Analytical Chemists Push The Cellular Envelope

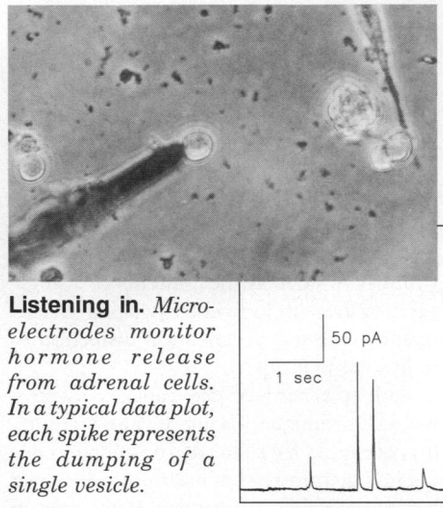
As chemists find ways to eavesdrop on the chemical chatter of single cells, neuroscientists are perking up their ears

WHEN GRADUATE STUDENT DAVID LESZCZYSZYN rushed into Mark Wightman's office two winters ago with a scratchy trace of data from their experimental microelectrode, the investigators were crestfallen. They had hoped that the sensor might catch chemical whispers coming from a single cell derived from a cow's adrenal gland. Instead, they saw a lousy-looking trace with so many ups and downs that it was hard to make any sense of it. "We thought we were looking at noise," recalls Wightman, an analytical chemist at the University of North Carolina (UNC) at Chapel Hill.

They thought wrong. As they looked more closely at the quivery trace, their disappointment gave way to the thrilling suspicion that the microelectrode was recording the cell's chemical chatter in even greater detail than they had hoped. Subsequent experiments by Wightman, Leszczyszyn, Jeffrey Jankowski, and other co-workers confirmed their hunch. Each spike in the electrode signal corresponded to the dumping of the chemical cargo (in this case the hormones adrenaline or noradrenaline) of a minuscule subcellular vesicle into the liquid medium around the cell. And each cargo amounted to no more than a billionth of the volume of a water droplet, containing perhaps a million molecules of hormone.

That's only one of the minute chemical signals on which Wightman and a handful of other analytical chemists are now managing to eavesdrop. By stationing their microscopic probes near living cells or separating individual cells into their chemical ingredients and analyzing them one by one, they are giving biologists the data they need to sharpen their picture of how living cells communicate and respond to stimuli. In the process, they are turning single-cell analytical chemistry into a subspecialty poised for takeoff. One measure of its growing status: a symposium titled "Analytical Chemistry at the Level of a Single Nerve Cell" at this year's Pittcon (formerly the Pittsburgh Conference), the gargantuan annual exposition of instruments and methods for analyzing the variety of chemistry in living things, industry, and the environment that will be held next month in New Orleans.

The most avid customers of the new bioanalytical techniques on show at Pittcon may well come from the ranks of neuroscience. After all, signaling is the stock-in-trade of nerve cells, which relay or withhold electrical signals depending on incoming and outgoing chemical and physical stimuli. For years, neuroscientists have been tuning in to that electrical activity in single cells. But they have lacked the refined techniques needed for monitoring the underlying biochemical choreography in individual cells. As a result, they traditionally have had to pool many cells to get enough substance to detect, an enterprise that averages out any intercellular differences in the sample population.



Listening in. *Microelectrodes monitor hormone release from adrenal cells. In a typical data plot, each spike represents the dumping of a single vesicle.*

Now analytical chemistry will enable them to "look at individual cells and study their chemical differences," Wightman asserts. For example, David Sulzer and Stephen Rayport of the Center for Neurobiology and Behavior at Columbia University have recently begun a collaboration with analytical chemist Andrew Ewing of Pennsylvania State University to study the cellular effects of amphetamines. "We've been looking at the electrical activity of brain cells for years, but with these methods we'll also have the ability to eavesdrop on the neurotransmitter dynamics underlying this activity," says Sulzer.

The strategy that Wightman and some of his colleagues will be describing at Pittcon relies on microelectrodes that can be maneuvered close to living cells to sense changing concentrations of certain electrically active compounds. Made of conductive carbon fibers just a few microns across, sealed almost to their tips in insulating glass capillaries, Wightman's electrodes can be swept through a range of chemical potentials to scan the chemical environment near a cell. Each compound reveals itself by gaining or losing electrons—processes known respectively as reduction and oxidation—at particular chemical potentials, generating characteristic signals.

In another search mode, the electrodes can be tuned to trace changing concentrations of a single compound. The trick is to hold their potential constant, at the compound's characteristic oxidation potential. Changes in the current through the electrode then indicate how the molecule's concentration is changing over tens of milliseconds. "This allows us to monitor things in real time," says graduate student Jankowski.

But not all important neurochemicals lose or gain electrons readily enough to be detected directly, says Werner Kuhr of the University of California at Riverside, another Pittcon participant. The solution his group is trying: Have other molecules generate the signal. Kuhr and his colleagues are adorning carbon microelectrodes with enzymes that react specifically with nonelectroactive targets, thereby generating a chemical product that can be detected with electrodes. A coating of the enzyme glutamate dehydrogenase, for example, can render an electrode sensitive to glutamate, an important—but electrically unresponsive—neurotransmitter. When the modified electrode encounters glutamate, the enzyme converts it into alpha-ketoglutarate while an electron is transferred to another compound in the sample, a transaction monitored by the electrode. Ultimately, Kuhr hopes to develop miniaturized enzyme sensors that could monitor a whole panoply of neurotransmitters in living cells.

That kind of chemical surveillance may become feasible not just for whole cells but for specific parts of a cell, if Ewing has his way. Ewing is trying to shrink microelectrodes to diameters as small as 400 nanometers—small enough to place next to a synapse between two communicating nerve cells and monitor the chemical traffic there, he says. To do so, he and his colleagues slim down carbon fibers by etching them in a

flame, then deposit a thin insulating polymer on the fibers, leaving only the tip exposed. Making the minuscule electrodes uniform enough to yield interpretable data is no small challenge. But Ewing hopes that once he perfects his electrodes, neuroscientists will be able to use them rather like tiny stethoscopes that might be applied to one part of a cell after another to hear its chemical pulse.

The signals that can be picked up at a cell's surface are only part of its chemical repertoire, and some analytical chemists are devising techniques to go deeper. They want to dissect the chemical complexity of entire cells. Doing so requires a way to sort out a cell's constituents so they can be analyzed separately. And that's where chemist Richard Zare of Stanford University, another Pittcon attendee, comes into the picture. He combines a chemical separation method called capillary zone electrophoresis (CZE), which is suited to handling extremely small sample volumes, with exquisitely sensitive detection techniques to identify the separated molecules. "We're able to see on the order of a thousand molecules," Zare claims—a quantity that chemists who traffic in the stingiest quantities call a zeptomole. That kind of sensitivity, Zare thinks, may help him track down some of the chemical changes underlying key puzzles in neuroscience. "My interests are to find the chemical bases of memory and learned responses," Zare says.

The analytical principle on which Zare is hanging his hopes is the tendency of molecules bearing different electrical charges to migrate at distinct rates in an electrical field. By drawing a cell's chemical brew into an ultrathin, buffer-filled capillary tube and applying an electric field along the length of the tube, Zare and his colleagues can separate the brew's components. Spreading out like sluggish race horses as they migrate through the capillary, the compounds can be identified one by one as they pass a detector.

And just as electrode designers are shrinking their tools to measure signals from individual regions of a cell, Zare is extending his technique to handle volumes far smaller than a single cell. As an early demonstration, Zare and his colleagues homogenized 400 neurons from an Aplysia—a marine snail often used in studies of learning—then diluted the resulting mixture to a volume that might fill a thimble and added fluorescent tags that would bind to specific amino acids or peptides. Next the investigators diluted the tagged cellular extract a second time and injected about 10 nanoliters of the resulting weak broth (representing about 2 millionths of a single cell's contents) into the capillary.

Using a laser to excite fluorescence as the tagged molecules passed by and a sensitive charge-coupled device to pick up the faint signal, they were able to detect several peptides that could not have been represented by more than a few tens of thousands of molecules. Zare concedes that the demonstration could not reveal differences in the chemical personalities of the 400 cells, since the tiny sample combined traces of all 400. But with zeptomole sensitivity, he says, the technique should be capable of analyzing volumes as small as the contents of a single axon or even an individual synaptic vesicle, opening the way to chemical dissection of individual neurons.

"We've been looking at electrical activity for years, but with these methods we can eavesdrop on neurotransmitter dynamics."

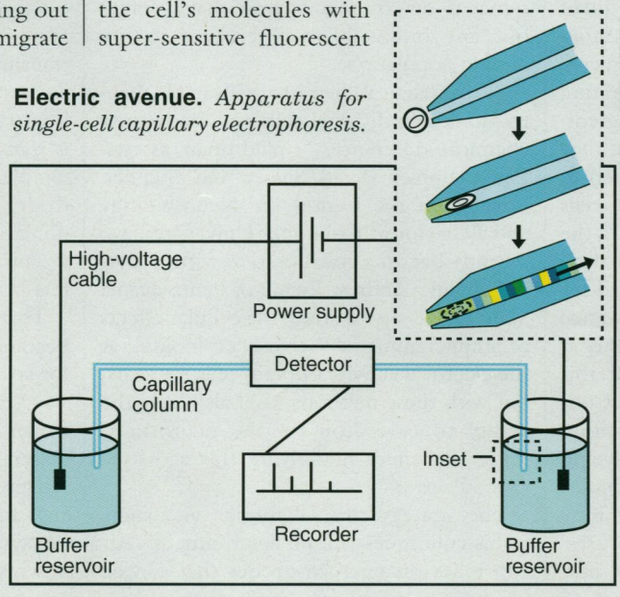
—David Sulzer

With a variant of the capillary electrophoresis technique, Ewing of Penn State and his colleagues have already succeeded in doing just that. Instead of making a cell extract before analyzing it, they impale the cell on the end of an especially thin capillary and draw out a sample of cytoplasm. That suck-a-cell strategy so far has ruled out tagging the cell's molecules with super-sensitive fluorescent markers, and it restricts the workers to electrochemical detection methods. But the strategy is still sensitive enough to detect chemical changes in cells before, during, and after stimulation, Ewing says.

In an even newer technique, which Ewing will describe at Pittcon, he draws an entire cell into a somewhat larger capillary, where he can monitor the release of neurotransmitters. Using that tactic, he and his colleagues found "good evidence for two different cellular compartments of neurotransmitter vesicles" in neurons from the common pond snail. With their analytical system, they were able to detect two distinct neurotransmitter signals, one appearing rapidly after a cell was stimulated and another appearing somewhat later. Ewing says these results are suggestive of a set of vesicles near the cell membrane and another set deeper inside. The finding no more than confirms evidence from microscopy, but Ewing thinks it foreshadows future, more substantive results.

After all, another single-neuron separation technique already has made a tantalizing contribution. Developed by Wightman's UNC colleague James Jorgenson, the technique—microcolumn liquid chromatography—exploits the different rates at which compounds seep through a slurry of tiny hydrocarbon particles in a slender column. When Jorgenson used the column to separate the extract from single cells taken from a cow adrenal gland, he came up with preliminary data challenging a long-held assumption that individual cells of the inner region of the adrenal gland secrete either mostly adrenaline or mostly noradrenaline. In a test of 22 cultured adrenal cells, the researchers found eight containing mostly noradrenaline, ten containing mostly adrenaline, but at least four with a significant amount of each.

Simply as feats of analytical chemistry, such preliminary successes are likely to earn Jorgensen, Wightman, Zare, and their colleagues badges of merit from the analytical chemist community when they present their results next month at Pittcon. Measuring ever more minuscule amounts of chemicals, in ever smaller samples, in ever shorter times with ever better accuracy and precision is the analytical chemist's professional imperative, after all. But the single-cell analysts may soon be playing to a wider audience of biologists. Marvels neuroscientist Sulzer: "For the first time, we can tell in real time some of the dynamic chemistry going on in these [brain] cells." ■ IVAN AMATO



Electric avenue. *Apparatus for single-cell capillary electrophoresis.*

SOURCE: WIGHTMAN ILLUSTRATION: D. DEFRANCESCO