

so outstanding. Biologists would always like to be able to look at specimens in their natural forms at huge magnification. But living cells can't be viewed with an electron microscope, the most powerful tool for visualization, because the sample must be in a vacuum chamber. So biologists try to stop the biological action by various methods of preserving their preparations.

To catch the events in the release of transmitter, the elements of the nerve ending must be immobilized in a few tenths of a millisecond. Standard techniques of preserving, which use chemicals to cross-link the proteins in the sample, take much too long, 15 to 20 milliseconds. Heuser, therefore, decided to stop the action by freezing his specimens.

But freezing introduced an additional problem—ice crystals can destroy the structure of cells. Heuser needed to freeze his samples so rapidly that any ice crystals would be too small to appear in the electron microscope.

To accomplish this feat, Heuser and co-workers built a rapid-freeze machine. Its design is based on the principle that the fastest way to freeze tissue is to press it against a cold metal block.

"We mount the tissue on a plunger and let it drop through the air. We stimulate it just before it hits the cold metal plate," Heuser explains. "Smacking tissue against cold metal is the fastest way to draw out heat. It takes us 0.3 milliseconds to freeze even the deepest parts of the tissue.

"I wanted to see vesicles popping open," Heuser says. "But this technique could also be used for studying muscle contraction or movement of rhodopsin in rod outer segments hit with light."

And Heuser did succeed in seeing vesicles pop. His pictures also illustrate a process that he hypothesized years ago as membrane recycling.

The diagram shows that to release transmitter contents, the membranes of the vesicles merge with the cell's membrane, then gradually flatten to become indistinguishable from the rest. That extra membrane is later recovered when the cell membrane pinches in to form new spheres that combine in larger structures and then redivide into new vesicles. Heuser estimates each vesicle goes through this process about 100 times each day.

"These membrane movements," Heuser says, "maintain the day-by-day economy of the synapse by recycling membrane for re-use after vesicle discharge."

Heuser explained one current theory about what triggers transmitter discharge. When the nerve is not signaling, both the cell's outer membrane and the vesicle membranes are negatively charged and tend to keep apart from each other. A nerve impulse opens gates in the cell membrane that let calcium ions come in. The work of Rodolfo Llinas at the Uni-

versity of Iowa has described these gates (SN: 2/22/75, p. 123). The positively charged calcium ions neutralize the membrane charge, ending the energy barrier to the contact between vesicles and membrane. About 0.2 milliseconds after calcium enters, according to Llinas's most recent experiments, the vesicles collide with the membrane and release their contents.

Another neurobiologist, Samuel Barondes of the University of California at San Diego School of Medicine, reports evidence of a way in which the release of transmitter could be altered by experience. Barondes is studying large nerve cells in the sea slug.

"If we stimulate a cell at a slow rate for about one minute," Barondes says, "it becomes a better releaser of neurotransmitter for a long period. After 100 stimulations, the cell is better for half an hour."

Barondes found that the rate of the cell's return to its normal release characteristics was dependent on temperature in a revealing manner. When the temperature was lowered to 10°C, there was a sharp decrease in the recovery rate.

"This abrupt change probably reflects the fluidity of the cell membrane," Barondes explains. "The lipid molecules in the membrane go from a fluid to a crystalline state. It's like butter, which turns from hard to soft over a small temperature range. When a membrane melts, it means its molecules can move about."

To test this idea, Barondes added alcohol, an agent that makes the membrane more fluid and acts as an antifreeze. As predicted, the rate of recovery of normal transmitter release increased. Alcohol thus had an effect opposite that of cold temperatures.

"These findings . . . suggest that the rate is limited by the membrane fluidity of some nerve terminal component," Barondes and co-workers wrote in *NATURE* (260:797). "The change in efficiency of transmitter release may itself be a change in presynaptic membrane organization (for example, a change in the number or effectiveness of 'vesicle attachment sites')."

The cell membrane is made up of two layers of lipid molecules with protein molecules interspersed among them. The vesicles are thought to merge only with portions of the membrane that are free of protein. Such regions have been observed with the electron microscope.

Repetitive stimulation, Barondes suggests, may somehow result in larger patches of protein-free membrane. The larger the patches, the greater the probability of vesicles fusing with the membrane. During recovery, Barondes says, the large protein-free patches would decay back to resting size.

The new techniques of electron microscopy may soon allow investigators to see directly the changes involved in these alterations of the nervous system. □

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