Biological Applications for Voltage Sensitive Dyes

Physiologists find that optical probes of cell voltage have advantages over conventional electrodes

By scanning a laser beam across the dye-treated surface of a gerbil brain, researchers are quickly able to locate tissue undergoing electrical changes like those that occur during epileptic seizures. These and other electrical phenomena in normal or diseased cells can now be measured with optical techniques by exploiting a group of dyes that change color in response to cell voltage changes.

The dyes are a potentially powerful new tool for physiologists, since cell voltage is an important indicator of cell function and of the interactions between cells. With these dyes it is possible to measure voltage changes in cells or cell fragments that are too small to survive being impaled by traditional electrical probes. Surface tissues of the brain or heart can be surveyed without cumbersome arrays of electrodes. Some scientists are working on techniques that should permit them to monitor simultaneously the activities of 100 or more individual nerve cells in an invertebrate brain—a feat virtually impossible with conventional techniques. By following so many cells at once, investigators hope to unravel the complicated interactions of the nerve cells, thus detailing the nervous system's contribution to comparatively complex behavior patterns.

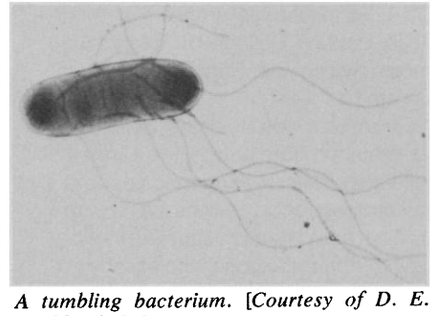
The dyes change color (absorption or fluorescence, or both) in response to cell voltage—the electrical potential difference between the cell cytoplasm and the medium surrounding the cell. This membrane potential arises because ions such as sodium and potassium, which can move across the membrane, are kept at different concentrations inside and outside the cell. Normal membrane potentials are between −10 and −100 millivolts, with the cell interior negative.

Slow or sustained changes in this membrane potential are rather nonspecific indicators of metabolic health. Oxygen deprivation and poisons that act on cell metabolism cause cells to depolarize, that is become less negative inside. In addition, some toxins shift membrane potential up or down by altering the permeability of the membrane to one or more types of ions.

Rapid changes in membrane potential play a central role in the functioning nervous system. Cell voltages may swing from a "resting potential" of −90 millivolts to a peak of +40 millivolts and back again in a matter of a few milliseconds. These "nerve impulses" are one of the currencies for information exchange in the nervous system.

Traditionally, researchers attempting to decode the complex interactions of nerve cells have had to place fine electrical probes in or near each cell that they wish to monitor. It is possible, but difficult, to use this technique to follow the simultaneous activity of ten or so identified nerve cells. However, the slightest movement of the tissues may cause one or more electrodes to destroy its intended target. The overall probability of success decreases sharply as the number of cells and electrodes increases.

With optical probes, the process is less delicate. For example, Lawrence B. Cohen and his colleagues at the Yale University School of Medicine have been able to monitor without ambiguity the activity of about 35 individual nerve cells in a dye-treated barnacle ganglion (nerve center). An image of the ganglion is focused onto an array of 100 photodetectors. By computer processing the signals from the detector array, Cohen and his co-workers are able to detect changes in absorption of light by individual nerve cells within the ganglion. Since these optical changes are related to electrical events, the activity and interactions of individual cells can be monitored. Cohen believes the technique can be extended to handle several hundred cells. Brian Salzberg at the University of Pennsylvania and Amiram Grinvald at the Weizmann Institute in Israel, who collaborated with Cohen in developing these optical methods, are working on applying the techniques to monitor activity in thin branches of nerve cells—dendrites—and in regenerating nervous systems.



A tumbling bacterium. [*Courtesy of D. E. Koshland, Jr.*]

While dyes that respond to changes in membrane potential have been known for about a decade, only recently have ones with an essential combination of low toxicity, adequate signal strength, and quick response been discovered. These dyes, such as the merocyanine-rhodanine dye used by Cohen, typically respond to voltage fluctuations in less than 10 microseconds. In nerve tissue, absorption changes are on the order of 0.0001 percent per millivolt: small but usable; and the lowest dye concentrations used, about 0.2 millimolar, seem to have only transient pharmacological effects on the barnacle nervous system.

Scientists studying swimming behavior in bacteria are using a cyanine dye, diS-C_3-(5), to probe membrane potential changes associated with addition of either desirable or noxious chemicals to the culture medium. These experiments, which may lead to a better understanding of how single-celled organisms control their movements, are not possible with conventional electrophysiological techniques. Bacteria are simply too small and too active to impale with electrodes.

According to Daniel Koshland at the University of California, Berkeley, chemical substances that depolarize the bacterium also increase the likelihood that a swimming bacterium will stop, tumble end-over-end briefly, and swim off in a different direction. The frequency of tumbling events apparently plays an important role in the ability of the bacterium to remain in a favorable chemical environment. Some substances, however, do increase the frequency of tumbling without depolarizing the bacteria.

The dye used in Koshland's study responds comparatively slowly to changes in cell voltage. Alan Waggoner at Amherst College, Joseph Hoffman at the Yale University School of Medicine, and Steven Hladky at Cambridge University have determined that diS-C_3-(5) and other "slow" dyes penetrate to the cell's in-

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teriors where nonfluorescent aggregrates form. The total fluorescence of a suspension of cells changes with cell voltage because the relative proportion of dye inside the cells depends on membrane potential. However, it may take several seconds or more for the dye concentrations to equilibrate. Thus, fluorescence changes are likely to be much slower than are changes in membrane potential. Slow dyes have the advantage of giving rather large optical responses—0.1 to 1 percent change in fluorescence per millivolt of depolarization. The signals are large enough that ordinary spectrometers can be used to assay changes in cell voltage. Already these slow dyes have been used in a host of studies on suspensions of cells, cell fragments, and organelles such as mitochondria.

At the Johnson Research Foundation in Philadelphia, scientists have been scanning tissue surfaces optically looking for fluorescence changes due to slow changes in cell voltage and metabolism which occur in heart or brain tissue deprived of oxygen, or in brain tissue during epileptic seizures. This work may help surgeons locate damaged, diseased, or oxygen-deprived tissues and evaluate

New Electronic Display Screens in the Offing

A variety of novel techniques are being exploited in prototype display screens for the electronics and communications industries. The new devices—now in different stages of development—offer brilliant color, high contrast, and "printed page" quality in panels that are large and thin. Power consumption is low and information, once displayed, does not need to be written again to keep it from fading. Such displays could eventually offer serious competition to the cathode-ray tube, now the technology of choice for television and computer graphics displays.

Mechanisms of some of the new displays resemble those of biologically active dyes that researchers use to measure voltage changes in cells and tissues (see story). One display is called the "Gyricon" by its developers Nicholas Sheridan and Michael Berkovitz at the Xerox Corporation's Palo Alto research center. Patches of the display surface—picture elements—change color from black to white or back when short voltage pulses of one polarity or the other are applied to transparent electrodes on either side of a thin, synthetic membrane. The color in the membrane is provided by microscopic two-colored spheres that rotate within the matrix of the membrane when voltage is applied to the electrodes. Magnetic spheres and pulsed magnetic fields are the bases of another type of the "twisting ball" display.

An electrophoretic mechanism has been used in prototype displays developed by Barry Singer and co-workers at Philips Laboratories in Briarcliff Manor, New York. In these devices, electrically charged pigment particles are suspended in a colored solvent between two electrodes. The suspension is viewed through the front electrode, which is semitransparent. Pigment particles migrate to the front or back electrode depending on the polarity of the voltage across the electrodes, so that either the pigment color or the solvent color is displayed. According to Singer, color and contrast similar to the familiar red on yellow Kodak film carton are easily achieved.

Another group of displays is based on colored electrochemical reactions. Margie Nicholson and co-workers at Rockwell International's Electronics Research Center in Anaheim are testing a rare-earth diphthalocyanine complex in an electrochemical cell. A thin layer of this substance is deposited on the electrode, forming one wall of an electrolyte-filled cell. Depending on the magnitude and polarity of the voltage applied between this electrode and a second electrode outside the field of view, the dye turns violet, blue, green, or red. At Bell Laboratories, an iridium oxide coating that changes from clear to gray and back is being tested by Joseph L. Shay and his colleagues. Yet another class of materials is being studied by Frank Kaufman and Edward Engler at IBM's Yorktown Heights research center. Kaufman is testing a number of inexpensive organic polymers that have multicolor capability. One advantage of the polymers, he says, is that they can be deposited from solution onto the electrode surface, thus avoiding the expense of vacuum deposition.

Displays based on colored electrochemical reactions are called "electrochromic" in the trade. Color changes in these displays result from charge transfer reactions similar to those that occur in batteries. An entirely different mechanism is involved in the color changes of the "electrochromic" dyes used by scientists to measure voltage changes across lipid membranes. These dyes change color in direct response to applied electric fields. While some progress has been made toward exploiting field-sensitive dyes for communications applications, the effect is probably too weak to be useful in display panels.

Predictably, each new approach has its advantages and disadvantages. A major limitation shared by all of these new displays is long response time. If it takes 30 milliseconds to turn one display point or element from white to black, then individiual circuits would be required to control each element if TV rates of 30 frames per second were desired for a 512 by 512 element matrix display. Speed can often be increased by raising the driving voltage, but high voltage circuits are significantly more expensive than standard integrated circuits. Accurate color rendition is another major problem; for many uses three or more colors are sufficient only if they can be combined to produce a continuum of natural colors, such as flesh tones.

There are, however, some strong selling points for the alternative displays. Energy consumption is consistently low. Between a microjoule and a millijoule is required to change the color of a square centimeter of display surface. Additional energy is not required until the image must be changed, perhaps minutes later if text is being displayed. For comparison, to light a square centimeter of a color TV tube requires about 20 millijoules every second. Another advantage over the cathode-ray tube is in image quality. The new displays produce images that are jitter-free and daylight-readable and look very much like a printed page.

Large thin display panels in which these novel mechanisms are used may soon be technically and economically feasible. However, it is likely to be a while before the devices actually appear on the market, and longer still before the cathode-ray tube is seriously challenged in computer and television displays.—F.F.H.

the success of corrective measures such as the rerouting of arterial blood vessels.

According to Lindsay Bashford of the Johnson Foundation, researchers there are able to follow mitochondrial activity by measuring normal changes in the fluorescence of flavoproteins and pyridine nucleotides, substances that occur naturally in mitochondria. Also, they have developed an oxonol dye which they think responds to membrane potential. The dye can display fluorescence changes of 0.01 to 0.5 percent per millivolt, reaching half the final value in 20 to 50 milliseconds. Bashford, Britton Chance, and their colleagues at the Johnson Foundation paint gerbil brain tissue with this dye. Then a laser beam—focused to a spot 18 to 50 micrometers in diameter—rapidly scans the brain surface. Fluorescence spectra of the illuminated tissue are recorded every 50 microseconds. In practice, a 6 to 10 square millimeter area of the cortex is surveyed once a second. With this technique, Chance and his co-workers are able to make two-dimensional images of the changes in cortical cell voltage during chemically or surgically induced waves of depolarization. Similar "spreading cortical depressions" occur in humans during epileptic seizures.

Many of the dyes now in use do not respond predictably from tissue to tissue, so that quantitative measurements require tedious calibration procedures. Differences in dye response may be due to different membrane composition. According to Sally Krasne at the University of California, Los Angeles, membrane surface charge, which depends strongly on composition, affects the optical response of certain types of dyes to changes in cell voltage. Krasne suggests that it may be possible to predict a dye's response in different tissues if allowances for variations in membrane surface charges can be quantified.

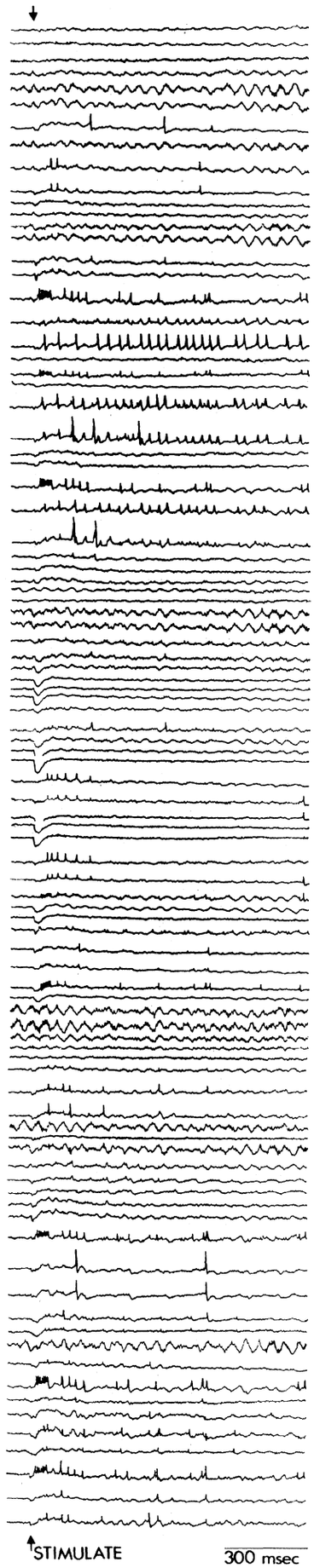
In another approach toward developing more universal optical probes, Leslie Loew at the State University of New York, Binghamton, is trying to identify and synthesize "electrochromic" dyes. These dyes bind to cell membranes and change color in direct response to changes in local electric field strength. The color change does not require that dye molecules pass through the membrane, reorient themselves on the membrane, or move from aqueous to membrane-bound phases as other dyes do, so that electrochromic dyes may respond more uniformly from tissue to tissue. Loew and his colleagues have developed a system, based on molecular orbital calculations, that predicts the magnitude of the electrochromic response. After testing their computer scheme on a variety of known electrochromic and nonelectrochromic dyes, they applied it to unsynthesized structures. Loew's group now has synthesized a theoretically promising dye and verified that it has a substantial electrochromic response.

Unfortunately, electrochromic dyes that have been tested on nerve cells are at least an order of magnitude less sensitive than the best available dyes. Under these circumstances, noise in the detector circuitry, vibrations of optical components, or even minute particles drifting about in the extracellular fluid become confused with the signal associated with changes in membrane potential.

The perfect dye has yet to be found. The search is for substances that show large optical responses to small changes in cell voltage. In addition, the dyes must not disturb the natural processes under study, be toxic to tissues, or cause light to damage cell membranes. More than a thousand dyes have been tested on biological systems. Cohen and his colleagues have tried some 800 of these on squid nerve fibers. Of all of the dyes, only a handful have proved useful to any significant extent. According to Cohen, Waggoner's work has been crucial to the development of useful optical probes. Besides providing dyes for researchers across the country, Waggoner's lab produces about 100 new dyes each year. In addition, he has been able to improve marginally useful dyes by modifying their chemical structures, thus producing most of the useful dyes now available.

Optical probes of membrane potential have proved their usefulness already. When more sensitive, less toxic dyes are developed, the impact of these techniques on cell physiology and neurophysiology may be considerable.

—FREDERICK F. HARTLINE



When the image of a dye-treated barnacle ganglion is focused on a 10 by 10 array of photodetectors, electrical activity of individual nerve cells in the ganglion can be inferred from the photodetectors' outputs. Here each trace shows the output of one detector. Changes in the absorbance of light by a single nerve cell can cause as many as 6 of the 100 detectors to respond simultaneously if the cell's image happens to impinge on that many. In this record, about 16 or 17 cells are active. The arrows indicate the time of an electrical shock given to nerve fibers leading into the ganglion. [Courtesy of Lawrence B. Cohen]

Additional Reading

L. B. Cohen and B. M. Salzberg, "Optical measurement of membrane potential," *Rev. Physiol. Biochem. Pharmacol.* **83**, 35 (1978).

A. S. Waggoner, "Dye indicators of membrane potential," *Ann. Rev. Biophys. Bioeng.* **8**, 47 (1979).