A Common Origin of Voltage Noise and Generator Potentials in Statocyst Hair Cells

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ABSTRACT Voltage noise, generator potentials, and hair movements in the *Hermissenda* statocyst were analyzed. Motile hairs on the cyst's luminal surface moved as rods through ±10° at 10 Hz when free and at 7 Hz when loaded with the weight of the statoconia (at 20°C). For hair cells oriented opposite to a centrifugal force vector, rotation caused depolarization and increase of voltage noise variance. The depolarizing generator potential and the increase in voltage noise variance were similarly reduced by perfusion with zero external sodium or chloral hydrate. Cooling, perfusion with zero external sodium or chloral hydrate reduced the movement frequencies of the hairs but increased their range of motion. The same treatments reduced voltage noise variance and increased input resistance of the hair cell membrane. The results indicate that voltage noise and hair cell generator potential have a common origin: exertion of force on statocyst hairs by the weight of statoconia. The collision of statoconia with the motile hairs, not the hairs' bending, produces most of the voltage noise.

INTRODUCTION

Transduction by sensory receptors necessarily begins at the interface of cellular structure and the external world and extends through successive steps of energy transformation within the sensory cell. For mechanoreceptors, mechanical energy causes distortion of cell membrane. This distortion, in turn, gives rise to a conductance change(s) which is accompanied by a shift in membrane potential (Eyzaguirre and Kuffler, 1955; Goldman, 1965; Alkon and Bak, 1973; Alkon, 1975). Previous work on the insect hair (Thurm, 1968) and the Pacinian corpuscle (Quillam and Sato, 1955; Loewenstein and Rothkamp, 1958) and other receptor organs has indicated possible sites for membrane distortion and conductance changes.

Statocyst cilia have long been thought, through their interaction with the statoconia, to mediate the response of hair cells (Charles, 1966). Recently depolarizing generator potentials were elicited in statocyst hair cells by gravitational and displacement stimuli (Alkon and Bak, 1973; Alkon, 1975; Wiederhold, 1974, 1976). Studies on these generator potentials indicated that they arose when statoconia (crystals suspended within the statocyst) experienced a gravitational

force and then presumably produced bending of motile hairs which line the inner surface of hair cell bodies. This bending was thought to produce depolarizing waves (voltage noise) which were also observed to occur spontaneously at lower frequency and with smaller peak to peak amplitudes (Alkon, 1975; Wiederhold, 1976).

A quantitative analysis of this voltage noise has been made (DeFelice and Alkon, 1977 *a*, *b*). This analysis demonstrated that in response to rotation the change in the variance of the voltage noise was approximately proportional to the change in the average membrane potential. It was also shown that changes in membrane potential due to rotation had the opposite effect on the variance of the voltage noise as did changes in membrane potential due to current injection.

These observations suggested that hair cell generator potentials could be considered as the sum of random voltage fluctuations according to a shot model used for acetylcholine-induced noise at the neuromuscular junction (Katz and Miledi, 1972). Further support for such a model would be provided by independent evidence for a common origin of voltage fluctuations and hair cell generator potentials.

To acquire such evidence, we first examined the relationship between the movements of the statoconia, the movements of the hairs, and the voltage noise within the statocyst. A variety of conditions were found to cause a reduction or sometimes a cessation of hair movements simultaneously with a cessation of statoconia movements. These treatments also drastically reduced, but not entirely eliminated hair cell voltage noise variance. Steady generator potentials produced in the hair cell by rotation (cf. Alkon, 1975) were also affected by the above conditions which altered intrinsic hair cell motility, statoconia movements, and associated base-line noise. These and additional experiments will be seen to indicate that the hair cell noise and generator potential arise from the same mechanotransduction process and involve the same conductance changes. This evidence for a common origin of hair cell noise and generator potentials is consistent with certain features of conceptual models previously proposed for transduction of gravitational stimuli by hair cells (Charles, 1966; Alkon, 1975; Wiederhold, 1976).

METHODS

Preparation

Hermissenda crassicornis were provided by Mr. Michael Morris of the Peninsula Marine Biological Supply Co. (Monterey, Calif.). Animals were maintained with 12 h of daily light (cf. Alkon, 1974) in a flow-through seawater aquarium at the Marine Biological Laboratory, Woods Hole, Mass. at 12°C. The circumesophageal nervous system of *Hermissenda* was dissected and isolated as previously described (cf. Alkon and Bak, 1973).

For most experiments described here, hair cell synaptic interactions and impulses were eliminated by cutting their axons at their point of entry into the cerebropleural ganglion ("cut nerve" preparation, cf. Alkon, 1975). Impulse generation was still possible when the hair cell axons were cut 10–20 μm distal to their point of entry into the cerebropleural ganglion.

Rotation of the Circumesophageal Nervous System

Details of mounting the preparation for rotation and intracellular recording have been previously described (Alkon, 1975). In the present study, rotation of the preparation was produced by a turntable which consisted of an aluminum disk, 1.9 cm thick and 39.5 cm in diameter, powered by 1/20-hp motor (B and B Motor and Control Corp., New York). The preparation was placed 18 cm from the center of rotation. Additional features of the rotation apparatus were also as previously described.

Intracellular Recording

As in previous studies, before making intracellular recordings the nervous system was incubated in a solution of a digestive enzyme (1–5 min protease; 0.2–1.0 mg/m^3; type VII, Sigma Chemical Co., St. Louis, Mo.). Recordings from cells with no enzyme incubation were not observably different from recordings from enzyme-treated cells.

Intracellular recordings were made with glass micropipettes filled with 4 M potassium acetate (resistance of 40–100 MΩ). The electrode was connected via a silver wire to the input stage of a high impedance amplifier. The reference electrode was a silver/silver chloride wire. A Wheatsone bridge circuit was used to pass current through the recording electrode. Current was monitored by recording the potential drop across a 10-MΩ resistor in series with the electrode. Further details concerning the amplifier used for potential recordings are given in Alkon (1975).

Membrane resistance was measured by injecting negative and positive current ramps (1–2.5 s duration) as well as square current pulses through the bridge circuit. Ramp injection allowed a more rapid measurement of a wide range of voltages, since dV/dt is close to zero. (The ramp durations were much longer than the longest time constant of the hair cell membrane: ≤ 45 ms, cf. Alkon and Bak, 1973.)

Perfusion Solutions

All of the experiments, except those using rotation, were performed with continuous perfusion of seawater solutions at a rate of 2.5 ml/min. The experimental chamber had a total volume of 0.6 ml. Hyperosmotic solutions (1,600–2,800 mosmol) were made by adding choline-Cl, NaCl, Tris-Cl, or glucose to normal seawater. The composition of the Na-free seawater solution was: 50 mM $MgCl_2$; 10 mM KCl; 10 mM $CaCl_2$; 525 mM Tris-Cl or choline-Cl. The pH was adjusted to 7.6–7.8 by 10 mM Hepes buffer. In some experiments the sodium was substituted by 130 mM putrescine and 600 mM glucose. Chloral hydrate solutions of 3–15 mg/ml were used. In the rotation experiments, solutions were changed by means of a calibrated microsyringe attached to the rotating table and connected to the Vaseline chamber via a fine polyethylene tube.

Temperatures in the range of 6–30°C (±0.5°C) were controlled by having the perfusion solutions flow through a stainless steel coil of a counter-current heat exchanger containing warm tap water or ice water. Temperature was monitored throughout the experiments by a small thermistor probe in the immediate vicinity of the preparation. Unless otherwise specified, all experiments were performed at 18–20°C.

Records

All of the voltage records presented were taken from a Brush 440 pen recorder (Gould, Inc., Cleveland, Ohio) which had flat frequency response from DC to 40 Hz when operating at the maximum of its deflection, and up to 100 Hz at about one-fifth of that deflection. Voltage records were also made simultaneously on a tape recorder for noise analysis. Membrane time constants and resistance were also measured from photographs of the oscilloscope display of hair cells' voltage.

Voltage Noise Analysis

Samples (30 s) of hair cell voltage noise were recorded on FM tape (model 3960, Hewlett-Packard Co., Palo Alto, Calif.) at 3.75 inches per second. The signal was recorded at a band width of DC-1000 Hz. Autocorrelation functions were computed off-line from digitized data (A to D converter, Hewlett-Packard model 5465A) on a Fourier Analyzer (Hewlett-Packard model 5451A) after filtering at 0.1–100 Hz. Sampling was done every 5 ms for periods of 20.48 s. Frequencies in the range 0.1–100 Hz were analyzed. The records were reproduced from the oscilloscope display by an X-Y recorder. Noise variance was taken as the first point of the autocorrelation function ($\tau = 0$).

Light Microscopy and Stroboscopic Measurements

Live preparations were observed and photographed with a universal transmitted light microscope (Carl Zeiss, Inc., N. Y.). The microscope could be tilted 90° to enable simultaneous visualization of free hairs and hairs which experienced the weight of the statoconia.

Experiments in which temperature and concentration of chloral hydrate were varied were performed under constant perfusion, and visualization was accomplished by a water immersion objective (×40). In these latter experiments the microscope could not be tilted and therefore only the frequencies of the statoconia movements were measured. The movement frequencies of the hairs and the statoconia were determined with a stroboscope (Strobotac, type 1531-AB, GenRad, Concord, Mass.). Strobe light was transmitted through an oil immersion condenser (with Nomarski optics, × 1,250) onto the preparation. The observer varied the strobe frequency from high to low and vice versa. The lowest frequency at which individual hairs appeared stationary was recorded. When this lowest or base frequency was doubled the hairs also appeared as stationary.

Each observation was repeated several times by the same observer and was repeated by at least two observers. The mean strobe frequencies obtained by the different observers were used for our calculations. Movement frequency of the statoconia was determined in the same way. Occasional "slow" movements of the statoconia were disregarded. When movement was considerably reduced by treatments, such as chloral hydrate, the frequency of the hairs or statoconia was determined under steady illumination by counting the number of oscillations during fixed time intervals.

Scanning Electron Microscopy

The circumesophageal nervous systems were fixed immediately after dissection with 2% glutaraldehyde and paraformaldehyde in 0.12 M cacodylate buffer, pH 7.4, and 4% sucrose for 4 h. After fixation, the tissue was dehydrated with serial dilutions of ethanol. While in absolute ethanol the luminal surface of the statocyst was exposed by making a dorsoventral cut through the statocyst with a razor blade. The alcohol was then replaced by liquid CO_2 in a critical point apparatus (Tousimis) and the tissue subsequently dried by the critical point method according to Anderson (1951). The specimens were mounted on stubs with silver glue, coated with approximately 150Å gold in a vacuum evaporator (Tousimis Research Corp., Rockville, Md.) and examined on a scanning electron microscope (JSM-35, JEOL U.S.A., Electron Optics, Medford, Mass.).

RESULTS

Structure

The statocysts of *Hermissenda* consist of 12–13 disk-shaped hair cells which are 40–50 μm in diameter and 5–10 μm in thickness (Alkon, 1975). Scanning

electron micrographs of normal specimens show the statocyst to be a sphere, 80–110 μm in diameter, surrounded by strands of a connective tissue bundle which also envelops each of the two *Hermissenda* eyes and optic ganglia. Each hair cell has approximately 120–150 hairs of uniform length (~ 8–10 μm). These hairs were motile as previously observed (Alkon, 1975). The hairs cover the entire luminal surface of the statocyst. The density of the hairs is 12–15 per 100 μm^2, with each hair being ~ 3μm from its nearest neighbor (Fig. 1 A). Examination of the inner surface of the statocyst, under the scanning electron microscope, in areas which were not damaged by the cutting, revealed that the hairs were fixed at different angles with respect to the plane of the hair cell's luminal surface (Fig. 1 A). Similar structure has been observed for the *Aplysia* statocyst (McKee and Wiederhold, 1974).

Examination of live preparations with the light microscope (using Nomarski optics) revealed that each hair cells' inner surface (which is in fact part of the inner surface of the statocyst) is convex. The hairs were found to be straight and perpendicular to the cell surface and therefore not all the hairs point toward the center of the statocyst (Fig. 2). Fixation with glutaraldehyde did not alter the hairs' orientations. Such fixation, however, did permit better photographic reproduction because of the hairs' immobility (Fig. 2). Individual statoconia (3–16 μm in diameter) could be found in contact with the hairs, although not attached to them, at their distal ends when the hairs were exactly perpendicular to the cell's surface (see also Fig. 1 B). As previously described (Alkon and Bak, 1973; Detwiler and Alkon, 1973), 150–200 statoconia, grouped within a spherical cluster, are continually moving, as visualized under a dissecting microscope. If we consider the hairs' density and the size of each statoconium, we can estimate the number of hairs interacting with one statoconium to be 1–20. Occasionally *Hermissenda* statocysts containing only one statoconium were found either on one or both sides of the animal. The structure of statocysts with one statoconium was different from the normal structure (Fig. 3). It consisted of a sphere (50 × 70 μm in diameter) with a much smaller inner lumen (20–40 μm in diameter) because of its unusually thick walls (10–15 μm). The hairs (10–15 μm long) covered the luminal surface of the statocyst, with each hair only 1 μm apart from its nearest neighbor. The single statoconium (~9 μm in diameter) was interacting continually with some of the hairs of hair cells situated throughout 360° around the cyst.

Movement of Hairs and Statoconia

Unloaded hairs, i.e., those hairs in the upper part of the statocyst in a tilted preparation (cf. Fig. 4), appeared to move, under Nomarski optics (×1,250), as rods whose free ends swing, at 10 Hz (20°C), 9–10° to each side of a line perpendicular to the plane of the hair cell's inner surface. With stroboscopic flashes at 20 Hz, as well as at 10 Hz, these unloaded hairs appeared to cease their movement whereas at flash frequencies lower than 10 Hz they always appeared to continue their movement.

Loaded hairs, i.e., those hairs in the lower part of the statocyst in a tilted preparation, appeared to move with a fundamental frequency of ~ 7 Hz (Table I). These hairs experienced the weight of the statoconia (cf. Fig. 4). The

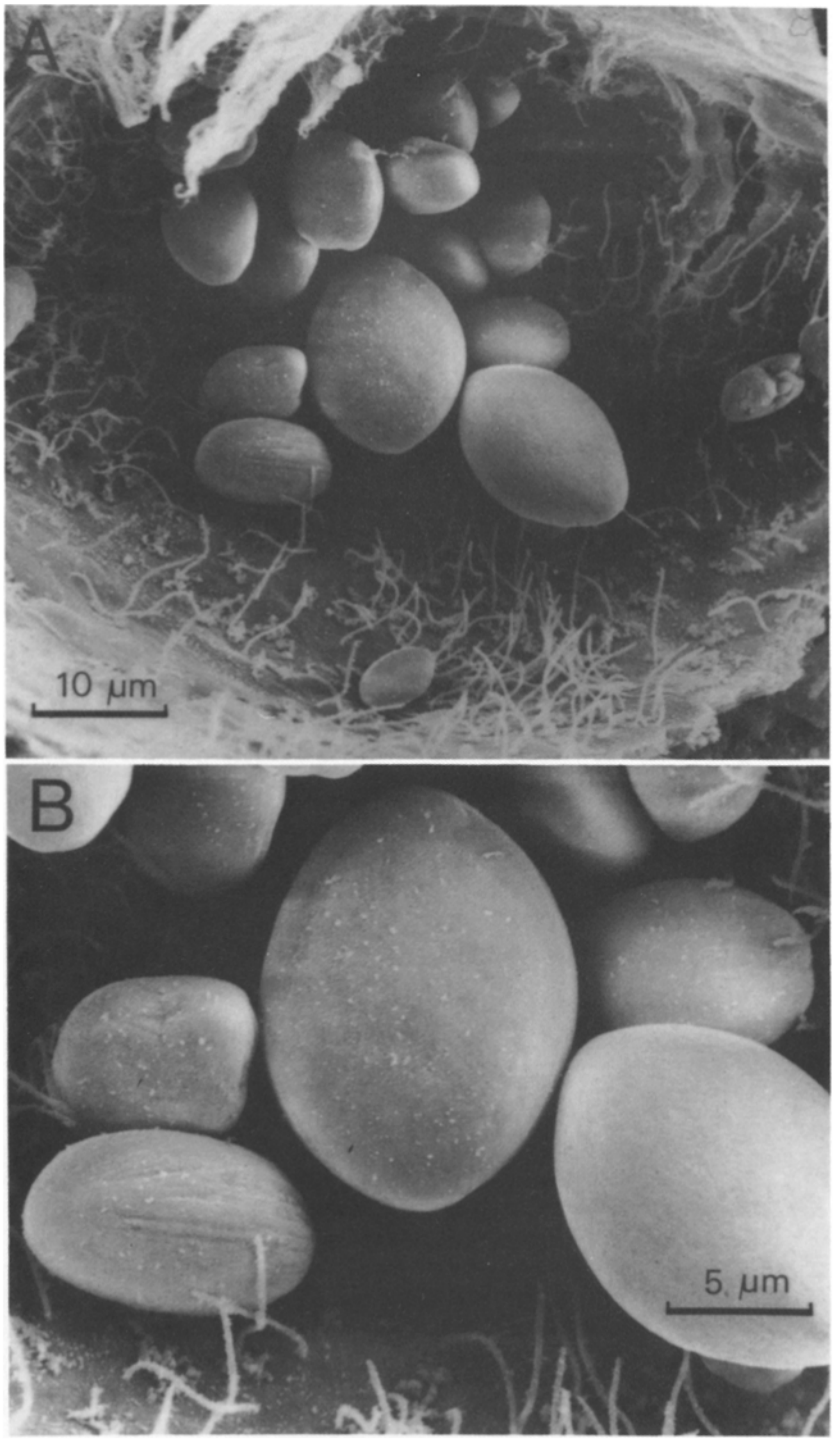


FIGURE 1. Scanning electron micrograph of *Hermissenda* statocyst. (A) On the luminal surface of the statocyst, hairs make contact with statoconia. (B) Higher magnification of the above.

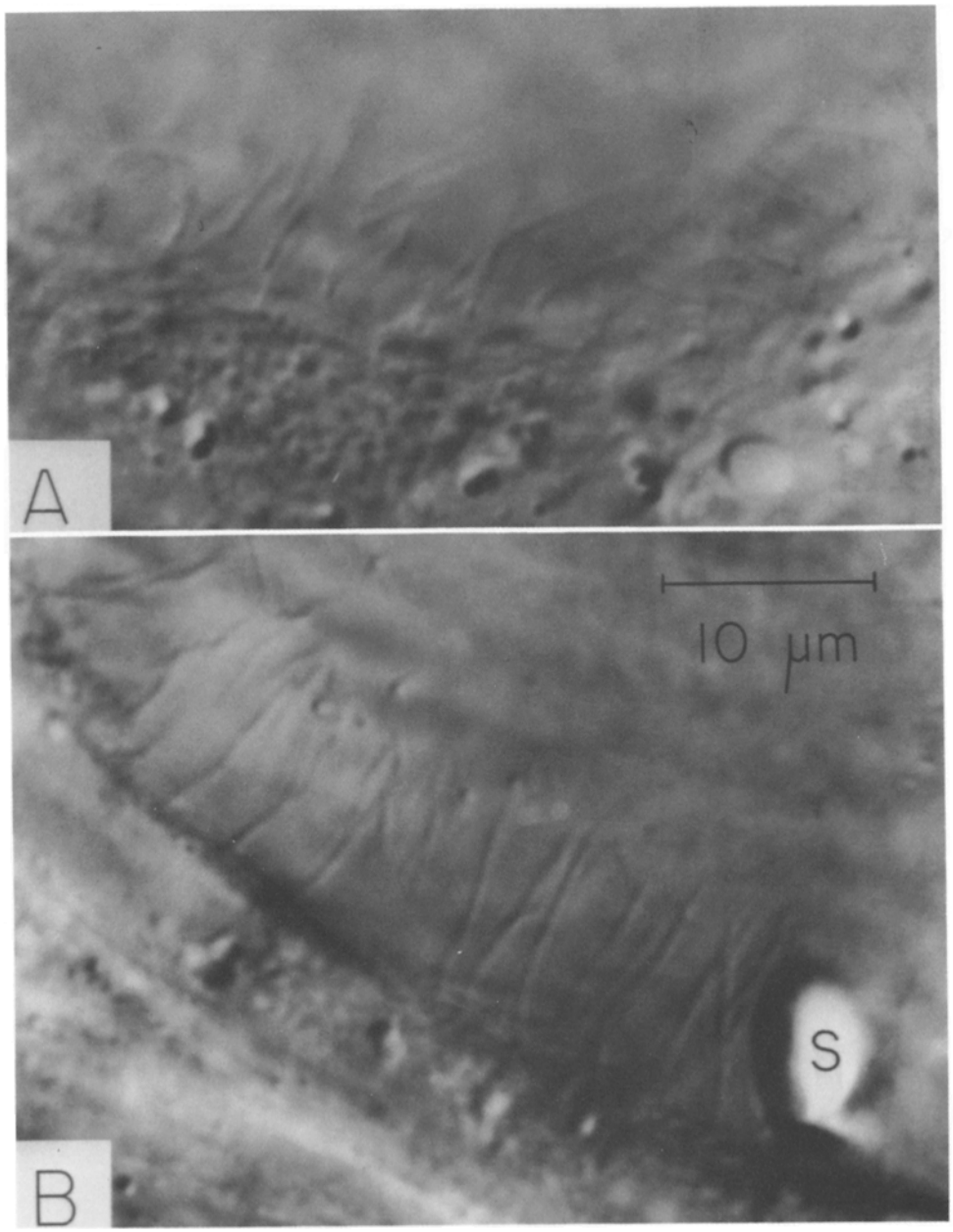


FIGURE 2. Visualization of motile hairs in *Hermissenda* statocyst. Hairs were visualized with Nomarski optics (× 1,250) in an intact statocyst placed on a microscope tilted 90°. (See also Fig. 4 E-G). (A) Vital preparation. Apical half of the motile hairs could not be photographed but could be visualized. (B) Preparation fixed with 0.5% glutaraldehyde. The full lengths of the hairs, now immobilized, are apparent in the photograph. A single statoconium (S) is seen among the hairs.

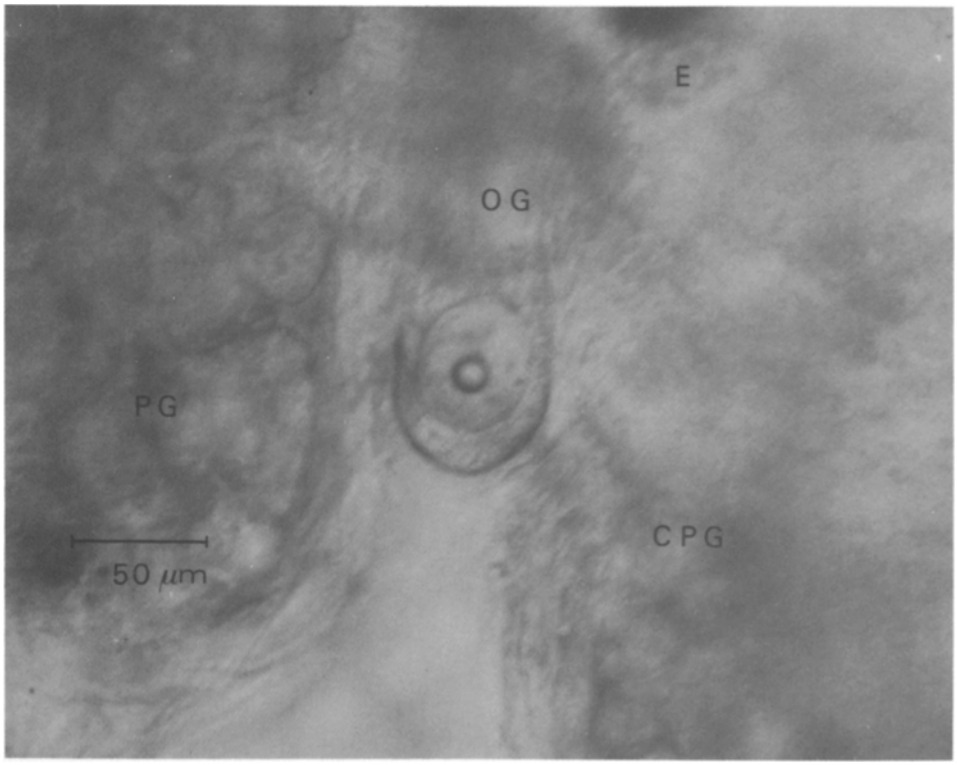
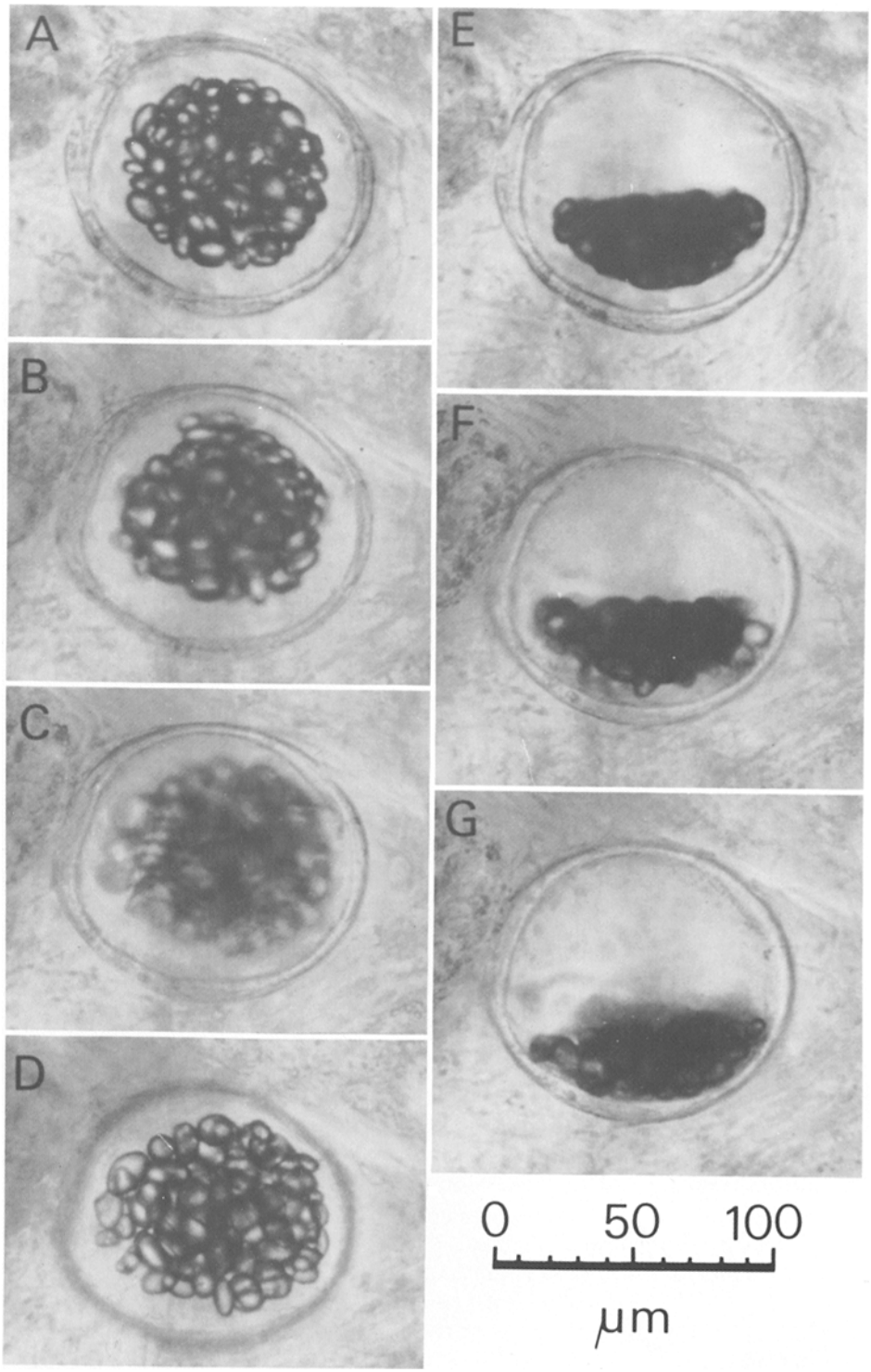


FIGURE 3. Light micrograph of statocyst with a single statoconium. CPG, cerebropleural ganglion; PG, pedal ganglion; OG, optic ganglion; E, eye.

movement frequency of each hair was essentially constant as determined by successive measurements by at least two observers (see Methods). Within the resolution of the stroboscopic technique, the movement frequencies of all neighboring hairs were essentially the same. Each hair was seen to move only in one spatial plane which was different for neighboring hairs. There was no obvious synchrony of hairs' movements, although some synchrony could not be entirely ruled out.

The movement frequency of statoconia adjacent to the loaded hairs was also ~ 7 Hz. Statoconia not adjacent to statocyst hairs (i.e., the statoconia towards the center of the cluster) moved with much lower frequencies and were not used for these measurements. The statoconia adjacent to the hairs appear to be

FIGURE 4. The effect of chloral hydrate (15 mg/ml) on statoconia distribution. A-D viewed dorsally, E-G same preparation, viewed after tilting the microscope 90°. Note that, with longer exposure to chloral hydrate, the statoconia are more dispersed and out of focus (as viewed dorsally) because they fall toward the bottom of the statocyst, and touch the hair cells' inner surface (as seen by tilting the microscope). This indicates progressive collapse of the hairs. No statoconia or hair movement are observed for panels C, D, and G. (A, E) Control; (B, F) 2.5 min of treatment; (C, D, G) 5 min of treatment. Depth of focus increased from C to D.


A
B
C
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E
F
G
0 50 100
μm

propelled by them. Although the loaded hairs often appeared to move with the adjacent statoconia, the statoconia were seen to frequently move away from some hairs to encounter other neighboring hairs and other statoconia.

As previously reported (Alkon, 1975), less frequent (<1 Hz) slow movements of the cluster of statoconia could be observed. These movements were also observed for very small statoconia (3–5 μm diameter) which sometimes fell between the loaded sensory hairs (Fig. 2). Presumably, the measured movement frequency for these smaller crystals (~ 7 Hz, Table I) represented the dominant frequency of a single loaded hair (also 7 Hz) or a small group of neighboring hairs. The more infrequent slow movements of such a crystal could represent the less frequent and less effective forces of neighboring hairs making less direct contact with the crystal.

In single statoconium cysts, the movement frequency of loaded and unloaded hairs was ~ 5 Hz (Table I). The single statoconium also appeared to move with this frequency. There was also no obvious synchrony of the hairs' movements although they all moved with approximately the same frequency. In these single

TABLE I
MOVEMENT FREQUENCY OF HAIRS AND STATOCONIA
(20°C)

	Frequency*	n‡
	Hz	
Free hair	10.7 ± 1.2	23
Hair under pressure (1.0 g)	7.5 ± 0.4	10
Bulk statoconia	6.9 ± 0.9	18
Individual statoconium	7.4 ± 0.7	8
Hair in statocyst with single statoconia	5.4 ± 0.37	4

* Mean ± SD.
‡ Number of measurements by individual observers on different preparations.

statoconium cysts, although the frequency of hair movements was lower (than in the normal cysts), the angle of the hairs' movement was approximately doubled (18–20° to each side of a line perpendicular to the plane of the hair cell's inner surface).

Factors Affecting Hair and Statoconia Movements

Hypertonicity has been shown to reversibly eliminate ciliary motility (Kilburn et al. 1977). We found that increased osmolarity (addition of 600–1700 mosmol of choline-Cl, Tris-Cl, NaCl, or glucose to normal seawater) reversibly eliminated hair movements in the *Hermissenda* statocyst. When preparations were cooled (Fig. 5) the frequency of movement of the statoconia was reduced (Q_{10} = 1.24) and their range of movements increased. Chloral hydrate (6–7 mg/ml), which is known to deciliate protozoans (Ogura and Takahashi, 1976), gradually reduced both the free hair and statoconia frequency of movement, initially different, to the same level (Fig. 6 A). During this reduction of movement frequency, the range of movement of each hair increased gradually up to 70–80° to each side

(compared to 10° in the control). The effect of chloral hydrate was more pronounced with increased concentrations (Fig. 6 B). Concentrations of 10–15 mg/ml could eliminate completely the movement of the statoconia. Chloral hydrate also had a dramatic effect on the rigidity of the hairs. In normal preparations the distance between the statoconia and the luminal surface of those hair cells whose hairs experience the full weight of the statoconia was 8.5–9 μm (Fig. 4 E). Such a distance is only slightly less than the full length of the hairs themseelves (10 μm). Thus, the hairs support the statoconia with little increased bending, indicating that the hairs are quite rigid. But when the preparation was treated with chloral hydrate, gradual "collapse" of hairs was observed and finally the statoconia were seen directly apposed to the hair cells' inner wall (Fig. 4 A-G).

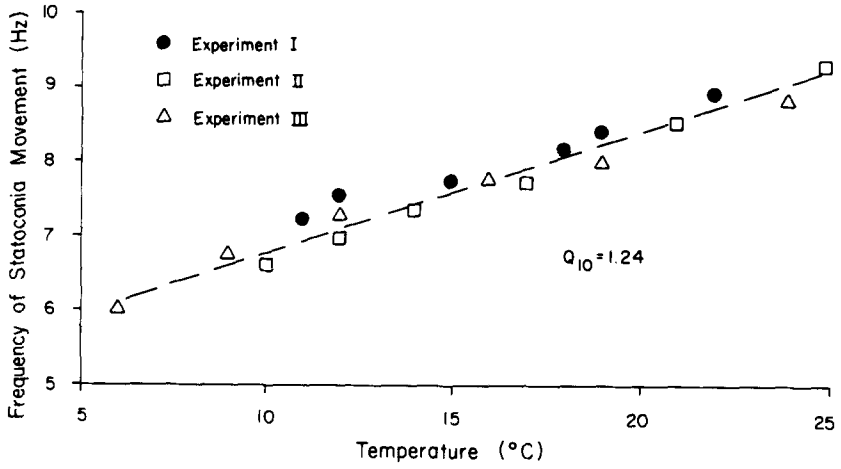
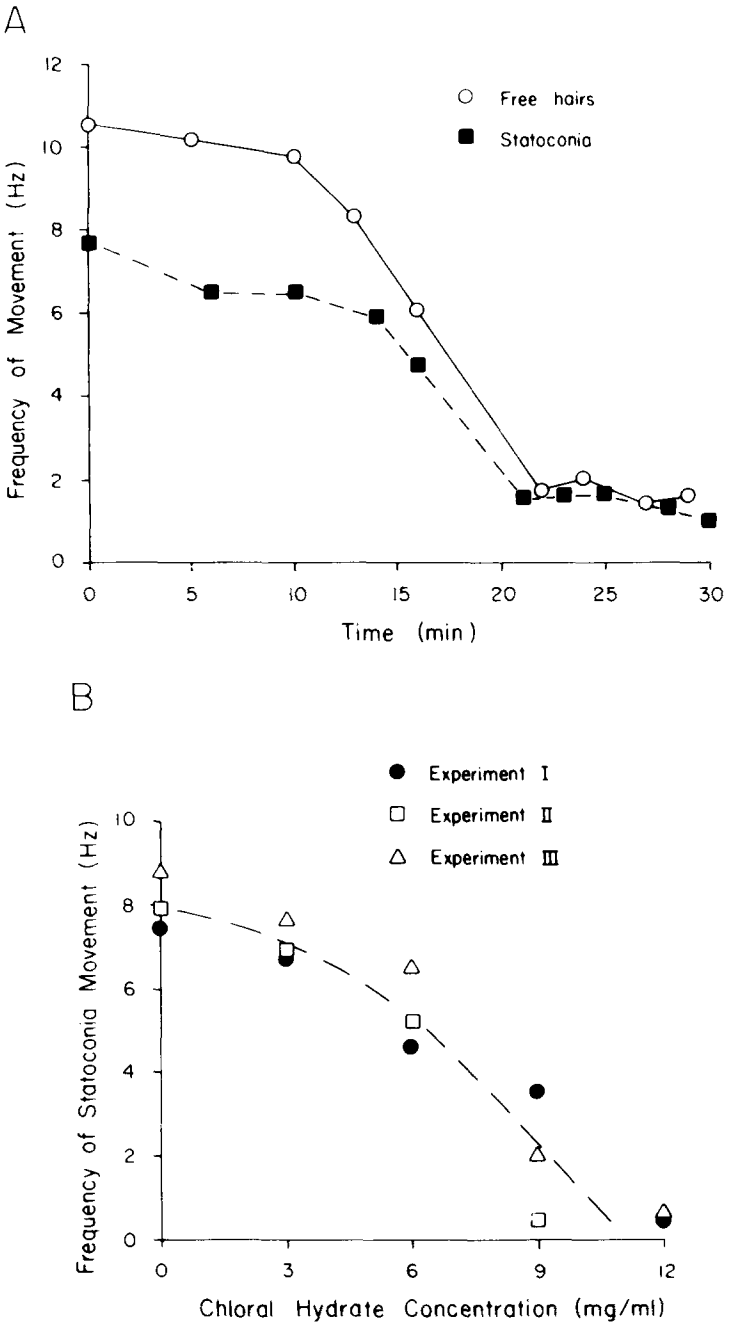


FIGURE 5. The effect of temperature on statoconia and hair motility. In three different experiments the movement frequency of the statoconia was measured by stroboscopic determination. Values at each temperature are means of ten measurements. Q_{10} of motility-temperature relation is 1.24.

Stimulus Effects on Voltage Noise

For hair cells with luminal surface oriented opposite to the direction of the centrifugal force vector, small rotational velocities (generating 0.2–0.4 *g*) could produce an increased number of depolarizing waves in the voltage noise associated with slight increases of variance and slight changes in the steady-state level of the hair cell membrane potential. This is illustrated in Fig. 7 for a hair cell whose axon was cut 10–20 μm distal to its point of entry into the cerebropleural ganglion. Thus, its synaptic interactions were eliminated while its impulses remained. For larger constant rotation velocities (generating 0.5–2.0 *g*), greater increases in the variance of the noise were superimposed on substantial depolarization of the hair cell (Figs. 7 and 8). As previously reported (DeFelice and Alkon, 1977 *b*) the increase of variance for this response to

rotation was approximately proportional to the depolarizing change in membrane potential. It should be emphasized that all variance measurements were obtained for voltage noise of hair cells whose axons were cut proximal to their points of entry into the cerebropleural ganglion. Thus, synaptic interactions,



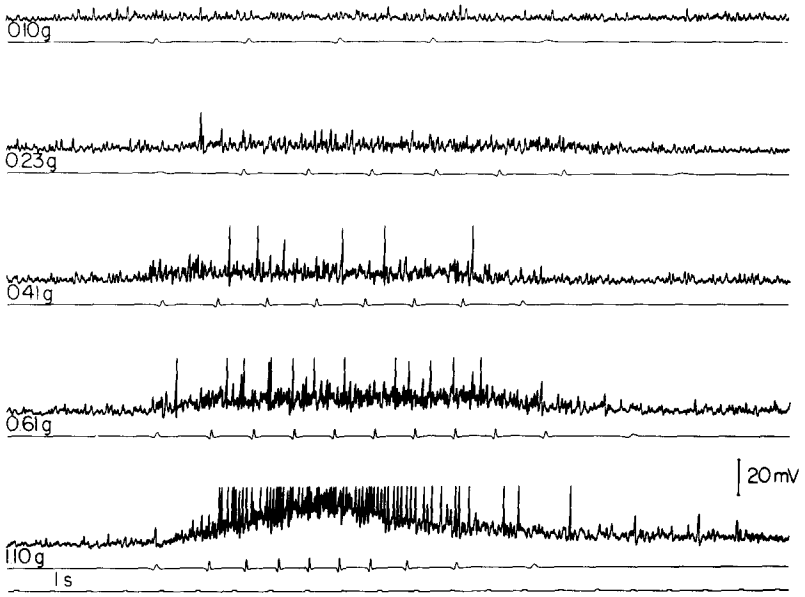


FIGURE 7. Response to rotation of hair cell with luminal surface opposite to the centrifugal force vector. The axon of the hair cell was cut to eliminate all synaptic but not impulse activity. Maximal centrigual force is indicated at the left of each lower trace monitoring the rotation. The amplitude of monitor signal is proportional to the angular velocity of the turntable. The interval between monitor signals equals the period of the turntable's rotation. Impulse peaks are not included in the bottom record.

impulses, and depolarizing local potentials (cf. Alkon, 1975) were all eliminated for these cells (cf. Table II, Fig. 8). Similar changes in base-line noise were produced by rotation of intact hair cells with luminal surface oriented opposite to the centrifugal force vector (Alkon, 1975).

For hair cells with luminal surface oriented in the same direction as the centrifugal force vector, a decrease in noise variance occurred under the same conditions of rotation. With the turntable used in the present study, sufficient force could be generated so as to almost eliminate base-line noise in hair cells oriented in this way. Thus, for those cells whose hairs have ceased to interact with the statoconia (which have been moved by rotation to the opposite side of the statocyst), the inherent motility of the hairs themselves was associated with little base-line noise.

FIGURE 6. The effect of chloral hydrate on hair motility. (A) Frequency of movement of free hairs (at top of statocyst in a 90° tilted preparation) and frequency of statoconia movements as a function of time in chloral hydrate (7 mg/ml). Note the rapid reduction of free hair movement frequency and its approximate equality to the statoconia movement frequency at end of treatment. (B) Increasing concentrations of chloral hydrate. Frequency values were obtained after 5 min in each concentration, and each value was the mean of four measurements.

Factors Affecting Voltage Noise

The same treatments which affected the frequency of hair movement (see above) were also studied with analysis of voltage noise. Increased osmolarity, chloral hydrate, and lowering temperature all greatly reduced the noise variance and increased the input resistance of the hair cell membrane (Table II, Fig. 8). Increased osmolarity with choline chloride ($n=4$), sodium chloride ($n=6$), or

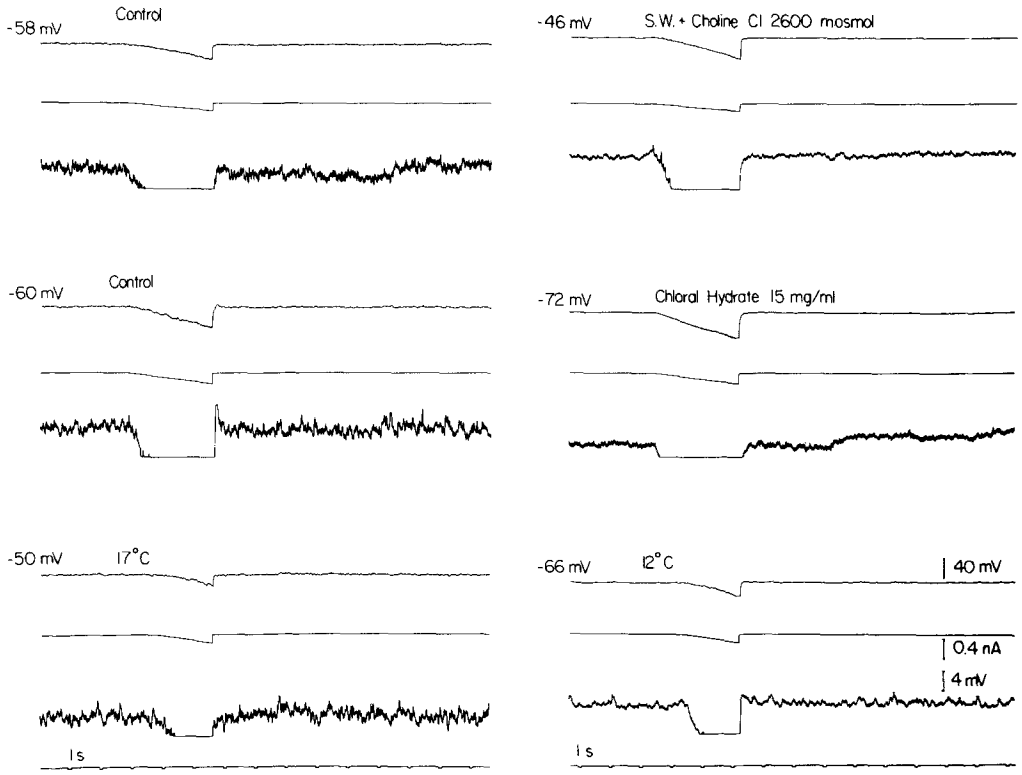


FIGURE 8. The effect of hyperosmolarity, chloral hydrate, and cooling on hair cell voltage noise and membrane conductance. Records on the left are controls for three different cells. The voltage is recorded at low gain in upper traces and simultaneously at higher gain in lower traces. Current ramp injection is monitored in middle traces. The resting membrane potential is indicated at the left of each record. Note the reduction of voltage noise associated with a decrease in membrane conductance with all treatments. Hair cells have synaptic and impulse activity eliminated by cutting their axons.

glucose ($n=6$) caused small and inconsistent changes of membrane potential. Perfusion with chloral hydrate usually hyperpolarized the membrane (-4.3 ± 4.8 mV mean $\pm$SD, $n=13$). A decrease of temperature (19 → 9°C) was usually associated with depolarization of the membrane ($+1.6 \pm 1.8$ mV per 1°C, $n=15$) which was occasionally preceded by a brief hyperpolarization (Fig. 8). These changes in base-line noise and membrane properties were completely reversible

TABLE II

TREATMENT EFFECTS ON HAIR CELL MEMBRANE POTENTIAL, INPUT RESISTANCE, AND VOLTAGE NOISE

Cell no.	Treatment	Δ Membrane potential	ΔR_{in}*	Voltage noise variance	
				Control	Treatment
		mV	%	$\times 10^{-9} V^2$	
	Direct current nA				
1	−1.2	−60	+54	0.90	8.60
2	−1.5	−60	+40	0.54	2.40
3	−2.2	−40	+32	0.65	2.72
10‡	−1.0	−60	+62	0.28	0.78
11‡	−1.3	−40	+44	0.60	3.00
23	−1.0	−20	+98	0.48	1.56
	Cooling °C				
1	18 → 14	+6	+17	0.90	0.19
2	19 → 13	+22	+100	0.54	0.50
3	19 → 11	+24	+105	0.65	0.47
9	19 → 10	+28	+86	1.14	0.8
23	18 → 8	+20	+45	0.48	0.15
	Chloral hydrate *mg/ml (min)*				
4	9 (2.5)	−5	+37	1.26	0.60
5	9 (6.0)	−4	+10	0.37	0.14
6	6 (3.5)	−8	+53	6.51	1.20
7	6 (4.0)	−4	+24	1.80	0.16
	Hypertonicity Δ *mosmol (min)*				
8	NaCl, 1,800 (4)	+10	−14	0.33	0.25
11‡	NaCl, 1,200 (3)	+16	+5	0.60	0.24
18	glucose, 600 (5)	−4	+12	2.36	0.40
19	glucose, 600 (6)	+6	+8	1.10	0.07
21	glucose, 900 (3)	−2	+24	3.76	0.26
	NaCl, 1,200 (3)	+6	+10	1.52	0.22
	$[Na]_o$§ = 0 *min*				
9	5	−20	+41	2.12	0.40
11‡	6	−12	+63	0.60	0.20
12‡	6	−10	+17	0.44	0.21
14	6	−12	+9	3.76	0.30
15	4	−10	+36	2.40	0.36

* Input resistance.

‡ Hair cell from statocyst containing single statoconium.

§ External sodium concentration.

with changes in temperature and were usually reversible after the other treatments provided normal conditions were resumed with 4–6 min. (When hair cells with intact axons were studied, action potentials were unaffected by the 4–6-min treatment periods used for the "cut nerve" preparation just described.)

The generator potential of hair cells was suggested to be associated with a conductance increase to an ion having an equilibrium potential more positive than the resting level (Alkon and Bak, 1973; Alkon, 1975). The effect of zero external sodium on the noise was therefore examined. Zero external sodium in the perfusing artificial seawater hyperpolarized the membrane (-11.7 ± 4.6 mV mean $\pm$SD, $n=9$) and abolished almost completely the noise variance (see Table II, Fig. 9). Membrane potential fluctuations were also eliminated with zero external sodium for *Aplysia* hair cells (Gallin and Wiederhold, 1977). Zero external sodium had no observable effect on the movement frequencies of the hairs and statoconia.

One additional "condition" was associated with reduction of baseline noise in hair cells. This consisted of "reducing" the number of statoconia to one. Such "reduction" was accomplished naturally when occasionally *Hermissenda* statocysts containing only one statoconium were encountered. Base-line recordings from hair cells in these statocysts showed lower variance of voltage noise (Table II).

Factors Affecting Hair Cell Generator Potentials

Low extracellular sodium eliminated the depolarizing response (generator potential) of hair cells to rotation (Fig. 9). Perfusion with chloral hydrate greatly reduced and finally eliminated this depolarizing response (Fig. 10). The effects of low sodium and chloral hydrate on the generator potential closely paralleled the effects on the hair cell noise (Figs. 8, 9, and 10). No additional noise was generated by rotation toward the end of the chloral hydrate treatment while some residual base-line noise still persisted.

It was also possible to elicit generator potentials from hair cells in statocysts partially collapsed by making several small holes in the statocyst with a microelectrode tip. The diameter of these partially collapsed statocysts was reduced by 20–40%. In such statocysts little or no space remained between the statoconia and the statocyst's wall. Generator potentials elicited under such conditions required much larger forces than usual, were reduced in maximum amplitude, and were not accompanied by an increase of voltage noise variance.

Previous observations showed that hair cell generator potentials are increased by injection of small negative currents and reduced by larger currents (Alkon, 1975). These observations were confirmed in the present study and extended to include a wider range of hair cell membrane potential (Fig. 11). As previously described (DeFelice and Alkon, 1977 *b*) the variance of the voltage noise also increased with hyperpolarization for a wide range of hair cell membrane potential.

Factors Affecting Hair Cell Conductance

Membrane conductances associated with the hair cell generator potentials and with the voltage noise were also determined. Negative current pulses were injected intracellularly at rest and during rotatory stimulation. These current

pulses were superimposed on steady negative current injection with which the hair cell membrane potential was shifted to more negative values. These measurements revealed that conductance increased during rotation for a range of resting hair cell membrane potential (0–70 mV). However, conductance

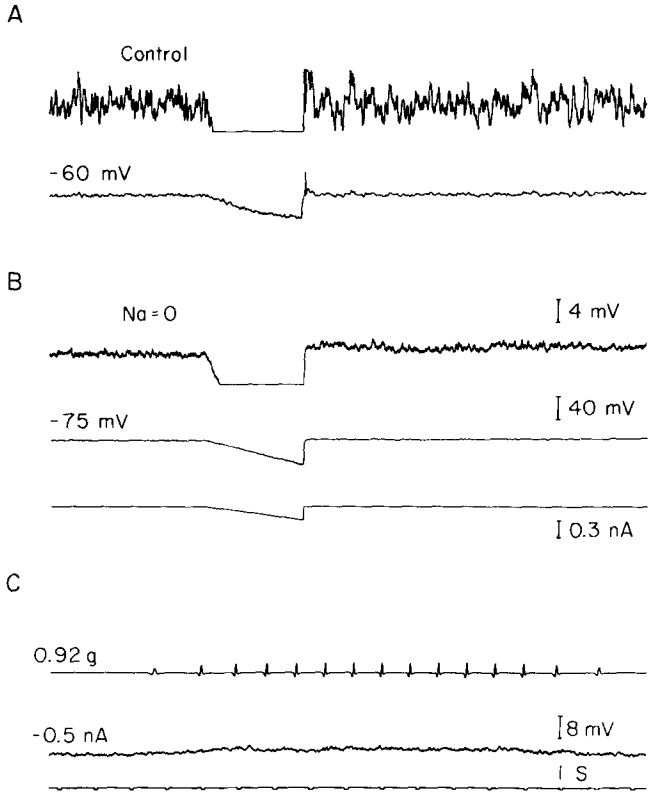


FIGURE 9. Effect of zero external sodium. (A, B) Cut nerve preparation with action potentials still present. Voltage was recorded, using one microelectrode, at high (upper traces) and low gains (lower traces). Voltage gain calibration for (A) and (B) is indicated in (B). (A) Control voltage noise and membrane resistance. (B) Perfusion with zero external sodium for 5 min hyperpolarized the cell by 15 mV (resting membrane potential is indicated at the left of traces), substantially reduced the voltage noise, and slightly increased (5–10%) membrane resistance. Current is monitored by the lowest trace of B. (C) Another cut nerve preparation, with action potentials eliminated. Perfusion with zero external sodium for 6 min eliminated most of the generator potential normally caused by rotation (as monitored by upper trace). Little or no increase of voltage noise was caused by rotation. Cell was hyperpolarized (−35 mV) with −0.5 nA direct current (which normally increases the generator potential amplitude).

showed little change during rotation at more negative membrane potentials (Fig. 10). The *I-V* curves of most of the cells which have been studied showed up to fourfold decrease in conductance between membrane potentials of −50 to −75 mV (Fig. 11). Because the resting potential of hair cells showed wide

variation, different amounts of hyperpolarization were necessary to reach this range of membrane potential for different cells (cf. Table II). The membrane potential could be shifted by steady negative current injection to levels more negative than −75 mV (Fig. 12). Depolarization of the hair cell membrane from these levels into the voltage range −50 to −75 mV was associated with a decrease of membrane conductance. This was true for depolarization (from these negative levels) produced by rotation or reduction of the negative current being injected (Fig. 12). In such conditions, the increase of conductance associated with the generator potential probably still occurred but was masked by the nonlinear behavior of the hair cell membrane.

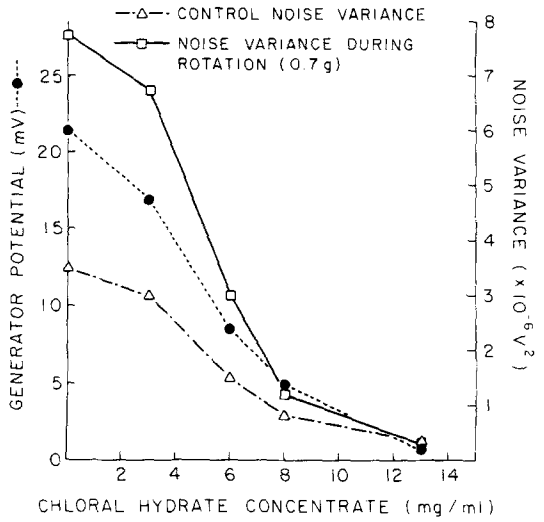


FIGURE 10. The effect of chloral hydrate on voltage noise and generator potential of hair cell with luminal surface oriented opposite the centrifugal force vector. Concentration increased gradually for the same hair cell (with cut static nerve). The response to a force of 0.7 *g* was measured after 5 min for each concentration. Note that the resting voltage noise variance almost completely disappeared. A very small generator potential associated with no increase in noise variance could be elicited in response to rotation with highest concentration of chloral hydrate.

Because of the nonlinearity of the hair cells' current-voltage relations, the observation of changes in the noise with rotation was reexamined. For membrane potentials more positive than −50 to −60 mV, the voltage noise variance increased during rotation. The percent change of the voltage noise variance was always greater than the percent change of an associated increase of membrane conductance. For membrane potentials more negative than −60 to −70 mV the voltage noise variance also increased during rotation. This latter increase was associated with a net decrease of membrane conductance during rotation (Fig. 12). These observations indicate that the change of voltage noise variance during rotation cannot be simply due to a net change of membrane conductance.

A decrease of membrane conductance (Figs. 8 and 9, Table II) also accompanied reduction of hair cell noise by increased osmolarity (−18 ± 12%, mean ± SD, $n=16$), chloral hydrate (−22 ± 7%, $n=13$), cooling ($Q_{10}=2$), and low external sodium (−25 ± 17%, $n=9$).

Effects of Membrane Time Constants on Voltage Noise

The charging curve of the resting hair cell membrane was shown to follow two time constants: one, 12 ms; the other, 45 ms (Alkon and Bak, 1973). Because of its 45-ms time constant, the hair cell membrane can be expected to filter (at ~ 4 Hz) some of the voltage noise before and during the variety of treatments

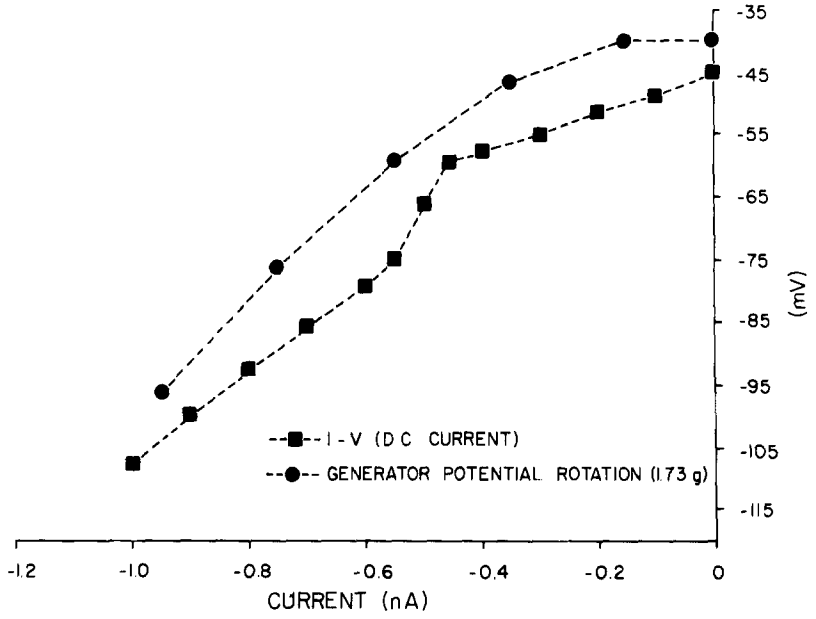


FIGURE 11. Current-voltage relation of hair cell at rest and during rotation. The *I-V* curve was obtained by DC current injection. Note the increase in slope resistance in the range −55 to −75 mV and the corresponding changes in the generator potential amplitude (which is equal to the voltage difference between the two curves). Generator potentials were evoked by rotation with force of 1.7 *g*.

presented above. Such filtering could also partially account for reduction of noise variance produced by the treatment when the membrane resistance, and thus the membrane time constant, was increased, as it often was by cooling, chloral hydrate, zero external sodium, and hyperosmolarity.

To assess the effect of the 45-ms time constant, the following experiments were conducted. Injection of direct current was used to displace the hair cell membrane potential. This displacement was associated with changes in hair cell membrane resistance as well as time constant (Figs. 11 and 12). During a given treatment the average time constant of the membrane (as well as its resistance) could be restored to control (or less than control) levels by shifting the

membrane potential with current injection. For example, cooling (18.5 → 8.5°C) depolarized the hair cell membrane, increased its input resistance, and greatly reduced the variance of the voltage noise (Fig. 8). The change in variance still occurred at 8.5°C when the hair cell membrane potential was restored to the original level by current injection. With such current injection at 8.5 °C the membrane input resistance was approximately equal to that for a hair cell at 18.5°C when −0.10 nA was injected. Yet the variance under these latter conditions increased substantially (compared to the variance at 18.5°C with 0.0 nA). Thus, the changes in variance observed with cooling are most likely not due to filtering effects of the hair cell membrane as a result of changes in membrane potential, time constant, or input resistance.

In a similar type of experiment, the hair cell membrane potential was displaced (by negative current injection) to a region of the *I-V* curve where

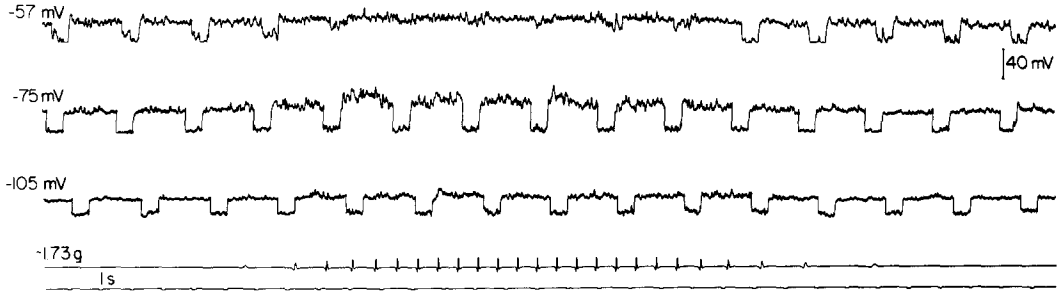


FIGURE 12. The effect of membrane potential on hair cell membrane resistance at rest and during rotation. Hair cell's luminal surface was opposite the centrifugal force vector and its axon was cut. Maximal centrifugal force produced by rotation was 1.73 *g* throughout the experiment. The amplitude of monitor signal (second trace from the bottom) is proportional to the angular velocity of the turntable. The interval between monitor signals equals the period of turntable's rotation. Membrane conductance was measured by injected square pulses (0.1 nA for the upper record and 0.4 nA for the other two records). Hair cell membrane potential, indicated at the left of each voltage trace, was shifted by DC current injection.

rotation produced no net change of conductance. Under these conditions rotation did not change the membrane time constant but it still increased noise variance.

DISCUSSION

Common Origin of Voltage Noise and Generator Potentials

Several observations strongly suggest that the hair cell generator potentials and voltage noise have a common origin. Rotation changes the voltage noise variance and membrane potential (generator responses) in a similar manner (DeFelice and Alkon, 1977 *b*; Fig. 7). Both the noise and the generator potentials show a similar dependence on membrane potential (Fig. 11). In addition, within a limited range of membrane potential, both the noise and depolarizing generator potentials are associated with an increase of membrane conductance (Figs. 11

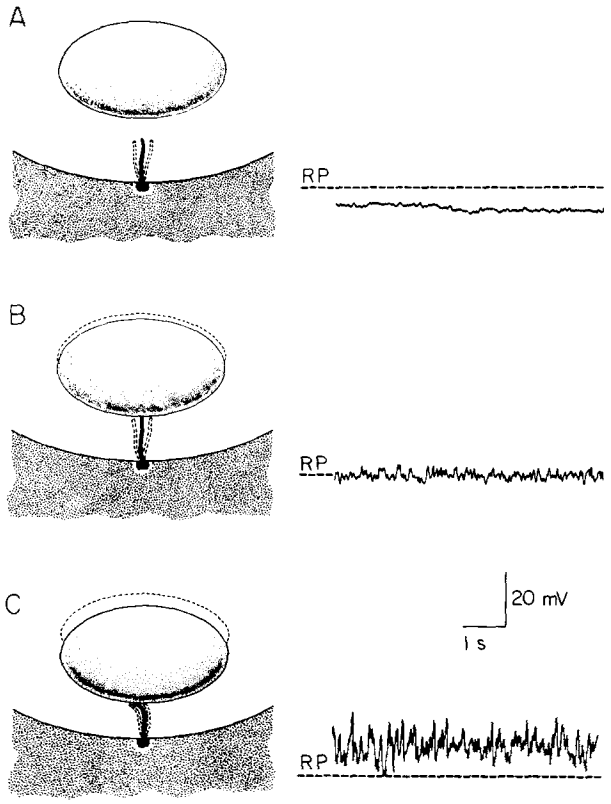


FIGURE 13. Conceptual model for hair cell voltage noise and generator potential production. The hair is inherently motile (range of motion indicated by dashed lines). (A) When the hair cell (oriented in the same direction as the centrifugal force vector) experiences a negative centrifugal force, the hair moves freely through a maximum range of motion producing little voltage noise (see noise record on right). (B) When the hair cell on that statocyst equator visualized when looking dorsoventrally is exposed to zero centrifugal force, the hair has some interaction with the statoconium. The statoconium exerts little net force on the hair although collisions of the statoconium with the rigid hair increase the voltage noise variance and frequency (voltage noise record on right). (C) When the hair cell (with luminal surface opposite the force vector) is exposed to a substantial centrifugal force (e.g., 1.0 *g*), the statoconium exerts a considerable net force on the hair, limiting its range of movement and reducing its movement frequency as well as dramatically increasing the voltage noise variance (see noise record on right). The increase of voltage noise variance and frequency results, by summation of individual events, in a depolarizing generator potential. Dashed line indicates level of resting membrane potential (RP) before a negative centrifugal force (A) or a positive centrifugal force exerted by rotation. All noise records were taken from actual hair cells under the conditions described.

and 12, Table II). Both the noise and generator potentials are eliminated by perfusion with zero external sodium (Fig. 9). Finally, the effect of chloral hydrate on the noise closely parallels the effect on the generator potentials (Fig. 10).

Additional observations suggested that the voltage noise, like the generator potentials, arises from interaction of hairs and statoconia. Gravitational stimuli caused depolarizing generator potentials as well as increases of voltage noise variance. Gravitational stimuli also caused increased interaction of hairs and statoconia (Fig. 4). Hyperosmolarity, cooling, and chloral hydrate decreased voltage noise variance. These same treatments also decreased the frequency of collision of hairs with statoconia.

Model of Hair Cell Noise Production

Voltage noise, recorded from hair cells with luminal surface oriented in the same direction as the centrifugal force vector decreased markedly during rotation. The hairs of these cells could not possibly interact with the statoconia (Fig. 4) during rotation. Yet these hairs themselves have an increased (by 40%) movement frequency when not in contact with the statoconia (Table I). These results indicate that the motion of the hairs themselves, although necessary, is not sufficient to produce substantial voltage noise. This interpretation gains further support from the effect of chloral hydrate on hair movement. The amplitude of hair movement greatly increased (whereas the voltage noise variance and frequencies decreased) during perfusion with chloral hydrate. This was also the case with cooling.

It is thus the collision of statoconia with the motile hairs which produces most of the noise. The small amount of residual noise recorded from those hair cells whose hairs are freely moving could arise from small membrane distortion associated with the hair movement, or alternatively, could reflect membrane leakage conductance(s). It should be emphasized that the statoconia, which are too large to undergo distinguishable Brownian motion, have no inherent motility of their own. As indicated by the present experiments then, the statoconia move as a result of either or both forces exerted by gravity and generated by the hairs.

These considerations are consistent with certain features of previously proposed conceptual models (Alkon, 1975; Wiederhold, 1976) of how voltage noise arises from collision of statoconia with the hairs. When sufficient force moves a statoconium away from a hair, the hair moves freely (Fig. 13) through ± 10° (limits of range of motion are represented by dashed lines). Visualization of normal vital preparations indicates that this lateral motion in a plane occurs within a range of 20° about an axis perpendicular to the luminal surface of the hair cells. Thus, freely moving hairs of cells on the top of the statocyst would not collide with statoconia which have been moved by gravity to the bottom of the statocyst (Fig. 4). Recordings from hair cells whose hairs do not collide with statoconia show minimal noise (Fig. 13 A).

With a small net gravitational force exerted on the statoconia, the statoconia in turn exert force on the hairs of those cells with luminal surfaces oriented opposite to the direction of the force vector. Recordings from these cells, stimulated by a small net force, show a small increase of noise variance with a slight steady depolarization (Figs. 7 and 13 B). With greater net force, further increases in variance are recorded and the sustained depolarization becomes

more pronounced (Figs. 7 and 13 C). The hairs must exert at least an equal but opposite force inasmuch as they are able to support the full weight of the statoconia, and even to accelerate them while retaining their own (the hairs') motility. This has been verified by direct visualization of hairs in vital preparations.

Thus, not only do the statoconia exert a force resisted by the hairs, they also limit the range of motion of the hairs and decrease their movement frequency (Fig. 6 A, Table I). Inasmuch as the noise persists and even increases in amplitude with large forces, although with a decreasing range of the hairs motion (indicated in Fig. 13 by dashed lines), it can be inferred that actual bending of the hairs is not critical for producing the noise. Instead, the force exerted on the hairs by the statoconia appears to be essential for increasing voltage noise variance. Further, support for this inference was found in the experiments in which the movement frequency of the hairs was reduced or eliminated (cooling, hypertonicity, chloral hydrate). The reduction in movement frequency was always associated with an increase in angle of lateral motion although the noise variance with such treatments was always reduced. An additional insight into this question was offered by the study of statocysts with a single statoconium. The noise variance from hair cells in these statocysts was lower than for hair cells in normal statocysts (Table II). In addition, the movement frequency of the hairs was 30% less than the normal although the angle of motion was doubled, and the density of the hairs was three- to fourfold greater.

If the force exerted on the hairs by the statoconia is essential for increasing the noise amplitude, how and where might such force act? There are at least two possible loci along the cilium where the force of the statoconia resisted by the force generated by the hair could reduce membrane distortion and consequent conductance change(s): (*a*) the point of contact between the hair and statoconium and (*b*) the point of insertion of the hair into the hair cell which supports it. Conductance changes at or surrounding the ciliary basal bodies, rather than on the cilia themselves, seem more likely. Such a locus for conductance changes is suggested by the observation that the basal segments seem to move together with their cilia. It is also suggested by the conclusion that the cilia impart substantial force to the statoconia (see above) and therefore must be fairly rigid structures. If such an inference is correct, an analogy could be drawn between the responsiveness of the motile sensory cilia of *Hermissenda* hair cells and the responsiveness of the motile effector cilia of the gill of *Mytilus edulis*. These latter cilia were found to be mechanically sensitive only at the ciliary bases (Thurm, 1968). Similar observations were reported recently for the grasshopper proximal femoral chordotonal organ (Moran et al., 1977).

Mechanism of Generator Potential Production

Because we have presented evidence that the hair cell generator potentials and noise have a common origin, the arguments made for the noise might also apply to the generator potentials. Thus, the depolarizing generator potentials would depend on the force exerted by the statoconia on the hairs and not on hair

bending. This force would then be expected to cause sustained membrane distortion and consequent conductance changes at the ciliary base rather than on the ciliary shafts.

That the generator potentials do not arise from lateral hair displacement itself (toward the plane of the inner hair cell surface) was further indicated by the experiments with collapsed statocysts. In collapsed statocysts, the statoconia remain approximated to the inner surfaces of the hair cells. The hairs, then too, must remain bent by the statoconia against the inner surface of the hair cells. With "flattened" hairs, a generator potential, albeit substantially reduced, can still be produced repeatedly by rotation without any associated increase in the noise. If displacement alone of the hair (toward the hair cells' inner surfaces) and not the force of the statoconia on the hairs were responsible for the generator potential, a steady depolarization which would not be reversible, repeatable, or associated with rotation would be expected.

Nature of Generator Conductances

It was shown above that the conductance increase, presumably produced by membrane distortion, is at least in part an increase in conductance to sodium for both the generator potential and increases in noise variance elicited by rotation stimuli. This is consistent with past reports in which generator potentials produced by displacement as well as by rotation stimuli were increased by steady hyperpolarization of the hair cell (Alkon and Bak, 1973; Alkon, 1975). Further hyperpolarizations caused by injection of large negative currents, however, reduced the conductance increase and generator potential elicited by rotation. This last observation was confirmed in the present study when negative current pulses were used to measure membrane conductance at progressively more negative hair cell membrane potentials with and without rotation (Fig. 12). Injection of large negative currents caused reduction of generator potentials and their associated conductance changes. This reduction with large negative currents approximately paralleled a reduction of increases of variance of the hair cell noise, i.e., the increases of variance elicited by rotation.

The dependence of voltage noise increases, the generator potentials, and conductance changes (all elicited by rotation) on hair cell membrane potential must be determined, at least in part, by the equilibrium potential for sodium and the nonlinearity of the *I-V* curve. Other processes, however, may influence such a potential dependence. A conductance increase to an ion in addition to sodium, e.g., to potassium, might also be important for the generator mechanism (cf. Goldman, 1965; Alkon, 1975). In addition, we cannot, at this point, rule out the effect of membrane potential on the ciliary movement (Naitoh, 1966) which, in turn, affects the generator potential.

It may be recalled that membrane conductance was also dependent on temperature ($Q_{10} = 2$). The observed Q_{10} was higher than the Q_{10} for membrane conductance of myelinated axons, $Q_{10} = 1.2-1.3$ (Frankenhaeuser and Moore, 1963), and squid axon $Q_{10} = 1.1-1.4$ (Hodgkin and Keynes, 1956). It seems, therefore, that the temperature effect on membrane conductance may be associated with additional reduction of the continual conductance fluctuations associated with the noise (see above), either by reducing the hair motility ($Q_{10} =$

1.3, Fig. 5) and (or) by reducing the rate of change of conductance of an elementary noise event. A similar effect was suggested for the generator potential by Detwiler and Fuortes (1975), although they disregarded changes in membrane passive conductance, possibly because only a small range of temperatures was considered.

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