The High Culture of Neuroscience

Advances in growing specified neurons in the lab are opening the way for remarkable insights into how neuronal networks operate in the intact animal

RESEARCH RESULTS AREN'T THE ONLY THINGS that move science forward; often, an advance in technique can open a door onto an entirely new class of experiments. One recent example of this process arises in neuroscience, where methods developed in the last decade for growing identified neurons in culture have made it possible to ask new, incisive questions. Using those techniques, one team of researchers has observed how synapses form on identified neurons that are associated with learning in a marine mollusk. And, as they report in this issue of *Science*, another team has managed for the first time to get a simple neural network (whose rhythm drives breathing in a different marine mollusk) to perform in culture just the way it seems to perform in the living animal.

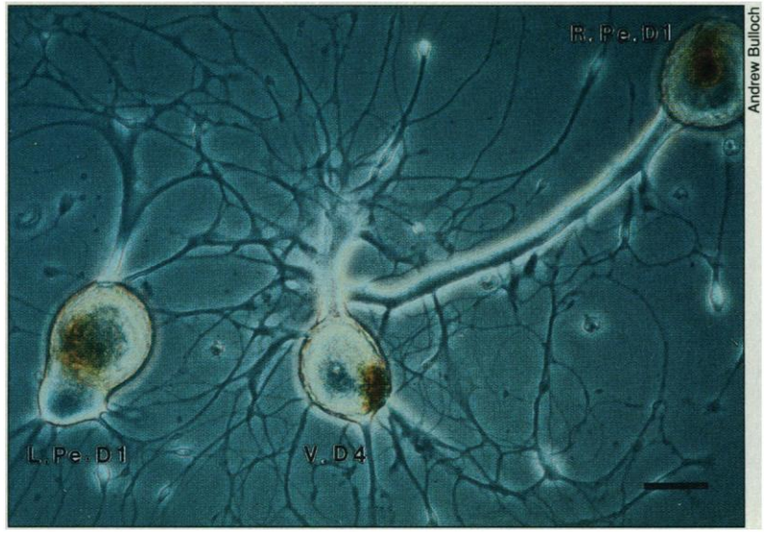
In neuron culture "you can get simplicity, you can get clarity of analysis, and you can do experimental manipulations that you can't do otherwise," says Eric Kandel of Columbia University, who has been using cultured neurons to study learning and memory in the marine mollusk *Aplysia*. Kandel explains that "in culture [you can be sure] you're not dealing with some system property that involves 30 to 40 other cells."

Doing the kind of work Kandel refers to requires picking out neurons whose function in the animal is known, removing them, and studying how they behave in culture. Much of this work on identified neurons in culture is being done on mollusks because they are one of the few groups of invertebrates with neurons large enough to be easily identified and plucked out.

The first steps in this direction were taken in the 1970s, when several groups managed to pick identified neurons from mollusks and leeches. But there was a hitch: the neurons didn't grow well enough for extensive experimentation. That problem was solved for mollusks in the early 1980s, when Stanley Kater, then of the University of Iowa, and Samuel Schacher of Columbia University began enriching their neuron culture medium with a brew of molluscan fluids and factors. Since that advance, the experimental results have been rolling in.

One of the fields benefiting most from the new culture techniques is the study of central pattern generators (CPGs for short). These networks drive simple rhythmic behaviors, such as breathing and walking, and they work by oscillating between two or more stable states. A CPG that governs breathing, for instance, may oscillate between a pattern of activity that stimulates the muscles controlling exhalation and a second pattern that drives inhalation. These rhythms stem from inherent properties of individual neurons—and also from the way the neurons are linked. Just how those properties and connections yield rhythmic firing is now an area of intense interest in neuroscience.

"If we can get at some of the underlying elements [of pattern generators] and ask questions about them, then we can put the pieces back together and understand more about the system as a whole," says Hillel Chiel of Case Western Reserve. As a postdoc at AT&T Bell Laboratories, Chiel worked with AT&T researcher David Kleinfeld to take a first step toward studying CPGs in culture.



A lotta nerve. *A team from the University of Calgary grew these neurons from the marine snail* Lymnaea *in culture and showed they will form a network that behaves as it does in the intact animal.*

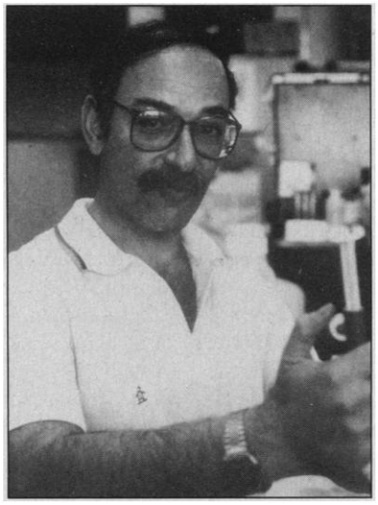
Kleinfeld and Chiel, whose work was recently published in the *Biophysical Journal* (April 1990, p. 697), found that certain pairs of *Aplysia* neurons exhibit intriguing behavior in culture. They form inhibitory connections, and each cell can inhibit the activity of the other. That allows the pair to adopt either of two stable states; in each state one neuron is active and its activity keeps its partner quiet. Stimulation of the quiet cell causes the pair to flip to the opposite state and maintain it.

Chiel points out that in this case the behavior of the neurons is an artifact of culture: his cells don't exhibit precisely that activity in the animal. But mutual inhibition *is* a common characteristic of neurons in CPGs, and the pairs of *Aplysia* cells provide a nice model for studying this aspect of CPGs. "Maintaining a stable pattern of activity is essential" to pattern generation, says Chiel, who adds: "This was a chance to look at that in a very simple system." Using this system, he and Kleinfeld were able to examine the amount of stimulation that is required to maintain or change states. But Chiel notes that the pairs of cells cannot generate their own oscillating pattern—the researcher must intervene to flip them from one state to the other.

And that's where this field stood only a few months ago. Yet the work on CPGs is moving very quickly and, in an exciting recent development, a group at the University of Calgary has taken the next step: they have produced, in culture, a simple network that generates its own oscillatory pattern. As they report on page 282 of this issue of *Science*, Naweed Syed, a postdoc with Andrew Bulloch and Ken Lukowiak at the University of Calgary, removed three nerve cells from the CPG that controls breathing in the pond snail *Lymnaea* and cultured them. Syed found that the three cells linked up and could be induced to fire in a self-sustained, rhythmic pattern similar to the

rhythm seen in vivo. This is the first case of a nerve cell network generating such a pattern in culture; it offers hope that cell-culture experiments will help identify the cellular properties and interactions that drive this—and other—CPGs.

Syed says he turned to neuron culture because in vivo techniques weren't sufficient to answer the fundamental question of how many (and which) neurons are needed to produce the breathing rhythm. Working in the intact *Lymnaea* nervous system, with William Winlow at the University of Leeds, he couldn't rule out the possibility that other cells were necessary. So he joined forces with Bulloch, who had experience culturing molluscan neurons. Putting the cells in culture is "the ultimate test" of whether they are sufficient to generate a pattern, says William Kristan, who studies central pattern generators in the leech at the University of California, San Diego.



Green thumb. *Samuel Schacher helped work out conditions for growing identified molluscan neurons in culture.*

The *Lymnaea* cells passed the test: The three neurons proved sufficient to generate the right rhythm. The system behaves a little like Kleinfeld's and Chiel's neuron pairs. One cell triggers expiration—and keeps a second cell quiet while it does so. The second cell triggers inspiration—and keeps the first quiet in turn when it is active. The key difference from Kleinfeld's system (which can't flip itself from one state to the other) is the presence of the third cell, called the large dopamine neuron, whose activity causes a rhythmic switching between states.

This system not only allowed Syed to show that the three neurons are sufficient to yield the basic rhythm, it enabled him to pick the system apart and understand some of its details. For example, Syed found he could get oscillation without the dopamine cell by applying dopamine spritzes at 3-second intervals. That experiment suggests the dopamine cell exerts its effect simply by rhythmically releasing dopamine—without other neurotransmitters or modulatory effects.

Such experiments, involving addition of neurotransmitters or other substances, would be practically meaningless in the intact nervous system, Bulloch says. The reason is that the complexity of the intact system makes it difficult to pin down causes and effects. For instance, some as yet unidentified neuron could have been responding to dopamine and then having a secondary effect on the target cell.

Although CPGs have recently received a lot of attention, they aren't the only networks benefiting from the advantages of identified neuron culture. Another example is the work Kandel, Schacher, and their colleagues at Columbia University are doing on a network of identified neurons that are involved in learning and memory in *Aplysia*.

Like a turtle drawing its head into its shell, *Aplysia* will, in response to a light touch on its siphon, defensively withdraw its gill. If the touch is paired with an electric shock to the tail, the animal will become more sensitive—and will continue to overrespond to siphon touches even when no tail shock is given. Having identified the network of neurons responsible for the gill withdrawal, Kandel and his co-workers are exploiting that process as a simple model for learning. Shock-training, they find, strengthens connections between some of the neurons in the network, intensifying the signals passed between them.

In collaboration with Kandel's group, Schacher cultured two key neurons from the network: a sensory neuron that receives the signal from the siphon and a motor neuron that causes the gill to withdraw when it is directly stimulated by the sensory cell. Schacher showed that in culture these neurons form synapses that can be strengthened by applying serotonin, the neurotransmitter known to strengthen connections between neurons in the intact animal after a learning session.

Schacher's work confirmed that serotonin works directly to produce the synapse-strengthening changes in the sensory cell, which would have been difficult to demonstrate in the intact animal. In addition, it made possible a variety of experiments into the nature of those changes—experiments that couldn't be contemplated in vivo.

Using cultured neurons, David Glanzman of Kandel's group, working with Schacher, showed, in a paper published recently in *Science* (17 August, p. 799), that serotonin enhances the connections between the two neurons partly by causing the sensory cell to branch out and form new synapses. Such changes had been suggested by earlier work in which comparisons were made between trained and untrained animals. The drawback of those experiments, however, was that they required a comparison between different animals and assumptions about what the trained animal's neurons had looked like prior to training. "What we've done now is take that one step further—and shown what happens if you look at the same cells over time," Schacher says. "[In culture] we can actually see the changes."

Schacher's group has now removed the motor neuron to see whether its presence is needed for the sensory cell's branching growth. And indeed, when the motor cell is missing, serotonin no longer causes the sensory neuron to branch, suggesting some feedback from the target cell is needed. Says Kandel: "Those are experiments you can't do in vivo."

Both the work on CPGs and Kandel's work on learning show that neuron culture has great advantages for studying simple networks. Yet the researchers who work with neuron culture point out that it does entail the risk that observations made in culture are not directly relevant to what goes on in the living creature. Some neurons change radically in culture, forming connections they ordinarily wouldn't, making neurotransmitters they usually don't, and even setting up aberrant dynamic relationships.

"You have to be careful that [the neurons you are studying] behave the same in vitro as in vivo," Bulloch cautions. Schacher points out that his experiments in culture confirm and extend results obtained in the intact nervous system, providing some assurance that "the cells more or less behave as they should" in culture. In Syed's experiments, Bulloch says, "everything seems to be the same [as it is in vivo]. All the evidence is in favor of these three neurons being sufficient [for pattern generation] in the animal."

But that may not be enough, warns Eve Marder of Brandeis University, who studies CPGs in crustaceans. It's important, she notes, to keep in mind that the complex influences eliminated by moving neurons into culture are important in the animal.

Yet even that disparity can be turned to advantage, says San Diego's Kristan. If the cultured network behaves more or less like its in vivo counterpart, he says, you can bet you have the essential parts. And when you find ways in which it doesn't correspond to the in vivo system, you need to go back to the organism and look for other components. "You want to know what the minimum is to get the effect," Kristan says. "Then you can add back the complexities." And that capacity—for finding the minimum needed to generate the network's effect—is the great beauty of neuron culture.

■ MARCIA BARINAGA