NEUROSCIENCE

Neurons and Silicon Get Intimate

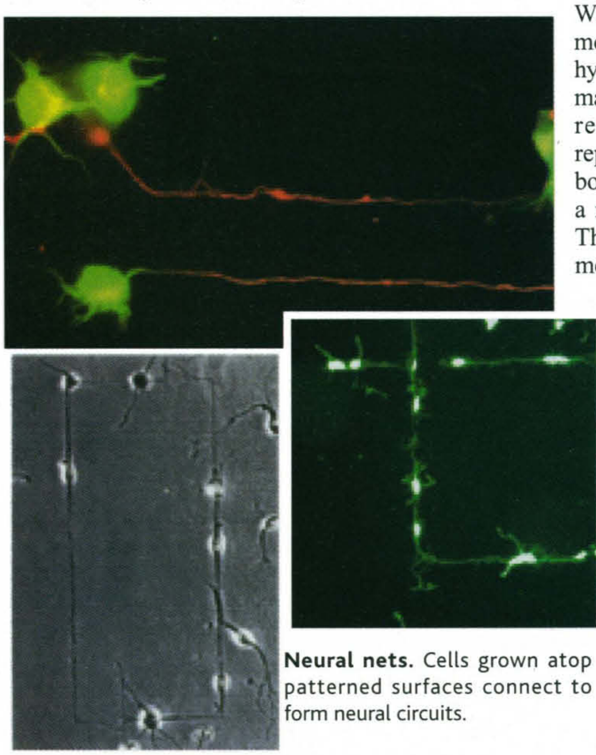
Researchers may be a long way from making a cyborg, but they are coaxing neurons to grow in patterns that could form a link between biology and silicon circuits

You don't have to look very far into the annals of science fiction, be it *Star Trek*, *Star Wars*, or the dark thrillers of futurist William Gibson, to find examples of cyborgs and other marriages of computer hardware with biology. Real-world researchers attempting to pull off this union have had only meager success. But at the American Chemical Society meeting in Anaheim, California, last month, a handful of research groups reported closing in on a key enabling technology: simple networks of neurons atop transistors and other microelectronic devices that can communicate with cells and listen in on their chatter.

These networks, usually just a few cells patterned into a rectangle or other circuitlike configuration, aren't likely to give even a pocket calculator a run for its money anytime soon, although researchers can't help speculating about such possibilities as hybrid computers, prosthetics, and sensors. Says chemist James Hickman of George Washington University in Washington, D.C., "We're getting to the point now where we can actually think about making devices." But well before hybrid circuits realize their promise outside the lab, they can serve another crucial purpose, says George Whitesides, a cell patterning pioneer at Harvard University. With such circuits, he says, "you can begin to set up really good tests of fundamental neurophysiology." In particular, say Whitesides and others, they will allow neuroscientists to explore basic questions such as how the nature of a neuron's connections with its neighbors affects its ability to fire.

Although neuroscientists have spent decades using needlelike electrodes to eavesdrop on the firing patterns of single neurons, they have been far less successful at monitoring networks of neurons. And even where they have come up with techniques to do more complex monitoring—such as using arrays of electrodes—"it's hard to know what anything means," says Peter Fromherz, a physical chemist at the Max Plank Institute for Biochemistry in Martinsried, Germany. The network of connections between neighboring neurons is typically so complex, he points out, that it is nearly impossible to decipher what signals any given cell is responding to.

Building neuronal networks from the ground up, one neuron at a time, and communicating with them via microelectronics offers a way of paring down this complexity. In 1991, Fromherz and his colleagues first reported in *Science* (31 May, p. 1290) that they were able to grow a leech neuron atop a silicon-based field effect transistor (FET), which monitored the neuron's activity. FETs pass a tiny electrical current through a semiconductor channel between two electrodes. When a small voltage is applied to a third "gate" electrode above the channel, it makes the channel more conductive and thus allows more electrons to flow. Fromherz and his colleagues showed that the cell's firing could alter the gate voltage and thus the current through the transistor. In 1995, they turned the tables, showing that a charge-releasing capacitor could provide a tiny electric jolt tailored to fire a cultured neuron sitting above it.



Neural nets. Cells grown atop patterned surfaces connect to form neural circuits.

CREDITS: (CLOCKWISE FROM TOP) B. WHEELER/UNIV. OF ILLINOIS; H. CRAIGHEAD/CORNELL UNIV.; J. HICKMAN/GEORGE WASHINGTON UNIV.

Now, researchers are moving to more complex systems: small groups of cells, coaxed into simple circuit patterns with the help of cell-friendly substances laid out on a glass or silicon surface like tape marks on a stage. In the first efforts, groups including Hickman's have patterned collections of cells on simple glass substrates, without microelectronic listening devices built in. At the meeting, Hickman detailed his team's latest success in creating a simple neuronal circuit by using conventional lithographic techniques, akin to those used to pattern computer chips, to lay out a chemical pattern that guided hippocampal neurons from the brains of rats into a simple rectangular circuit.

The researchers started by coating a glass surface with a molecule-thin layer of DETA, a neuron-friendly organic compound. They then shone ultraviolet light onto it through a thin, stenciled metal mask: Wherever the light hit the surface, it removed the DETA, leaving a residue of hydroxyl groups, while the DETA remained intact in the masked areas. The researchers then added a neuron-repelling, Teflon-like substance, which bound to the hydroxyls. That gave them a rectangular-shaped pattern of DETA. They then coated the glass with culture media spiked with neurons and watched as a pair of cells migrated to the DETA and then sent out their tendril-like axons along the cell-friendly material, eventually making connections to one another.

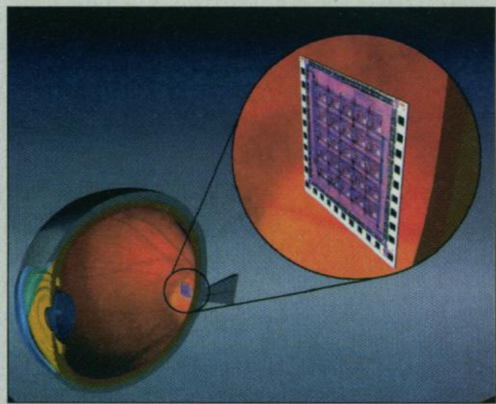
Finally, Hickman's team showed that the neurons were in synaptic communication: They stimulated one cell with a needlelike electrode, causing it to fire, and then measured a similar firing in the neighboring cell with another sensing electrode. "It shows that we can begin to make simple structures and control how individual neurons connect to each other," says Hickman. Next up, he says, he hopes to create such neural circuits on actual microelectronics.

A trio of other groups reported at the meeting that they're already pushing into that territory. In this case the teams—led by Harold Craighead at Cornell University in Ithaca, New York, Andreas Offenhäuser at the Max Plank Institute for Polymer Research in Mainz, Germany, and Bruce Wheeler at the University of Illinois, Urbana—all used a technique known as microcontact printing to pattern their neurons. This printing technique—developed by Whitesides and his team at Harvard—relies on traditional lithography to etch microscopic features into a silicon wafer or anoth-

Bypassing Nervous System Damage With Electronics

Although the effort to marry neurons and microelectronics into hybrid circuits is still in its infancy (see main text), neural prostheses that artificially stimulate the nervous system to partially restore lost vision, hearing, or movement are, paradoxically, much further along. In part, that's because they need only stimulate groups of cells rather than contact individual neurons. Heading the list of successes are cochlear implants, which use implanted electrodes to stimulate auditory nerves and provide rudimentary hearing to the deaf and have already been received by over 20,000 people. And the U.S. Food and Drug Administration recently approved related implants to improve bladder control and restore hand grasping abilities to quadriplegics.

Propelled by these successes as well as by a bevy of new tools coming from advanced microelectronics technology, "the bandwagon is starting to move," says Richard Norman, a bioengineer at the University of Utah, Salt Lake City. "A large number of groups are starting to work in this area." Still, he adds, the ultimate goal of making advanced neural prostheses that can fully restore a patient's motion or vision is "a bit of a long shot." The obvious problem is communicating with the body's complex set of neurons. In just the eye, for example, 1 million nerves carry stimuli from light receptors in the retina to the brain. Stimulating all those nerves independently remains, for now, an impossibility.



Second sight. Implanted chip bypasses damaged light sensors to restore some vision.

Surprisingly, however, much has been accomplished with relatively crude electrical inputs. Cochlear implants, for example, connect at most 22 electrodes to auditory nerves in the cochlea that respond to different sound frequencies. When a tiny microphone outside the ear picks up sound, it passes the sound to a signal processor behind the ear that analyzes it and signals an implanted electrical pulse generator to stimulate the appropriate electrodes in the cochlea. Although this doesn't provide perfect hearing, people with the implants typically pick up enough sound to carry on a conversation.

Other researchers are now trying to forge related technology to restore sight by stimulating cells in the retina, the optic nerve, or the brain's visual cortex. At Johns Hopkins University School of Medicine in Baltimore, Maryland, for example, Mark Humayun and his team have temporarily implanted a 3-millimeter-wide array of 25 electrodes atop the retina of one eye in each of two elderly patients with retinitis pigmentosa. (This hereditary condition slowly degrades the eye's light sensors, known as rods and cones, eventually leaving patients totally blind.) An external unit sends electrical signals to the electrodes via wires passing through a tiny slit in the eye.

In an upcoming issue of *Vision Research*, Humayun and his colleagues describe how the retinal stimulation allowed both patients to perceive complex shapes, such as squares and letters. The team is already working to create more complex arrays and control circuitry that would transmit signals through the skin via radio waves so that the arrays could be permanently implanted in the eye. "There's fundamentally no reason why you can't take a blind person and get them to see coarse features consistent with mobility," says Humayun.

Devices that provide rudimentary muscle control have also made strides. P. Hunter Peckham and his colleagues at Case Western Reserve University in Cleveland, Ohio, for example, have shown that a series of eight implanted electrodes that directly excite different muscle groups in the forearm and hand can restore hand gripping movement to quadriplegics. In a recently commercialized version of the device, patients control their hand movements by thrusting their opposite shoulder forward and backward, activating implanted sensors that then relay the information to a signal processor and electrical-pulse generator implanted just below the collarbone.

Peckham says that he and his colleagues are currently working on adding electrodes so as to restore fine motor control of the hands and arms. The team is also making steady progress with a variety of other neural prostheses, such as one that helps paralyzed patients stand and even walk, as well as an advanced version of a bladder-control device now on the market. Says Peckham, "It's almost unlimited what you can conceive of being able to accomplish." –R.F.S.

er rigid material. The patterned wafer then serves as a mold to cast a rubbery stamp.

The teams "inked" their stamps in solutions spiked with cell-friendly compounds and stamped them on various substrates, creating patterns that would guide the growth of cells. Craighead and Wheeler, for instance, stamped patterns of compounds such as laminin, a protein found in the extracellular matrix between cells in the body, onto glass or silicon surfaces to position cells over electrodes embedded in the material. Offenhäuser's group, meanwhile, stamped out a gridlike pattern of a laminin fragment called PA-22 to orient his cultured neurons atop silicon FETs.

The researchers aren't sure whether the neurons in these networks are actually sending signals to each other. Still, there are some early signs of success. Offenhäuser, for example, reported that after his team created patterned cell networks and then let the cells grow in a culture medium for 5 to 10 days, the cells fired spontaneously, which typically occurs only if neurons have made synaptic connections to their neighbors. "This is our hint that we have synaptic connections," says Offenhäuser. Wheeler says his team has seen similar signs. Now all the teams hope to trigger one cell to fire and watch the impulse propagate to its neighbors.

If the new hybrid circuits do pan out, their eventual use is anybody's guess. "Ultimately we want to use this for prosthetic devices, sensing, and interfacing with the nervous system," says Craighead. "But the idea of applying this to a therapeutic use is quite far away," as are hybrid computers. One of the biggest problems is that neurons cultured by themselves typically die within a month. Still, Hickman points out that researchers at the University of Virginia have shown that when they culture neurons alongside support cells known as glial cells, the neurons survive for more than a year. "This field is just getting started," says Hickman. "We're at the same spot where they were 50 years ago with the transistor. Nobody at that time could envision making a PC."

–ROBERT F. SERVICE

CREDIT: M. HUMAYUN/JOHNS HOPKINS UNIV.