NEUROSCIENCE

The Remembrance of Blinks Past

One of the hottest areas of neuroscience is the quest to understand where memories are stored in the brain. One potential site that has been the subject of much controversy is the cerebellum. Some have argued that this structure at the base of the brain, which is involved in coordinating motion, also stores memories involving movement. But other researchers have strongly disagreed. In this issue of *Science* (p. 989) Richard Thompson and his colleagues at the University of Southern California present evidence pinpointing a memory to a small area of the cerebellum. Yet some critics of the idea of cerebellar memory remain unconvinced.

Thompson's work centers on a simple conditioned reflex, using rabbits as an experimental model. Puff air in a rabbit's eye, and the animal will blink. And if the puff is consistently preceded by a tone from a loudspeaker, the rabbit soon learns to blink in response to the tone alone.

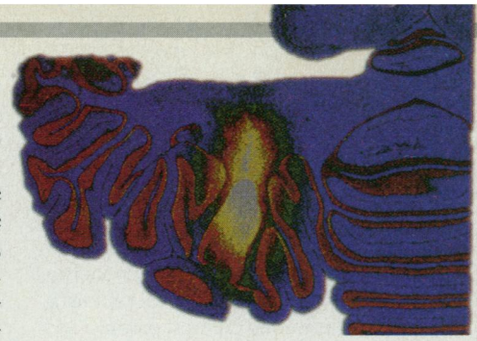
In the 1980s Thompson and others showed the simple type of learning that connects the puff and the blink could be blocked by damage to part of the cerebellum. But the possibility remained that the learning occurs somewhere else, and cerebellar damage was merely a roadblock in a neural pathway, preventing learned information stored beyond the cerebellum from reaching the brain-stem neurons that actually direct the movements.

To get around that problem, Thompson, with co-workers David Krupa and Judith Thompson, used a drug called muscimol to block temporarily different parts of the neural pathway from the cerebellum to the brain stem while they trained the rabbits. Since muscimol's effects wear off, the researchers could discover if they'd blocked memory formation or merely prevented transmission.

In separate experiments, the team used muscimol to deaden either a nucleus in the cerebellum or a structure in the brain stem called the red nucleus, which sits just beyond the cerebellum. When trained under the influence of the drug, all the rabbits barely blinked an eye when they heard the tone. When the muscimol wore off, rabbits whose red nucleus had been drugged blinked away when the tone sounded; they had learned the response perfectly, and the drug had merely blocked transmission of the information from higher up the path. But when the block had been in the cerebellum, the rejuvenated rabbits showed no signs that they'd learned anything at all. That means, argues Thompson, that the memory has to be stored in the cerebellum.

"It's a very clear-cut result, beautifully documented in this paper," says Stephen Lisberger, a neuroscientist at the University of California, San Francisco. As long as there are no unknown neural detours between the cerebellum and the red nucleus, he says, Thompson's conclusion is probably right. Larry Squire, who studies learning and memory at UC San Diego, agrees: "This is probably the best evidence yet that there is localization to the cerebellum." It presents a great object lesson, he adds, for how to seek memory sites elsewhere in the brain.

This enthusiasm, however, isn't universal. John Harvey of the Medical College of Pennsylvania and John Welsh of the New York University School of Medicine have argued that in earlier Thompson experiments the way he tested the animals may have biased the results. Although others say those concerns are resolved in the current paper and other recent work, Harvey and Welsh insist they still see "technical problems" with the new research. They refused to discuss their objections, saying they were preparing a technical rebuttal to Thompson's paper. So learning and memory fans should expect further debate in the cerebellar corner of their field.



KRUPA, THOMPSON AND THOMPSON

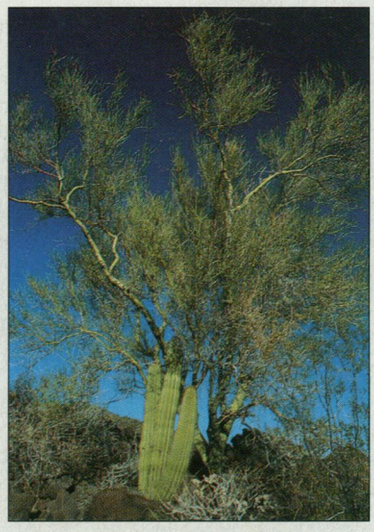
Storage room. Memories seem to be stored in a portion of a rabbit's cerebellum *(light area)*.

–Marcia Barinaga

FORENSIC SCIENCE

Botanical Witness for the Prosecution

Sometime late on the night of 2 May 1992, a woman was killed and her body abandoned in the Arizona desert. A beeper, found near the body, pointed police to the man now on trial for the murder. But the key piece of evidence may be something far subtler and more scientific: DNA sequences from a few seed pods found rattling around the back of the same man's truck. In April of this year, Judge Susan Bolton of the Superior Court of Arizona's Maricopa County ruled that DNA profiles linking the seed pods to a Palo Verde tree near where the body was found could be admitted as evidence in the murder trial.



DOUG SOKELL/VISUALS UNLIMITED

Rugged individual. Palo Verde trees show a lot of genetic variation.

Bolton's decision appears to mark a scientific and judicial first. Although DNA profiles from samples of human tissue are widely used in criminal trials for rape and murder, the Maricopa case appears to be the first use of plant DNA in a criminal case. Those following the status of DNA profiles in the courtroom say the decision is a sign that additional novel applications of the technique are likely to appear as investigators become more aware of its possibilities and begin looking for other kinds of biological evidence from which DNA can be extracted.

When the Maricopa County Sheriff's office first asked molecular geneticist Tim Helentjaris of the University of Arizona, Tucson, to look into the possibility of using DNA profiles to try to match the seed pods in the defendant's truck to an individual specimen of the Palo Verde tree—a bizarre, often leafless tree that can photosynthesize through its branches—at the crime scene, Helentjaris replied that he wasn't sure the job could be done. For one thing, he didn't know whether he could get enough DNA for the analysis from the seed pods. And he also worried that the plants wouldn't have sufficient genetic variability to identify an individual through its DNA profile. But as he pursued his research, Helentjaris learned that Palo Verde trees show a high degree of genetic variation—which made it possible for the pods to take the stand in the trial.

Helentjaris, whose lab has been mapping plant genomes, analyzed the Palo Verde DNA with a technique known as Randomly Amplified Polymorphic DNA, or RAPD, a technique involving the PCR gene amplification method. RAPD uses generic DNA primers that contain as few as 10 bases and thus bind to many sites in the genome. Under the proper binding conditions, each primer produces a reproducible profile of amplified fragments. Helentjaris says that by using multiple

primers he could easily identify the DNA of individual trees.

Indeed, in blind tests, Helentjaris matched the DNA of the seed pods found in the defendant's truck to that of the key tree at the crime site. In one test, he picked the correct tree out of a "lineup" that included the other 11 Palo Verde trees at the crime site; in a second he was able to distinguish that tree from 18 other Palo Verde samples supplied by the sheriff's department. While the seed pods can't place the defendant at the crime site, Helentjaris says, they suggest his truck was there.

The Palo Verde seed pods aren't the only sources of nonhuman DNA that are now making their way into the court system. Just last month, for example, California Fish and Game officials used DNA profiles to match a buck trophy thought to have been poached from Clint Eastwood's ranch to the entrails the hunters had left on the property. And some expert observers think the method may expand into other uses, such as tracing contaminated food products to their sources, and identifying sources of polluted water supplies. Seed companies are also using DNA profiles to keep track of their inbred crop lines.

But if DNA forensics do expand in that way, it will only heighten the controversies surrounding the method, which primarily concern the need for standards for labs doing the tests and the methods used to estimate the frequency of a particular DNA pattern in the population (*Science*, 20 December 1991, p. 1721). As the technique's applications diversify, the debate over standards and means of interpreting the data will no doubt become even more intense.

–Carol Kaesuk Yoon

Carol Kaesuk Yoon is a free-lance writer based in Ithaca, New York.

COLD FUSION

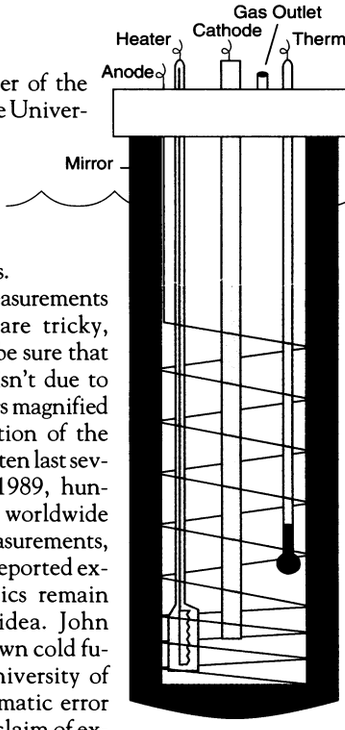
Pons and Fleischmann Redux?

Much of the scientific world wrote off Martin Fleischmann and Stanley Pons not long after the dramatic press conference in which they touched off the cold fusion drama. But though their claims have been widely rejected and their competence questioned, the two continue to work quietly in the French Riviera town of Sophia Antipolis, at the European facility of the Institute of Minoru Research Advancement (IMRA), which is owned by an affiliate of Toyota. And last week the erstwhile celebrities tried to claim a place in the scientific mainstream by presenting their latest cold fusion results in the 3 May *Physics Letters A*, a peer-reviewed journal.

But history may be repeating itself. Despite the paper's respectable venue, a round of calls by *Science* to active and retired players in the cold fusion arena elicited a strong sense of déjà vû. Physicists and chemists who have seen the paper, which reports surges of heat in the familiar cold fusion setup—palladium electrodes immersed in heavy water—say it's old news. Like the report that sparked the furor of 1989, they say, the new work contains baffling assumptions and complicated arguments, fails to document key controls, and leaves the field where it has been since the beginning: in a state of confusion, albeit hovering around the remote possibility of an intriguing new phenomenon in solid state science.

As they did 4 years ago, Pons and Fleischmann ran an electrochemical current for weeks on end between palladium and platinum electrodes in a bath of heavy water spiked with current-carrying lithium ions. During the procedure, the current splits the heavy water molecules, releasing deuterium ions that jam into the palladium in large numbers. That much is accepted on all sides. But Pons and Fleischmann argue that something more is going on, because in the new experiments, as in the original ones, continuous temperature measurements suggest the cells produce more heat than can be explained by the flow of current. At the insistence of *Physics Letters A* editor Jean-Paul Vigier of the Pierre and Marie Curie University in Paris, however, the paper never invokes "nuclear fusion" as an explanation, though it does rule out chemical mechanisms.

Such long-term measurements of heat production are tricky, however; it's hard to be sure that the apparent excess isn't due to subtle systematic errors magnified by the extreme duration of the experiments, which often last several months. Since 1989, hundreds of researchers worldwide have made similar measurements, and some have even reported excess heat. But skeptics remain cool to the whole idea. John Huizenga, a well-known cold fusion critic at the University of Rochester, says systematic error could underlie every claim of excess heat—by Pons and Fleischmann as well as by all the researchers who followed their lead.



Hot cell. Will a palladium cathode in a vessel of heavy water bring electrochemistry to a boil again?

That may explain why Pons and Fleischmann go on to present what they think is a more intuitive demonstration of excess heat than long-term measurement: simply clocking how long it takes hot deuterium-charged palladium rods to boil off a roughly known amount of heavy water. They know the rate at which electric power is going into the cell, and they can calculate how much power should be consumed in boiling off the water. The power input falls short by a factor of about 4, they say. And that, they infer, indicates that some heat-generating process must have kicked in to make up the difference.

The reported surge of heating is much larger than other workers in the field have claimed. To be sure, the boiling lasts only minutes, but the total amount of heat released over the 25-day span of the experiment (including the boil-off period) still comes out to 6% more than the input of power could explain, notes Michael McKubre of SRI International. He says his own experiments, which he is just now restarting following an explosion that killed a colleague early last year, have shown a 3% energy excess.

But even researchers who believe something unusual, if not fusion, is going on in the deuterium-loaded palladium say the boil-off method is far from conclusive. And one active cold fusion researcher, Richard Oriani of the University of Minnesota, says he found the paper too difficult to assess with any confidence.

As for skeptics like chemist Nathan Lewis of the California Institute of Technology and physicist Richard Petrasso of the Massachusetts Institute of Technology, both of whom were among the most active and vocal critics during the fiery initial days of cold fusion, they find the new results only too familiar. Says Petrasso: "I'm neither interested enough, nor do I have the time, to look at it." Lewis did look at the paper but finds "nothing in it to make me change my views," he says. "They never say how reproducible [the claimed phenomenon] is; they lack controls; it's the same old stuff." Pons and Fleischmann could not be reached for comment before press time.

One thing all sides can agree on is that the new paper isn't going to change long-held positions in the cold fusion debate. As before, says McKubre, "Most people would say [Pons and Fleischmann] either don't know what they are doing, they are frauds, or they are right."

–Ivan Amato