BIOLOGICAL CLOCKS AND NERVE CELLS

Eventual understanding of the mechanism of man's memory may owe a great deal to current experiments using a large ocean mollusk called a sea hare, in which certain single nerve cells have been found to act as biological clocks, producing patterns of electrical pulses that reflect natural cycles in the sea hare's environment. Felix Strumwasser, associate professor of biology, by instrumenting and studying the electrical activity of the animal's extremely large nerve cells (up to one-half millimeter diameter), has found that certain individual cells produce pulse bursts at the time of transition from night to day and day to night. In addition, cyclic variations in the time at which the pulse bursts occur correspond to similar variations in the bi-monthly ocean tides.

The sea hares can be entrained to artificial light/dark cycles in the laboratory in about three days. Once studies of the nerve cells begin, these artificial cycles govern electrical activity, even though the light cycling is discontinued.

These electrical pulses, which are a little less than a tenth of a volt, are recorded through an electrode made of a finely drawn glass tube filled with a saline solution, which is inserted through the cell wall. Electrical activity continues for about 48 hours after the ganglion containing the nerve cells is removed from the sea hare; however, longer cycles can be investigated, since it has become possible to culture the ganglion for periods of at least one month after removal. Moreover, because the same cells are identifiable in other sea hares, nerve cells from a colony of animals taken from the same location can be studied "in tandem" for longer periods of time.

The particular cells used are in an abdominal ganglion, which is concerned with feeding, respiration, excretion, reproduction, and protection — some of which must rely on time of day and tides.

Pulse rates vary from a few per minute during quiet periods to about 40 per minute at "dawn," and remain that high for about three hours. There is less activity corresponding to "dusk," indicating that it is probably less important to the sea hare.

The mechanisms by which information can be stored chemically within the cell, then converted to an electrical discharge at the cell membrane at some later time, then transferred between cells chemically are not yet known. They are key questions in biology. There are, however, indications that messenger RNA is involved in one aspect of the read-out process, and Dr. Strumwasser is investigating this involvement.

He believes that the electrical impulses are induced by build-up of an excitatory chemical substance in the nerve cells that is triggered by a timed production of messenger RNA. Nerve cells in the sea hare, as well as those in other animals, are unusually rich in RNA. In many of the sea hare's nerve cells, the DNA-rich nuclei occupy about half the cell body's space. Yet these nerve cells do not divide in the sea hare (nor in the human brain). Nor do they export many different chemical agents, since each nerve cell is known to produce only one chemical transmitter substance. Therefore, the large amount of nucleic acid must be used for other purposes.

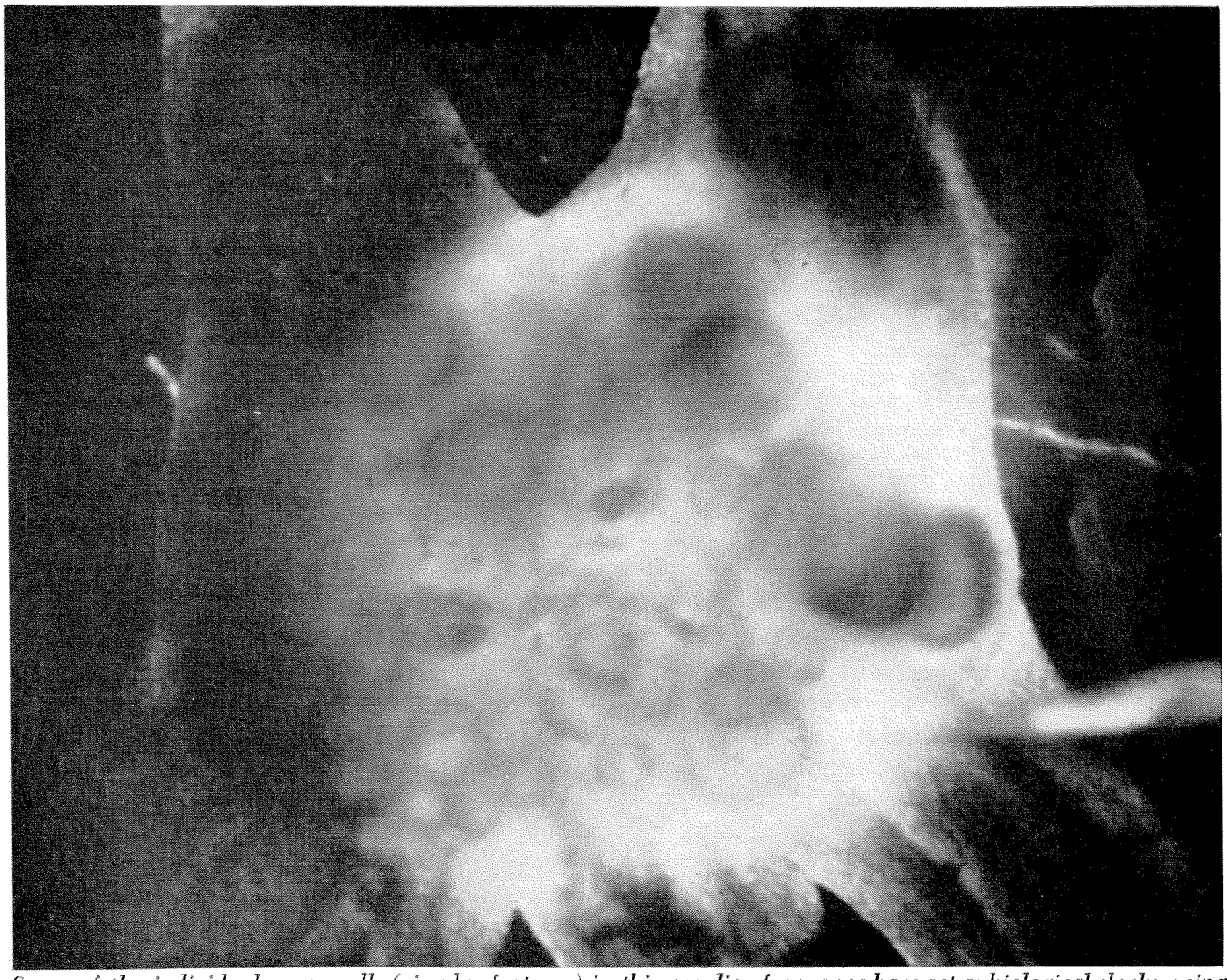
Evidence that there is a timed release of a stored excitatory substance has been found with several different experiments. In one, the temperature of the nerve cell's salt water bath was increased from 58° to 76° for an hour. When the heat was applied during the cell's projected night cycle, it caused release of the excitatory substance, resulting in a premature expression of the pulse burst that, in the absence of heat treatment, would have occurred at the light/dark transition. Heat applied in the light cycle produced little or no effect, possibly because there is no excitatory substance built up in the cell at that time.

Another experiment showed a direct link with RNA activity in the cell. Actinomycin D is a substance that, by binding to DNA, can inhibit the synthesis of RNA. It is also possible that when it binds to the DNA it displaces messenger RNA, which then moves out into the cytoplasm. When

this substance was injected into a nerve cell during the dark period, it caused a large pulse burst almost immediately and, as in the case when heat was applied in the dark period, eliminated the expected response at the time of light/dark transition. When injected during the light period, it caused only a small burst, after which all electrical activity stopped, as if the cell had died. However, 24 hours later there was another large burst, timed not with the light/dark transition, but with the injection of actinomycin D. The nerve cell had been reset by the drug.

Dr. Strumwasser began this work at the Walter Reed Army Institute of Research in Washington, D.C., and has been continuing it at Caltech under sponsorship of the Air Force Office of Scientific Research and the National Aeronautics and Space Administration. He is now beginning experiments to determine if the amount of messenger RNA in the cell's cytoplasm increases just before and during the heightened electrical activity.

One of the interesting techniques he is now trying makes use of autoradiography, in which uridine (one of the bases in RNA) treated with tritium is injected into the cell, where it is incorporated into RNA being manufactured. Ganglia are then frozen at various stages of the electrical cycle and cut into thin sections. The sections are coated with photographic emulsion, in which silver ions are reduced by the emission of electrons from the radioactive RNA. The result is a visual record, in the form of black dots, of RNA concentrations. Comparison of sections made at different times shows the RNA movement within the cell. If the quantity of messenger RNA does increase at the time of electrical activity, it will indicate RNA involvement in recall, one of the important components of memory.



Some of the individual nerve cells (circular features) in this ganglion from a sea hare act as biological clocks, using electrical pulses to communicate with other cells. A micro-electrode (entering a cell from the lower right of this picture) detects this electrical activity, which is then recorded and correlated with the time of environmental cues previously given to the sea hare. The width of the ganglion shown here is about three millimeters.