

A Dual Synaptic Effect on Hair Cells in *Hermisenda*

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ABSTRACT Type A photoreceptors can produce an initial hyperpolarizing wave followed by a delayed long-lasting increase in firing which is usually accompanied by a small depolarizing wave. The initial hyperpolarizing wave arises from an increase in conductance while the depolarizing wave was shown to arise from a decrease in conductance. The data presented indicate that both effects produced by the type A photoreceptors in ipsilateral hair cells are synaptic.

INTRODUCTION

Hair cells in the statocyst of *Hermisenda* have been shown to respond to electric and photic stimulation of photoreceptors in each of the animal's two eyes (Alkon 1973 *b*). An initial hyperpolarizing wave followed by a delayed but long-lasting increased firing frequency occurs in hair cells in response to illumination of the ipsilateral eye. The delayed increase in firing frequency is usually accompanied by a small depolarizing wave. The initial hyperpolarizing wave varies markedly for different hair cells and often is not observable at all. This depolarizing response of hair cells was absent in hair cells of animals which had been previously exposed to associative training (Alkon, 1974, 1975). It, therefore, seemed of particular interest to gain further insight as to how the depolarizing response is generated.

In this report data are presented which demonstrate that type A photoreceptors (see below) can produce a dual effect in hair cells: an initial inhibition followed by a delayed excitation. The data presented indicate that both effects are synaptic but do not rule out the intervention of one or more interneurons in the mediation of these effects.

METHODS

Recording and Stimulation Techniques

Hermisenda were provided by Dr. Rimmon Fay of the Pacific Bio-Marine Supply Co., Venice, Calif. The eyes, statocysts, and optic ganglia of *Hermisenda* are located symmetrically under the integument at the junction between the pedal and cerebro-

pleural ganglia. A transverse cut immediately beneath the anterior portion of the animals causes the integument to retract exposing the entire circumesophageal nervous system. This nervous system (ganglia and sensory organs) was then dissected and immersed in artificial seawater at 15–21°C.

A connective tissue sheath enveloping the circumesophageal nervous system was partially digested with Pronase (0.3–0.5 mg/cc) (Calbiochem, San Diego, Calif.), a nonspecific protease, for 15–25 min to facilitate insertion of the microelectrodes. The micropipettes were filled with 4 M potassium acetate and had a resistance of 70–100 m Ω . Conventional methods were used to record electrical potentials of the penetrated cells. A bridge circuit was employed in the experiments involving use of extrinsic currents. Illumination was provided by a quartziodine incandescent lamp. The intensity of light between 4,000 and 8,000 Å which reached the preparation from this source was about 6×10^8 ergs cm⁻² s⁻¹.

RESULTS

It has been previously shown (Alkon, 1973 *a*; Alkon and Fuortes, 1972) that in each eye there are two type A and three type B photoreceptors. The type B photoreceptors have an average spike amplitude of 15 mV, are spontaneously active in darkness, are sensitive to dim flashes, and are mutually inhibitory. The type A photoreceptors, located in the ventral-anterior portion of the eye, have an average spike amplitude of 45 mV, are quiet in darkness, are insensitive to dim flashes, and have little or no synaptic interaction.

It was previously found (Alkon, 1973 *b*) that a train of impulses in a type A photoreceptor was followed by a delayed increase in firing and slight de-

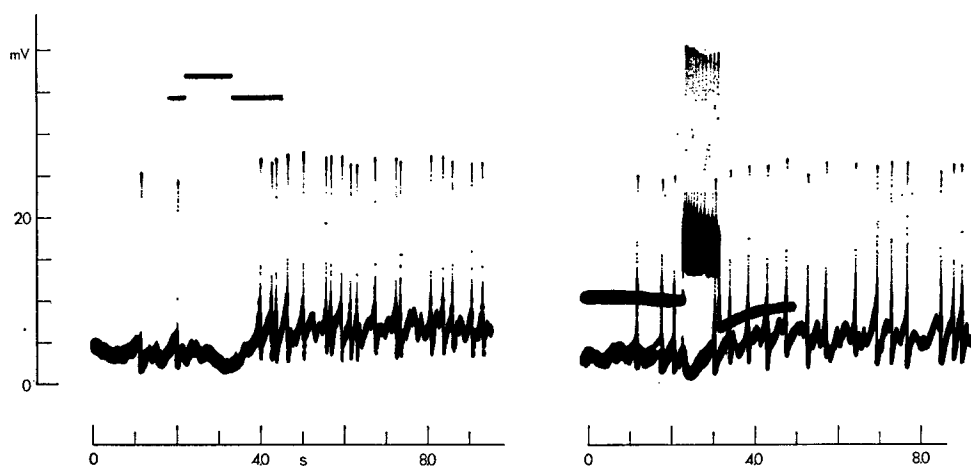


FIGURE 1. Response of hair cell to illumination of the ipsilateral eye (record on left) and a train of impulses in an ipsilateral type A photoreceptor (records on right). Bar on left indicates duration of flash (intensity 6.0×10^8 ergs/cm²-s). A 0.6-nA depolarizing pulse was given to a type A photoreceptor penetrated simultaneously with the hair cell. Base-line activity of hair cell: 67 impulses/min.

polarization in some ipsilateral hair cells. Additional penetrations of type A photoreceptors simultaneously with ipsilateral hair cells reveal that a hyperpolarizing wave often precedes the depolarizing wave in hair cells excited by type A photoreceptors (Figs. 1, 2). The frequency of firing which follows the initial hyperpolarization in the hair cell increases with increasing frequency of firing of the presynaptic type A photoreceptor (Fig. 3), as does the magnitude of the hyperpolarizing wave.

As was already mentioned, frequently, the increase in hair cell activity produced by type A cells is not preceded by a clear hyperpolarizing wave. These depolarizing responses of hair cells to impulse trains of type A cells, as well as the hair cell responses to an ipsilateral flash, without a preceding

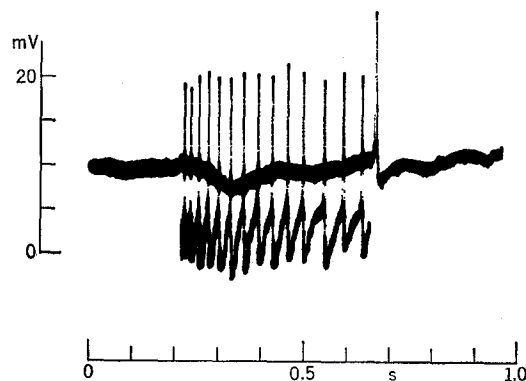


FIGURE 2. A 0.7-nA depolarizing pulse to a type A photoreceptor (lower record) causes a short latency hyperpolarization in an ipsilateral hair cell (upper record). This hyperpolarization is followed by a long latency depolarizing wave which is beginning at the end of sweep.

hyperpolarizing wave have the same latency and time-course, are similarly graded, and are affected by currents in the same way as those responses with a preceding hyperpolarizing wave (Fig. 1 vs. Fig. 6 *b*). This suggests that the preceding hyperpolarizing wave cannot always be observed perhaps due to differences in hair cell activity, recording technique, and/or the ability of the synaptic potential to spread to the electrode recording in the cell soma.

The frequent presence of the preceding hyperpolarizing wave suggested that the hair cell excitation caused by the type A photoreceptor is the result of rebound excitation. It was previously found (Alkon, 1973 *b*) that a brief hyperpolarizing pulse in hair cells is followed by a prolonged (10–20 s) increase in hair cell activity. The time-course of the depolarizing wave after a hyperpolarizing current pulse is comparable to the time-course of the hair cell depolarizing response to light. Hyperpolarizing current pulses of increas-

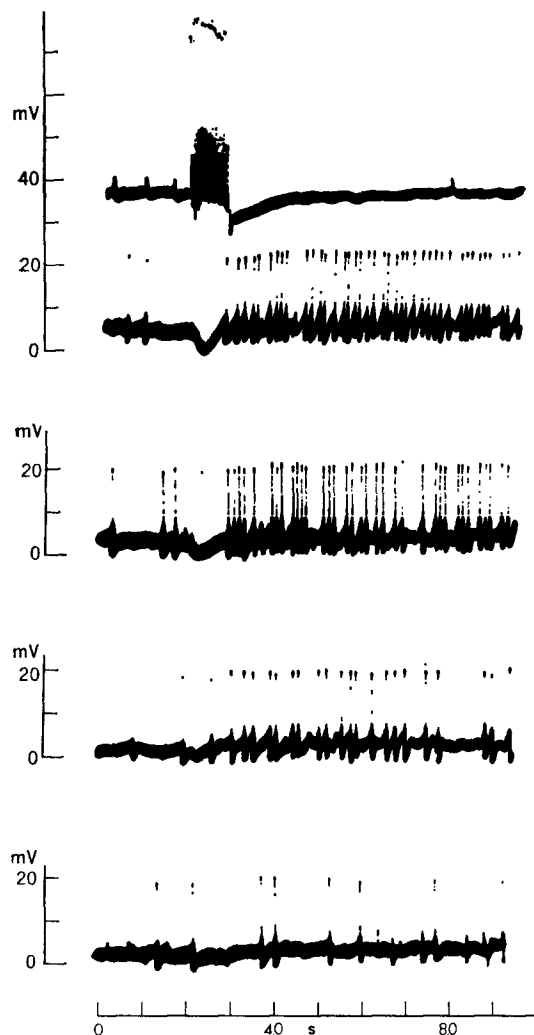
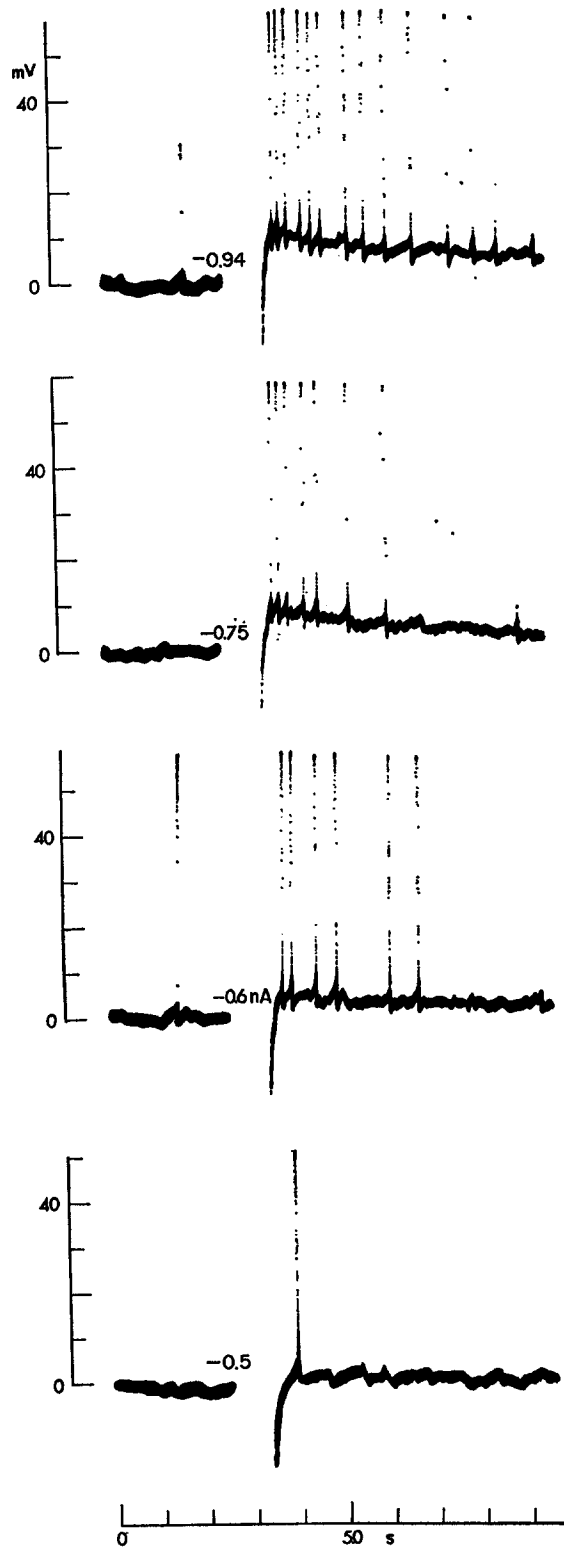
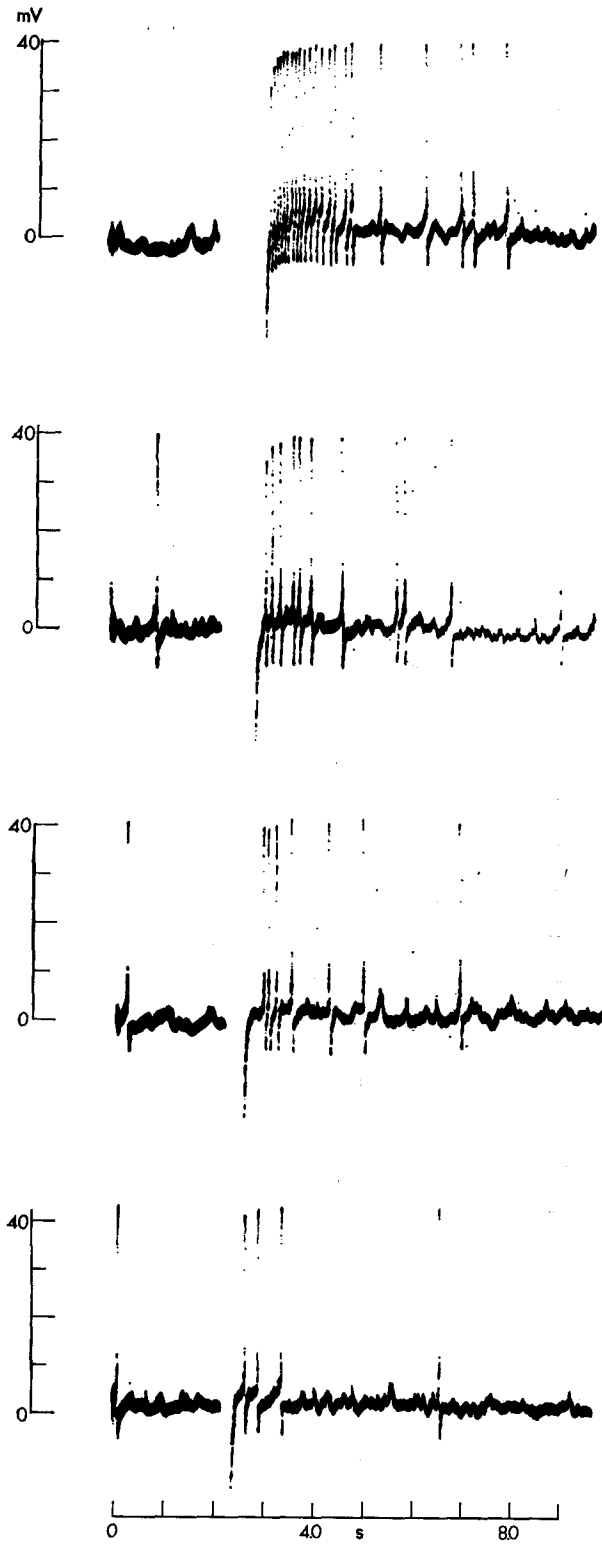


FIGURE 3. Responses of hair cell to increased firing of ipsilateral type A photoreceptor (top trace). Depolarizing current input to photoreceptor increases from bottom trace (0.25, 0.4, 0.6, 0.7 nA). The initial hyperpolarization becomes more prominent and the subsequent increased firing of the hair cell more marked with higher firing frequency of the type A photoreceptor. Base-line activity of hair cell: 60 impulses/min.

ing magnitude (for a certain range of intensity) in hair cells are followed by depolarizing waves of increasing magnitude and duration (Fig. 4). Hyperpolarizing current pulses of increasing duration in hair cells are also followed by depolarizing waves of increasing magnitude and duration (Fig. 5). These

FIGURE 4. Depolarizing waves in a hair cell after hyperpolarizing current pulses. Pulses all have 1.0-s duration and increase in magnitude from bottom trace. Waves become larger and longer with increasing current input. Base-line activity of hair cell: 15 impulses/min.





hair cell responses to negative current pulses are similar to those observed for crustacean stretch receptors (Terzuolo and Washizu, 1962).

That the rebound excitation produced by hyperpolarizing current pulses increases with negative displacement of the hair cell membrane potential (Fig. 6) indicates that it arises from a conductance increase of an ion with a positive equilibrium potential. It is clear, however, that displacement of the hair cell membrane potential in the negative direction decreases (Fig. 6) and eventually abolishes the depolarizing response of the hair cell to light. The initial synaptic hyperpolarizing wave is also reduced and finally abolished with more negative hair cell membrane potentials (Alkon, 1973 *b*). This last observation suggests that the initial hyperpolarizing wave arises from an increase in conductance of an ion with a negative equilibrium potential. This interpretation is confirmed by the finding that resistance decreased (and thus conductance increased) during the synaptic hyperpolarizing wave (Fig. 7).

The decrease of the depolarizing response with more negative hair cell membrane potentials might be explained by a reduction and eventual abolition (with more negative hair cell membrane potentials) of the synaptic hyperpolarizing wave which precedes and could produce the rebound excitation. Alternatively, the decrease of the depolarizing response with more negative hair cell membrane potentials might occur because the depolarizing response arises from a conductance decrease of an ion with a negative equilibrium potential. A final possibility is that hyperpolarizing the hair cell brings the membrane potential below the threshold for a local response responsible for the depolarizing response of the hair cell.

Additional experiments, in fact, show that the depolarizing response arises in large part by neither a rebound nor a local response mechanism. Resistances of hair cells were measured before and during the depolarizing response to light. This was accomplished by injection of negative current pulses of increasing magnitude through a balanced bridge circuit. Resistance was found to increase slightly (and thus conductance to decrease) during the depolarizing response (Fig. 8). The observed increase in resistance might be somewhat reduced by rectification which is associated with a decreased resistance in depolarized hair cells (Alkon and Bak, 1973). Resistance was observed to increase during depolarizing responses produced either by a flash to the ipsilateral eye or by an impulse train in an ipsilateral Type A photoreceptor elicited by a positive current pulse.

FIGURE 5. Depolarizing waves in a hair cell after hyperpolarizing current pulses. Pulses are all 0.7 nA in magnitude and increase in duration from bottom trace (200, 400, 800, 100 ms). Waves become larger and longer with increasing pulse duration. Base-line activity of hair cell: 20 impulses/min.

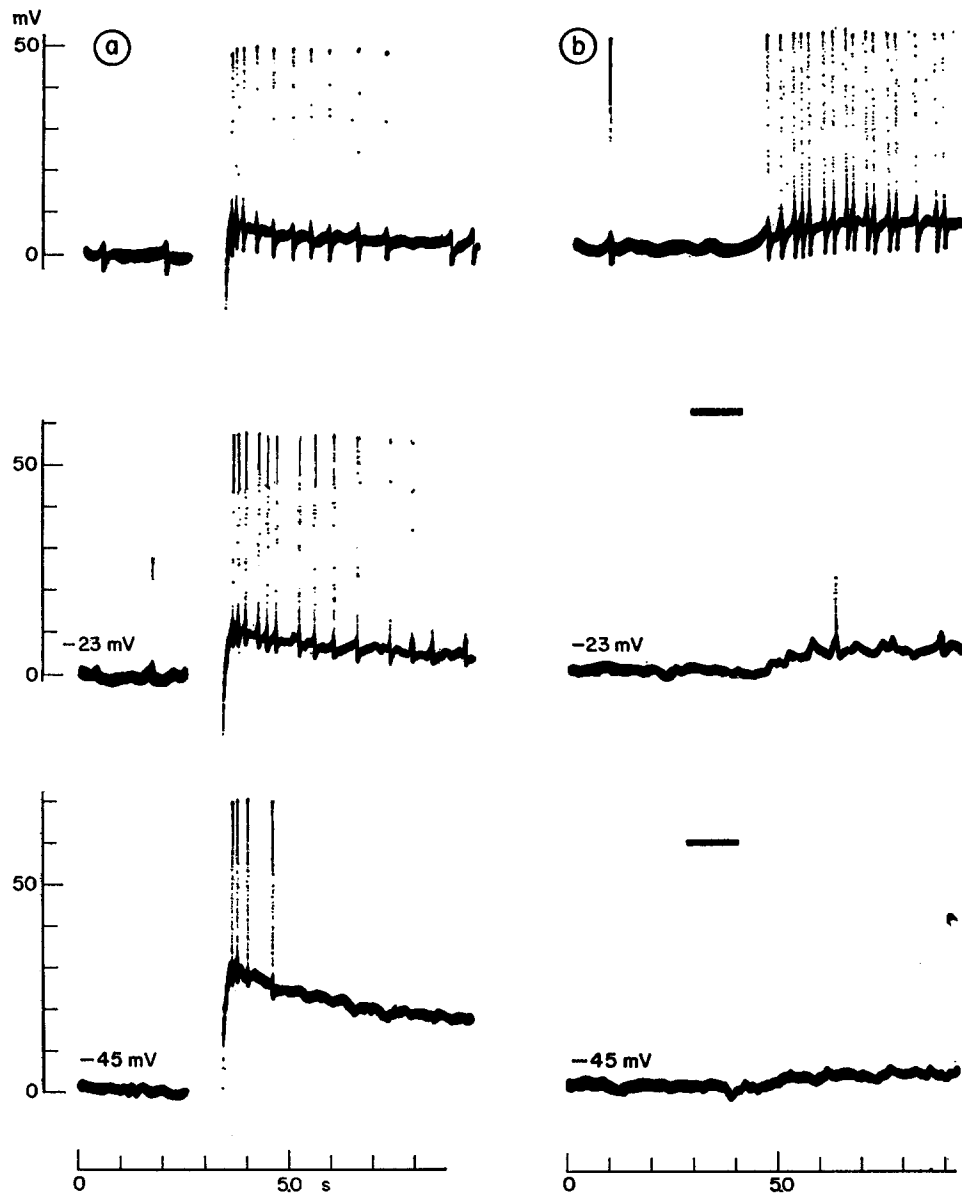


FIGURE 6. (*a, b*) Effect of negative displacement of hair cell membrane potential on depolarizing waves after a hyperpolarizing pulse, (0.9 nA, 1.0-s duration) and after illumination of the ipsilateral eye. Bars on right indicate duration of flash. Numbers refer to hair cell membrane potential in *a* and *b*. Base-line activity of hair cell: 20 impulses/min. Current used in middle and lower records: 0.2 and 0.4 nA, respectively.



FIGURE 7. Effect of negative current pulses on hair cell during the initial hyperpolarizing wave in response to illumination of the ipsilateral eye. The current pulses (0.3 nA) cause a smaller potential change during the hyperpolarizing wave. The initial hyperpolarizing wave was followed by a delayed long-lasting depolarizing response (not pictured). Bar indicates duration of flash.

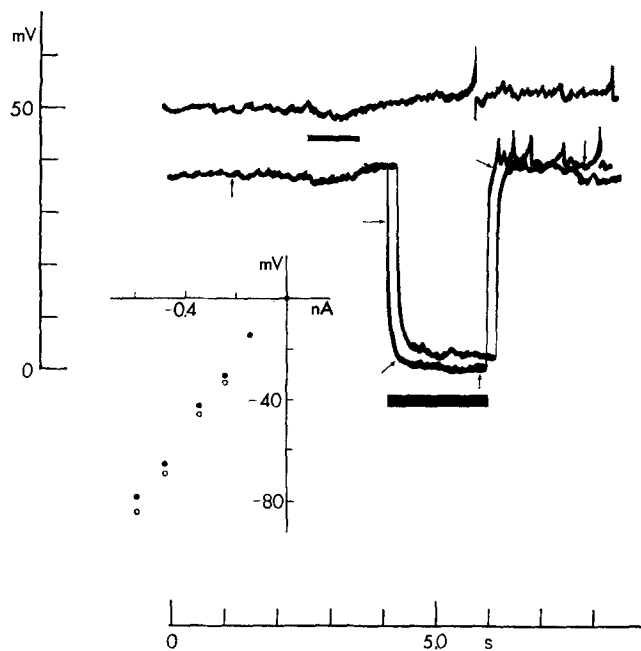


FIGURE 8. Resistance of hair cell during depolarizing response to illumination of ipsilateral eye. Top trace: depolarizing response of hair cell to flash (indicated by top bar). Lower trace: response (indicated by arrows) to same flash and 0.30-nA negative current pulse (indicated by lower bar) during the depolarizing response. The same current pulse repeated with hair cell in dark is included (without arrows). Inset: current-voltage plot of negative current pulses to the hair cell in dark (closed circles) and during the depolarizing response to light (open circles).

In another type of experiment, the duration of an impulse train in a type A photoreceptor was found to determine the duration of increased firing (Fig. 9) of some ipsilateral hair cells. Even with prolonged trains (30 s or more) the hair cell showed a large increase in firing frequency which quickly diminished with cessation of the type A impulses. Thus the type A receptor

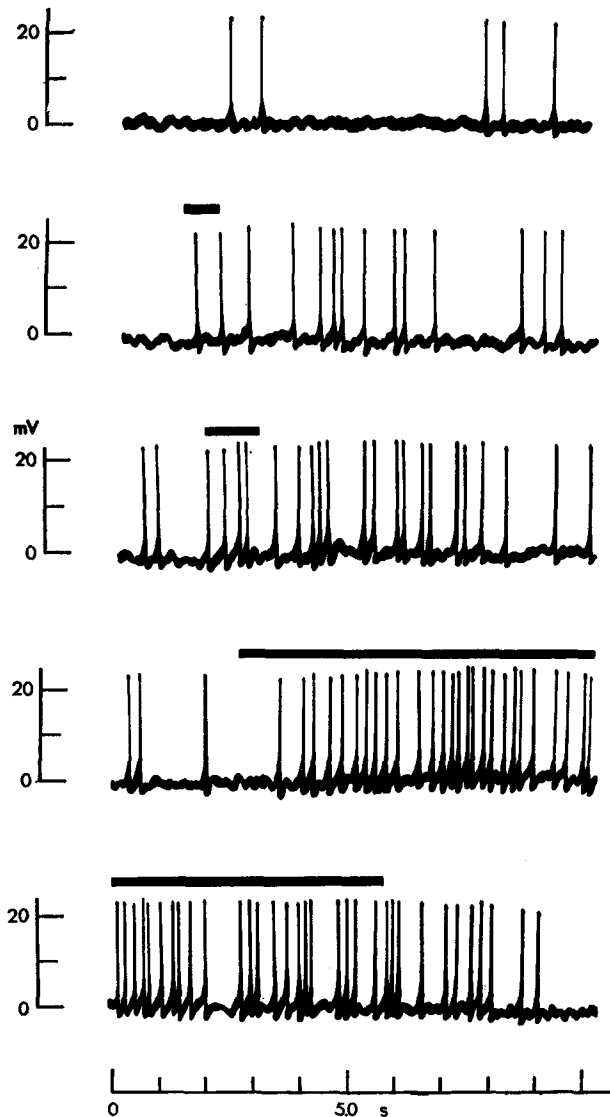


FIGURE 9. Hair cell responses to impulse trains in ipsilateral type A photoreceptor. Bars indicate duration of depolarizing current (0.6 nA) in type A photoreceptor. Top trace: spontaneous activity of hair cell. Lowest trace: current in type A photoreceptor turned off after 30 s.

can tonically excite the hair cell. Such a tonic excitation cannot be explained by a rebound mechanism.

DISCUSSION

Origin of Hair Cell Light Responses

It was previously suggested (Alkon, 1973 *b*) that the depolarizing response of hair cells to illumination of the ipsilateral eye could be produced by a combination of: (*a*) excitation from ipsilateral type A photoreceptors, (*b*) disinhibition by ipsilateral optic ganglion cells, (*c*) rebound excitation after the hyperpolarizing input of ipsilateral type B photoreceptors, (*d*) a local response mechanism. Additional information concerning these possibilities was presented in this report. It was shown that a train of impulses in a type A photoreceptor produced a long-lasting depolarizing wave which was frequently preceded by a hyperpolarizing wave. Similar biphasic synaptic responses have been observed in *Aplysia* (Kehoe, 1967; Wachtel and Kandel, 1967) and the sympathetic ganglion (Weight and Votava, 1970). The presence of this preceding hyperpolarizing wave suggested, however, that the depolarization which followed could be produced by rebound excitation. The duration of the depolarizing wave which follows a hyperpolarizing pulse to a hair cell supports this reasoning.

Using steady negative displacement of the hair cell membrane potential, it was shown that the rebound excitation produced by a hyperpolarizing current pulse to the hair cell could arise from an increase in conductance of an ion with a positive equilibrium potential. The depolarizing response of the hair cell, however, produced by a flash to the ipsilateral eye or by an impulse train in an ipsilateral type A photoreceptor, was shown to arise from a decrease in conductance of an ion with a negative equilibrium potential. Type A photoreceptors, in addition, were shown capable of tonically exciting ipsilateral hair cells. Rebound excitation, therefore, cannot account for the hair cell depolarizing response. Nor can it account for the contribution to the response made by the type A photoreceptors. The type A photoreceptor could excite the hair cell directly, or indirectly by disinhibition of the hair cell (cf. Wilson and Burgess, 1962). The type B photoreceptors certainly can excite the hair cells by disinhibition through a known interneuron: the ipsilateral optic ganglion cells. If the type A photoreceptors do excite the hair cells by disinhibition, an unknown interneuron would be required. Furthermore, this hypothetical disinhibition by type A photoreceptors would require a very long-lasting inhibition (approximately 20 s) of the interneuron. This long-lasting inhibition would be required because a 1.0-s impulse train in a type A photoreceptor can produce a 20-s depolarizing response in the hair cells. The other possibility is that the decrease in conductance is

responsible for a direct excitation of the hair cell produced by the type A photoreceptor. Such a mechanism of excitation has previously been suggested in the sympathetic ganglion (Weight and Votava, 1970) and more recently in the land snail, *Helix aspersa* (Paupardin-Tritsch and Gerschenfeld, 1973).

SUMMARY

(a) Impulse trains in type A photoreceptors were observed to produce a dual effect on some ipsilateral hair cells. This effect consisted of an initial hyperpolarizing wave followed by a delayed long-lasting increase in firing. The increase in firing was usually associated with a small depolarizing wave. The initial hyperpolarizing wave varied markedly in magnitude.

(b) The depolarizing wave caused by rebound excitation after a negative current pulse in the hair cell increased with more negative membrane potentials in the hair cell. The opposite was true of the depolarizing wave in response to illumination of the ipsilateral eye.

(c) Resistance was observed to increase during depolarizing responses produced either by a flash to the ipsilateral eye or by an impulse train in an ipsilateral type A photoreceptor elicited by a positive current pulse.

(d) Resistance was observed to decrease during the initial hyperpolarizing wave in the responses of hair cells to ipsilateral illumination.

(e) The duration of an impulse train in type A photoreceptors was found to determine the duration of increased firing in some ipsilateral hair cells.

(f) The data presented indicate that both effects produced by the type A photoreceptors in ipsilateral hair cells are synaptic. Although the role of rebound excitation and a local response mechanism was shown to be at best minimal, the data do not rule out the intervention of one or more interneurons in the mediation of the dual synaptic effect.

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BIBLIOGRAPHY

- ALKON, D. L. 1973 *a*. Neural organization of a molluscan visual system. *J. Gen. Physiol.* **61**:444.
- ALKON, D. L. 1973 *b*. Intersensory interactions in *Hermisenda*. *J. Gen. Physiol.* **62**:185.
- ALKON, D. L. 1974. Associative training of *Hermisenda*. *J. Gen. Physiol.* **64**:70.
- ALKON, D. L., 1975. Neural correlates of associative training in *Hermisenda*. *J. Gen. Physiol.* **65**:46.
- ALKON, D. L., and A. BAK. 1973. Hair cell generator potentials. *J. Gen. Physiol.* **61**:619.
- ALKON, D. L., and M. G. F. FUORTES. 1972. Responses of photoreceptors in *Hermisenda*. *J. Gen. Physiol.* **60**:1.
- KEHOE, J. S. 1967. Pharmacologic characteristics and ionic bases of a two-component post-synaptic inhibition. *Nature (Lond.)*. **215**:1503.
- PAUPARDIN-TRITSCHE, D., and H. M. GERSCHENFELD. 1973. Neuronal responses of 5-hydroxytryptamine resulting from membrane permeability decreases. *Nat. New Biol.* **244**:171.
- TERZUOLO, C. A., and Y. WASHIZU. 1962. Relation between stimulus strength, generator potential and impulse frequency in stretch receptor of crustacean. *J. Neurophysiol.* **25**:56.

- WACHTEL, H., and E. R. KANDEL. 1967. A direct synaptic connection mediating both excitation and inhibition. *Science (Wash. D. C.)*. **158**:1206.
- WEIGHT, F. F., and J. VOTAVA. 1970. Slow synaptic excitation in sympathetic ganglion cells: evidence for synaptic inactivation of potassium conductance. *Science (Wash. D. C.)*. **170**:755.
- WILSON, V. J., and P. R. BURGESS. 1962. Disinhibition in the cat spinal cord. *J. Neurophysiol.* **3**:392.