Grand Gamble on Fruit Fly Learning

Cold Spring Harbor is betting millions that the genetic workhorse Drosophila *can provide a good model system for studying memory, but many in the field remain skeptical*

NEUROBIOLOGIST TIM TULLY HAS ENDURED years of tedium branding simple memories into fruit flies' brains—a process that requires repeatedly placing the insects in plastic tubes, wafting chemical vapors over them, then administering electric shocks to teach them to shy away from the odors. For all its monotony, however, the routine promised exciting results: insights into the genetic underpinnings of learning and memory.

Gnat-sized *Drosophila melanogaster*, a workhorse in genetics, has won devotees in neuroscience because of its surprising ability to form simple memories. But the tedious Pavlovian conditioning required to train the insects has caused many a case of experimental burnout among budding fly neuroscientists. Recently, however, Tully had a better idea: a "Teaching Machine." At Brandeis University, he began developing a computerized system into which he could load a swarm of untrained flies, push a few buttons, and automatically train the insects, leaving him time to dream up his next experiments.

Tully's machine should soon be in heavy use at Cold Spring Harbor Laboratory (CSH), where he joined the staff this month (September) to help launch an ambitious new neuroscience program on *Drosophila*. For Tully, it will be the chance of a lifetime. The brainchild of lab director Jim Watson and assistant director Bruce Stillman, the program will occupy a third of a new $22.5-million neuroscience center at CSH, have an annual operating budget of about $1 million, and give its three principal scientists, including Tully, access to the lab's considerable expertise in molecular biology.

The goal of the new group is to zero in on specific mutations that prevent the flies from learning and remembering their simple odor-avoiding trick. If the scientists can do that, they will have validated an important model system—the fruit fly—for use in exploring the genetic basis of learning and memory. That would be a boon to neuroscience. But it's a big if, since many neuroscientists view CSH's new fly program as an odds-against gamble. Indeed, Stillman concedes that "a lot of neurobiologists advised us against" committing large sums to the fly program.

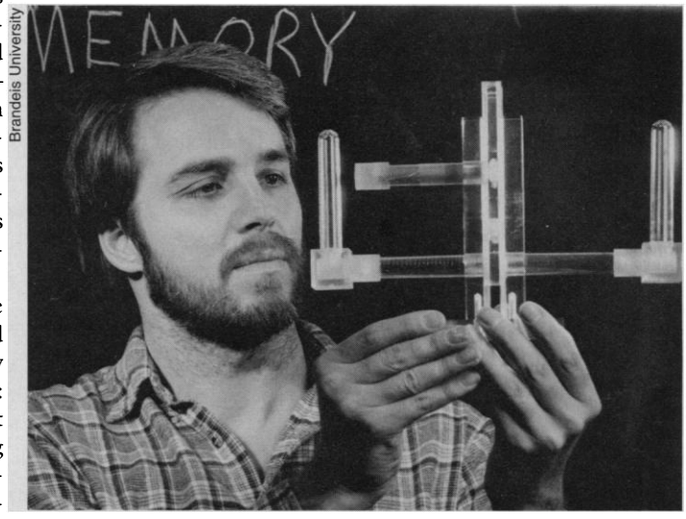
The skepticism stems from a view—widely held among learning and memory researchers—that *Drosophila* hasn't lived up to its early promise for studying cognitive phenonema. Although mutants with apparent learning defects were identified in the 1970s, the field hasn't moved very far since then. Indeed, not all researchers agree that those early mutant strains actually had specific learning defects—as opposed to defects in other areas (sensory systems, for example). What's more, identifying the specific genetic defects has proved frustratingly difficult in most cases.

And yet in spite of these obstacles, many in the field aren't writing off the new CSH fly lab. One reason: Jim Watson's excellent track record at pulling big gambles. Says widely respected Salk Institute neuroscientist Charles Stevens: "Jim Watson has a good sense of what people can do. I think it's a good bet."

Both the excitement at Cold Spring Harbor—and the skepticism of others in the field—stem from the high but largely frustrated expectations raised when the fly was introduced as a model for learning in the mid-1970s. That was when, in the laboratory of biology professor Seymour Benzer at the California Institute of Technology, researchers discovered flies could be trained by simple Pavlovian methods.

The most persistent of the researchers in Benzer's lab working on those techniques was William "Chip" Quinn, now at the Massachusetts Institute of Technology. Quinn's method (an improved version of which Tully is automating) was to expose 40 or so of the insects to an odor in a plastic tube, then shock them with a conductive mesh lining the tube. Typically, about 65% of the flies later avoided the shock-associated odor. The learned avoidance didn't last long: Over a few hours the memory of the shock seemed to fade out in most flies. But the finding provided a handy test for learning and memory deficiencies.

Using that test to screen chemically mutated flies, over the next few years researchers isolated five strains that had essentially normal olfaction and aversion to shock, but abnormally low ability to associate odors with shocks. The mutants had somewhat different deficiencies—most were poor learners, but one, named *amnesiac*, showed nearly normal learning ability followed by rapid forgetting.



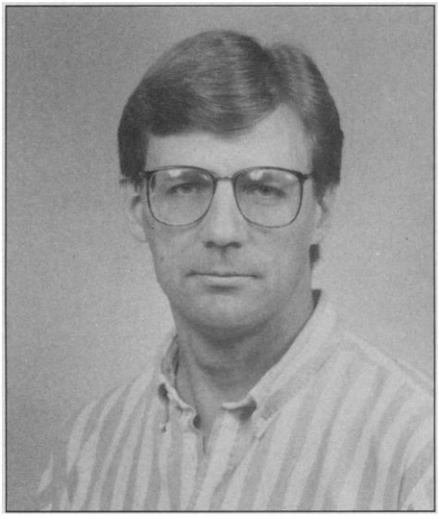
Trainer of the flying circus. *Tim Tully holds up part of the apparatus he uses to train fruit flies.*

Excitement grew when another of the five, dubbed *dunce*, was shown to have a defective gene for an enzyme regulating levels of cyclic AMP (cAMP). Fly researchers were ebullient because earlier work with the sea slug *Aplysia* had shown cAMP is a pivotal part of a "second messenger" signaling pathway in nerve cells that helps form associative memories. A similar striking connection was made in the early 1980s, when a gene defect in another of the mutants, named *rutabaga*, also was shown to affect the cAMP pathway.

It was a bang-up start, but not long afterward, the magic ran out. No more mentally deficient strains were found. Work on most of the existing mutants was stymied by lack of chromosomal markers needed to pinpoint the defective genes. Research on the few genes and proteins that had been identified was hindered by the fact that scientists didn't know where and when the genes were activated in *Drosophila*'s brain, and by other

problems. In 1989 fruit fly researcher Martin Heisenberg of the University of Würzburg in Germany declared in a review of the research area that the "goldrush...is over."

After the end of the goldrush, it's going to take some pretty compelling results to change the minds of a lot of neuroscientists—including some who are centrally placed. "I don't see that [the fly model] merits enormous funding," says Daniel Alkon, chief of neural systems research at the National Institute of Neurological Disorders and Stroke. The fruit fly work, Alkon argues, is dogged by two major technical problems: an inability to locate the cells where purported memory-affecting genes



Reinforcements. *Ron Davis is joining the CSH fly program from Baylor.*

are active and uncertainty about whether such genes actually modify learning and memory, as their discoverers claim. The mutant flies might, for example, have subtle olfactory defects that could account for their deficiencies in associating smells with shocks. "It's very hard just to analyze normal olfactory function" in flies, adds Alkon.

And Salk's Stevens, although he's a fan of Watson's managerial capacities, still has doubts about the fly. The new fly program, he says, "faces extremely difficult problems," particularly the one mentioned by Alkon—establishing clearcut cause-and-effect links between genes and memory-related behavior. But Stevens adds that "history shows you can find very specific genes that affect very specific functions."

For their part, Cold Spring Harbor scientists readily concede that the skeptics have some good points. Tully recently acknowledged in a review article in *Trends in Neuroscience* the "sinister" possibility that Alkon is right: Fly researchers may have mistaken subtle motor or other physiological problems in certain mutants for cognitive ones. Adds Stillman: "We have kind of stuck our necks out" with the *Drosophila* program. "But we had a strong feeling that we should do something on cognition. We also decided it would be very valuable to have a good genetic system to integrate" with the new neuroscience center's two other main research areas—the human brain and the structures of neural molecules. "If one chooses to study genetics and cognition, *Drosophila* is about the only thing available," Stillman argues.

The program's proponents also predict that it will provide technical and scientific advances over the next year or so that will dispel doubts about its promise. One area where strides are being made is in localizing the effects of some of the putative "memory" genes in the fly brain. Neurobiologist Ronald Davis, who is joining the program from Baylor University, has led studies indicating where the *dunce* gene comes into play. Using antibodies to its enzyme product, his team found that it's expressed at elevated levels in a *Drosophila* brain structure called the mushroom body. That finding represents an intriguing link with higher animals, because the mushroom body is in some ways analogous to the hippocampus in mammals and, like the mammalian structure, is thought to integrate sensory input and help form memories.

Davis and colleagues have buttressed the idea that the *dunce* gene has an important cognitive role in studies on homologous genes they've identified in mice, rats, and humans. In one study, they showed that the enzyme product of a rat version of the gene is inhibited by an experimental antidepressant drug called rolipram, suggesting the gene affects mood in mammals.

The CSH team also hopes to identify other single-gene mutations that are clearly cognitive and to pin down those genes' mechanism of action. Part of their hope springs from the use of transposons, "jumping genes" that can be used to insert a piece of DNA into one of the fly's genes at random and thereby disable it. By incorporating a marker (which turns the fly's brain tissues blue, for instance, or produces a recognizable eye color) into the transposon, they can quickly screen for single-gene mutations and locate the mutant genes' anatomical sites of action. That accelerates the process of generating mutants whose gene defects are known to affect brain cells, and the researchers are betting they'll soon find interesting cognitive defects among such flies with the Pavlovian screening test.

Now that interesting mutants can be generated quickly, says Davis, "behavioral screening is the limiting factor" in establishing which of them have true cognitive defects. And that's where Tully's work on training and screening flies comes in. Partly to show doubters that clearcut, one-to-one correspondences between fly genes and cognitive effects can be established, Tully is developing a new screening test based on "nonassociative" memory: habituation to repeated cues.

Normal flies jump up and buzz around when exposed to noxious chemicals, says Tully. But they jump less and less when habituated by repeated exposures. According to unpublished experiments he recently conducted, at least some of the purported associative memory mutants show the usual jump responses, but markedly lessened ability to become habituated. That unambiguously suggests they have memory-chemistry defects, Tully argues—because if flies had physiological problems, such as a tendency to tire quickly when jumping, they would become habituated faster than normal. "To explain the aberrant habituation as a sensory or motor problem, you'd have to concoct a very complex and unlikely scheme," he adds.

Such habituation tests could help answer the criticism raised by Alkon and others that those working with flies haven't always rigorously distinguished learning and memory defects from other types of deficiencies. Habituation tests, however, won't help elucidate how associative memory defects operate. But Tully's new teaching machine might, partly by standardizing fly training so that associative memory experiments yield more clearcut, easily repeated results. After sets of flies are loaded into its chambers, a personal computer releases odors into it and shocks the insects in programmable cycles.

The system also promises to facilitate long-term memory studies that previously have been thwarted by fast fadeout of flies' conditioned responses. By repeatedly conditioning normal flies over several hours—a process too labor-intensive to do on a large scale without automation—Tully and Brandeis colleagues recently have gotten 70% of the insects to avoid a shock-associated odor for at least a day. Avoidance responses can still be detected after 4 days, he adds.

These technical and conceptual advances won't win over the doubters immediately. But, along with other tricks that Tully and Davis say they have up their sleeves, they make up a program that the researchers and their Cold Spring Harbor backers hope will bring fly learning studies out of the stage of being regarded as trickery and into the realm of respected science. As Tully says, fly learning and memory research "has always been regarded in science as a lot of cute hocus-pocus. Now it finally may be coming of age." ■ DAVID STIPP

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