mice of the same age. Indeed, their connections resembled those in juvenile mice.

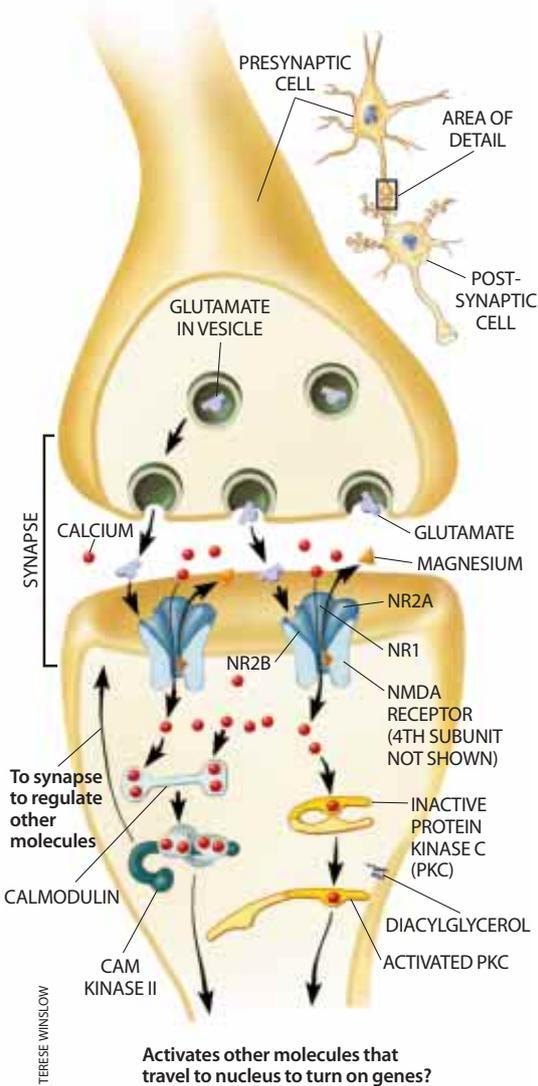
What Smart Mice Can Do

Next, Ya-Ping Tang and other members of my laboratory set about evaluating the learning and memory skills of the mice that we had named *Doogie*. First, we tested one of the most basic aspects of memory, the ability to recognize an object. We placed *Doogie* mice into an open box and allowed them to explore two objects for five minutes. Several days later we replaced one object with a new one and returned the mice to the box. The genetically modified mice remembered the old object and devoted their time to exploring the new one. Normal mice, however, spent an equal amount of time exploring both objects, indicating that the old object was no more familiar to them than the new. By

MAKING DUMB AND SMART MICE involves tampering with a protein called the NMDA receptor that is important for learning and memory. But the NMDA receptor plays crucial roles elsewhere in the body, so the author and his colleagues used snippets of DNA (on switches in the diagram) to manipulate the genes for various subunits of the receptor *only* in the brain. The smart, or *Doogie*, mice have extra subunits in their brains; the dumb, or conditional knockout, mice lack a different NMDA receptor subunit in their brains.

mice to see whether such an alteration improved the animals' learning and memory. If it did, that result—combined with the previous ones—would tell us that the NMDA receptor truly is a central player in memory processes.

This time I focused on different parts of the NMDA receptor, the NR2A and NR2B subunits. Scientists have known that the NMDA receptors of animals as



TWO NEURONS MEET at a junction called a synapse. A leading hypothesis of how memories form involves proteins called NMDA receptors, which sit on the surfaces of postsynaptic cells. NMDA receptors, which are tiny pores through which calcium can pass, can link two events in time—a prerequisite for laying down a memory—because they open only when they receive two signals. The first signal is the binding of glutamate released by the presynaptic cell; the other is electrical stimulation by input from another neuron that expels magnesium from the channel of the receptor. The inrush of calcium activates biochemical cascades that eventually strengthen the synapse.

helped them to learn, we employed a classic behavioral experimental paradigm known as fear-extinction learning.

In the fear-extinction test, we conditioned the mice as we did before in a shock chamber, then placed the animals back into the fear-causing environment—but without the paw shocks—again and again. Most animals take five repetitions or so to unlearn the link between being in the shock chamber and receiving a shock. The *Doogie* mice learned to be unafraid after only two repetitions. They also learned not to fear the tone faster than the normal mice.

The last behavioral test was the Morris water maze, in which the mice were required to use visual cues on a laboratory wall to find the location of a submerged platform hidden in a pool of milky water. This slightly more complicated task involves many cognitive factors, including analytical skills, learning and memory, and the ability to form strategies. Again, the genetically modified mice performed better than their normal counterparts.

Our experiments with *Doogie* mice clearly bore out the predictions of Hebb's rule. They also suggested that the NMDA receptor is a molecular master switch for many forms of learning and memory.

Although our experiments showed the central role of NMDA receptors in a variety of learning and memory processes, it is probably not the only molecule involved. We can expect many molecules

that play a role in learning and memory to be identified in the coming years.

Everyone I have encountered since the publication of our results has wanted to know whether the findings mean we will soon be able to genetically engineer smarter children or devise pills that will make everyone a genius. The short answer is no—and would we even want to?

Intelligence is traditionally defined in dictionaries and by many experimental biologists as “problem-solving ability.” Although learning and memory are integral parts of intelligence, intelligence is a complex trait that also involves many other factors, such as reasoning, analytical skills and the ability to generalize previously learned information. Many animals have to learn, remember, generalize and solve various types of problems, such as negotiating their terrain, foreseeing the relation between cause and effect, escaping from dangers, and avoiding poisonous foods. Humans, too, have many different kinds of intelligence, such as the intelligence that makes someone a good mathematician, an effective CEO or a great basketball player.

Because learning and memory are two of the fundamental components of problem solving, it would not be totally surprising if enhancing learning and memory skills led to improved intelligence. But the various kinds of intelligence mean that the type and degree of enhancement must be highly dependent on the nature of the learning and memory skills involved in a particular task. Animals with an improved ability to recognize objects and solve mazes in the laboratory, for instance, might have an easier time finding food and getting around from place



Genetic engineering will never turn mice into geniuses capable of playing the piano.

to place in the wild. They might also be more likely to escape from predators or even to learn to avoid traps. But genetic engineering will never turn the mice into geniuses capable of playing the piano.

Our finding that a minor genetic manipulation makes such a measurable difference in a whole set of learning and memory tasks points to the possibility that NR2B may be a new drug target for

repeating the test at different intervals, we found that the genetically modified mice remembered objects four to five times longer than their normal counterparts did.

In the second round of tests, Tang and I examined the ability of the mice to learn to associate a mild shock to their paws with being in a particular type of chamber or hearing a certain tone. We found that the *Doogie* mice were more likely to “freeze”—an indication that they remembered fear—than were normal mice when we returned the animals to the chamber or played them the tone several days later. These tests suggested to us that the *Doogie* mice had better memory. But were they also faster learners?

Learning and memory represent different stages of the same gradual and continuous process whose steps are often not easy to distinguish. Without memory, one cannot measure learning; without learning, no memory exists to be assessed. To determine whether the genetic alteration of the *Doogie* mice

THE SEARCH FOR A MEMORY-BOOSTING DRUG

Smarter Mice Are Only the First Step

treating various age-related memory disorders. An immediate application could be to search for chemicals that would improve memory by boosting the activity or amount of NR2B molecules in patients who have healthy bodies but whose brains have begun to be ravaged by dementia during aging. Such drugs might improve memory in mildly and modestly impaired patients with Alzheimer's disease and in people with early forms of other dementias. The rationale would be to boost the memory function of the remaining healthy neurons by modulating and enhancing the cells' NR2B activity. Of course, designing such compounds will take at least a decade and will face many uncertainties. The possible side effects of such drugs in humans, for example, would need to be carefully evaluated, although the increased NR2B activity in the *Doogie* mice did not appear to cause toxicity, seizures or strokes.

But if more NR2B in the brain is good for learning and memory, why has nature arranged for the amount to taper off with age? Several schools of thought weigh in on this question. One posits that the switch from NR2B to NR2A prevents the brain's memory capacity from becoming overloaded. Another, which I favor, suggests that the decrease is evolutionarily adaptive for populations because it reduces the likelihood that older individuals—who presumably have already reproduced—will compete successfully against younger ones for resources such as food.

The idea that natural selection does not foster optimum learning and memory ability in adult organisms certainly has profound implications. It means that genetically modifying mental and cognitive attributes such as learning and memory can open an entirely new way for the targeted genetic evolution of biology, and perhaps civilization, with unprecedented speed. SA

How close are researchers to devising a pill to help you remember where you put your car keys? The short answer is "not very." But that doesn't mean they aren't working on it—and hard. Less than eight months after Joe Z. Tsien of Princeton University (the author of the preceding article) and his colleagues reported genetically engineering a smarter mouse, Tsien has teamed up with venture capitalist Charles Hsu to form a company based on the discovery.

The newly incorporated firm is called Eureka Pharmaceuticals, and its home for the time being is Hsu's office at the Walden Group in San Francisco. The company's first order of business is to use gene technology called genomics to identify molecules that are potential targets for drugs to treat central nervous system disorders such as memory loss and dementia. "We believe the tools that Joe and his colleagues have developed can be translated pretty quickly into a basis for discovering therapies for human disease," Hsu says. Hsu is the CEO of Eureka; Tsien is the company's scientific adviser but will remain at Princeton.

Eureka's first target is the so-called NMDA receptor—which Tsien and his co-workers manipulated genetically to make their smart *Doogie* mice—although the company will also look for other targets. The receptor is essentially a pore that allows calcium to enter nerve cells, a prerequisite for strengthening the connection between two nerve cells. Such strengthening is thought to be the basis for learning and memory.

Over the past decade, several pharmaceutical companies have tested as possible stroke drugs various compounds that decrease the activity of the NMDA receptor. When the brain is starved of blood, such as happens when the blood clot of a stroke blocks an artery, nerve cells can release too much glutamate, a chemical the cells use to communicate. In a phenomenon called excitotoxicity, the excess glutamate binds to NMDA receptors on other nerve cells, allowing a tsunami of calcium to flood into the other cells. Together with the lack of oxygen, this causes the cells to die.

So far, however, the search for NMDA-receptor blockers that could serve as stroke drugs has been "incredibly disappointing," comments neuroscientist Robert C. Malenka of Stanford University. The problem, he explains, is finding a chemical that binds to precisely the right spot on the NMDA receptor and in just the right way, without causing other neurological effects. (After all, the illicit hallucinogenic drug phencyclidine—also known as PCP or "angel dust"—also binds to the receptor.)

The lack of success with NMDA-receptor blockers against stroke—together with the possibility that agents that bind to the receptor might be toxic—has blunted some scientists' enthusiasm for developing drugs that might boost learning and memory by activating the receptor. "Nobody is seriously considering upregulating the activity of the NMDA receptor to boost memory, to my knowledge," Malenka says. "But maybe some clever person will come up with that magic drug that will tweak the receptor just so."

A more likely scenario—and one being pursued by Tsien—might be developing drugs that subtly modulate the activity of the NMDA receptor, without binding to it directly, according to Ira B. Black of the University of Medicine and Dentistry of New Jersey. Black studies a naturally occurring chemical called brain-derived neurotrophic factor (BDNF), which increases the likelihood that parts of the NMDA receptor will have a phosphate group tacked onto them. NMDA receptors with phosphate groups are more likely to be active than those without such groups.

Still, most neuroscientists concur that the search for a drug that enhances learning and memory without side effects will take time.

—Carol Ezzell, staff writer

The Author



JOE Z. TSIEN has been an assistant professor in the department of molecular biology at Princeton University since 1997. He came to the U.S. in 1986 after graduating from East China Normal University in Shanghai and working for two years as an instructor at East China University of Science and Technology in Shanghai. He received his Ph.D. in biochemistry and molecular biology in 1990 from the University of Minnesota. He has consulted for several biotechnology companies seeking to develop therapies for age-related memory disorders. The *Doogie* mouse was a hit in his seven-year-old son's class during show-and-tell.

Further Information

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