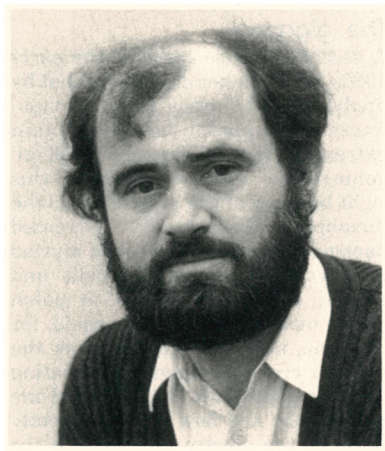
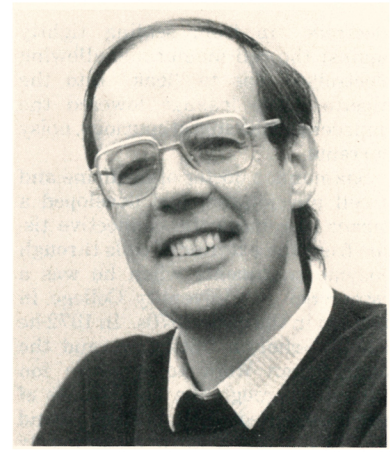
NEHER AND SAKMANN WIN PHYSIOLOGY NOBEL FOR CELL MEMBRANE STUDIES

Many biological functions—such as nerve impulses, muscle contraction, vision and even conception—have their basis in a sudden change in cell membrane permeability. Specific membrane proteins act as gates or agents of active transport, regulating the flow of molecules and ions between the cell and its environment. Members of one class of membrane proteins, the ion channels, open in response to electrical, chemical or mechanical stimuli to allow the spontaneous flow of ions down a concentration or potential gradient across the cell membrane. In 1976 Erwin Neher and Bert Sakmann reported using a tiny yet simple electrode, called a patch clamp electrode, pressed up against a frog muscle cell to record for the first time the opening and closing of a single ion channel[1] in a biological membrane. For their invention, refinement and application of the patch clamp and of the electronics for recording the minute currents it samples, Neher and Sakmann, now at the Max Planck Institutes in Göttingen and Heidelberg, respectively, have been awarded the 1991 Nobel Prize in Physiology or Medicine. In honoring the two, the Nobel Assembly at the Karolinska Institute cited "their discoveries concerning the function of single ion channels in cells."

All living cells are surrounded by a membrane with an oily or waxy lipid core, which is impermeable to water-soluble substances such as ions, metabolites and wastes, explains biophysicist Howard Berg of Harvard. A variety of highly specific transport proteins that span this layer have evolved; by selectively controlling the transfer of ions and molecules across the cell membrane they regulate cell volume and pH, transfer metabolites and wastes, and generate ion gradients, which allow nerve and muscle cells, charged like little capacitors, to fire.



Erwin Neher



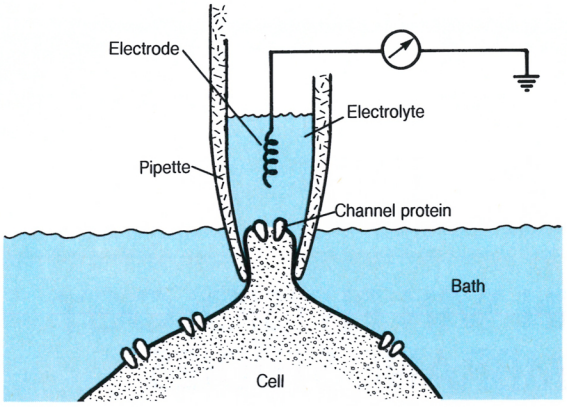
Bert Sakmann

The concept of voltage-gated ion channels in biological membranes dates back to the work of Alan Hodgkin and Andrew Huxley, who in 1952 explained nerve conduction on the basis of sodium and potassium ion channels. In the 1960s studies in which toxins were used to poison nerve cells revealed that it took only a very small amount of toxin to inhibit nerve action, suggesting that ion conduction occurred through sparsely distributed, highly conducting sites. Harold Lecar, a pioneer in the study of ion channels now at the University of California, Berkeley, suggests that "excitable membranes of the nervous system are analogous to semiconductor junctions, where channel proteins are the impurities, the 'dopants' which endow membranes with their interesting conduction properties."

Single-channel currents

By 1970 several investigators had shown that synthetic lipid-bilayer membranes doped with bacterial proteins responded to applied voltage changes with a discretely fluctuating conductance. This response indicated an opening and closing of ion channels. "The expectation was that biological membranes would have similar channels," explains Neher, a membrane biophysicist, "so we set out to look for them."

At that time the standard technique for measuring cell currents, whole-cell recording, involved impaling a cell with a microelectrode that had a sharp, 0.1–0.5-μm tip. This technique produced background noise roughly two orders of magnitude greater than the signal researchers expected to measure for single-channel currents, which is on the order of picoamperes. "For low-noise recording," Neher notes, "you need a high input impedance." One way to increase impedance is to sample a physically small signal source. The patch clamp, by isolating a very small patch of membrane, does just that. (See the figure on page 18). In the patch clamp technique a glass pipette with a fire-polished tip tapered to 3–5-μm diameter, and containing electrolyte solution and a Ag/AgCl electrode, is pressed against the exterior of a cell wall, making it possible to observe currents from no more than a handful of channels. However, in early experiments, connective tissue covering the cell prevented this tiny



Patch clamp electrode, with a tip 3–5 μm in diameter, allows measurement of ion currents through a single channel on a cell membrane.

electrode tip from sealing tightly against the cell membrane, allowing electrolyte ions to "leak" into the pipette. This leakage lowered the impedence and caused spurious, noisy currents.

Sakmann, a doctor of medicine and a cell physiologist, had developed a means to remove the connective tissue from the exterior of cells through enzyme treatments when he was a researcher at University College in London in the early 1970s. In 1972 he joined Neher in Göttingen, and the team began work to measure ion currents through the membranes of enzyme-treated cells. They found that by placing the tip of an optimally shaped pipette against the surface of an enzymatically cleaned cell, they could obtain a pipette–membrane seal with 20–30-megohm resistance. This advance allowed the first successful single-channel recording—the measurement of a channel opening stimulated by the neurotransmitter acetylcholine,[1] completed while Neher was a postdoc at Yale University.

Neher and Sakmann's results helped affirm a number of hypotheses about channel behavior that had been based on earlier, whole-cell recordings—noisy superpositions of thousands of channel responses from which channel kinetics had to be culled indirectly through statistical noise analysis. Their recordings, rectangular current pulses of fixed amplitude (3.4 pA) and random duration (a few milliseconds), confirmed the gate-like behavior of the channel. Their findings also suggested that the channel remains open as long as acetylcholine remains bound to the channel receptor. (The receptor, the channel's "sensor," is a region on the surface of the channel protein with a high affinity for a specific stimulating molecule.) And their observation of random channel opening and closing upheld the notion that the receptors act independently of one another.[2]

The 'gigaseal'

A second advance came in the early 1980s, when Neher discovered that by applying light suction with a scrupulously clean pipette he could obtain extremely high-resistance (10–100 gigohm) seals.[3] Dubbed "gigaseal," this tight bonding of the pipette tip to the membrane dramatically decreased background noise and opened myriad new ways to manipulate cells and control cell environments in patch clamp measurements. One could, for example, now tear patches from the cells to create a membrane coating over the pipette tip. This allowed one to measure the flow of ions directly between the pipette interior and the bath, giving added control over the measurement conditions. One could also, through strong suction, rupture the patch to make an opening into the cell, permitting electrical or diffusional access to the cell interior through the pipette while maintaining a tight seal to the entire cell.[2] Another advantage of the gigaseal, says Frederick Sigworth of Yale, who was a postdoc for Neher at the time of its invention, is that it enables the experimenter to impose large voltage differences between the pipette interior and the bath; this allowed him and Neher to record an electrically stimulated channel selective to sodium ions.[4]

The gigaseal has opened a wide range of cell types for electrophysiological study, not just those "paradigm tissues from which you can easily make electrical recordings," observes Lecar. He calls the 1980s "an ion-channel era," during which the patch clamp recording of channels coincided with the development of other techniques in molecular biology, and saw a surge of research on the molecular basis of membrane transport.

Sakmann and Neher have continued their electrophysiological studies. Sakmann, to determine how different parts of the channel proteins contribute to transport, works with animal cells whose membranes have been reconstituted with genetically altered ion channels. Neher exploits the sensitivity of the gigaseal to measure minute capacitance changes in the cell membrane during secretion. In secretion a vesicle (a small, fluid-filled, membrane-bound sac within the cell) containing the substance to be secreted fuses with the cell membrane, slightly increasing its area and hence its capacitance.

Berg calls the patch clamp "a major technical advance, which, like the cyclotron, made possible a whole new realm of study." Ion channel studies have helped elucidate the mechanisms of several diseases, such as cystic fibrosis and epilepsy, and neuromuscular disorders, like Lambert–Eatons disease. A number of venoms and drugs, such as local anasthetics, act directly on channel receptors. The awarding of the Nobel Prize to Neher and Sakmann, says Berg, "was absolutely inevitable."

Sakmann worked as a research assistant at the Max Planck Institute in Munich before his two-year stay at University College in London. He returned to Germany in 1973 to receive his MD from the University of Göttingen and continued his work as a research assistant in neurobiology at the Max Planck Institute in Göttingen, where he began his collaboration with Neher and held a number of posts, including director of the department of cell physiology. In 1989 he moved to the Max Planck Institute in Heidelberg to assume his current post of director of the department of cell physiology. He is also a professor on the medical faculty of the University of Heidelberg.

Neher received his MS in physics in 1967 from the University of Wisconsin, Madison. In 1970 he received his PhD in physics from the Institute of Technology in Munich. He spent several years as a postdoc at the Max Planck Institute in Göttingen and at Yale. In 1976 he returned from the US to Göttingen where he became director of the membrane biophysics department in 1983 and honorary professor in 1987; he continues to hold both positions.

—Ellen J. Zeman

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