

TO SIMULATE MEMORY FORMATION, an electrophysiological recording apparatus can both stimulate and record electrical signals from a 400-micron-thick slice of rat hippocampus.

MAKING MEMORIES STICK

Some moments become lasting
recollections while others just evaporate.

The reason may involve the same processes that shape our brains to
begin with **BY R. DOUGLAS FIELDS**

In the movie thriller *Memento*, the principal character, Leonard, can remember everything that happened before his head injury on the night his wife was attacked, but anyone he meets or anything he has done since that fateful night simply vanishes. He has lost the ability to convert short-term memory into long-term memory. Leonard is driven to find his wife's killer and avenge her death, but trapped permanently in the present, he must resort to tattooing the clues of his investigation all over his body.

That disturbing story was inspired by the real case history of a patient known in the medical literature only as "HM." When HM was nine years old, a head injury in a bicycle accident left him with debilitating epilepsy. To relieve his seizures that could not be controlled in any other way, surgeons removed parts of HM's hippocampus and adjoining brain regions. The operation succeeded in reducing the brain seizures but inadvertently severed the mysterious link between short-term and long-term memory. Information destined for

what is known as declarative memory—people, places, events—must pass through the hippocampus before being recorded in the cerebral cortex. Thus, memories from long ago that were already stored in HM's brain remained clear, but all his experiences of the present soon faded into nothing. HM saw his doctor on a monthly basis, but at each visit it was as if the two had never met.

This transition from the present mental experience to an enduring memory has long fascinated neuroscientists. A person's name when you are first introduced is stored in short-term memory and may be gone within a few minutes. But some information, like your best friend's name, is converted into long-term memory and can persist a lifetime. The mechanism by which the brain preserves certain moments and allows others to fade has recently become clearer, but first neuroscientists had to resolve a central paradox.

Both long- and short-term memories arise from the connections between neurons, at points of contact called synapses, where one neuron's signal-emitting extension, called

an axon, meets any of an adjacent neuron's dozens of signal-receiving fingers, called dendrites [see box on opposite page]. When a short-term memory is created, stimulation of the synapse is enough to temporarily "strengthen," or sensitize, it to subsequent signals. For a long-term memory, the synapse strengthening becomes permanent. Scientists have been aware since the 1960s, however, that this requires genes in the neuron's nucleus to activate, initiating the production of proteins.

Memory researchers have puzzled over how gene activity deep in the cell nucleus could govern activities at faraway synapses. How does a gene "know" when to strengthen a synapse permanently and when to let a fleeting moment fade unrecorded? And how do the proteins encoded by the gene "know" which of thousands of synapses to strengthen? The same questions have implications for understanding fetal brain development, a time when the brain is deciding which synaptic connections to keep and which to discard. In studying that phenomenon, my lab came up with an intriguing solution to one of these mysteries of memory. And just like Dorothy, we realized that the answer was there all the time.

Genetic Memory

EARLY MOLECULAR BIOLOGISTS discovered that genes play a role in the conversion of a memory from short- to long-term. Their experiments with animals trained to perform simple tasks demonstrated that learning required new proteins to be synthesized in the brain within minutes of training, or else the memory would be lost [see "Memory and Protein Synthesis," by Bernard W. Agranoff; SCIENTIFIC AMERICAN, June 1967].

For a protein to be produced, a stretch of DNA inside the cell nucleus must be transcribed into a portable form called

messenger RNA (mRNA), which then travels out to the cell's cytoplasm, where cellular machinery translates its encoded instructions into a protein. These researchers had found that blocking the transcription of DNA into mRNA or the translation of mRNA into a protein would impede long-term memory formation but that short-term memory was unaffected.

Because one neuron can form tens of thousands of synaptic connections and there could not possibly be a gene dedicated to each one, cellular neuroscientists sought to explain how the cell nucleus was controlling the strength of these individual connections. They theorized that an unknown signaling molecule must be generated by a synapse when it was sufficiently stimulated.

With its connection temporarily strengthened, this synapse could hold the memory for a short time while the signaling molecule departed, wending its way to the nucleus of the nerve cell. There this

messenger molecule would activate appropriate genes needed to synthesize proteins that would permanently strengthen the synaptic connection. Yet a second problem was how this protein, once it was manufactured in the cell body of the neuron, could then find the one synapse among thousands that had called for it.

By the mid-1990s, memory researchers had a more detailed picture of events [see box on page 78]. Several of them had shown that a transcription factor named CREB played a key role in converting short-term memory into long-term memory in animals as distantly related as flies and mice. Transcription factors are master proteins inside the cell nucleus that find and bind to specific sequences of DNA. They are thus the ultimate on/off switches that control a gene's transcription. So CREB activation within a neuron leads to gene activation, leading to manufacture of the mysterious synapse-strengthening proteins that transform a short-term memory into a long-term one.

In 1997 elegant experiments by Uwe Frey of the German Federal Institute for Neurobiology, Gene Regulation and Plasticity and Richard G. M. Morris of the University of Edinburgh further showed that whatever these "memory proteins" were, they did not need to be addressed to a particular synapse. They could be broadcast throughout the cell but would only affect the synapse that was already temporarily strengthened and make that connection permanently stronger.

These revelations still left at least one more burning question: What is the synapse-to-nucleus signaling molecule that determines when CREB should be activated and a memory preserved? Around this time, my colleagues and I found ourselves approaching the same problems as the memory researchers from a different perspective. In my laboratory at the National Institute of Child Health and Human Development, we study how the brain becomes wired up during fetal devel-

HOW DOES A GENE "KNOW" WHEN TO STRENGTHEN A SYNAPSE PERMANENTLY?

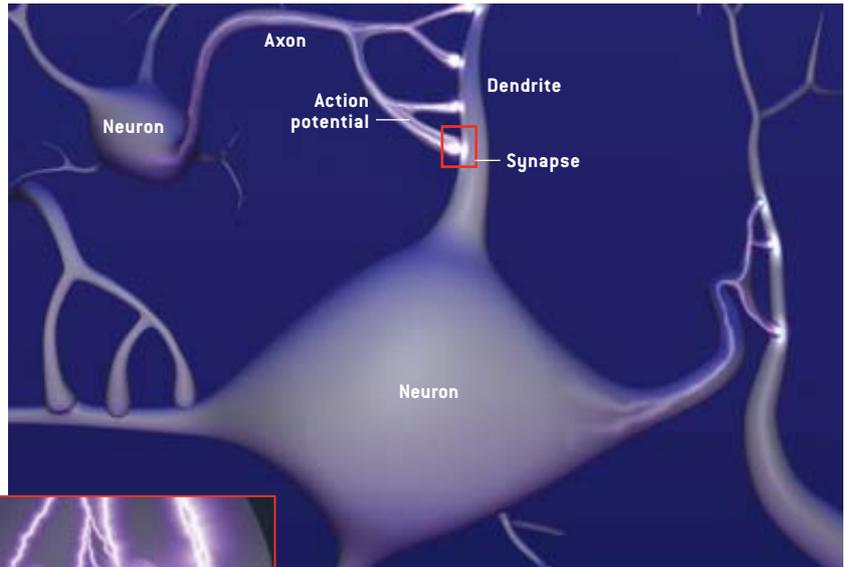
Overview/*Hardwiring Memory*

- Individual nerve cells somehow know which memories to preserve in the form of lasting connections to other nerve cells and which to let fade. Similarly, the developing brain somehow chooses certain neural circuits to preserve and others to discard.
- Both processes require electrochemical signals from the far edges of a nerve cell to activate genes in the cell nucleus and the genes to direct an answer back to the cell's extremity.
- Like many of life's decisions, the neuron's choice to cement a connection comes only after its importance has been demonstrated.

MAKING MEMORIES

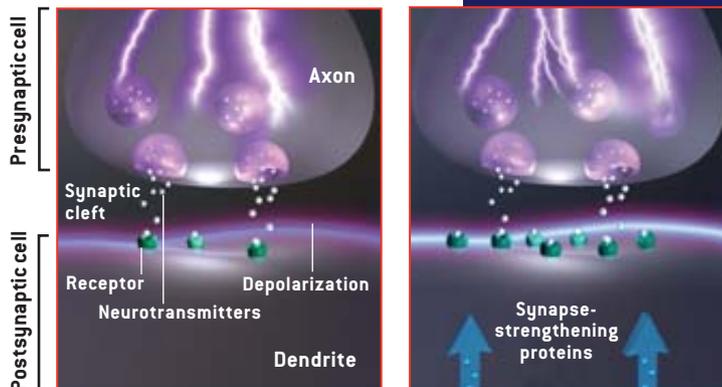
Memories are created when nerve cells in a circuit increase the strength of their connections, known as synapses. In the case of short-term memories, the effect lasts only minutes to hours. For long-term memories, the synapses become permanently strengthened.

Signaling itself contributes to memory formation. Messages begin to travel between one neuron (the presynaptic cell) and another when an electrical pulse known as an action potential (*below*) travels down an extension of the first neuron called an axon to its tip.



SIGNALING AT THE SYNAPSE

At the axon terminal (*inset below*), the pulse causes synaptic vesicles in the presynaptic neuron to release chemicals called neurotransmitters into a gap, or synaptic cleft, between the axon and a dendrite on the second, postsynaptic neuron. The neurotransmitters bind to receptors on the dendrite, triggering a local depolarization of the postsynaptic cell's membrane that is described as a "firing" of the synapse.



SYNAPSE STRENGTHENING

After a synapse fires briefly at high frequency, it becomes more sensitive, experiencing a greater voltage swing in response to subsequent signals. This temporary strengthening of the synapse is the basis of short-term memory. Although the process is poorly understood, investigators know that permanent strengthening to form long-term memories requires the postsynaptic cell to manufacture synapse-strengthening proteins (*left*). These proteins might add more receptors and otherwise remodel the postsynaptic part of the synapse as well as possibly influence the presynaptic cell's responses.

opment. So while memory researchers were wondering how mental experience could affect genes, which could in turn affect certain synaptic connections, we were wondering how genes could specify all the millions of connections in the developing brain in the first place.

We and other developmental neuroscientists already suspected that mental experience might have some role in honing the brain's wiring plan. The fetal brain could start out with a rough neural circuitry that was specified by genetic instructions. Then, as the young brain developed and tested those connections, it would preserve the most effective ones and eliminate the poor ones. But how, we wondered, does the brain identify which connections are worth keeping?

Building a Brain

AS FAR BACK AS 1949, a psychologist named Donald Hebb proposed a simple rule that could govern how experience might bolster certain neural circuits. Inspired by the famous Pavlovian dog experiments, Hebb theorized that connections among neurons that fired at the same time should

become strengthened. For example, a neuron that fired when a bell sounded and a nearby neuron that fired when food was presented simultaneously should become more strongly connected to each other, forming a cellular circuit that learns that the two events are connected.

Not every input to a nerve cell is strong enough to make that cell fire a signal of its own. A neuron is like a microprocessor chip in that it receives thousands of signals through its dendrites and constantly integrates all the input it receives from these connections. But unlike a microprocessor that has many output wires, a neuron has only one, its axon. Thus, a neuron can respond to inputs in only one way: it can either decide to send a signal on to the next neuron in the circuit by firing an impulse through its axon, or not.

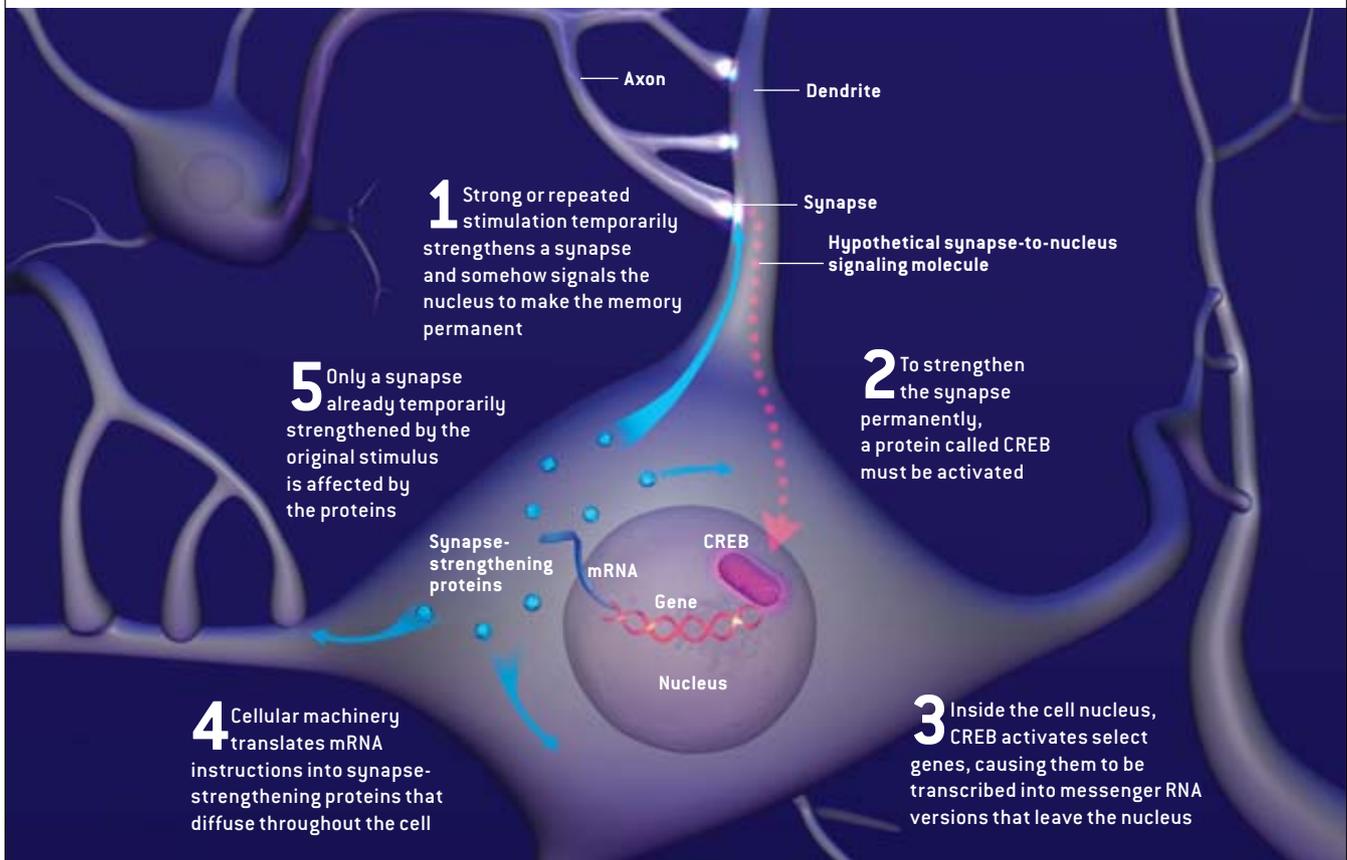
When a neuron receives such a signal, the voltage of the membrane on its dendrite changes slightly in the positive direction. This local change in voltage is described as a "firing" of the neuron's synapse. When a synapse fires in brief, high-frequency bursts, the temporary strengthening observed in short-term memory formation occurs. But a single

HOW GENES MAKE MEMORIES STICK

That gene activation leading to protein manufacture is necessary for long-term memory formation was discovered in the 1960s. But this realization raised further questions: How does a gene in the nucleus “know” when to generate the proteins that permanently strengthen a synapse, thereby turning a short-term memory into a long-term one, and when to remain silent, allowing the short-term memory to simply

fade away? Is there an undiscovered synapse-to-nucleus signaling molecule that tells the cell when to make synapse-strengthening proteins?

And once these proteins are manufactured in the cell body, how do they “know” which synapse among thousands to strengthen? By the mid-1990s, elegant experiments had provided some answers to the puzzle.



synapse firing briefly is generally not enough to make the neuron fire an impulse, technically termed an action potential, of its own. When many of the neurons' synapses fire together, however, their combined effort changes the voltage of the neuronal membrane enough to make the neuron fire action potentials and relay the message on to the next neuron in the circuit.

Hebb proposed that, like an orchestra player who cannot keep up, a synapse on a neuron that fires out of sync with the other inputs to the neuron will stand out as odd and should be eliminated, but synapses that fire together—enough so as to make the neuron fire an action potential—should be strengthened. The brain would thus wire itself up in accordance with the flow of impulses through developing neural circuits, refining the original general outline.

Moving from Hebb's theory to sorting out the actual mechanics of this process, however, one again confronts the fact that the enzymes and proteins that strengthen or weaken syn-

aptic connections during brain wiring must be synthesized from specific genes. So our group set out to find the signals that activate those genes.

Because information in the nervous system is coded in the pattern of neural impulse activity in the brain, I began with an assumption that certain genes in nerve cells must be turned on and off by the pattern of impulse firing. To test this hypothesis, a postdoctoral fellow in my lab, Kouichi Itoh, and I took neurons from fetal mice and grew them in cell culture, where we could stimulate them using electrodes in the culture dish. By stimulating neurons to fire action potentials in different patterns and then measuring the amount of mRNA from genes known to be important in forming neural circuits or in adapting to the environment, we found our prediction to be true. We could turn on or off particular genes simply by dialing up the correct stimulus frequency on our electrophysiological stimulator, just as one tunes into a particular radio station by selecting the correct signal frequency.

Time Code

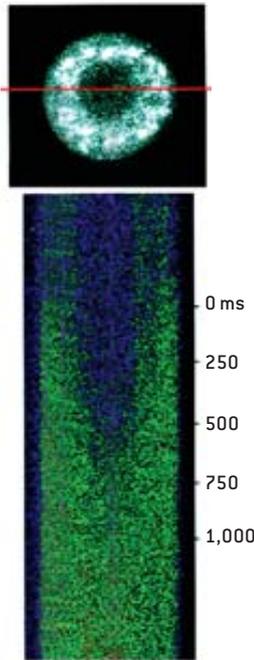
ONCE WE OBSERVED that neuronal genes could be regulated according to the pattern of impulses the cell was emitting, we wanted to investigate a deeper question: How could the pattern of electrical depolarizations at the surface of the cell membrane control genes deep in the nucleus of the neuron? To do so, we needed to peer into the cell cytoplasm and see how information was translated on its way from the surface to the nucleus.

What we found was not a single pathway leading from the neuron's membrane to its nucleus but rather a highly interconnected network of chemical reactions. Like the maze of roads leading to Rome, there were multiple intersecting biochemical pathways crisscrossing as they carried signals from the cell membrane throughout the cell. Somehow electrical signals of varying frequencies on the membrane flowed through this traffic in the cytoplasm to reach their proper destination in the nucleus. We wanted to understand how.

The primary way that information about the neuronal membrane's electrical state enters this system of chemical reactions in the cytoplasm is by regulating the influx of calcium ions through voltage-sensitive channels in the cell membrane. Neurons live in a virtual sea of calcium ions, but inside a neuron the concentration of calcium is kept extremely low—20,000 times lower than the concentration outside. When the voltage across the neuronal membrane reaches a critical level, the cell fires an action potential, causing the calcium channels to open briefly. Admitting a spurt of calcium ions into the neuron with the firing of each neural impulse translates the electrical code into a chemical code that cellular biochemistry inside the neuron can understand.

In domino fashion, as calcium ions enter the cytoplasm, they activate enzymes called protein kinases. Protein kinases turn on other enzymes by a chemical reaction called phosphorylation that adds phosphate tags to proteins. Like runners passing the baton, the phosphate-tagged enzymes become activated from a dormant state and stimulate the activity of transcription factors. CREB, for instance, is activated by calcium-dependent enzymes that phosphorylate it and inactivated by enzymes that remove the phosphate tag. But there are hundreds of different transcription factors and protein kinases in a cell. We wanted to know how a particular frequency of action potential firing could work through calcium fluxes to reach the appropriate protein kinases and ultimately the correct transcription factors to control the right gene.

By filling the neurons with dye that fluoresces green when the calcium concentration in the cytoplasm increases, we were able to track how different action-potential firing patterns translated into dynamic fluctuations in intracellular calcium. One simple possibility was that gene transcription might be regulated by the amount of calcium rise in a neuron, with dif-



CALCIUM IONS glow in a cross section of a neuron (left, with dark nucleus at center) filled with calcium-sensitive dye. The author used scanning laser confocal microscopy to track pulses of calcium influx following each action potential fired by the cell. Cross sections of the first view taken at two-millisecond intervals, then stacked (below left), provided a time-lapse representation of intracellular calcium density (green and red).

ferent genes responding better to different levels of calcium. Yet we observed a more interesting result: the amount of calcium increase in the neuron was much less important in regulating specific genes than the temporal patterns of calcium flashes, echoing the temporal code of the neural impulse that had generated them.

Another postdoc in my lab, Feleke Eshete, followed these calcium signals to the enzymes they activate and the transcription factors those enzymes regulate, and finally we began to appreciate how different patterns of neural impulses could be transmitted through different intracellular signaling pathways. The important factor was time.

We found that one could not represent the pathway from the cell's membrane to its DNA in a simple sequence of chemical reactions. At each step, starting from calcium entering the membrane, the reactions branched off into a highly interconnected network of signaling pathways, each of which had its own speed limits governing how well it could respond to intermittent signals. This property determined which signaling pathway a particular frequency of action potentials would follow to the nucleus.

Some signaling pathways responded quickly and recovered rapidly; thus, they could react to high-frequency patterns of action potentials but could not sustain activation in response to bursts of action potentials separated by long intervals of inactivity. Other pathways were sluggish and could not respond well to rapid bursts of impulses, but once activated, their slowness to inactivate meant that they could sustain signals between bursts of action potentials that were separated by long intervals of inactivity. The genes activated by this pathway would therefore respond to stimuli that are delivered repeatedly, but infrequently, like the repetition necessary for committing new information to memory.

In other words, we observed that signals of different temporal patterns propagated through distinct pathways that were favorably tuned to those particular patterns and ultimately regulated different transcription factors and different

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genes. For instance, our measurements showed that CREB was rapidly activated by action potentials but sluggish in inactivating after we stopped stimulating the neuron. Thus, CREB would sustain its activation between repeated bursts of stimuli separated by intervals of 30 minutes or more, similar to the intervals of time between practice sessions required to learn new skills or facts.

Given CREB's role in memory, we could not help but wonder if the signaling pathway we were studying to understand brain development might not also be relevant to the mechanism of memory. So we devised a test.

Memory in a Dish

IF THE PART OF THE BRAIN that was removed from the patient HM, the hippocampus, is dissected from a rat and kept alive in a salt solution, microelectrodes and electronic amplifiers can record the electrical impulses from individual synaptic connections on a neuron. By administering a burst of electrical shocks to a synapse, causing it to fire in a specific pattern, that synaptic connection can be strengthened. That

is to say, the synapse produces about twice as much voltage in response to subsequent stimulations after it has received the high-frequency stimulus.

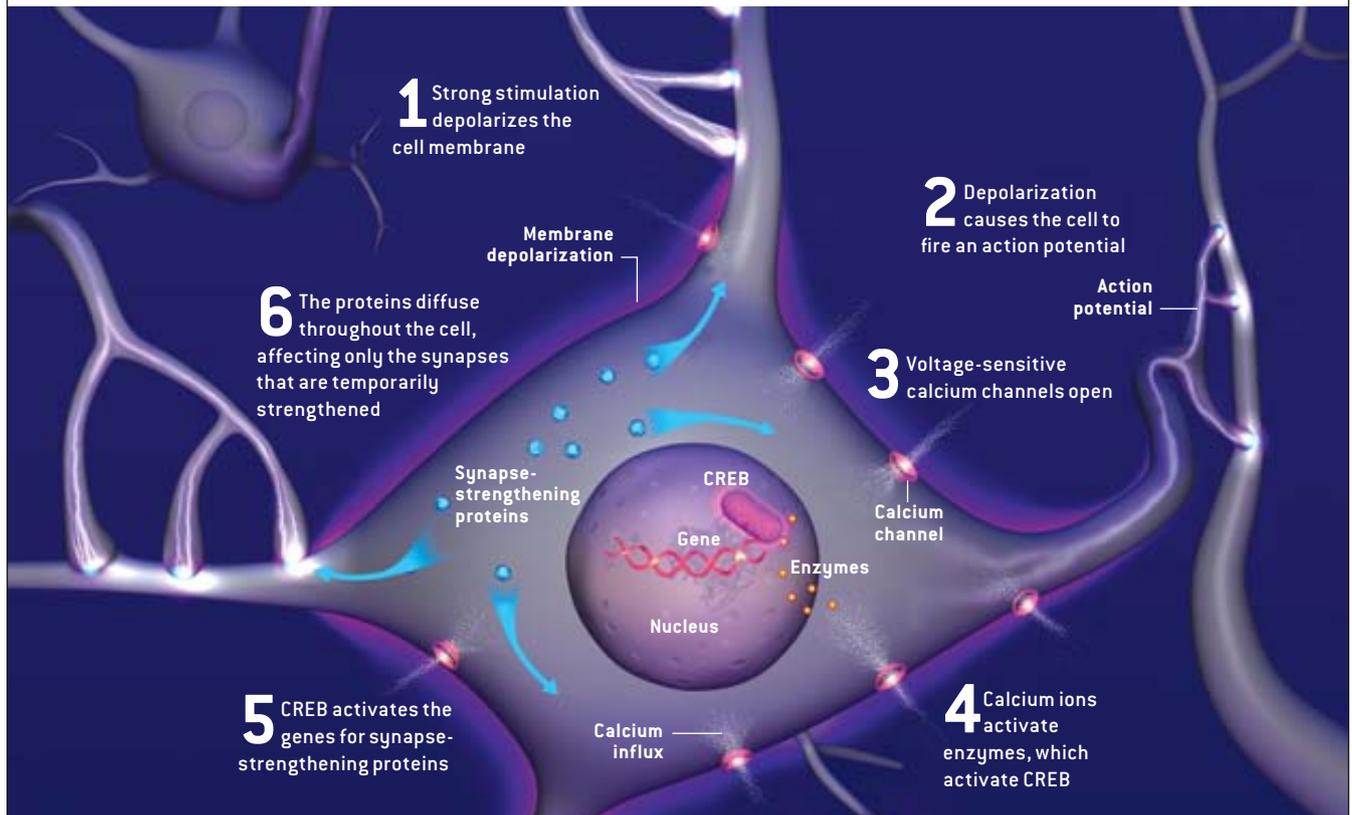
This increased strength, termed long-term potentiation (LTP), can be, despite its name, relatively short-lived. When test pulses are applied at a series of intervals after the high-frequency stimulus, the voltage produced by the synapse slowly diminishes back to its original strength within a few hours. Known as early LTP, this temporary synaptic strengthening is a cellular model of short-term memory.

Remarkably, if the same high-frequency stimulus is applied repeatedly (three times in our experiments), the synapse becomes strengthened permanently, a state called late LTP. But the stimuli cannot be repeated one after the other. Instead each stimulus burst must be spaced by sufficient intervals of inactivity (10 minutes in our experiments). And adding chemicals that block mRNA or protein synthesis to the salt solution bathing the brain slice will cause the synapse to weaken to its original strength within two to three hours. Just as in whole organisms, the cellular model of short-term memory is not

HOW GENES "KNOW" WHEN TO STRENGTHEN A SYNAPSE

Experiments by the author show that a theoretical synapse-to-nucleus signaling molecule is unnecessary. Strong stimulation, either from the repeated firing of a single synapse or from the simultaneous firing of several synapses on a cell, depolarizes the cell membrane, causing the cell to fire action potentials of its own, which in turn causes voltage-sensitive

calcium channels to open. The calcium ions interact with enzymes that activate the transcription factor CREB, which activates the genes for manufacturing synapse-strengthening proteins. The cell's nucleus "listens," in effect, to the cell's output—firing action potentials—to determine when to permanently strengthen a synapse and make a memory last.



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dependent on the nucleus, but the long-term form of memory is.

Indeed, Frey and Morris had used this technique to show that synapse-strengthening proteins would affect any temporarily strengthened synapse. First, they stimulated a synapse briefly to induce early LTP, which would normally last just hours. Then they fired a second synapse on the same neuron in a manner that would induce late LTP in that synapse: three bursts separated by 10 minutes. As a result, both synapses were permanently strengthened. The stronger stimulus sent a signal to the nucleus calling for memory-protein manufacture, and the proteins “found” any synapse that was already primed to use them.

Based on our work showing how different patterns of impulses could activate specific genes, and recalling Hebb’s theory that the *firing* of a neuron was critical in determining which of its connections will be strengthened, we asked whether a signaling molecule sent from the synapse to the nucleus was really necessary to trigger long-term memory formation. Instead we proposed that when a synapse fired strongly enough or in synchrony with other synapses so as to make the neuron fire action potentials out its axon, calcium should enter the neuron directly through voltage-sensitive channels in the cell body and activate the pathways we had already studied leading to CREB activation in the nucleus.

To test our theory, postdoc Serena Dudek and I administered a drug known to block synaptic function to the brain slice. We then caused neurons to fire action potentials by using an electrode to stimulate the neurons’ cell bodies and axons directly. Thus, the neurons fired action potentials, but the synaptic inputs to these neurons could not fire. If a synapse-to-nucleus signaling molecule was necessary to trigger late LTP, our cellular model of long-term memory formation, then this procedure should not work, because the synapses were silenced by the drugs. On the other hand, if the signals to the nucleus originated from the neurons firing action potentials, as in our developmental studies, silencing the synapses should not prevent activation of the memory-protein genes in the nucleus.

We next processed the brain tissue to determine if the transcription factor CREB had been activated. Indeed, in the small region of brain slice that had been stimulated to fire action potentials in the complete absence of synaptic activity, all the CREB had a phosphate molecule added to it, indicating that it had been switched to the activated state.

We then checked for activity of the gene *zif268*, which is known to be associated with creation of LTP and memory. We found that it, too, was turned on by the hippocampal neuron firing, without any synaptic stimulation. But if we performed the same stimulation in the presence of another drug that blocks the voltage-sensitive calcium channels—

which we suspected were the actual source of the signal from the membrane to the nucleus—we found that CREB phosphorylation, *zif268* and a protein associated with late LTP called MAPK were not activated after the neurons fired.

These results clearly showed that there was no need for a messenger from the synapse to the nucleus. Just as in our developmental studies, membrane depolarization by action potentials opened calcium channels in the neuronal membrane, activating signaling pathways to the nucleus and turning on appropriate genes. It seems to make good sense that memory should work this way. Rather than each synapse on the neuron having to send private messages to the nucleus, the transcriptional machinery in the nucleus listens instead to the output of the neuron to decide whether or not to synthesize the memory-fixing proteins.

FINALLY, WE BEGAN TO APPRECIATE THAT THE IMPORTANT FACTOR WAS TIME

Molecular Memento

PERHAPS UNDISCOVERED synapse-to-nucleus signaling molecules do participate in some way in the memory process, but our experiments indicate that they are not absolutely necessary. As predicted by Hebbian rules of learning, the firing of a neuron, resulting from the combined excitation of all synaptic input to the cell, is the necessary event for consolidating memory.

This understanding offers a very appealing cellular analogue of our everyday experience with memory. Like Leonard in *Memento* or any witness to a crime scene, one does not always know beforehand what events should be committed permanently to memory. The moment-to-moment memories necessary for operating in the present are handled well by transient adjustments in the strength of individual synapses. But when an event is important enough or is repeated enough, synapses fire to make the neuron in turn fire neural impulses repeatedly and strongly, declaring “this is an event that should be recorded.” The relevant genes turn on, and the synapses that are holding the short-term memory when the synapse-strengthening proteins find them, become, in effect, tattooed. SA

MORE TO EXPLORE

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