

The Synapse Revealed

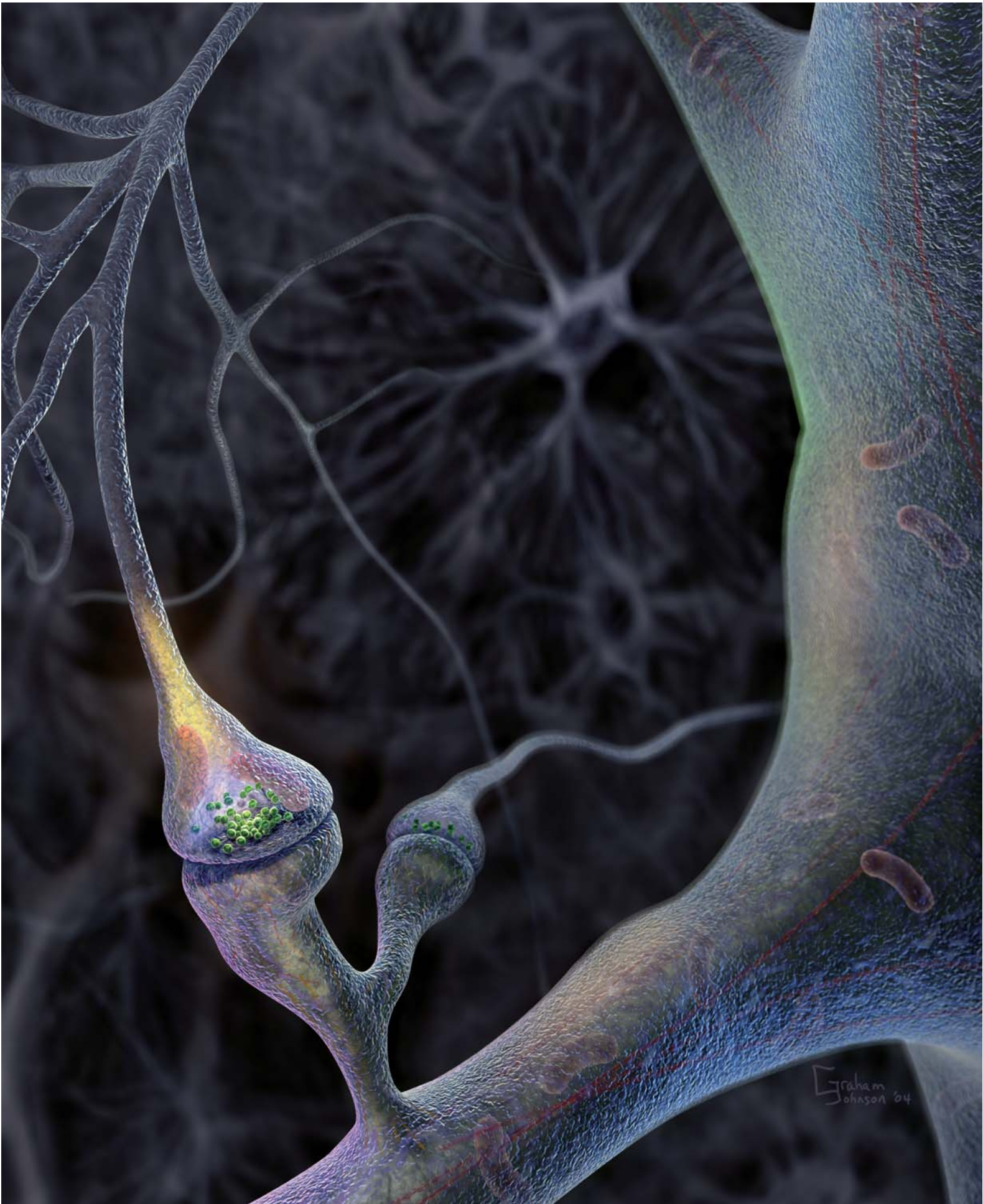
The molecular details of this neural structure give clues on perception, behavior, memory, and thinking.

● By STEVE OLSON

AS YOU READ THESE WORDS, your brain is changing. Through a maze of pathways far more complex than any computer network, your brain is distributing copies of what you are seeing to more than a dozen separate processing centers, where words are being recognized, analyzed, and understood. Simultaneously, the neurons in your brain are undergoing subtle changes where they connect with each other, so that if you read these words again—whether a minute or a year from now—you're likely to remember at least something of what they said.

Virtually any cognitive task requires that the brain function on many different levels, from the individual molecules in cell membranes to far-flung neural circuits that encompass many cerebral regions. But at the center of the action is the “synapse”—the nar-

Central to the brain's web of neural circuitry, the synapse is the pivotal site from which all thought and behavior is generated.



row cleft between neurons where one brain cell sends signals to another. This neural structure is essential to perception, behavior, memory, and thinking. “Without synapses, the brain would be just a collection of isolated neurons,” says New York University neuroscientist Joseph E. LeDoux, author of *Synaptic Self: How Our Brains Become Who We Are*. “Synapses are the gateway for information flowing into the brain, the means of communication within the brain, and the means by which behavior emerges from the brain.”

The word “synapse” is more than a century old, having been coined in 1897 by the English physiologist Sir Charles S. Sherrington. (Actually, Sherrington wanted to call the junction between nerve cells a “syndesmo,” but a classicist acquaintance persuaded him to combine the Greek *syn*, meaning “together,” with *haptein*, for “to clasp.”)

Through his study of reflexes, Sherrington helped demonstrate that the flow of information through synapses is a one-way affair: Nerve impulses pass from the axon of one neuron through a synapse to the dendrite of another neuron. But not until the 1950s did neuroscientists prove that synaptic transmission occurs through chemicals called neurotransmitters that are released by axons and detected by dendrites. As recently as the early 1980s, scientists knew very little about the detailed molecular mechanisms at the heart of synaptic processes.

Today, the synapse is rapidly yielding up its secrets. Researchers are identifying the chemical signposts that guide growing neurons to establish connections with each other. They are figuring out the molecular mechanisms by which brain cells change in response to experience. They are beginning to understand what goes wrong in diseases like Alzheimer’s and Parkinson’s and how the neural ravages of age might be countered. “Our rate of progress is exponentially fast,” says Morgan Sheng, an HHMI investigator at the Massachusetts Institute of Technology (MIT). “Ten years ago we only knew what some of the key components of synapses are. Now we know what *most* of them are.”

Rapid progress in turn has created unprecedented optimism. Researchers are confident that if they can figure out how synapses work, they can begin to piece together the molecular mechanisms of the mind. “I don’t pretend for one minute that a comprehensive understanding of how a synapse works will tell you about higher cognitive functions,” says Sheng. “On the other hand, the great strength of molecular studies is that they provide you with tools that you can use to go into the brain and ask systems-level questions.”

Many of these “systems-level questions” involve memory and the ability to learn new skills and information. But the ultimate prize is much greater. By understanding how synapses function and change over time, neuroscientists hope to shed light on the processes of reasoning, emotion, and maybe even consciousness. “A lot of us in the field think we’ve come to a real turning point,” says HHMI investigator Richard L. Huganir at the Johns Hopkins University School of Medicine, who studies the function of particular proteins in synapses. “We’re trying to go from

the molecular details all the way up to the global effects of behavior. That’s an ambitious goal, but I think we’re getting there.”

● OUT OF SYNC, LOSE THE LINK

Three floors above Sheng’s lab, postdoc Robert A. Crozier—like Sheng, a researcher at the Picower Center for Learning and Memory at MIT—is about to begin his first experiment of the day. Watching a television monitor mounted beside his elaborately rigged microscope, he searches for just the right cell in a slice of rat visual cortex, cut from a brain earlier that morning.

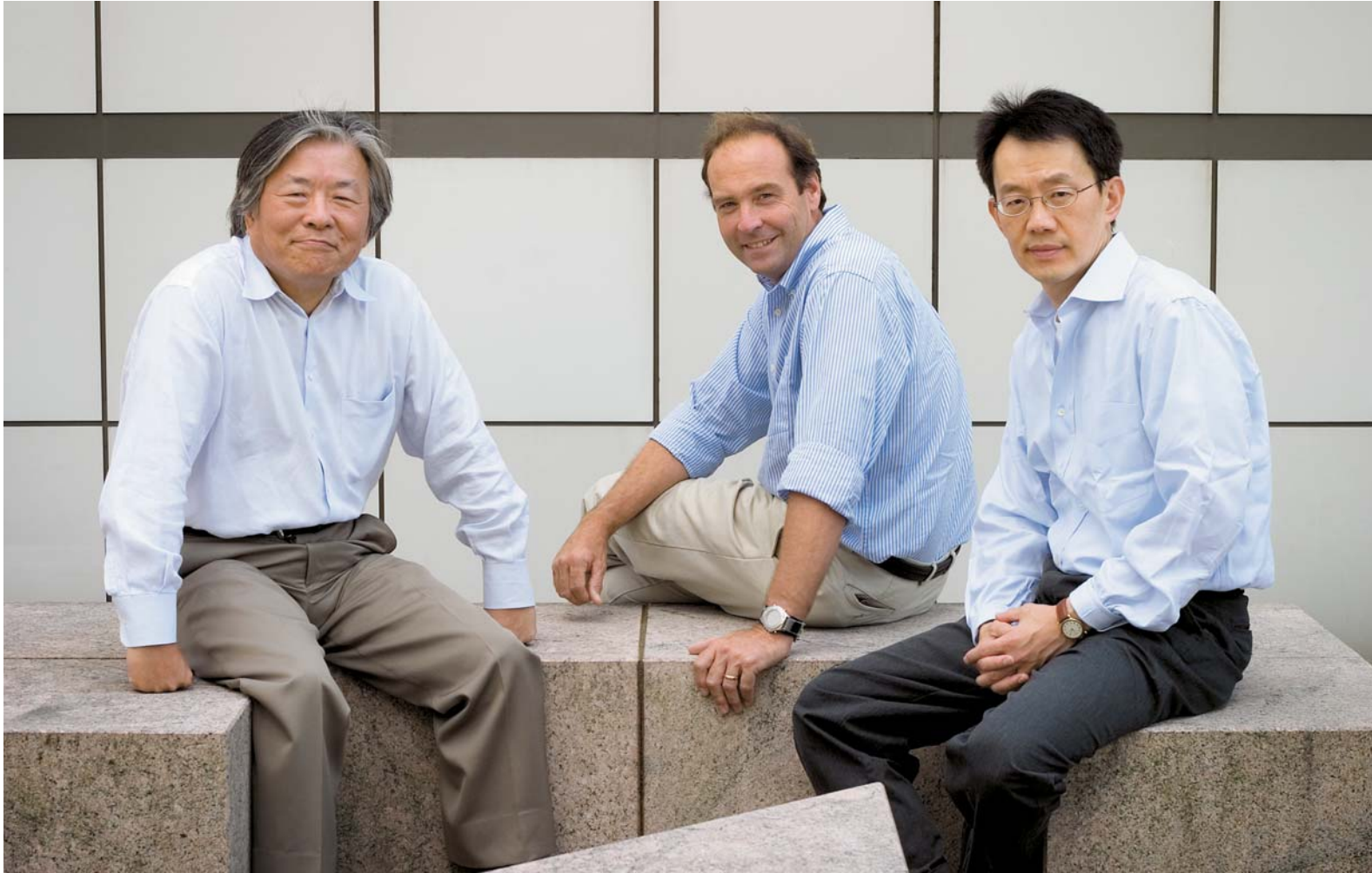
“You want to avoid the cells that have a sort of crispy look,” he says. “Those are cells that are dying. A healthy cell has a soft, fluffy appearance.” Choosing a viable candidate, he pops the pressure tube of a micropipette into his mouth and, spinning the fine-movement manipulators on either side of the microscope, positions the tip of the pipette against the wall of the cell. “This is the part you don’t see in the scientific papers,” Crozier comments. With a quick puff on the mouthpiece, the micropipette punctures the cell membrane, infusing the cell with a chemical that blocks the action of an enzyme known as protein kinase A (PKA). “That looks good,” he says. “At the beginning of the day, this is fun. If I’ve been doing it for a few hours and haven’t gotten a good recording, my language gets more colorful.”

Electrophysiologists like Crozier have been probing the behavior of neurons for more than a century, since German physiologist Emil du Bois-Reymond began investigating what he called “animal electricity.” But today’s scientists are focused on targets that would have seemed impossibly precise to their forebears. PKA plays a pivotal role in the massively complex set of chemical reactions that occurs when a neurotransmitter crosses a synapse and is detected by a receiving dendrite. It opens additional receptors on the dendrite, helps alter the dendrite’s physical structure, and participates in the cascade of reactions that eventually leads to the synthesis of new proteins in the neuron.

Crozier’s work is part of a decades-long effort to understand what is known as “synaptic plasticity”—the changes

Investigators from MIT’s Picower Center for Memory and Learning. Below, postdoc Robert Crozier. Opposite, HHMI investigators Susumu Tonegawa (l), Mark Bear (c), and Morgan Sheng (r).





In the 1980s, ignorance about synapses was so vast that it was hard to formulate meaningful questions. —MARK BEAR

that occur in synapses because of events we experience or thoughts we produce. Neuroscientists have long hypothesized that memories must be stored in the form of physical or functional changes in synapses. As LeDoux succinctly puts it, “We are our synapses.” But hard proof that such changes occur was surprisingly elusive. Not until the 1970s did researchers identify the first form of such plasticity, which they called long-term potentiation (LTP). When a rapid train of strong nerve impulses is sent down an axon, the synapses connecting that axon to the receiving dendrites of other nerve cells can be strengthened (or “potentiated”). For at least the next several hours, a nerve impulse passing through those synapses will generate a stronger response in the receiving neurons than was the case before the LTP occurred.

Crozier works with Mark F. Bear, an HHMI investigator at MIT who specializes in a complementary form of plasticity known as long-term depression (LTD). When a less rapid, weaker train of nerve impulses is sent down a neuron, the connection between that cell and receiving neurons can be weakened. “LTD was ignored for a long time,” says Bear. “But we were believ-

ers, and that conviction led us to persist in looking for a model for LTD.”

Bear’s interest in LTD dates back to his graduate school days at Brown University in the early 1980s, when he became interested in a classic experiment done two decades earlier by David H. Hubel and Torsten N. Wiesel. The two researchers sewed shut one eye of an infant kitten and showed that when the eye was reopened several weeks or months later, it did not respond to light. The kitten’s brain had rewired itself so that the eye no longer functioned.

What molecular mechanisms were responsible for such a remarkable neuronal change, Bear wondered. He started working on the problem, but in those days, he recalls, “our ignorance about the basic mechanisms of synaptic transmission in the brain was so vast that it wasn’t even possible to formulate very good questions.” Then, while doing a postdoctoral fellowship with Wolf Singer at the Max Planck Institute for Brain Research in Frankfurt, “everything changed,” he recalls. Researchers had discovered a particular kind of receptor in synapses that appeared to trigger LTP. Bear found that the recep-



The challenge [now] is to synthesize reductionist and holistic approaches to create a unified view of mental processes. —ERIC KANDEL

tor also seemed to be playing a role in the weakening of synaptic transmission. When he blocked the action of the receptor with a chemical compound, he could dramatically reduce the loss of function when the eye of a laboratory animal was sewn shut.

As a new professor at Brown University, Bear's first priority was to understand how this receptor was exerting its effects on vision. In 1992 he discovered, through experiments in brain slices, that appropriate stimulation of the receptor caused LTD. Perhaps LTD was contributing to the loss of vision in Hubel and Wiesel's kittens.

As Bear's lab at Brown grew, LTD remained a centerpiece of his research. With Brown physicist (and 1972 Nobel laureate) Leon N. Cooper, he developed a theory about how the loss of vision from one eye could produce a rewiring of the brain. They proposed that the neural reorganization resulted not from an absence of light but from a mismatch between the signals the brain was receiving from the open eye and those from the closed eye. To test the idea, Bear and his colleagues injected a long-lasting anesthetic into the eye of a kitten. When the eye was anesthetized, it sent no signals to the brain, so there was no mismatch between the incoming signals. Sure enough, when the anesthesia wore off, the anesthetized eye worked almost as well as before.

At Columbia, Eric Kandel (l) and Steven Siegelbaum study diseases, neural development, behavior, and learning.

“The prevailing view used to be ‘use it or lose it,’” says Bear. But a more accurate conclusion may be “neurons that fire out of sync lose their link. It’s not the absence of activity that is important to induce blindness, but the presence of activity that is not useful.”

In 2003, Bear moved from Brown to MIT, where he and his coworkers succeeded in revealing a mechanism at least partly responsible for the loss of vision. When a neuron receives mismatched signals, synapses lose receptors just as they do during LTD. If the loss of receptors is sufficiently prolonged, Bear suspects, the synapse eventually will disappear.

● UNEXPECTED CONSEQUENCES

These findings could have important implications for treating vision problems. For example, when children have visual imbalances such as the condition known as lazy eye, ophthalmologists typically patch the strong eye to help the weak eye get better. “But it’s usually a zero-sum game, because the strong eye gets weaker while the weak eye gets stronger,” says Bear. “There

was a debate in the literature about whether it would be better to allow some light to strike the patched eye, because then you would get at least some retinal activity.” Bear and his colleagues advocate blocking as much light as possible to minimize the mismatched signals reaching the brain.

Bear’s research into LTD has had other, and sometimes quite unexpected, consequences. His lab also has investigated a form of LTD triggered by the activation of another receptor on receiving neurons. One of the proteins affected by this receptor is the one that’s missing in fragile X syndrome, the most com-

mon cause of inherited mental retardation in humans. “I gave a talk at a Hughes meeting on the subject, and as it happened I was sitting next to a guy named Steve [Stephen T.] Warren, who was a Hughes investigator at the time and is now the editor of the *American Journal of Human Genetics*. Steve was the world expert on fragile X syndrome, and after my talk he leaned over to me and said, “That was a nice talk. Would you like to look at the fragile X knockout mouse?” Warren had developed a genetically engineered mouse that lacks the same protein missing in fragile X syndrome. Like its human counterparts, the mouse exhibited a number of behavioral and physical abnormalities—including an extremely suspicious characteristic of its dendrites. Most dendritic synapses perch on the tip of squat, hair-like projections called spines that project from the dendrites. In humans and mice with fragile X syndrome, these spines are

Prions on My Mind

In 2001, Eric R. Kandel walked into Susan L. Lindquist’s office and asked her a question out of left field. “Do you think prions could be involved in storing memory?”

Normally considered deformed renegades that mercilessly rob the brain of functions, prions might seem like unlikely candidates for safeguarding memories. “I nearly jumped out of my chair because I was speculating the same thing,” recalls Lindquist, a noted prion researcher who was then an HHMI investigator at the University of Chicago and has just stepped down after 3 years as director of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts.

Kandel, an HHMI investigator at Columbia University College of Physicians and Surgeons and a 2000 Nobel laureate, told her about a puzzling prion-like property in a protein that seemed to play an essential role in long-term memory storage. Kandel’s previous research had described a memory-forming cascade set in motion by CREB (cAMP response element binding protein). CREB functions as a molecular switch that turns short-term memory to long-term memory. A learning experience flips that switch, allowing proteins and untranslated messenger RNAs (mRNAs) to be dispatched throughout the cell body. However, he found that many mRNAs remain dormant until another learning experience acts as an alarm clock, arousing the mRNAs to build the proteins that strengthen just those synapses where long-term memories form.

Kandel was intent on discovering what molecular mechanism acted as the alarm clock for dormant mRNAs. He further wanted to understand why that mechanism was set in motion at just those synapses where a specific, enduring memory forms. Like many neuroscientists, his lab used the foot-long snail *Aplysia* as a model organism because its exceptionally large neurons are relatively easy to study and manipulate.

Kausik Si, a postdoc in his laboratory, had pinpointed CPEB (cytoplasmic polyadenylation element binding protein) as the selective trigger that woke up the dormant mRNAs at those pre-loaded synapses. Kandel’s team wanted to know how CPEB maintains the continuing protein synthesis that stores a memory long after the learning experience has passed.

While investigating that question, Si noticed that the CPEB found in neurons differs from that of other cell types. Normally, CPEB has phosphorylation sites that activate mRNAs, which increases their levels of protein expression. In snails as well as in flies and mammals, however, the CPEB in neurons does not have those sites. Instead, it has a strange functional domain that is unusually rich in the amino acid glutamine and lacks any secondary structure.

Si knew of only one other protein type with a similar domain—prions.

Prions cause a protein to alter its shape and then enforce a similar, self-perpetuating conformation on similar proteins. Si and Kandel wondered whether prion-like, self-perpetuating qualities in CPEB could be what keep a memory vivid over time. That’s why Kandel popped his question to Lindquist and later collaborated with her to pursue it.

Lindquist explains that the “funky” prion domains attach to one another, forming immobile, insoluble clumps. The rest of the proteins dangle along the side of the clump, like charms on a bracelet. This state can cause them to lose their beneficial function and gain a toxic function that leads to neurological disorders such as mad cow disease.

However, Lindquist had shown that the dangle sections of yeast prions can remain functional—and behave beneficially—in cultures. Theoretically, she reasoned, prion qualities could enhance a protein’s function if, for instance, it needs to be anchored to a site and work cooper-

atively to sustain a process—such as storing a memory at a synapse.

To test whether the *Aplysia* neuronal CPEB does act like a prion, Si attached its weird domain to a yeast protein. Using a color assay that Lindquist had developed, he watched the protein morph into a self-perpetuating prion before his very eyes. Then Si devised an assay for the whole *Aplysia* CPEB when expressed in yeast. It, too, appeared to behave like a prion.

Moreover, while *Aplysia*’s CPEB was very active in the prion clusters, it barely functioned in the nonprion state. Perhaps, the researchers hypothesized, CPEB needs to be in a prion-like state to sustain the perpetual protein synthesis necessary for storing memories. And perhaps that is why certain events—like repeating the times tables, practicing piano, and crashing a car—can become so unforgettable. They trigger enough CPEB production so that some copies of the proteins convert into prions to perpetuate themselves at the synapses where the long-term memory forms.

Kandel, Lindquist, and Si proposed this model of memory storage in the December 26, 2003, issue of *Cell*, accompanied by a Kandel and Si article on the role of CPEB in *Aplysia* long-term memory storage. “It’s a very nice finding,” Kandel says. “We’re now going back to the *Aplysia* nervous system, where we’ve shown that this protein is required for the maintenance of long-term memory. We want to find out if the prion domain causes self-perpetuation in neurons as it does in yeast, and, if so, if it is the mechanism that maintains memory in *Aplysia* neurons.”

Lindquist and Kandel speculate further that a similar prion mechanism might be involved in other contexts, such as developmental processes and cancers, where cells maintain a continuing function. This research therefore might lead to improved treatments for a range of disorders.

—CATHRYN M. DELUDE

long and spindly, not short and solid as they are in unaffected individuals.

Bear enthusiastically accepted Warren's offer, beginning a collaboration that has produced major advances in the understanding of fragile X syndrome. Bear's lab has been able to show that the loss of fragile X protein essentially results in runaway LTD. Further, the receptors linked to this protein take part in a number of other neurological and metabolic processes that are strikingly askew in fragile X patients, who often have epilepsy, extreme anxiety, obsessive-compulsive disorder, and intestinal problems. "It was astonishing how far we could go in accounting for the symptoms of fragile X by assuming exaggerated signaling of this receptor," says Bear. He and his collaborators have begun to explore whether blocking the receptor might allay some of the symptoms of fragile X syndrome, although because of the key role of the receptor in the nervous system, this approach warrants caution. Still, when mice prone to convulsions—a common feature of fragile X syndrome—are treated with blockers of the receptor, their condition is substantially improved. "Maybe if we intervene early enough we even could prevent some of the symptoms from occurring in the first place," says Bear.

Bear had no idea, when he began his investigations of vision, that someday he would confront one of the most tragic of human genetic malfunctions. "It's exciting and gratifying for me to see that the study of LTD may have relevance to diseases of the nervous system," he says. "This is the best example in my work of how basic research can lead in unanticipated directions."

● LIKE CHRISTMAS LIGHTS

In his laboratory at the California Institute of Technology (Caltech), just down Ventura Freeway from the huge movie studios of Burbank and Pasadena, postdoc Gentry N. Patrick is also making movies—but his films chronicle the changes that go on every second inside our heads. Every two minutes, the half-million-dollar confocal microscope at Patrick's side takes an image of the nerve cells growing in a dish mounted on the microscope's stage. When Patrick combines the images into a time-lapse sequence, the cells' dendrites glow and twitch like a string of Christmas tree lights in a steady breeze.

Patrick and his faculty adviser, HHMI investigator Erin M. Schuman, are probing one of the enduring mysteries of synaptic plasticity. How are the short-term impressions that fly through our minds every waking moment (the words of this sentence, the temperature in the room, a colleague's voice) converted into long-term memories that we can recall days, months, or even years later? Many experiments have shown that the formation of long-term memories requires the synthesis of proteins in neurons, but the details of this process have remained largely unknown. Neuroscientists traditionally have held that protein synthesis occurs in the cell bodies of neurons and not in the dendrites or axons. But if long-term memories are encoded by changes in individual synapses, how do the necessary proteins get from the cell body to the particular synapses where they are needed?

Schuman and her colleagues at Caltech have been studying an iconoclastic idea: Maybe synapses change themselves in part by synthesizing proteins locally rather than waiting for them to arrive from the cell body. To investigate this hypothesis, they infect nerve cells grown in culture with a virus containing a bioengineered flu-

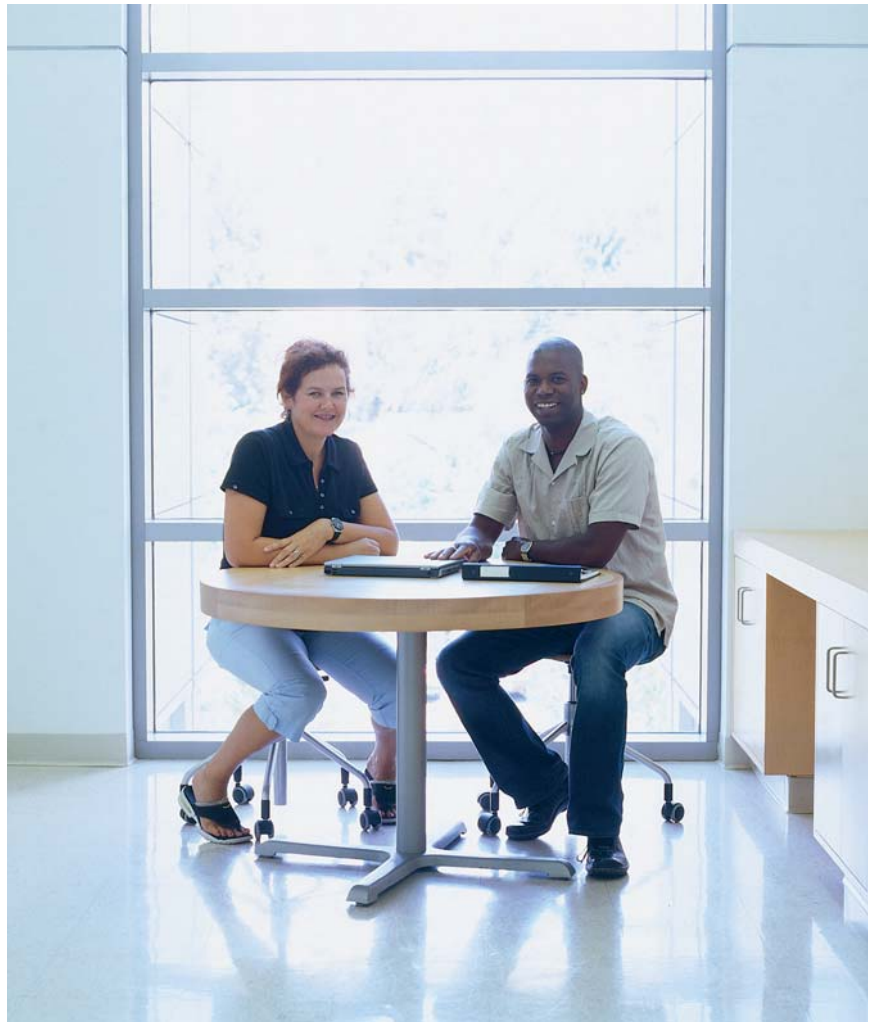
orescent molecule. Then they watch as the cells are stimulated with a growth factor or other compound. Wherever the nerve cell is actively making proteins (or, in a separate set of experiments, degrading proteins), the fluorescent molecule glows a bright green. "What we do is look at the essentials of how synapses work and change," says Schuman. "We start with intriguing ideas and then follow them."

Schuman says she "stumbled into" the study of local protein synthesis. As a new professor at Caltech in the mid-1990s, she and a colleague, Hyejin Kang, were studying a particular form of LTP in brain slices when, in one set of experiments that sought to block the potentiation with a chemical compound, they noticed that the blocking mechanism seemed to be occurring in the dendrites rather than in the cell body.

"We got completely captivated by the notion of local synthesis of proteins," she says. "If the function of synapses is controlled by modification of proteins, then a way to change the protein complement of a synapse could be to construct or degrade proteins at the synapse."

Schuman's fluorescent images have provided the first definitive proof that protein synthesis occurs in dendrites.

At Caltech, Erin Schuman and Gentry Patrick explore the enduring mysteries of synaptic plasticity.



That finding, in turn, has raised a host of additional questions. “We’d like to understand the logic of the system,” she says. “When will a synapse use a local source of a protein and when will it use a protein trafficked from the cell body, because some of the same proteins are both trafficked and made locally.” Local synthesis of proteins also may play a role in diseases of the brain. For example, the protein missing in fragile X syndrome is made locally in dendrites, and other diseases also may arise from defects in local protein synthesis. “Some of the thinking in the last few years about Alzheimer’s disease and Parkinson’s,” says Schuman, “is that the synapse itself is the problem rather than a manifestation of a problem elsewhere.”

Other investigators have been examining forms of plasticity in other parts of synapses. For example, at Columbia University College of Physicians and Surgeons, HHMI investigator Steven A. Siegelbaum has been focusing not on the receiving neuron but on the transmitting neuron. “The communication between cells in a synapse has to be well-coordinated,” says Siegelbaum. “By focusing on the presynaptic component, you have a way of asking what a synapse as a whole is doing.”

Siegelbaum and his colleagues at Columbia inject a fluorescent dye that is taken up by the transmitting neurons so that, when they release neuro-

dow in the top of its head. “With the microscope we look right through here,” she says. “Every four days we take a new set of images.”

Upstairs, a series of photographs on Wilbrecht’s computer shows three months’ worth of changes in part of Number 25’s brain. Thin, tentative-looking spines sprout from dendrites and reach toward surrounding cells. Some of the spines soon wither away, but others thicken and stabilize, as if they embody memories too valuable to lose. “We want to study the cell biology of the synapse as an animal learns,” says HHMI investigator Karel Svoboda, head of the lab where Wilbrecht works at Cold Spring Harbor Laboratory.

In what colleagues consider a technological tour de force, the Svoboda team has developed a way of watching the brain as it learns. Their technique employs a bioengineered mouse line that expresses a particular molecule in a very small fraction of neurons. They mark this molecule with fluorescent dye, so that a microscope can home in on specific dendrites. Using dental adhesive, they implant a plastic window in the mouse’s head over the part of the brain that receives sensory information from the whiskers on the mouse’s face. As particular whiskers are clipped and then regrow, Svoboda and his colleagues can watch as a neuron reacts first to the absence of sensory input and then to its renewal. For example, a few days after a whisker

In diseases like Alzheimer’s, the synapse itself may be the problem rather than a manifestation of a problem elsewhere. —ERIN SCHUMAN

transmitters, the dye is released too. The team has found that particular kinds of stimuli cause presynaptic neurons to undergo a long-term change that results in the release of greater amounts of dye, and presumably of the accompanying neurotransmitter. The researchers have even discovered a form of presynaptic LTD in which activation of receptors on the surface of the receiving neuron leads to a feedback signal that reduces release of neurotransmitter from the transmitting neuron.

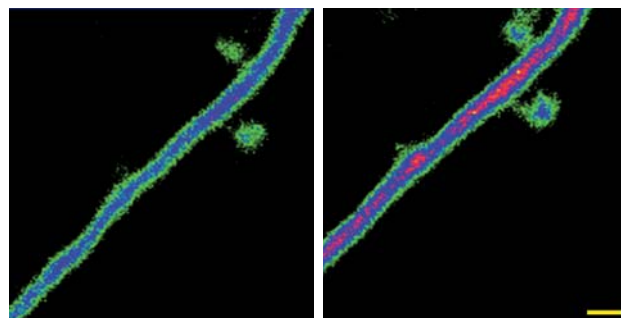
That plasticity should occur in the presynaptic as well as postsynaptic neuron is not surprising, says Siegelbaum. As neuroscientists learn more about the function of synapses, they are recognizing more and more kinds of plasticity—some localized to particular parts of the brain, and some that occur throughout. “If you read about the brain in a textbook, it seems static,” says Siegelbaum. “But it’s actually a very dynamic thing.”

Understanding that dynamism at the molecular level is the most important goal in the neurosciences today, according to Thomas C. Südhof, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas who studies the generation of synapses and the release of neurotransmitters. It’s also a daunting goal—more than 1,000 proteins function in presynaptic synapses, typically as part of complex networks in which any given protein can serve more than one function.

“Understanding these protein networks and overlapping functions will be a major challenge,” he says. “The key, absolutely essential component, I believe, is to integrate different disciplines—physiology, genetics, structural biology, and biochemistry.”

● HOW AN ANIMAL LEARNS

“This is my favorite, Number 25,” says Linda Wilbrecht, plucking a small brown mouse from a plastic bin where it was playing with three other mice. The scientist holds the mouse up to the overhead light, revealing a small win-



Increased brightness of fluorescence along the length of the dendrite and within dendritic spines (r) reflects a boost in localized protein synthesis after treatment with a protein synthesis stimulant.

is cut, the neuron receiving input from that whisker steps up its rate of spine formation. It appears to be reaching out to its neighbors, searching for new inputs to replace those it has lost.

To Svoboda, these *in vivo* observations of living neurons have suggested a compelling model of how the brain grows and changes. During development, the axons and dendrites of nerve cells extend throughout the brain and make connections. But after puberty, the growth of nerve processes largely stops. “There may be some change at the level of the axons and dendrites in the adult brain, but it’s limited,” says Svoboda. “We’ve never seen branches appear or disappear.”

In the adult animal, plasticity appears to occur largely through the growth of new synapse-carrying spines from existing dendrites. For example, Svoboda and his coworkers have examined the changes that occur in the brains of mice when their whiskers are cut in a checkerboard pattern. In the brain regions cor-

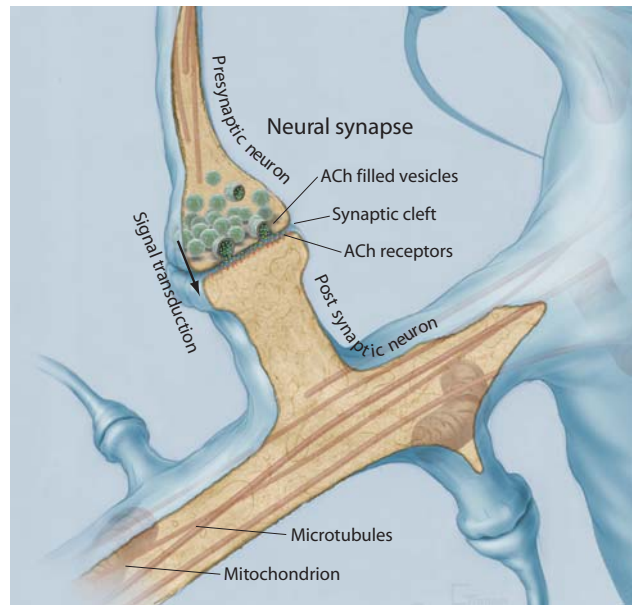
responding to the cut whiskers, the synapses soon begin to respond more strongly to the remaining whiskers. This occurs not through the expansion of dendrites and axons, Svoboda explains, but through the growth of new spines between established dendrites and axons. The same thing probably happens in humans when we learn a new skill, like typing or playing the piano: The area of our brain responsible for our less-used fingers gradually expands as new spines link axons and dendrites that previously had few or no connections.

“Adult plasticity may mostly involve these local changes,” says Svoboda. “That could be the fundamental difference between the developing and the adult brain.”

Svoboda’s work has generated great excitement among neuroscientists because it addresses one of the central problems in the field: How do the actions of individual molecules and synapses contribute to changes in behavior? According to Susumu Tonegawa, an HHMI investigator at MIT (and director of the Picower Center for Learning and Memory there), “We are trying to understand not just the molecular and biochemical events taking place in the brain. We are trying to identify the neuronal circuitry and mechanisms underlying behavior and cognition. To do that, we need experiments that link processes at different levels of complexity.”

Tonegawa has been instrumental in developing the most widely used technique for exploring these links. During the 1970s and 1980s he was an immunologist working on the generation of antibody diversity, research for which he received the 1987 Nobel Prize in Physiology or Medicine. In the early 1990s he decided to switch fields and become a neuroscientist. In immunology, he often had studied genetically engineered knockout mice that lacked the gene for a particular protein. As a neuroscientist, he began to explore the effects of missing or altered genes on animal behavior.

A key challenge was to make the knockout technology more specific. “The proportion of genes that are expressed in the brain is huge compared to other biological systems—more than 50 percent,” he says. “So we had to add an additional technology where you can target gene manipulations to a specific type of cell or the specific phase of an animal’s development.” Through a manipulation of the introduced gene’s expression, Tonegawa and his colleagues developed a way to turn off a gene just in the forebrain, for example, and only in the adult animal. They then set about exploring the effects of these very



Passage of neurotransmitters, such as acetylcholine (ACh), across the synaptic cleft at the right time and in the right amounts is key to brain function. Tiny vesicles ferry the chemical messages from sending axon to receiving dendrite.

selective genetic lesions.

One of the most provocative molecular pathways they have investigated involves an enzyme known as calcineurin—a regulatory protein that is active both in the immune system and in synapses, where it contributes to the

growth of spines and to changes in synaptic strength. When the gene for calcineurin was knocked out in mice, they exhibited severe deficits in short-term memory. In exploring a maze for food, for example, the mutant mice would forget they had already fetched the food in a particular passage just a moment before and would explore it again. Further studies revealed other kinds of deficits. The animals were socially withdrawn; instead of sleeping together, as mice usually do, they would sleep in opposite corners.

Tonegawa and his colleagues believe these deficits bear a striking resem-

Memory Circuits

Think about your most vivid memories—a favorite relative, the first day at a new school, a triumph (or failure) in sports. Odds are that most of them are associated with strong emotion.

Previous research has proven what common sense dictates: that the events we remember best are the ones that evoked a strong emotional reaction. We also remember negative information better than positive information. Now neuroscientists are beginning to reveal the brain circuits responsible for these intense memories.

At the Massachusetts Institute of Technology’s Department of Brain and Cognitive Sciences, HHMI predoctoral fellow Elizabeth A. Kensinger,

working with professor Suzanne Corkin, took functional magnetic resonance imaging pictures of volunteers’ brains as they were learning various kinds of words. Some words were designed to have negative connotations but not be emotionally arousing, such as “sorrow” and “mourning.” Other words, such as “rape” and “slaughter,” were both negative and emotionally charged.

Learning the two categories of words tended to trigger activity in different regions of the brain. The nonarousing words lit up regions in the front of the brain and in the hippocampus, a structure in the brain’s interior that is often active when the brain is learning or remembering new information. The

arousing words, in contrast, lit up the hippocampus and the amygdala, a separate region of the brain involved in fear and other emotional responses.

“What this study shows is the network of regions involved in these memory tasks,” says Kensinger. “Other techniques are needed to tell you which part is sending and which is receiving.”

Kensinger and Corkin are now looking at whether arousing and nonarousing words with positive connotations produce the same patterns. They are also beginning to investigate whether the brains of younger people and older people work differently when dealing with emotionally wrought memories.

—STEVE OLSON

blance to schizophrenia, a devastating disease that attacks approximately 1 in every 100 humans. People with schizophrenia also have problems with short-term memory and tend to withdraw socially. Even more suggestive, genetic studies of families with an especially high incidence of schizophrenia have revealed a link between the disease and the gene on chromosome 8 that encodes calcineurin.

Tonegawa and several colleagues are preparing to set up a small biotechnology company to begin looking for compounds that can alter the calcineurin pathway and possibly ameliorate the symptoms of schizophrenia. “The antipsychotic drugs that are on the market now mostly target dopamine receptors, and those drugs have serious limitations,” he says. “We are trying to intervene with a totally different target.”

● A WINDOW ON THE MIND

“You’ll see a word appear in the middle of the screen,” postdoc Denise Head tells a visiting journalist who is snugly ensconced inside the magnetic resonance imaging (MRI) machine. “If it’s an abstract word, push the button on the left-hand side. If it’s a concrete word, something you can see or touch, push the button on the right side.”

In the adjacent control room, a computer monitor displays images of 12 virtual slices through the research subject’s brain. As the subject sorts the words

found in Alzheimer’s patients that certain kinds of memory processes, such as those involved in learning new skills, remain surprisingly intact.

More broadly, Buckner sees his images of the brain at work as one part of a more comprehensive view of the brain. “We need to correlate these observations across different levels of analysis, from the structural to the functional to the behavioral,” he says. “That way, we can ask causal questions about how one level relates to the others.”

● UNIFIED APPROACH

Throughout the 20th century, scientists investigating the brain took one of two broad approaches, says Eric R. Kandel, an HHMI investigator at Columbia University College of Physicians and Surgeons. The reductionist approach sought to understand the brain in terms of its constituent parts—the cells and molecules of which it is composed. The holistic approach analyzed the brain’s ultimate effects: human behaviors and thinking. Study of the synapse helps to unify these two domains. “The synapse has been a very productive target for research,” says Kandel. “We have learned a great deal about receptors, receptor insertion into membranes, neurotransmitter release, and modulation of neurotransmitter release by endogenous inputs. Although we do not as yet have a complete understanding of how synapses work and how they are regulated for plasticity,

A good portion of the front of your brain appears to be devoted to networks that control other networks. —RANDY BUCKNER

into categories, the regions of his brain that are doing the work light up with the effort. “This is one of the best tests of memory we’ve found,” says Randy L. Buckner, an HHMI investigator at Washington University in St. Louis and leader of the experiment Head is conducting. “Putting a word in a category forces you to think about that word in the context of other words you know.”

Buckner and his colleagues, using a technique known as functional MRI, or fMRI, are watching the brains of humans as they think. When part of the brain is activated, oxygenated blood rushes to supply the firing neurons with energy. The scanner detects these changes in blood flow, opening a window on the mind.

Buckner has been investigating perception, brain changes during aging, and memory in particular. He and his coworkers have found, for example, that deciding whether a word is abstract or concrete typically activates specific areas on the left side of the brain that are associated with language, as would be expected with this task. But it also activates areas in the front of the brain that appear to control the process of making decisions about the nature of the word. Furthermore, some frontal regions are activated while comparing words, and a partially overlapping set of frontal regions is activated when comparing new faces with known faces. According to Buckner, “a good portion of the front of your brain appears to be devoted to networks that control other networks.”

Buckner and his colleagues are investigating this question in part by examining brain function in hundreds of individuals, including people with forms of dementia such as Alzheimer’s disease. By comparing brain function with memory capacity and the strategies people use to remember, the researchers hope to identify positive and negative mental changes that occur over time. This epidemiological approach may also suggest ways of intervening to stem memory losses. For example, Buckner and his coworkers have

learning, and memory, we do have a satisfactory understanding.”

At the same time, the holistic approach has been reinvigorated as neuroscientists have begun to understand human behaviors and diseases in terms of actions at the synaptic level. “Insofar as you can relate how changes in synaptic transmission relate to behavior, you are moving from a reductionist to a holistic viewpoint,” says Kandel.

The challenge for the 21st century, according to Kandel, is to achieve a synthesis of the reductionist and holistic approaches to create “a new, unified, and intellectually satisfying view of mental processes.” This unification would produce tremendous advances in the effort to reduce human suffering. It also would mark a truly great milestone of modern science: a culmination of our quest to understand ourselves. ■

Synapses at Janelia Farm

Many of the questions discussed in this article are likely to get attention in research at HHMI’s Janelia Farm Research Campus, now under construction. After holding a series of workshops that included several of the investigators in the story, HHMI established two areas for initial research at Janelia Farm:

- The identification of general principles that govern how information is processed by neuronal circuits
- The development of imaging technologies and computational methods for image analysis

No doubt some of the future findings on synapses—and perhaps the next generation of questions for further work—will be informed by investigations at Janelia Farm.