Technical Advances Power Neuroscience

New methods—for cell culture, genetic manipulation, and imaging—are offering insights into the brain that weren't available even a couple of years ago

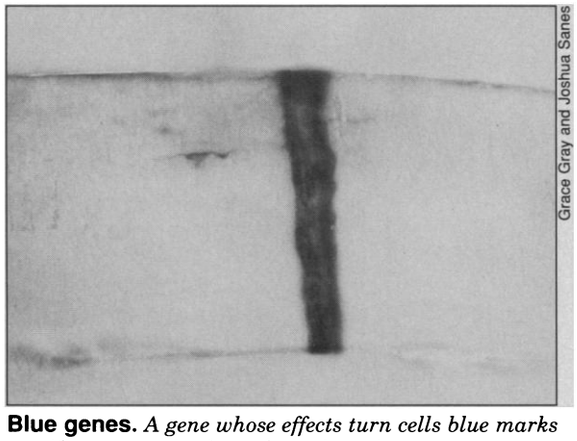
FACED WITH AN ORGAN AS COMPLEX AS THE brain, even the most highly skilled neuroscientists must feel at times like the blind men encountering the elephant: each technique yields intriguing—but frustratingly incomplete—information about the subject as a whole. Yet neuroscientists are endlessly resourceful in developing new ways of attacking their problems. At this year's meeting of the Society for Neuroscience, held in St. Louis, it was clear that, through such resourcefulness, which makes new techniques available almost continually, the "blind men" are acquiring a more sensitive touch that is bringing them closer to their goal of comprehending the beast.

Among the new advances discussed at this year's meeting were genetic tricks that aid both the study of neuronal development and the creation of neuronal cell lines, means for observing the activity of hundreds of neurons at once, and imaging technology for spying on the thinking human brain. These methods are providing countless new insights that were out of reach as recently as a year or two ago.

One area in which researchers have made recent leaps forward is the quest for ways to create immortal cell lines from specific types of nerve cells. A cell line is like having "cells on tap," says David Anderson of the California Institute of Technology. It provides an endless supply of cells, opening the door to biochemical and physiological experiments that aren't possible with the small numbers of nerve cells that can be taken from an animal. But for years this goal seemed hopelessly beyond reach because mature neurons, which never divide, are not easily coaxed to revert to the continuously dividing state necessary for creating a cell line. The solution, achieved in a number of laboratories, proved to be to get the neurons at a vulnerable stage of development, before they "grow up."

Caltech's Anderson, along with postdoc Susan Birren, used a neuronal cell line to address a fundamental question in neuronal development: how chemical factors influence a neuron's fate. The cells they chose to study take one of two developmental paths, becoming either neurons in the sympathetic nervous system or secretory cells in the adrenal gland. Researchers suspected that the choice is influenced by molecules in the cells' surroundings. Exposure to nerve growth factor (NGF), went the theory, could make the immature cells become neurons, but if they wandered close to the adrenal gland, they might fall under the influence of cortisol (an adrenal hormone) and become secretory cells instead. That hunch remained untested in embryonic cells because no one could get enough of them.

Anderson developed a scheme for purifying the cells, and immortalized them with special retroviruses engineered by Connie Cepko of Harvard University and Ronald McKay of the Massachusetts Institute of Technology. He then quickly uncovered a key element of the puzzle that others had missed: the cells can't respond to NGF until they are first primed by fibroblast growth factor (FGF), which induces them to make NGF receptors. Only then can they come under the influence of NGF and complete their conversion to sympathetic neurons. Infant neurons that escape this fate apparently move on to the adrenal gland, where cortisol causes them to become secretory cells. These results in culture have prompted a search for FGF in the appropriate parts of the embryo.



Grace Gray and Joshua Sanes

Blue genes. *A gene whose effects turn cells blue marks specific neurons in the embryonic optic tectum.*

Richard Weiner of the University of California, San Francisco, Pamela Mellon of the Salk Institute, and their colleagues also made a neuronal cell line, in this case from the elusive population of brain neurons that control reproductive hormone levels by releasing pulses of gonadotropin releasing hormone (GnRH). Among the billions of neurons in the brain, says Weiner, "there are only 1500 neurons that release this hormone. It's always been an arduous task to study them." Physiologists have been eager—but unable—to find out what makes the neurons release GnRH in rhythmic pulses.

To find out, Weiner and Mellon first injected mouse embryos with a tumor-inducing gene that had been genetically engineered to be expressed in GnRH cells. Some of the mice from those embryos grew tumors of the GnRH cells; the researchers then harvested the tumor cells to found the line.

The cells appear very similar to the GnRH neurons, says Weiner. And they have already shed some light on the longstanding puzzle of pulsed release. In culture, the immortalized cells connect to each other and rhythmically release GnRH. That suggests the rhythm is inherent in the cells and doesn't require input from other brain regions. Weiner plans to use the cell line to test for drugs that disrupt the pulsing—and could therefore be new forms of birth control.

New techniques are helping researchers study the development of nerve cells in the animal as well as in culture. In the past, researchers have marked young cells with dyes, then followed their descendants in the developing organism. But those dyes often became diluted to invisibility after a few rounds of cell division, leaving researchers to dream of some more long lasting marker. Several labs, including that of Joshua Sanes of Washington University in St. Louis, have come up with a molecular version of indelible ink: viruses that enter the cells' DNA, marking the cells and all their progeny. The viruses are easily noted, because they carry the gene for beta-galactosidase—an enzyme that catalyzes a reaction that turns the cells blue in the presence of a synthetic substrate.

Sanes is using these "blue genes" to draw up a family tree for neuron precursors in

chicken brains. So far, he has learned that certain progenitor cells choose one of several different migratory routes into the brain, then turn either into one of several types of neurons or into support cells called glia, depending on the route they take. "That kind of information had not been obtainable by any other method," Sanes says.

Every neuroscientist knows that regulatory genes play a critical role in brain development. But which genes? And where do they act? In attempts to answer such questions, researchers who study vertebrates have been frustrated by their inability to knock out the function of a particular gene and see how the mutation alters development. "This is the only way you can actually determine the function of a gene," says Mario Capecchi of the University of Utah.

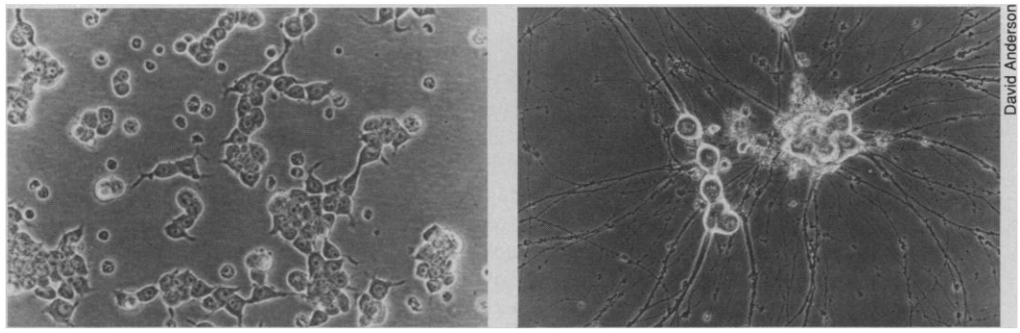
Until recently, such experiments were impossible in vertebrates because site-specific recombination, the kind necessary to mutate a chosen gene, is a very rare event. But in the past several years, Capecchi and others have developed schemes to select for that rare event, and at the neuroscience meeting Capecchi presented some of the first results showing that directed mutation can have dramatic effects on the nervous system.

The gene Capecchi chose to mutate, *int-1*, codes for a growth factor–like molecule thought to be a key developmental signal. The notion proved correct. Mice lacking the gene grow to adulthood but are missing all or part of the cerebellum, a brain structure that helps to coordinate movement.

The specificity of the defect resulting from the mutation came as a surprise, because *int-1* is expressed in many places in the developing nervous system. The result adds to a growing body of evidence for overlapping roles for developmentally important molecules—a kind of genetic safety net Capecchi believes will turn out to be a hallmark of developmental processes. "You don't want to be so susceptible that one genetic mistake kills the embryo," he says.

Although genes clearly play a central role in embryonic development, they aren't by any means the whole story. Indeed, in the nervous system, patterns of electrical activity are thought to guide the formation of the correct neuronal connections. But studying those activity patterns requires recording electrical activity from many neurons simultaneously—a technical feat that was impossible until quite recently. "For many, many years now, a big issue has been how are we going to study more than one neuron at at time," says Carla Shatz of Stanford University. "Now we've got some techniques that are making that possible."

One of those techniques is known as multiunit recording. Shatz's lab, in collaboration with Marcus Meister, a postdoc with Dennis Baylor at Stanford, has made use of a multielectrode array developed by Jerome Pine of Caltech, and adapted by Meister for recording from retinas of fetal cats and neonatal ferrets. Even before these animals open their eyes, spontaneous activity occurs in the nerve cells of the retina. This activity is thought to help shape the connections between the retina and the brain. Modelers of brain development have hypothesized that for this process to be carried out correctly, nearby retinal neurons need to fire together, while more distant neurons fire out of synchrony.



Different fates. *Embryonic cells exposed to an adrenal hormone become adrenal cells (left); those exposed to fibroblast growth factor and nerve growth factor become neurons (right).*

Shatz and her colleagues removed retinas from fetal animals and placed them on the tiny (1 millimeter) array, consisting of 61 electrodes. They were able to record the simultaneous activity of about 100 retinal neurons. What they saw—waves of activity spreading across the retina—could never have been observed using single-cell recording techniques. The wave-like pattern confirms the modelers' predictions. Cells are more likely to fire in synchrony with their close neighbors than with cells farther away.

Michael O'Donovan of the National Institute of Neurological Disease and Stroke presented a similar observation at the neuroscience meeting—but his came from the spinal column of chicks. He used a sensitive video camera and specialized dyes that fluoresce in response to the calcium influx that accompanies electrical activity.

Like the neurons of the retina, the spinal cord neurons that control walking begin to fire spontaneously while the chick is still in the egg, an activity that may help tune the neural connections that later control walking. O'Donovan loaded the neurons with a calcium-sensitive dye and saw a rhythmic pattern of activity apparently beginning with a cluster of small neurons near the motor neurons. Since these small neurons are hard to record from directly, O'Donovan says that without the dye he would not have known they were involved in the activity, let alone that they may initiate it.

The recent advances in neuroscience techniques apply not only to neurons, but also to whole brains as well. One example is a high-resolution electroencephalogram system (EEG) developed by Alan Gevins of EEG Systems Laboratory in San Francisco. Gevins' device records from 124 sensors on the subject's scalp and provides a tantalizing view of the human brain in thought. Although the EEG cannot pin down the actual sites of activity as precisely as static brain imaging methods such as positron emission tomography (PET) can, it complements PET with real-time recording that can keep up with the very rapid pace of brain activity. For example, Gevins has caught the brain in the act of mental preparation. In the split second of anticipation before subjects saw a signal to which they would have to respond, he saw activity in brain areas that would be activated a moment later during the execution of the task. The pattern, Gevins says, is "suggestive of a mental rehearsal."

In subjects performing a variety of mental tasks, Gevins sees rapidly changing centers of activity around the brain, localized with a precision never before possible with EEG. But as with many of the experiments displayed at St. Louis, the high-resolution EEG is not an endpoint. It is simply another advance—and one that calls for other new technologies to address the questions it raises, questions such as how the cells of those active brain regions are storing information and communicating it to one another.

Showing his audience a 19th-century photograph of a subject wearing a barbaric-looking "brain stimulating" device, Gevins made a comment apropos to all of the fast-moving, technology-driven areas of neuroscience: "I show you this not to make fun of it," he said, "but to make the point that in 10 years, or maybe 20, what we are doing now will look just as primitive." ■ **MARCIA BARINAGA**