# Research in Progress

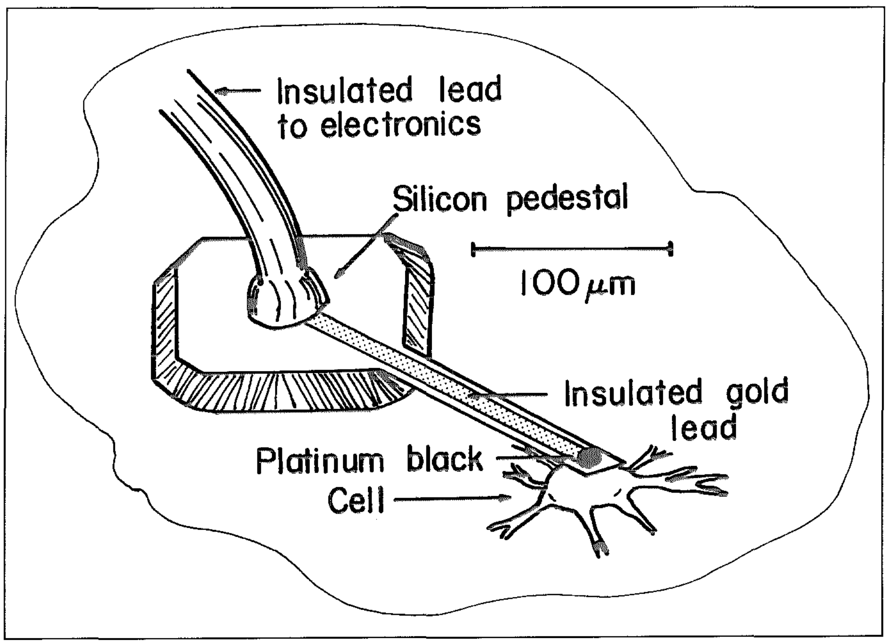
## The Neuron-Silicon Connection

NEUROPHYSIOLOGY is a tough business. If you want to study the electrical activity of nerve cells, you're placed on the horns of a dilemma. You can insert a fine glass electrode into a neuron to get detailed information on a single cell, but the electrode often kills the cell after a short period, and it's very difficult to insert two intracellular electrodes into neighboring neurons to study their interactions. Or you can use an extracellular electrode, which can be left in the brain of an active animal for much longer periods without doing harm and which can record the activity of neighboring neurons simultaneously. But an extracellular electrode is too distant to provide detailed information on the inner workings of any of the neurons in its vicinity.

Resolving this dilemma is the project of two Caltech researchers: Jerome Pine, professor of physics, and David Rutledge, associate professor of electrical engineering. Pine, a physicist turned neuroscientist, and Rutledge, an expert in microfabrication techniques, are collaborating on the development of microdevices that might eventually allow researchers to stimulate and record from dozens of individual neurons hooked up into complex networks. And beyond that, the devices might be implanted into humans to form an element of a high-precision neural prosthesis.

For now, the researchers would be happy to build a device that would allow long-term intracellular monitoring of a single neuron kept alive in a culture dish. Graduate student Wade Regehr is constructing a prototype "diving board electrode," illustrated here, using techniques developed for etching silicon surfaces in the fabrication of computer chips. A silicon pedestal will support a fine gold electrode suspended just 20 μm off the surface, less than a third the width of a human hair and just about the thickness of a cultured neuron. The electrode will be insulated with a mixture of silicon dioxide and silicon nitride, except for a contact hole about 5 μm in diameter. Regehr will electroplate the gold in this exposed area with platinum black, a material that looks something like cauliflower under a scanning electron microscope, to greatly increase the electrode's effective surface area.

Conventional intracellular electrodes are made of glass micropipettes, drawn down to extremely fine points and filled with conductive fluid. Such an electrode is gently poked into a cell, the cell membrane seals around the glass and, if things go well, researchers can stimulate the cell and record from it for periods ranging from minutes to hours. But a glass electrode requires bulky and expensive equipment to keep it steady so it doesn't rip open the delicate cell membrane. The diving board electrode should avoid this problem. Using a micromanipulator, an experimenter will position the diving board over a cell, glue the pedestal to the culture dish, and, with a brief burst of high voltage, blast a hole in the cell's membrane. The membrane may then seal itself around the silicon insulation, forming a stable intracellular connection. The $64,000 question is just how stable this connection will be. Says Pine, "We don't know whether the cell will tolerate it for a minute, an hour, a day, or for weeks." Even if a stable intracellular connection cannot be formed, an electrode just outside the cell membrane would form an intimate extracellular connection, still very useful for two-way com-




*Pine and Rutledge hope that the diving board electrode will allow long-term recording and stimulation of a neuron maintained in cell culture.*

munication with a neuron.

But the researchers, operating under the assumption that they *will* learn how to achieve a useful connection with a single cell, are already starting on the next stage of the project: building "neurochips" that will house at least 16 cultured neurons on a silicon wafer. Immature neurons will be injected into tiny wells, which will have electrodes at their bases. Undergraduate Heidi Langeberg is working on the delicate task of constructing the neurochips. One problem she's facing is that the thin, delicate silicon wafers must be etched from both the top (to produce the well) and the bottom (to produce the electrode). In addition, she must overcome the tendency of cultured neurons to migrate out of their wells. She's dealing with this by etching the top of the wafer into a fine gridwork of silicon dioxide bars that will overlay each well. The bars have to be far enough apart to allow for the injection of an immature cell and the outgrowth of axons and dendrites. But the bars have to be close enough together so that the cell body, after a day or two of growth, couldn't "walk" away from its electrode.

Since the wells in the neurochip will only be 100 μm apart, the axons and dendrites growing from the developing neurons should easily find each other and form connections. The ability to stimulate and record from all the neurons in the resulting network will be an important tool for neuroscientists. One of the most perplexing questions in neurobiology is how the brain changes in response to new information. Learning is thought to involve synaptic change — either the formation of new connections or the change in strength of existing ones. Says Pine, "It's hard to study that kind of question in an active animal because you can't record from many individual cells over a long time. We've chosen to answer the technical problem by recording in culture where we can easily have access to the same cells as time goes on. So that's the name of our game — to study synaptic change."

If the neurochips can be made to work, Pine and Rutledge are eager to try an idea, suggested by graduate student John Gilbert, that sounds like the stuff of science fiction. Says Pine, "We'd like to put a probe like this into the nervous system with cells in it that have been preselected to ask a specific question." For example, the probe could be loaded with the type of cortical cells that normally receive inputs from other brain regions. If these inputs hooked up to the neurochip cells, researchers would have a powerful means of studying the development and function of those connections.

And the neurochips might also be used to help create neural prostheses, perhaps for the control of artificial limbs. "People know how to make beautiful artificial limbs," says Pine, "but getting the appropriate control signals from the amputee is very difficult." The difficulty is in the interface between a person's desire to make a movement and the limb's machinery. A neurochip loaded with the proper cells could be implanted at the site of the amputation. This might then receive the same signals the brain uses to move a natural limb, and send those signals on to the artificial limb. Pine and Rutledge caution that this is highly speculative and, even if possible, is certainly many years away. But they may overcome the major hurdle on this road — a long-term neuron-silicon connection — within the next year or two. □ — *RF*

# Travels of a Terrane

The residents of Juneau, Alaska might be surprised to learn that they live on land that was once part of eastern Australia. But the Australians will have trouble asserting property rights; the 70,000 square kilometers of what geologists call the Alexander Terrane broke away from Australia 375 million years ago. The terrane traveled across the Pacific and stopped off the coast of Peru. Then it turned northward, brushed past California, and finally came to a halt only after it slammed into the North American continent.

This, at least, is the sequence of events pieced together by Jason Saleeby, associate professor of geology, and his former student George Gehrels, who is now on the faculty of the University of Arizona. By reading the stratigraphic, paleomagnetic, and fossil evidence plastered on the terrane like labels on an old steamer trunk, Saleeby and Gehrels have constructed a detailed history of this highly peripatetic land mass.

Gem-quality zircon was the key to understanding the detailed history of the Alexander Terrane. This mineral, which is found in small quantities in igneous rock, can be dated with great precision. When zircon is formed it contains rather high levels of uranium and virtually no lead. Radioactive isotopes of uranium turn to lead as they decay, so the amount of lead that has accumulated in a zircon sample is a reflection of the host rock's age. Since zircon has such a low proportion of lead contamination to begin with, Saleeby and Gehrels were able to provide a stratigraphic time line with a high degree of precision.

Using this time line, the researchers determined that the Alexander Terrane started its existence over 500 million years ago as a long chain of volcanoes much like the present-day Mariana, Solomon, or Tonga island chains. Such "island arcs" leave distinctive lava flows. The lava flows on the Alexander Terrane turn out to be the remains of volcanoes far older than volcanoes anywhere else in western North America.

The history of mountain building also indicates that the Alexander Terrane could not have originated in its present location. Geologists infer mountain building by evidence of major faulting, by the metamorphism of rocks, and by a distinctive type of sedimentation resulting from coarse rocks shed from high elevations. This sort of information has led to the conclusion that the Alexander Terrane has been the site of two major mountain uplifts, one 520 million years ago and one 430 million years ago. Eastern Australia had periods of mountain building at just those times, but western North America was quiescent then. On the other hand, western North America underwent a series of mountain-building episodes starting about 350 million years ago, a time when there's no evidence of such events in the Alexander Terrane.

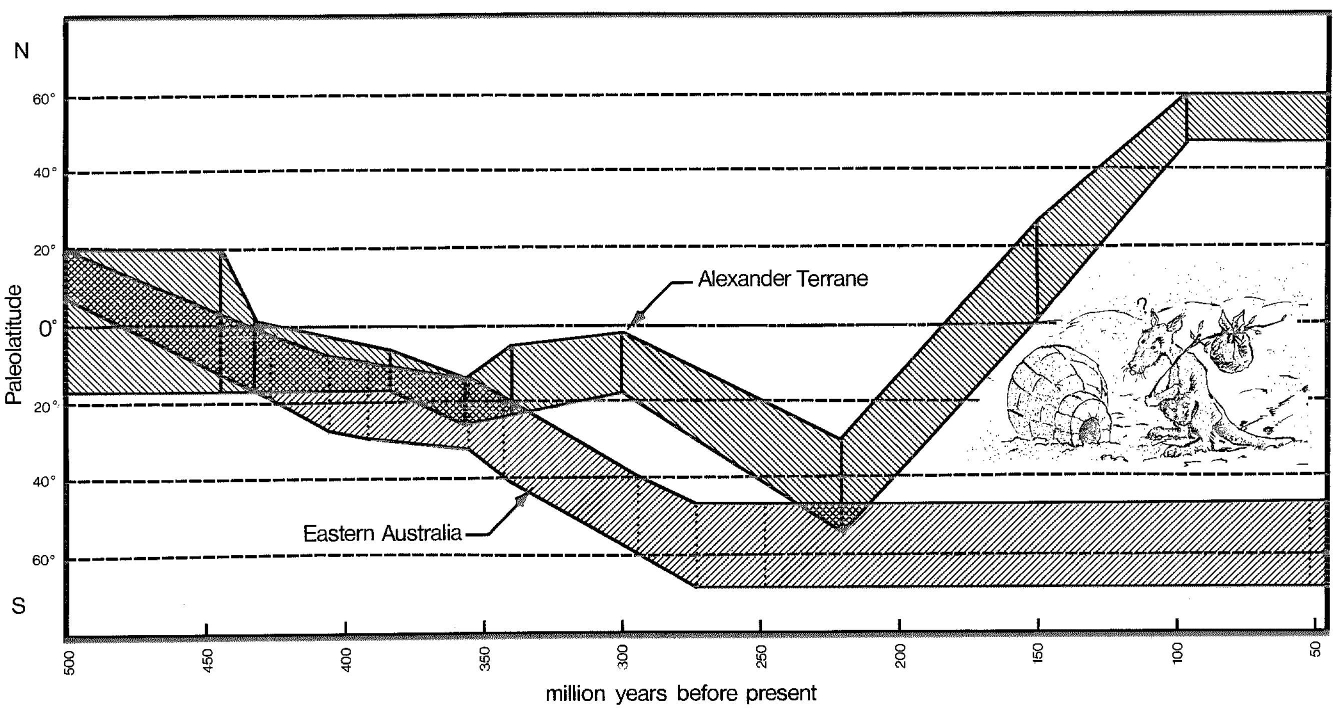
Paleomagnetic data provided the next piece of the puzzle. When a rock solidifies from the molten state, it contains many tiny magnets, all of which line up in the direction of the earth's magnetic field. Geologists can take cores from these rocks and determine the orientation of the magnetic material with the use of a magnetometer. By correlating this information with information on the rock's age, its original position on the horizontal plane, and the history of shifts in the earth's magnetic field, geologists can deduce the latitude at which a rock formed. Such information was used to construct the accompanying illustration, which indicates that the Alexander Terrane started out at the same latitude as eastern Australia, but started diverging some 375 million years ago. About 225 million years ago it began moving northward at 10 centimeters a year. This continued for 135 million years, at which time the terrane collided with the North American continent at its present latitude.

When it separated from Australia, the Alexander Terrane stretched like a piece of taffy, forming a subsurface ocean plateau. It remained an ocean plateau throughout nearly all of its travels, floating freely on the underlying mantle until it collided with North America. After the terrane was firmly welded to the continent, glaciers scoured fjords and channels, forming Prince of Wales Island and the other islands of the Alexander Archipelago.

The fossil evidence found on the terrane is consistent with this history and provides a third piece of the puzzle. The earliest fossils are land plants and animals with affinities to those of eastern Australia. When the terrane first became an ocean plateau, many small planktonic creatures called foraminifera settled out, forming small reefs. When the paleomagnetic evidence shows that the terrane moved south toward the coast of Peru, various marine bivalves begin appearing in the fossil record. The fossil bivalves proceed in a neat sequence from southern hemispheric species, to equatorial species, to species characteristic of northern temperate zones. Finally, about 100 million years ago, North American fauna began appearing.

The geological community has expressed much interest in these views of the Alexander Terrane's history, and the hypothesis is now in a state of rigorous testing. Saleeby and Gehrels are trying to determine whether the terrane traveled along with a neighboring land mass known as Wrangellia. They're trying to refine their dating of the collision and are attempting to determine the means by which much of the terrane was shoved underneath the North American cordillera.

The researchers are also testing some ideas that are more speculative. Saleeby believes, for example, that the renegade terrane may have snatched some of the host rocks of the California Mother Lode gold belt as it grazed the North American coast. "This is something that's going to draw a lot of debate because it flies in the face of established ideas of California geology," says Saleeby. But if the speculations are confirmed, these rocks would have formed the host for the Juneau gold belt. So the Alaskan gold rush may have actually been the California gold rush, just displaced 1,500 miles to the north. □ — *RF*




*According to the paleomagnetic evidence, the Alexander Terrane broke away from eastern Australia 375 million years ago. It traveled across the Pacific, stopped briefly off the coast of Peru, and then moved northward at about 10 centimeters per year. It reached its present latitude about 100 million years ago when it became welded to the North American cordillera.*