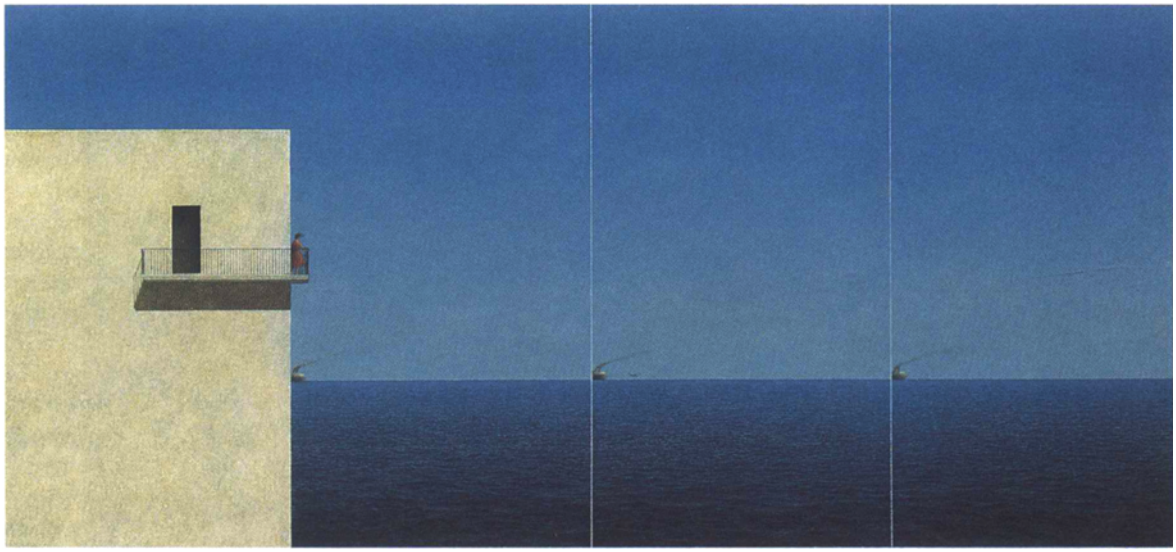
YOU MUST REMEMBER THIS

Finding the master switch for long-term memory

BY JOHN B. CONNOLLY AND TIM TULLY



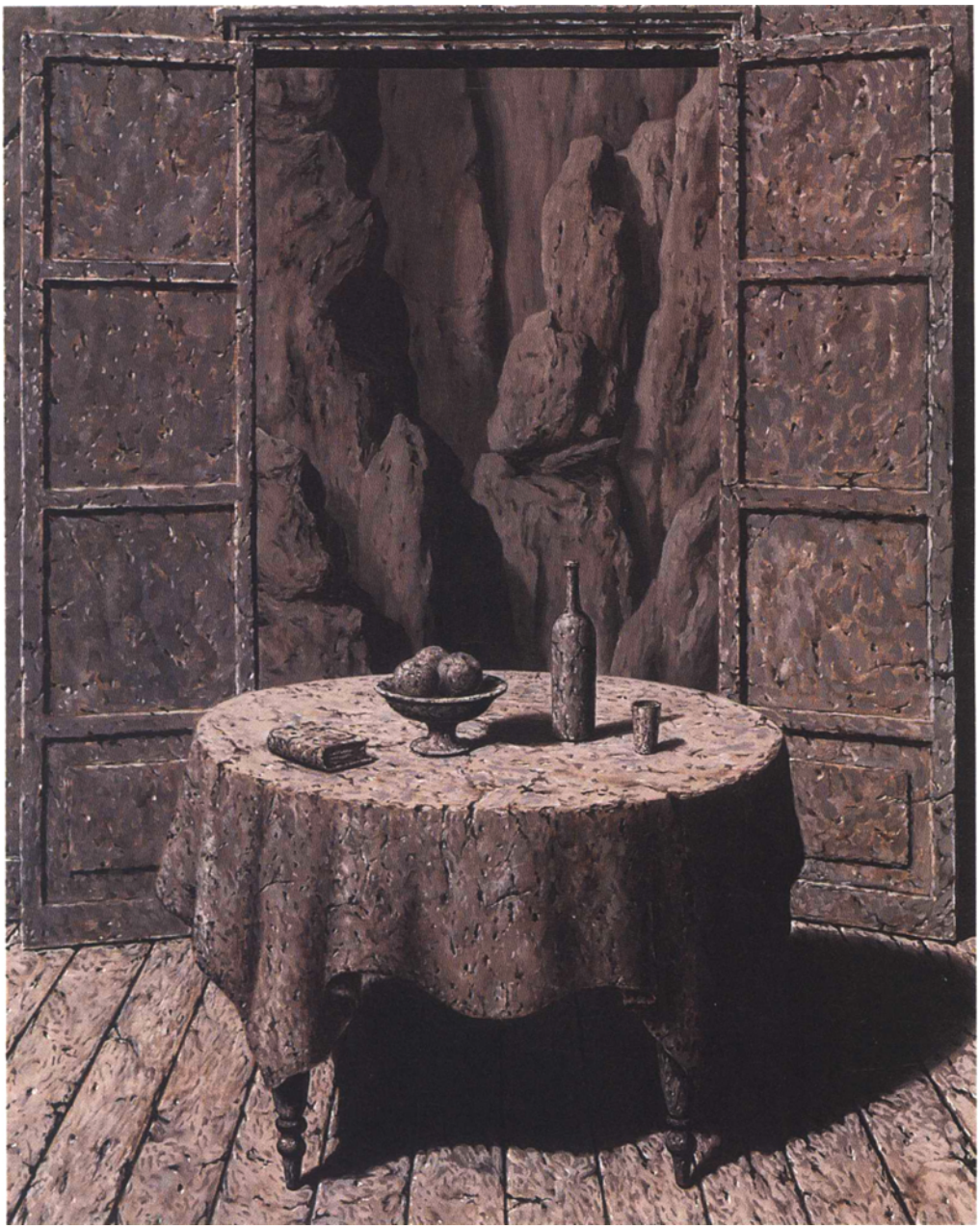
Alfredo Castañeda, Retablo of the Forgotten, *1994*

IN BOOK NINE OF HOMER'S EPIC THE *ODYSSEY* a hurricane carries the hero, Odysseus, and his fleet of ships far off course, to the land of the lotus-eaters. When the storm finally subsides, Odysseus sends two of his best men and a runner ashore to reconnoiter. The men fail to return, and so Odysseus sets off from his ships to rescue them. But the rescue party is ill prepared for what it finds on shore. The missing men are neither dead nor hostages; instead they survive in a dreamlike state, devoid of all recollections, feasting with the natives on the fruits and blossoms of the lotus flower. They have lost all memory of who and what they are: they have lost their psyches.

Homer clearly understood that memory is an integral part of who a person is. Previous experiences inextricably link a person to the perception of self and others, and they serve to color almost all behavior. Fortunately, the plight of Odysseus's men is temporary; dragged back to their ships at last, they regain their memories, their senses and their identities.

For people suffering from genuine memory disorders, however, the fates are not so kind. In 1968 the Russian neuropsychologist Alexander R. Luria published *The Mind of a Mnemonist,* a book devoted to the remarkable case of one Shereshevsky. Apparently Shereshevsky could remember everything he had ever encountered in his life. Luria described one occasion when he presented Shereshevsky with a contrived, complex mathematical formula. After several minutes' study Shereshevsky reproduced the formula with complete fidelity. Astoundingly, fifteen years later, when Shereshevsky was asked to generate the formula, he did so without error. Such a "gift"—commonly called photographic memory—was a double-edged sword for the Russian mnemonist. He had difficulty combining memories of the same individual and thus struggled with personal interaction. Indeed, Shereshevsky's memory so interfered with his ability to work that he ended his days as a "memory man" in a music hall.

Most people, fortunately, inhabit the more hospitable middle ground between lotus-eater and mnemonist. That felicitous state turns out to be—like many other dynamic biological processes—the net result of countervailing activities that either activate or repress. In our work at the Cold Spring Harbor Laboratory in Cold Spring Harbor, New York, we have devised ways of studying memory in *Drosophila melanogaster,* the common fruit fly. In principle, our experiments combine the classic experimental design of the turn-of-the-century Russian physiologist Ivan Petrovich Pavlov with late-twentieth-century genetic engineering. First we try to create associations between previously unrelated stimuli in the insects, measure the strength of the associations and determine how long the memory of the associations persists. Then we seek the genetic underpinnings of the associative process.



René Magritte, Souvenir de Voyage III, *1951*

WE HAVE CONFIRMED IN FLIES WHAT psychologists and neurobiologists have long suspected in larger animals: memories are formed in distinct phases, each new phase overlapping the preceding one. Short-term memory (STM) gives rise to middle-term memory (MTM), which under certain conditions becomes consolidated into a long-lasting memory. But more, by analyzing flies that carry single-gene mutations, we have discovered that each memory phase is closely associated with the function of certain discrete sets of genes. In particular, we have been able to identify a gene that can enhance and suppress long-lasting-memory formation in flies: We can make a fly into a mnemonist or a lotus-eater. Our work is the first example of a genetic manipulation that enhances long-lasting memory in any organism. Thus it begins to show what really makes memory tick.

But our findings may go far beyond the humble fruit fly. All animals, it seems, from invertebrates to vertebrates, form long-lasting memories in basically the same way. Furthermore, the genes identified in fruit flies also occur in the other staples of the biological laboratory—in mollusks, chicks, mice and rabbits—and in humans. To the extent that their functions have not changed over the long evolutionary span since insects and mammals diverged, those genes may hold the key to the understanding of memory in humans. Such a prospect offers both promise and threat. There is a hope that people with disorders of memory—Alzheimer's disease, for instance—could one day be helped with drugs developed through our genetic insights. But there is concern as well—concern that, as with all drugs ushered in from the new era of medical genetics, the use or abuse of a pill of memory or a shot of forgetfulness will be solely at the discretion of the drug administrator.

MOST PEOPLE ARE AWARE OF THEIR ABILITY to remember both recent and long-past events—the weather yesterday, as well as the toys and stuffed animals they had as children. But how, exactly, do they remember such events? Do memories exist as isolated facts or discrete processes, quanta of information stored in some well-demarcated region of the brain? Or are memories in some way holographic, diffused over the entire nervous system?

In the 1950s, after years of effort to find the seat of memory in the brain, the American psychologist Karl S. Lashley rejected the idea that memories are localized, and he conjectured that memories are "statistical features of temporal patterns." Since then, however, numerous studies of people whose brains are partly incapacitated have led to what is now the prevailing view among neurobiologists: that specific memories are indeed stored in specific sites. In 1904 the German biologist Richard Semon coined the term *engram* for the site of memory storage—where memory would physically manifest itself as a "change of the organic substance."

THE GENE WE IDENTIFIED CAN ENHANCE *or suppress long-term memory. We can make a fruit fly into a mnemonist or a lotus-eater.*

The classic case study of an epileptic man known as H.M. demonstrated one of the most striking features of memory storage: whatever underlies long-lasting memory appears to be physically distinct from learning and from short-term memory. In 1953, to quell his severe bouts of epilepsy, surgeons removed significant portions of the hippocampus, amygdala and temporal lobe of H.M.'s brain. Although the effects of the epilepsy were attenuated, the surgery left H.M. unable to transfer new information into permanent memory, though he could remember new information for a short time. As a result, H.M. lives in a perpetual present where "every day is alone by itself, whatever enjoyment I've had, and whatever sorrow I've had. . . ." His memories from before the surgery, however, remain intact.

Long-lasting memory forms in many animal species, and psychologists and neurobiologists have often pointed to two general features of the process. One feature is retrograde amnesia: newly acquired information can be lost if one is subjected to head trauma, shock treatment, hypothermia, anesthetics or insults that lead to unconsciousness. Typically, the amnesia reaches backward in time from the moment of the unconsciousness to an earlier moment before which memory is unaffected by the trauma. What appears to take place is that as time passes and new information gets "committed to memory," the memory becomes progressively resistant to disruption. For example, after being knocked unconscious in a sledding accident on Christmas Day 1968, one of us (Tully) could remember past events until as recently as the preceding December 12. The ensuing two weeks, however, including the gift exchange earlier on Christmas Day, were permanently lost. The appearance of that so-called anesthesia-resistant memory has generally been interpreted as the earliest manifestation of a stable long-lasting memory.

The second feature of long-lasting memory, derived from experiments on animals, is that its formation depends on protein synthesis. The emerging view among neurobiologists is that memory is ultimately stored as a permanent change in the way certain neurons communicate with each other in the brain. Neurons connect to each other by way of synapses, which they have in abundance. When long-lasting memory appears, existing synaptic connections seem to strengthen or grow. Proteins, synthesized within the neurons, are necessary raw materials for that process, in the same way bricks are necessary for extensions to a brick house.

The classic experiments demonstrating the need for protein synthesis in long-lasting-memory formation were done in 1963. Wesley C. Dingman, a psychiatrist at Chestnut Hill Hospital in Rockville, Maryland, and Michael B. Sporn, a pharmacologist at the Dartmouth School of Medicine in Hanover, New Hampshire, injected rats with a drug that inhibits protein synthesis. When the rats were injected just before being trained to negotiate a water maze, they quickly forgot what they had learned about the maze. But when the rats were injected only after the training period, they remembered more about the maze; and the later the injections, the more they remembered. After a certain interval, what they learned about the maze was "committed to memory," and the subsequent injection of the inhibitor drug had no effect. Thus a long-term memory (LTM) dependent on protein synthesis became progressively resistant to inhibitors after training.

BOTH FEATURES OF LONG-LASTING-MEMORY formation—its resistance to disruption by anesthesia and its dependence on protein synthesis—exist in the subject of our investigations, the fruit fly. We first train our flies, as Pavlov trained his dogs, to associate a neutral stimulus with a stimulus that usually elicits a strong behavioral response. To do so, we trap about a hundred flies at a time in a cylindrical chamber much like a test tube, whose inner surface is covered with an electrifiable grid. At one end of the chamber we attach an "odor cup" that gives off one of two odors: octanol, which smells like licorice, or methylcyclohexanol, which smells a lot like tennis shoes in July. By passing air through the chamber, we expose the flies to one of the odors, and we simultaneously electrify the grid on which they rest. We expose the flies to the second odor in the absence of electroshock, as a control.

Once the flies are trained to associate one of the odors with electroshock, we test them at various times afterward in a T maze. We place the flies at the junction of the T, between converging air currents that carry one odor or the other. Untrained flies show no preference for either odor; they distribute themselves in a fifty-fifty ratio in the two arms of the T maze. But the trained flies are far from indifferent: 90 percent of them avoid the shock-paired odor by running into the opposite arm of the maze. As time passes, however, the flies' memories slowly fade, and after

about a day their preference for the odors in the T maze reverts to indifference.

Although a day is a long time in the life of a fruit fly, it is not forever. We were able to induce long-term, permanent memory in our flies by drawing upon work done more than a century ago by the German psychologist Hermann Ebbinghaus. In his 1885 book, *Über das Gedächtnis* (On memory), Ebbinghaus reported his discovery that a list of nonsense syllables can be memorized more accurately if several training sessions are spaced out over time than if the training is crammed into a single long session. Schoolteachers, of course, have been aware of the phenomenon for years. Cramming before a test helps students only in the short term. They retain more if they parcel out their study time over several intervals.

We applied the same principle to our flies. We trained them in ten sessions, with a fifteen-minute rest interval between each session. The memories we were able to create in the flies then persisted indefinitely.

OUR PAVLOVIAN TRAINING SHOWED THAT fruit flies exhibit many of the kinds of memory seen in other animals. Most notably, memory forms in increasingly stable phases. Immediately after training, flies have a burst of short-term memory, which lasts for several minutes. STM is followed by middle-term memory, which lasts several hours and is followed by anesthesia-resistant memory (ARM).

We have a simple trick for showing that flies develop ARM: we "cold-shock" them at progressively longer intervals after one training session. We induce cold shock by placing the flies in a test tube and submerging the test tube in ice water until the flies become unconscious. After two minutes we warm them up again (they regain consciousness quickly, with no side effects), and we test their memories in the T maze three hours after the training. We find that if the flies are cold-shocked immediately after training, their three-hour memory is severely disrupted. As the interval between training and cold shock becomes progressively longer, however, the flies' memories three hours after training become more resistant to disruption. Those features indicate the appearance of ARM.

IN FRUIT FLIES, AS IN PEOPLE, *cramming helps only in the short term. Both species retain more if they rest between training sessions.*

Finally, we were able to generate long-term, nondecaying memory in our flies by repeating the training sessions and spacing them out. More, we have shown that for that final memory phase to form, proteins must be synthesized: when we fed our flies a protein-synthesis-inhibiting drug, we found, just as Dingman and Sporn had with their rats, that long-term memory failed to appear.

One finding that aroused our interest was that when we trained our flies in one massed training session without rest, the protein-synthesis inhibitor had no effect on the memories that developed. The drug seemed to work only when the training sessions were spaced out. Crucially, flies subjected to massed training seemed to acquire anesthesia-resistant memory, which also seemed unaffected by the protein-synthesis inhibitor. Those results were curious, because for thirty years neurobiologists and psychologists had assumed that the appearance of ARM and the requirement for protein synthesis were two aspects of the same process: the consolidation of long-term memory.

But how could we prove the obvious hypothesis: that contrary to the widespread belief, anesthesia-resistant memory and long-term memory are physically distinct? The key was the vast and detailed knowledge geneticists have gained in the past century about the genetics of the fruit fly. One strain of the fly, in particular, possesses a defective, mutant copy of a single gene known as *radish*. Flies with an intact *radish* gene developed anesthesia-resistant memory, but flies with the defective *radish* gene did not.

In a way, such a result was typical. Over time we and our colleagues at Caltech and the Massachusetts Institute of Technology have identified other strains of fly with mutations in single genes that are unable to form short-term or middle-term memories. But we also discovered that mutations in genes involved with STM or MTM disrupt all downstream memory phases. That observation suggested there is sequential processing, at the genetic level, of the various memory phases: STM induces MTM, which induces ARM, which leads to LTM.

But the *radish* mutant proved an important exception to that simple picture. Although flies with the mutant *radish* gene did not develop ARM, after spaced training they still developed memory that, apparently, was permanent. Could such permanent memory reflect the appearance of LTM, for which proteins must be synthesized? The answer is yes. When mutant *radish* flies were fed a protein-synthesis inhibitor, spaced training failed to give rise to long-term memory. The observation that the mutant *radish* gene disrupts ARM but not LTM demonstrates a clear genetic dissection of the two properties of consolidated memory: anesthesia resistance and the dependence on protein synthesis. Because the two kinds of memory can be independently disrupted, the ARM and LTM phases of memory appear to be parallel processes rather than sequential ones.

IF ARM AND LTM ARE SO DISTINCT, WHAT ARE the genes specific to the formation of long-term memory? The responses of normal (that is, nonmutant) flies to massed-training versus spaced-training regimens supplied the clue. We discovered that whereas spaced training of normal flies gives rise to both anesthesia-resistant memory and long-term memory, massed training leads only to ARM. Even after forty-eight massed-training cycles, done without intervals of rest, the flies still formed no long-term memories.

What is so important about the rest? Whatever proteins turn out to be associated with LTM, their concentrations somehow increase during the rest period. The concentration of a protein in a cell is controlled by the degree to which the gene coding for that protein is expressed. Typi-

cally, the expression of such a gene is controlled in turn by activator and repressor proteins that enable or prevent the information carried by the gene from being transcribed. A good guess about the control mechanism might be that the functional levels of both activator and repressor proteins increase during the training. The functional level of repressor protein should then fall more rapidly than that of activator protein during the rest. The differential buildup of activator protein would then account for the subsequent buildup of the proteins directly associated with LTM.

Workers in our laboratory have recently found precisely such a mechanism in the fruit fly. It has been known for some time that some of the earliest biochemical events involved in learning are mediated by the cyclic AMP, or cAMP, signal-transduction pathway within the cell. (The cAMP pathway is a well-studied messenger system.) The neurobiologist Eric R. Kandel and his colleagues at the Columbia University College of Physicians and Surgeons in New York have shown that learning in the mollusk *Aplysia* is disrupted by perturbing cAMP signaling. The signals mediated by cAMP closely resemble the observed phases in the formation of memory: both are sequential, and both lead to increasingly stable molecular changes within neurons.

It turns out that what is lacking in the genetically mutant flies that form no short-term memory are enzymes that either generate or break down cAMP. Thus the biochemistry of learning appears to be virtually the same in mollusks as it is in flies. Farther down the cAMP-signaling pathway is a molecule known as cAMP-responsive element-binding protein, or CREB. The molecule dictates whether a cell will make new proteins in response to cAMP signaling. And therein lay the clue for LTM formation in flies. If the early events in cAMP signaling were responsible for the early events of memory formation, perhaps molecules acting later in the same pathway mediated the protein synthesis on which long-term memory depends. If so, CREB was an obvious candidate.

AFTER YEARS OF PAINSTAKING molecular research, Jerry C. P. Yin, a colleague at Cold Spring Harbor Laboratory, identified a gene encoding CREB in flies, known as *dCREB2*. The *dCREB2* gene is complex and encodes many forms of CREB protein, including a repressor form to turn off CREB activity and an activator form to switch it on. Yin showed that the activities of each form are highly specific. Flies in which the repressor form of the protein was produced at artificially high levels showed no sign of long-term memory, though short-term, middle-term and anesthesia-resistant memory all formed normally. Even more dramatic, whereas normal flies required ten spaced-training sessions to acquire long-term memory, flies that synthesized higher than normal levels of CREB activator developed the usual amount of long-term memory after only one training session.

Such discoveries should hold in a general way for people. As the wags have it at Cold Spring Harbor: "Flies are flies and mice are people." The genome of a mouse is virtually identical to the

Memory board (lukasa), Luba, Zaire, early-twentieth century

genome of a person. Our laboratory colleague Alcino Silva studied memory formation in mice with a mutant CREB gene and observed an outcome similar to the one we observed in flies. Short-term memory remained normal, but long-term memory did not form. CREB seems to be the master memory switch in flies, mice and, by genetic implication, people.

There is tantalizing evidence that CREB misregulation may account for some human cognitive disorders. For CREB to activate the synthesis of new protein, it must team up with a molecule called CREB-binding protein (CBP). Recently, a link has been reported between disruption of the CBP gene in people and Rubinstein-Taybi syndrome. Among the clinical features of the syndrome are mental retardation and physical abnormalities of the thumbs and toes. Analyses have shown that Rubinstein-Taybi patients carry mutant forms of the CBP gene or have microdeletions in the region of the chromosome that includes that gene.

GENES DO NOT *DETERMINE* BEHAVIOR, *and, perforce, they cannot* determine *memories. But who would not rejoice if a memory pill became available?*

We have only begun to consider how CREB functions in humans. We speculate that CREB may act as an information filter for most tasks, ensuring that only recurrent events become committed to long-term memory. If so, many new questions arise: Exactly how does the differential activity between CREB repressors and activators unfold during the rest intervals between training sessions? What genes does CREB target? Where are the long-term memory cells, the elusive engrams? How does CREB activity in the nucleus of a neuron target only a small group of specific synapses while leaving unmodified thousands of other synapses in the same neuron.

Answers to such questions could lead to enormous human benefits. Understanding the workings of proteins encoded by memory-related genes may enable pharmacologists to develop drug treatments for patients suffering from memory lapses, including the terrible losses caused by diseases such as Alzheimer's. Because memory is such an integral part of human identity, the hope exists that the far larger group of people suffering from such mental disorders as anxiety and schizophrenia could also be helped.

YET THAT HOPE SHOULD BE TEMPERED WITH caution. Basic genetic research has taught biologists that the activity of a gene is rarely isolated from the environment or even from the rest of the genome. For example, as long ago as 1907 experiments with magnesium concentrations in water showed that the environment can dramatically alter gene expression. Living in water with high concentrations of magnesium, fish develop not two eyes, but one. The "nature versus nurture" debate in biology must be dismissed as oversimplified and anachronistic by contemporary genetic research.

Interaction among many genes, for instance, is the rule rather than the exception. If one further considers the role the environment plays in gene expression, the number of factors involved in such aspects of life as behavior, identity, memory and thought becomes astronomical. It makes little sense to characterize the "meaning" or "function" of a gene, when in the vast majority of biological circumstances its effect depends on its interactions in combination with hundreds of other genetic and environmental factors. In short, genes to not *determine* behavior.

That principle is particularly true in the formation of memory. The discovery of a master switch for long-term memory reveals nothing about which memories will be stored or what shape they will take in storage. Rarely are events experienced in isolation; generally, a person brings a good part of the past to bear on present circumstances. Memory making in the present is undoubtedly affected by the memories already established. Science may never be able to fathom the complexity of past, present and future events and interactions as they take place in the mind. The most immediate danger of memory pills, if any become available, is that most of their effects will be unpredictable.

The most serious worry about such a technology, though, may be what is entirely predictable. The burden of an overpowering memory, as the case of Shereshevsky shows all too clearly, may be unsupportable. Indeed, much is known about eidetic, or photographic, memory from studies of its presence in elementary-school children. As many as half such children possess it up to puberty, after which it disappears in all but a few. Eidetic memory probably helps a developing mind assimilate new facts in early life. But as children reach adulthood, eidetic memory gives way to the unconscious process of filtering, sorting, evaluating and overlooking that is necessary for living in a world in constant flux. Chronically circumventing that process through pharmaceutical memory enhancement could bring about inconceivable difficulties.

AT THE SAME TIME, FEW THINGS IN DAILY LIFE are more frustrating than the experience of forgetting. Who would not rejoice to be spared the embarrassment of blocking a person's name, of blanking on a speech, of stumbling over the performance of a song? What but cause for celebration would it be if aging did not bring the humiliation and loss of self that comes with deep forgetting? If we honor Homer for his humane understanding of memory, we also marvel at the virtuosity of his memory in performance. Who today could commit to memory 15,000 lines of verse, as Homer did? In an age of scripts and teleprompters, how wonderful it would be to travel light, to kick away the mental scaffolds and speak from memory, confident that our faculties will not fail. That, in part, is the promise and vision of our work. •

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