AFRRI SR74-18
AUGUST 1974

AFRRI
SCIENTIFIC REPORT

INTRACELLULAR RESPONSES TO PHYSIOLOGIC STIMULI FROM *APLYSIA* STATOCYST RECEPTOR CELLS

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AFRRI SR74-18

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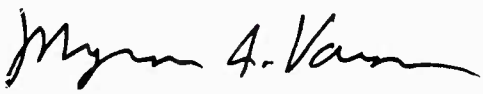

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ABSTRACT

By tilting a statocyst preparation with a receptor cell impaled by a microelectrode, depolarizing receptor potentials, action potentials and increased membrane conductance are observed during the application of the natural stimulus. The conductance increase has an equilibrium potential near -20 mV. Fluctuations in cell membrane potential increase in amplitude as the preparation is tilted in an excitatory direction.

I. INTRODUCTION

The statocyst of gastropod molluscs, such as Aplysia californica, functions in the righting response and contains receptor cells which produce action potentials when the preparation is rotated about a horizontal axis.[2,5] The Aplysia statocyst is a fluid-filled sphere containing approximately 600 to 1000 free statoconia.[2,4] The wall of the statocyst is composed mainly of 13 receptor cells, each bearing many cilia on its luminal surface. As the cyst is rotated, the statoconia roll to deflect the cilia of those receptor cells forming the bottom one-third of the cyst. It is hypothesized that this deflection leads to excitation of the receptor cells.

II. METHODS

We have recorded intracellular potentials from receptor cells with glass micropipette electrodes of 20- to 80-megohm resistance during exposure to the natural stimulus of tilting about a horizontal axis. The receptor cell input resistance was measured by passing current through the recording electrode using an active bridge circuit. The preparation consisted of the isolated circumesophageal ring of ganglia with one pedal ganglion removed to expose the statocyst on that side. The preparation was pinned with the rostroventral side of the ring up and was maintained in an uncirculated artificial seawater bath. The bath was held at 15°C by coolant flowing through a jacket surrounding the preparation chamber. Physiologic stimuli were provided by mounting the preparation, electrode holder and amplifier head stage on a tilting table. The table could be tilted 45° in either direction from horizontal. In addition, the top of the table could be rotated up to 90° so that any point on the statocyst wall could be moved through a 90° excursion by tilting. All cells from which recordings were made

were near or above the "equator" of the statocyst when the table was in the horizontal position.

III. FINDINGS

Upon penetration of a receptor cell, full resting membrane potential of -40 to -50 mV was attained within 1 to 2 minutes. Initial spiking usually ceased within the same time. Passive current-voltage relations for the cells were nearly linear from approximately -40 to +5 mV relative to the resting membrane potential. Input resistance varied from 10 to 110 megohms. The time course of charging, using -0.5 nA, 300-msec pulses, usually followed a single exponential with a time constant of 10 to 70 msec. Cells with input resistance less than 20 megohms and time constants less than 20 msec were rejected as having been injured during penetration. Spikes could be elicited by passing positive current, 0.2 to 0.5 nA for 50 to 200 msec usually being sufficient. Spikes elicited either by current or physiologic stimuli were from 60 to 110 mV peak to peak amplitude.

The response of a statocyst receptor cell varies with the direction of the tilt. If the table is tilted from the horizontal position so that the recorded cell is moved upward, i.e., away from the statoconia, either no change or a slight increase in negative membrane potential results (approximately 3 mV more negative in Figure 1A). When the recorded cell is tilted downward, a depolarizing response is obtained (Figure 1B). This depolarization can be as much as 20 mV at its maximum (15 mV maximum in the case of Figure 1B). The depolarization generally declines during a maintained tilt, but it is still evident 60 sec after a tilt is initiated. In the example of Figure 1, the depolarization declined to 7 mV after 60 sec (compare initial portion of Figure 1C to

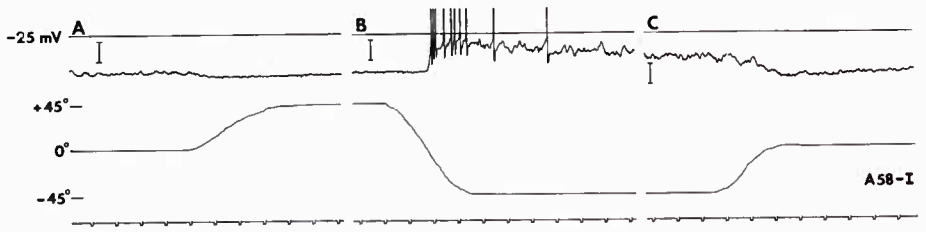

Figure 1. Responses of an Aplysia statocyst receptor cell to tilting. Upper trace: reference line of -25 mV (intracellular potential relative to bath). Second trace: membrane potential, calibration bar = 10 mV in each portion. Spikes were cut off photographically, actual spike amplitude: 70 mV peak to peak. Third trace: tilting table position $+45^{o}$ being maximum cell-up position and -45^{o} being maximum cell-down position. Table position was measured by means of a fixed potentiometer whose wiper was attached to the tilting platform. Bottom trace: 1-sec time markers. In this case the tilting table platform was positioned so that the point of electrode penetration was most distant from the axis of tilting, allowing a maximum vertical excursion of the recorded cell. The point of electrode penetration was near the equator of the cyst as viewed from above in the horizontal position. Thirty-five seconds omitted between A and B; 50 sec omitted between B and C.

middle portion of Figure 1B). Upon return to the horizontal position, the potential returns to its initial value (Figure 1C). Action potentials may occur during the early portion of the response to a downward tilt (only during the first 5 sec in Figure 1B). Discharge is usually not maintained during maintained tilt. The transient nature of the spike response may be due to the limited excursion of our tilting table. With a microelectrode in the cyst lumen, it is often possible to record maintained small extracellular spikes from several cells. It may be that if we could get our penetrated cells in a lower position, they would also exhibit maintained firing.

In the resting state the statocyst receptors exhibit fluctuations in membrane potential. These fluctuations vary from 1 to 15 mV in peak to peak amplitude. The amount

of fluctuation correlates with the position of the recording electrode on the cyst wall. When a penetration is made near the top of the cyst, the fluctuations are usually small whereas they are larger when penetration is near the equator of the cyst. When recording from cells with similar input resistances in the pedal or pleural ganglia, using the same electrodes, the potential fluctuations are typically less than 1 mV. The potential fluctuations in the statocyst receptor cells also change with the position of the tilting table (Figure 1). The fluctuations are moderate when the table is in the level position, minimal in the "cell-up" position, and maximal in the "cell-down" position. Presumably the fluctuations and the depolarization come about from the statoconia striking the cilia of the recorded cell. In the case of Figure 1, in the level position, a portion of the recorded cell may have extended far enough down to be in contact with some statoconia. In the cell-down position more of the cell's luminal surface and its cilia would have been covered by statoconia. This would be consistent with the large extent of the receptors noted in our anatomical studies.[4] When observed through a dissecting microscope, the statoconia are seen to move in a random fashion, even with the tilting table stationary. This random motion may be caused by collisions with the cilia, leading to the fluctuations in membrane potential. At moderate levels of potential fluctuation, discrete positive going "bumps" of 1-5 mV are frequently seen. If these were caused by collisions with one statoconium, the anatomical relationship of statoconia to cilia[4] would suggest that the contribution to potential changes from a single cilium would be on the order of 0.5 to 1 mV.

When observed with Nomarski optics, the movement of the statoconia is greater near the ciliated wall, suggesting that the movement is imparted by motile cilia.

Since all cilia appeared qualitatively similar in electron microscopy,[4] this suggests that the statocyst cilia are both sensory and motile. This has also been suggested for Aplysia limacina[2] and appears to be the case in Paramecium.[3]

The receptor cell membrane conductance increases during the depolarizing receptor potential (Figure 2). The potential changes developed by constant current pulses passed through the recording electrode decrease as the table is tilted from a cell-up to a cell-down position (Figure 2A). The time courses of potential and input resistance changes are similar (Figure 2B, C). As the membrane depolarized by approximately 5 mV, in the case of Figure 2, the input resistance dropped from approximately 57 to 32 megohms, or 44 percent. Data from similar experiments where larger depolarizations were elicited indicate that the conductance increase is part of a "channel" whose equilibrium potential is approximately -20 mV. Equilibrium potential was estimated from plots of cell input resistance versus membrane potential during a gradual depolarizing receptor potential (extrinsic polarizing currents were not applied). These plots were nearly linear and the potential of the extrapolated straight line at zero input resistance was taken as the equilibrium potential. This procedure gives the actual value of the "battery" associated with the transducing membrane only if the nontransducing membrane resistance does not change with membrane potential. If the membrane resistance were to decrease during depolarization, our estimate of equilibrium potential would be more negative than the true value although the plots indicate this error would be less than 10 mV.

Figures 1B and 2 reveal a delay of several seconds between the onset of tilt toward the cell-down position and development of the depolarizing receptor potential.

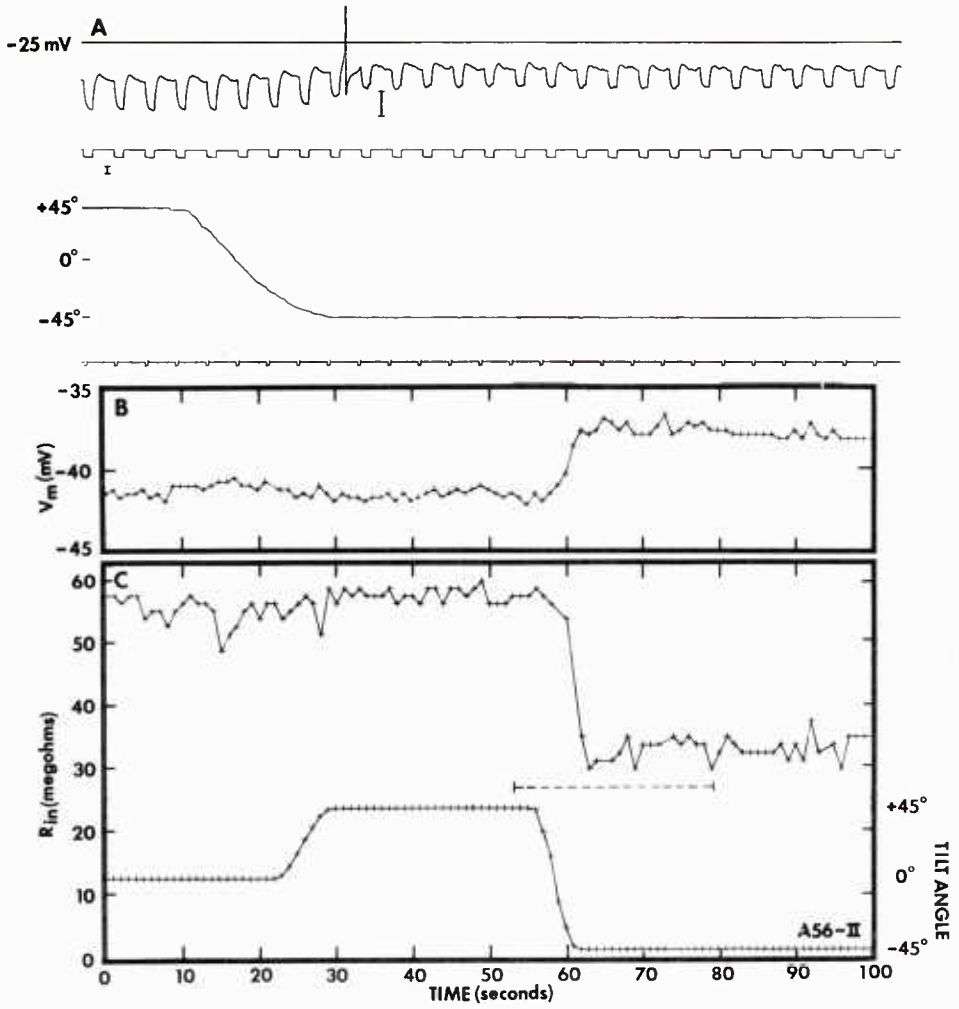

Figure 2. Measurement of change in cell input resistance during response to tilting. A. All traces except middle one as in Figure 1. Calibration bar for membrane potential equals 10 mV. Actual spike amplitude: 62 mV peak to peak. Middle trace: current applied through recording electrode, calibration bar for current = 0.2×10^{-9} A. B. Receptor cell membrane potential in millivolts during a series of tilts from horizontal to cell-up position to cell-down (tilting table position indicated in lower trace of C). C. Receptor cell input resistance (upper trace, left-hand scale) and tilting table position (lower trace, right-hand scale). The dashed line in C indicates portion of plot for which raw data are displayed in A. In B and C, plotted potential is that at the beginning of each current pulse; input resistance is calculated from the membrane potential displacement at the end of the current pulse, and table position is that at the beginning of each pulse. Current pulses were -0.2×10^{-9} A, 300-msec duration, presented 1/sec.

This is consistent with observation of statoconia movement in an excised statocyst. If the statocyst is dissected out, relatively free of connective tissue, a translucent sphere is obtained within which the statoconia can be seen from any angle. When such a cyst is rotated 90^{o} the statoconia roll along the inner cyst wall and do not come to rest at the bottom of the cyst for 4-5 sec. Thus presumably one receptor cell does not begin to depolarize until it is in a low position and the statoconia have rolled down to it to deflect its cilia.

One source of artifacts which can easily plague studies of mechanoreceptors is movement or bending of the recording electrode. High resistance micropipettes are very sensitive to bending: slight movements of the tip can change the electrode tip potential and resistance. If the electrode were flexed during a mechanical stimulus, part of what is interpreted as a physiological response could be due to changes in electrode properties. Mechanical stimuli could also cause relative motion of the electrode tip and the cell membrane, in which case the electrode could deform the membrane and change its resistance. In these experiments, records such as Figure 2B, C serve as a control for such artifacts. When the table is tilted 45^{o} in the cell-up position, no consistent change in potential or resistance is seen. Presumably in this case the cell, which has no or few statoconia in contact with it in the level position, is being moved away from the statoconia and no physiological response is to be expected. If the responses seen were from an artifact of either electrode bending or the electrode deforming the cell membrane, then it would not be expected that such a mechanical movement would be so nonlinear as to give large responses in one direction and none in the other.

Although intracellular recordings from statocyst receptor cells have been obtained in another mollusc,[1] the effects of physiologic stimuli on these cells have not been previously studied. The ciliated mechanoreceptor cells in the Aplysia statocyst respond to the physiologic stimulus of tilting with a depolarizing receptor potential. This depolarization can elicit transient spike responses and is associated with an increase of fluctuations in membrane potential. The depolarization and increased membrane conductance appear consistent with the statoconia loading the cell and increasing the conductance of a channel whose equilibrium potential is approximately 20 mV, inside negative.

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DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Armed Forces Radiobiology Research Institute
Defense Nuclear Agency
Bethesda, Maryland 20014

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP
N/A

3. REPORT TITLE

INTRACELLULAR RESPONSES TO PHYSIOLOGIC STIMULI FROM APLYSIA STATOCYST RECEPTOR CELLS

4. OESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*

M. L. Wiederhold

6. REPORT DATE
August 1974

7a. TOTAL NO. OF PAGES
11

7b. NO. OF REFS
5

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. NWED QAXM

c. Task and Subtask C 912

d Work Unit 13

9a. ORIGINATOR'S REPORT NUMBER(S)
AFRRI SR74-18

9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*

10. OISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Director
Defense Nuclear Agency
Washington, D. C. 20305

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