



Research With *Drosophila* Provides Clues to Enhancing Human Memory

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BOSTON—The sea slug, *Aplysia*, is not the only invertebrate with a memory that can provide clues to understanding how human memory operates. Although *Aplysia* was the organism of choice for the investigations into learning and memory of neurobiologist Eric Kandel, MD, of Columbia University, who recently shared the Nobel Prize in physiology and medicine, studies of the modest cognitive abilities of the fruit fly, *Drosophila*, also have been providing insight into the biologic and genetic basis of memory for decades.

Researchers such as Tim Tully, PhD, of Cold Spring Harbor Laboratory in New York, have focused their efforts on elucidating the alterations in neuronal synapses that provide a physical basis for memory in *Drosophila*. They have identified numerous genes associated with memory and learning in the organism, and lessons derived from their work are helping to improve understanding of human cognitive functioning and are pointing to new therapies that may help treat various forms of cognitive dysfunction, said Tully at the American Neurological Association's annual meeting here in October.

Although fruit flies are simple organisms with fewer genes in their genome and fewer neurons in their neural network than humans, they are still capable of an elemental form of learning, said Tully. Because many of the basic mechanisms of learning and memory have been shown to be evolutionarily conserved, research in one animal species sheds light on others.

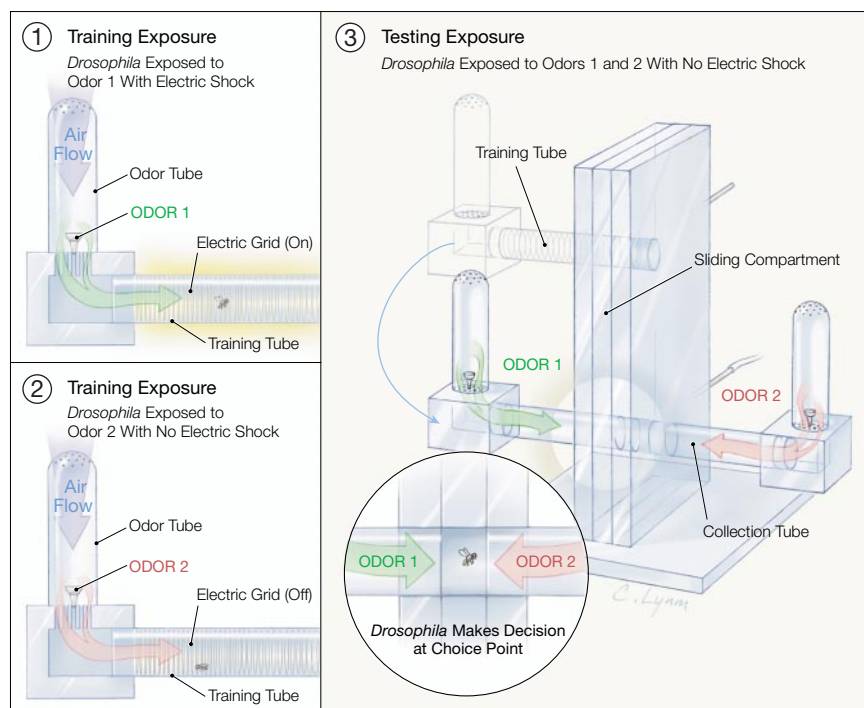
"We can do gene discovery in simple systems like the fly with every expectation that it is a fast track, an economy of scale, to come up with candidate genes involved in the process in humans," said Tully, who pointed out that the difference between organisms has to do with neural circuitry, not the underlying neural plasticity. In other words, "flies are Philco radios, and we are MacIntosh computers, but both run on transistors."

PAVLOV'S FLIES

The discovery of genes for behavior started in the laboratory of Seymour Benzer, PhD, at the California Institute of

Technology 30 years ago with *Drosophila*. In the 1980s, Tully, a student of one of Benzer's students, joined the search for behavioral mutants that demonstrated deficits in learning and memory.

"I applied a Pavlovian notion to this fruit fly task and essentially developed Pavlov's flies," he said. Pavlov had simplified learning into an elemental form—namely, that of a change in behavior produced by the temporal association of two stimuli. "Because it's so simple, we believe it's the elemental form of more complex types of learning that humans are capable of."



This teaching machine is used to isolate memory mutants—flies that cannot associate an odor with electric shock—among the *Drosophila* studied. About 100 flies enter it at one time.



Behavioral mutants were isolated on the basis of a failure to demonstrate associative learning between an odor and an electric shock. The experiment was carried out in a multichambered “teaching machine” in which flies can be exposed to odors wafting through on air currents and can receive shocks from an electrifiable floor. The unit even has a tiny elevator that transports the flies from one stage of the experiment to another.

Flies were loaded into a chamber, exposed to an odor, and administered a shock. Next, the chamber was cleared out and the flies were exposed to a second odor, but this time they received no shock. Finally, flies were transported via the elevator to another area of the unit where they were exposed to both odors and allowed to choose which they preferred. Most flies learned to move away from the odor associated with shock, but some did not. Controls were included in the experiments to show that flies were indeed learning and not just mimicking the behavior of other flies. Experimental design also demonstrated that the mutant flies’ inability to learn truly resulted from the inability to make a connection between stimuli, not from a defective sense of smell or inability to react to shock.

Once the behavioral mutants were identified, their biochemical and molecular composition was studied. One mutant of particular interest, said Tully, had a defect in the gene coding for cyclic adenosine monophosphate response element binding protein (CREB), which is a transcription factor involved in regulation of new gene expression. Because evidence throughout the animal kingdom suggests that long-term memory formation requires protein synthesis, whereas short-term memory formation does not, Tully’s group hypothesized that CREB might occupy a unique position in the pathway involved in turning on long-term memory.

MEMORY SWITCH

Subsequent studies showed that by disrupting CREB, the investigators could

impair long-term memory. Conversely, they showed that by genetically switching CREB to an “on” position, they could produce a fly with the equivalent of a photographic memory. Enhancing CREB did not produce more memory per se; rather, it reduced the amount of practice needed to convert short-term memory to long-term memory.

The fly with the enhanced CREB activity, quipped Tully, “was equivalent to those guys we PhDs hated in college—most of them went on to be MDs—who could read a page in the text and remember the facts with no additional practice, while the rest of us toiled repeatedly over the same material.”

This research indicates that activity in specific neuronal circuits can, under the right circumstances, activate the so-called CREB switch and change and regulate gene expression in the underlying neurons, stated Tully. And the change in gene expression ultimately results in synaptic growth that changes and fine-tunes the neural circuitry.

More recently Tully’s group has been attempting to identify drugs that enhance the CREB switch in cell culture in a manner analogous to the genetic flipping on of CREB. They have identified 10 drugs so far that not only increase regulation of the CREB gene but also enhance the regulation of endogenous CREB-dependent genes in primary neuronal cultures and in flies. Preliminary results indicate that a partial enhancement of memory can be achieved by a feeding regimen with one of the chemicals, said Tully. “The drug is beginning to produce long-term protein synthesis-dependent memory from training conditions that normally only produce short-term memory.” These experiments are now being carried out in rodent models and Tully said the results look promising.

Should these drugs be proven to work safely and effectively, they could be used to enhance long-term memory formation in humans. Tully pointed out there

is a growing body of evidence among cognitive psychologists that shows that specific experiences, or “brain exercises,” can be used to drive the normal process of synaptic refinement. While these brain exercises improve memory on their own, memory enhancement drugs could be used to augment these exercises by turning up the CREB switch.

BRAIN EXERCISE

“We think it might be possible to exercise your brain on a periodic basis, maximize the economy of that exercise by drug augmentation, and keep your brain fine-tuned longer into the aging process,” he predicted. These drug enhancers also may be useful in improving treatment for individuals with cognitive problems, he added. For example, they could lessen the time it takes a patient to recover from a stroke by reducing the amount of practice needed to achieve improvement in the area of deficit.

The search for other genes and pathways involved in memory formation continues in Tully’s laboratory. In this quest, DNA microarray technology is proving invaluable. Microarrays—postage stamp-sized devices dotted with snippets of DNA or RNA that are used to study gene expression—are helping researchers not only to identify more genes that are transcriptionally regulated during long-term memory formation, but also to compare gene regulation during memory formation in healthy brains with gene expression in the dysfunctional brains of single-gene mutant flies. The pharmacological effects on genomic responses in both single-gene mutants and normal insects given drugs can also be observed with microarrays.

As researchers identify more memory-related candidate genes in the fly that point to homologues in the human genome, fly genetics and genomics will help unravel what has seemed until recently an intractable puzzle—how human memory works. □



Anti-IgE Antibody May Help Treat Some Asthma Patients

Alice T. D. Hughes, MD

SAN FRANCISCO—A paradigm shift has occurred over the past four decades in the treatment of asthma, a disease that during that time has increased in prevalence worldwide, particularly in urban areas of the industrialized West.

This change reflects a greater understanding of the pathophysiology of asthma. Once considered a disease of reversible bronchoconstriction, asthma is now considered a chronic inflammatory disease with the potential to cause irreversible deterioration of pulmonary function. Anti-inflammatory agents, inhaled corticosteroids (ICSs) chief among them, have become mainstays of asthma therapy.

Attendees at the October meeting of the American College of Chest Physicians discussed the limitations of current therapy for allergic asthma and considered therapies on the horizon. Some promising data were reported from several phase 3 clinical trials of a recombinant monoclonal antibody against immunoglobulin E (IgE).

BLOCKING IGE

Patients with atopic disease produce IgE antibodies in response to particular allergens, explained Jeffery Tillinghast, MD, of Washington University School of Medicine. The IgE then binds to high-affinity receptors on mast cells and basophils, releasing mediators that contribute to the symptoms of allergic diseases, including allergic asthma. RhuMab-E25, a recombinant monoclonal antibody against IgE consisting of 95% human and 5% murine amino acids, blocks this high-affinity binding of IgE to mast cells and basophils, thereby preventing their degranulation. Current therapies such as ICSs and leukotriene modifiers act later in the allergic cascade, after the inflammatory mediators have been unleashed.

Anti-IgE antibodies were first described in 1993. They were developed by the biotechnology firms Genentech Inc and Tanox Inc nearly simultaneously, said Charles Johnson, MD, a senior clinical scientist at San Francisco-based Genentech. A joint decision was made to pursue the development, testing, and approval of the Genentech-developed antibody, rhuMab-E25. Novartis Pharmaceuticals has also been involved in the collaboration.

DATA FROM THREE TRIALS

Researchers presented data from three phase 3 randomized, double-blind, placebo-controlled, multicenter clinical trials of the anti-IgE antibody as an adjunctive therapy in patients taking ICS for chronic allergic asthma. All three trials had a similar design, with an initial 4-month phase in which patients were given the study drug or placebo while continuing to take stable doses of ICSs, followed by a critical 12-week phase in which physicians attempted to taper ICSs while continuing rhuMab-E25 or placebo.

The drug was given subcutaneously at dosages and intervals individualized according to patients' IgE levels and weight. In two of the trials, one of which was conducted in European as well as US centers, the subjects were adults. The third trial involved only children, in whom IgE mediation of asthma may be more important.

John Georgitis, MD, an investigator at Wake Forest University School of Medicine, Winston-Salem, NC, discussed this age-dependent difference in IgE mediation in a session on the molecular and cellular mechanisms of asthma pathology. "When you look at asthma in a child, it's predominantly IgE-mediated. There are times when it's not, but that's not the norm," he said. "Does it change over time? I think it does. In adults, there is a shift away

from an IgE-mediated response. In adults, the mast cell is not pivotal. It's easy for me to find atopic disease in children. It's difficult in adults."

The trials presented at this conference are not the first to examine the clinical efficacy of rhuMab-E25. Discussing the existing literature, John Fahy, MD, of the University of California, San Francisco, School of Medicine, summarized a study published last year (*N Engl J Med.* 2000;341:1966-1973) that demonstrated a modest improvement in symptom scores in patients treated with ICSs and either rhuMab-E25 or placebo. In that study, there was no statistically significant difference in the extent to which ICS dosage could be tapered in either group.

While the main outcome measures for the phase 3 trials of rhuMab-E25 presented at the meeting were safety end points, the speakers emphasized clinical end points, including whether adjunctive therapy with rhuMab-E25 improved subjective assessments of treatment effectiveness compared with placebo and whether adjunctive therapy had any utility as a steroid-sparing agent, a use that had not been conclusively demonstrated in earlier trials.

Data from the three trials indicated that a significantly greater reduction in ICS dosage could be achieved in adult and pediatric patients treated with rhuMab-E25 than in those treated with placebo. Jonathan Bernstein, MD, of the Bernstein Clinical Research Center in Cincinnati, reported that median reduction in ICS dosage at the end of the 28-week treatment period was 100% in children treated with rhuMab-E25 vs 67% in children treated with placebo ($P=.001$).

Edward Lisberg, MD, of the Asthma and Allergy Center of Chicago, presented comparable findings in adults, describing an 83% median reduction in ICS dosage in those treated with rhuMab-E25 vs a 50% reduction among patients treated with placebo ($P<.001$). Genentech's Johnson, who presented the work of W. James Metzger, MD, of East Carolina University School of Medicine in Greenville, presented data showing a median ICS



dosage reduction of 75% in patients treated with rhuMab-E25 compared with 50% in those treated with placebo ($P<.001$).

However, Philip Silkoff, MD, of the National Jewish Medical and Research Center in Denver, presented divergent data drawn from a subgroup of the pediatric trial. "There was no statistical difference in our ability to withdraw inhaled corticosteroids either in the placebo or the active group," he said.

Silkoff did find that exhaled nitric oxide (NO), a marker of airway inflammation, tended to increase during ICS withdrawal in pediatric patients receiving placebo, while remaining relatively constant in patients treated with rhuMab-E25, perhaps indicating that this agent was keeping inflammation at bay despite corticosteroid withdrawal. The observed differences in exhaled NO levels did not, however, reach statistical significance, he said.

EVALUATION AND EVENTS

Investigators in the pediatric trial and one adult trial examined global evaluations of treatment effectiveness as rated by patients and their physicians at the end of the treatment period. Tillinghast and Lisberg reported that response to treatment was rated significantly more favorably by patients who had received rhuMab-E25 and their physicians than by patients in the placebo group and their physicians. Assessments were fairly favorable in both groups, however, with most patients, even those in the placebo groups, rating their response to treatment as moderate or better.

Lisberg surmised that immunotherapy and sham immunotherapy might be perceived as having a favorable effect because the treatments are physician-administered. He further cautioned that "it's really difficult as an investigator to make these evaluations when you see multiple patients over time. Especially in pediatric trials, we want to give our patients the benefit of

the doubt. Children and investigators tend to overreport responses because none of us want to actually make our kids sicker."

During a question-and-answer session, Tillinghast, an investigator in one of the adult trials, was asked for data on more objective clinical end points, such as change in peak expiratory flow or forced expiratory volume in 1 second (FEV₁) during treatment with rhuMab-E25 vs placebo.

"Obviously, this study wasn't embarked on to look at patients' and investigators' global assessments. There were multiple end points. There was a decrease in bronchodilator use [in patients treated with rhuMab-E25] that was statistically significant. There were statistically significant decreases in daytime and nocturnal symptom scores. There was a statistically significant though modest improvement in FEV₁. These are some parameters to back up the subjective global assessment," Tillinghast responded.

As for safety end points, none of the investigators reported an increased frequency of adverse events in patients treated with rhuMab-E25 compared with placebo-treated patients. Johnson reported no drug-related adverse events, including reported cases of anaphylaxis or immune complex-mediated disease.

In an interview, Johnson acknowledged that in animal studies of the antibody, some cases of dose-related thrombocytopenia had occurred. These findings came to light in August 2000, Johnson said, 2 months after Genentech had submitted a biologics licensing application to the US Food and Drug Administration (FDA).

At that point, the FDA put trials of rhuMab-E25 on hold pending further review of animal and human data. The agency permitted the continuation of the open-label extension phase of the pediatric trial, the only ongoing trial of rhuMab-E25.

In a review of hematologic data from the human trials, investigators found six cases of transient thrombocytopenia in patients treated with rhuMab-E25 and no cases in placebo-treated patients, Johnson said, adding that in no case had the thrombocytopenia been sustained. He said hematologic parameters were monitored regularly in all patients.

Johnson does not expect concerns about thrombocytopenia to delay the FDA new drug approval process.

Another question for Tillinghast was whether there are any potentially negative consequences of a nonselective blockade of free IgE. He responded, "There is nothing beneficial about IgE other than keeping me in business." However, he acknowledged the role of IgE in fighting parasitic disease, saying, "If you were in Africa, it would be a different story."

FINDING A PLACE

Clinicians at the conference expressed uncertainty about where this new therapy, if approved by the FDA, is going to fit into the current asthma armamentarium.

Fahy suggested that patients with asthma and a comorbid allergic disease, such as allergic rhinitis, might be good candidates for the new therapy, as might patients whose disease is not adequately controlled with ICS therapy. He cautioned, "We don't have enough of a database yet to see where it's going to fit . . . we need comparative trials."

Furthermore, he said, "I think it's going to take us several years to figure out the role of anti-IgE in asthma and I have not formed an opinion about where this drug fits in asthma management—and I would encourage you not to form an opinion yet either. It's taken us a long time to figure out how the leukotriene antagonists fit, and we're still learning how inhaled corticosteroids fit, even though they've been on the market for 20 years." □