J. Exp. Biol. (1974), 60, 707–719
With 1 *plate and* 9 *text-figures*
Printed in Great Britain

THE ELECTROPHYSIOLOGY OF PHOTORECEPTORS IN THE NUDIBRANCH MOLLUSC, *TRITONIA DIOMEDIA*

BY RONALD CHASE

Department of Biology, McGill University, P.O. Box 6070, *Montreal* 101, *Quebec, Canada*

(*Received* 11 *October* 1973)

INTRODUCTION

Despite the continuing success of intracellular recordings from retinal cells in vertebrates, an understanding of information processing in these systems is hampered by the number of different cell types present in these eyes, their small size, and the complexity of interconnexions between them. To circumvent these problems, simpler preparations have been sought for the study of retinal integration. Recently the eye of the nudibranch *Hermissenda crassicornis* has been described as offering rather special advantages (Dennis, 1967; Alkon & Fuortes, 1972). The *Hermissenda* eye contains only five photoreceptors, each of which is large enough to permit stable non-injurious intracellular recording. Other cell types in the eye do not participate in the visual response. It has been shown that the receptors interact synaptically and the form of the photic response in each of the five cells has been related to the pattern of synaptic connectivity which exists between them.

In the present investigation, the eye of the nudibranch *Tritonia diomedia* has been examined in order to determine the degree to which the *Hermissenda* organization is unique among nudibranchs and, more generally, among the opisthobranchs. Whereas *Hermissenda* is largely an intertidal animal, very active and capable of free swimming, *Tritonia* is a bottom dweller at depths up to 100 m or more and non-swimming except for a rudimentary escape response. It was anticipated that these and other differences in the species' morphology and behavioural ecology might dictate divergent organization of the visual system. Knowledge relating to sensory processes in *Tritonia* should be especially useful in view of recent exploitation of the animal for studies of the neuronal control of behaviour (Willows, Dorsett & Hoyle, 1973*b*).

METHODS

Specimens were obtained either from Los Angeles (Pacific Bio-Marine Supply Co.) or from Puget Sound, Washington (courtesy of Dr A. O. D. Willows). They ranged in size from 3 in to 10 in long. The anatomical measurements indicated below are based on medium-sized animals, about 6 in in length. Prior to use, animals were maintained in aquaria at 10 °C and were exposed to a regime of 12 h of light (intensities varied from $7{\cdot}3 \times 10^{-2}$ μW/cm^2 to 1·02 mW/cm^2) alternating with 12 h of total darkness. Dissection of the eyes and the cerebral–pleural–pedal ganglia was accomplished, so far as possible, under red light. Each eye was pinned down in artificial sea water, usually in such a manner as to optimally expose the receptors which are located

posteriorly behind the lens. Hence in most experiments the stimulus light fell predominately on either the 'side' or the 'rear' of the eye. The preparation was maintained at approximately 13 °C within a light-tight Faraday cage. The experiments were conducted in a dimly lit room.

Intracellular potentials were recorded with glass micropipettes filled with 3·0 M-KCl and having resistances between 10 and 50 MΩ, as measured in artificial sea water. A coil of heavy-gauge Ag–AgCl wire served as a grounded reference electrode. The microelectrode was coupled to a high-impedance electrometer (W.P.I. model 4-ARM) which in turn led into an oscilloscope and a Brush model 440 chart recorder. A bridge circuit was employed when current was injected into cells through the recording electrode. In such cases, current strength was monitored using the terminal on the electrometer provided for that purpose. For extracellular recording, the optic nerve was partially de-sheathed and sucked up *en passant* into a drawn-out polyethylene tube. Electrodes were selected to provide a tight fit with the nerve while avoiding excessive stretch. Both active and indifferent electrodes were silver wires which were insulated with dark enamel except for about 3 mm of exposed tip which was plated with Ag–AgCl. The extracellular signal was amplified by a Grass P-16 DC pre-amplifier.

Wide-field illumination was provided by a tungsten filament light source located 27 in from the preparation. Duration of the stimuli was controlled by a mechanical shutter. Stimulus intensity was regulated by either varying the voltage supplied to the light source or by interposing neutral density Wratten filters. Stimulus timing and intensity were monitored by a silicon photodiode which was calibrated by the manufacturer, National Semiconductors Ltd.

For histological examination, eyes were fixed in 10% buffered formalin, embedded in paraffin, and cut to sections 2 μm thick. The tissue was stained with either hematoxylin and eosin or toluidine blue.

RESULTS

Morphology

The two eyes of *Tritonia* are located bilaterally near the base of the rhinophores, well beneath the integument. They are supported by connective tissue which varies from dense to loose in this region. Typically, three thin stands of connective tissue adhere to the eye and appear to fix its position. The eye is roughly spherical in shape, measuring about 250 μm in diameter. About one half of the eye consists of a lens which was always found directed anteriorly. The posterior half of the eye appears black, owing to the presence of pigment granules. The optic nerve leaves the rear of the eye and travels about 2·0 mm before joining with an anterior branch of the sixth cerebral nerve (Willows, Dorsett & Hoyle, 1973*a*). The distance from this junction to the cerebral ganglion is about 2·5 mm. Both of these distances vary between animals, partly as a function of gross size. In some specimens the eye appears to yield two or more separate nerves that eventually join separately with the cerebral nerve; however, electrical activity was not successfully recorded from any of the multiple nerves.

Histological examination of 2·0 μm serial sections from four eyes revealed three types of cells (see Pl. 1 *a*). Small epithelial cells are confined to the anterior end of the eye and form a corneal covering for the lens. The lens itself is clear and basophilic to stains. Posterior to the lens are found two classes of large cells, one of which contains

Text-fig. 1. Extracellular recording from the optic nerve showing the presence of five identifiable spikes. Calibration is 100 μV, 500 msec. Negativity upwards.

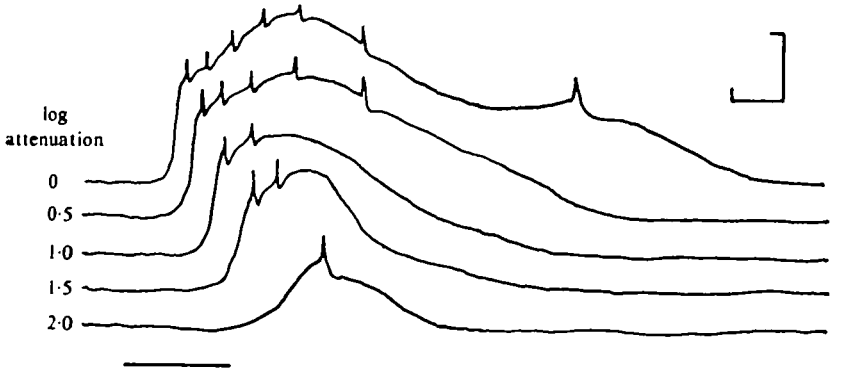
spherical black granules about 1 μm in diameter. These pigment cells form an irregularly shaped band of black pigment around the posterior part of the lens. Somata of the receptors lie external to the pigment, although arms of these cells extend towards the lens and in doing so interrupt the pigment band. Between the pigment and the lens lies a strip of material which has a corrugated appearance with lines running orthogonal to the edge of the lens. It is presumed to contain photosensitive rhabdomeres belonging to the receptors. The neurites of the receptors leave the eye in the optic nerve, a histological observation that has been confirmed by injection of receptor cells with Procion Yellow dye. The optic nerve measures 30–35 μm in diameter. It has not been examined in cross-section.

Nuclei of the receptor cells are generally ovoid in shape and extremely large, typically measuring 45–50 μm in the longest dimension. They are distinguishable from nuclei of pigment cells on the basis of size, staining reaction, and location with respect to the pigment granules. The nuclei of both cells, but particularly of the larger receptors, are characterized by the presence of spherical inclusions 1–4 μm in diameter which stain densely purple with toluidine blue. Assumed to be nucleoli, their number is approximately proportional to the size of the nucleus. As many as eleven have been seen in a thin section of a single nucleus (Pl. 1 *b*). The receptor perikarya are irregularly shaped and, together with receptor axons leaving via the optic nerve, fill the space external to the pigment cells in the posterior half of the eye.

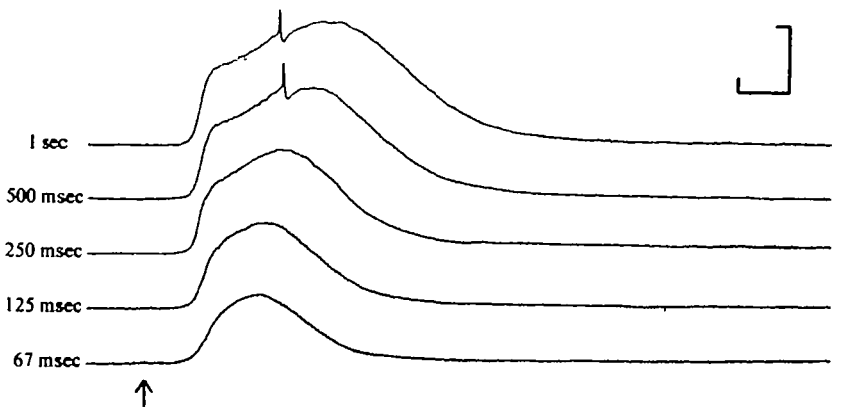
A count of nuclei revealed a total of five receptor cells in each of four eyes examined. Five is also the maximum number of unitary spikes identified in over 35 extracellular recordings from the optic nerve (Text-fig. 1). It was not possible to identify the same cell in different specimens by either histological or physiological means, nor was there any apparent consistency in the arrangement of the nuclei within the eye.

Intracellular recordings

Despite the relatively large size of the photoreceptors in *Tritonia*, intracellular recordings were difficult to achieve unless the connective tissue investment in the vicinity of the eye permitted adequate stabilization by pinning. Successful impalements lasting up to several hours were obtained from about 30 receptor cells. Penetration of a cell was signalled by the sudden appearance of a resting potential of between 30 and 50 mV. Some cells displayed a short train of injury spikes. Thereafter, spikes were seen in about 25% of the receptors. Spike amplitudes were never overshooting, the largest recorded being 12 mV. Typically spikes were seen only when the cell was



Text-fig. 2. Responses of a receptor cell to illumination at different intensities. Timing of the 1 sec stimulus is indicated by the horizontal line. Intensity of the stimulus in the top trace was 0·82 mW/cm^2; the intensity of stimuli for the other traces was attenuated in half-log steps as marked. The inter-stimulus interval was 5 min. Calibration is 10 mV, 500 msec. Traced from the original to eliminate 60 Hz interference.

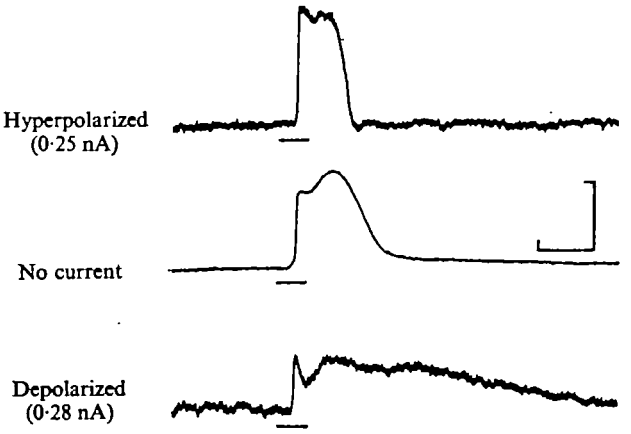


Text-fig. 3. Responses of a receptor cell to illumination of different durations. Stimulus intensity was 0·82 mW/cm^2. Onset of the stimulus is indicated by the arrow; durations were as marked. The inter-stimulus interval was 5 min. Calibration is 10 mV, 500 msec.

depolarized, either by injury, by illumination, or by extrinsic currents. Spike height decreased during strong depolarization, probably due to a loading affect of the non-active soma membrane (Text-fig. 2). Some of the penetrated cells showed no response to light whatsoever during at least 15 min. of observation. It is assumed that these recordings were from the pigment-containing cells of the retina.

Responses to illumination

Regardless of stimulus intensity (typically tested over a range of 3–5 log units), receptors responded to illumination by depolarization. In no cell did the membrane potential ever fall below the resting level during illumination. Response shape and amplitude depended upon the stimulus properties, the condition of light/dark adaptation, and perhaps individual differences among the receptors, although classification was not possible. Text-fig. 2 shows how responses vary with stimulus in-



Text-fig. 4. Effects of extrinsic currents on the amplitude of receptor responses to light. The stimulus intensity was 0·56 mW/cm^2; its timing is indicated by the horizontal line. Calibration is 15 mV, 10 sec.

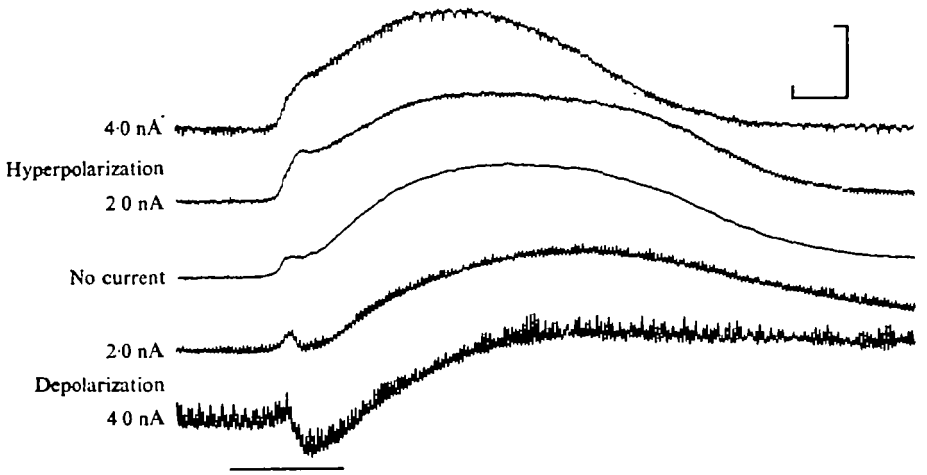
tensity, and Text-fig. 3 illustrates response features dependent on stimulus duration. Clearly, the maximum level of depolarization that is reached during a response and the duration of the response are both functions of the total stimulus energy. The largest depolarization observed in any cell was 25 mV. Response latency is inversely related to intensity over the range illustrated in Text-fig. 2, but is unaffected by duration until stimuli become shorter than about 20 msec, when responses are very weak. When a visual stimulus is sustained, the receptors maintain a steady level of depolarization, apparently indefinitely. As seen in Text-fig. 6, the amplitude of the steady depolarization varies with stimulus intensity, and it is often preceded by a larger, transient depolarizing response.

The transient response of photoreceptors (whether to brief or sustained stimuli) usually consists of two phases, as seen in Text-figs. 2 and 3. The first component of the dual response is fast, the second is more slowly rising and carries superimposed spikes when they are present (Text-fig. 2). Not all cells show a dual response. It is most prominent with relatively high stimulus energies and absent when the stimulus is weak. The rise time of each component also tends to vary directly with stimulus energy. When the slow phase is sometimes delayed, a brief plateau appears between the two components (controls in Text-figs. 4 and 5).

If the eye is left in total darkness after exposure to light, the membrane potential of the receptors slowly increases, as does the sensitivity to light. A change of sensitivity amounting to 2 log units has been observed over a period of 1 h. In eyes that are well dark-adapted the time necessary for repolarization after a response also increases. Because of the great sensitivity of the eye and the progressive effects of dark-adaptation with time, at least up to 1 h, it is difficult to obtain identical responses to two successive stimuli of equal energy.

Effects of extrinsic currents on the response

As illustrated in Text-fig. 4, the total response amplitude of a photoreceptor increased when the cell was hyperpolarized by extrinsic current delivered through the



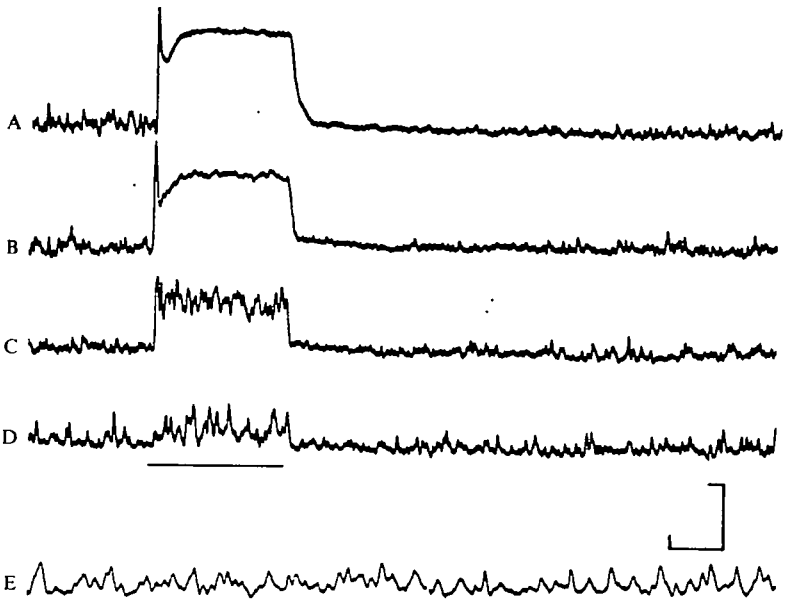
Text-fig. 5. Effects of extrinsic currents on the shape of receptor responses to light. The stimulus intensity was 0·82 mW/cm²; its timing is indicated by the horizontal line. Calibration is 10 mV, 500 msec.

recording electrode in the soma, and it decreased when the cell was depolarized. This result implies that membrane at or close to the soma undergoes an active permeability change in response to light.

Application of extrinsic currents could also produce systematic changes in the shape of the light response. These changes were particularly revealing with respect to the two component responses. In Text-fig. 5, hyperpolarization causes an increase in the size of the fast phase and progressively eliminates the delay before the second, slower phase. Depolarization, on the other hand, eliminates the fast phase and, with strong depolarizing current, replaces it by a hyperpolarizing wave. Although the noise in the records would obscure unitary IPSPs, these observations are consistent with the possibility that the excitatory response of the receptors is interrupted by active inhibition, for which the reversal potential is close to the resting potential. Further evidence relating to synaptic inhibition is presented below.

Interactions between cells

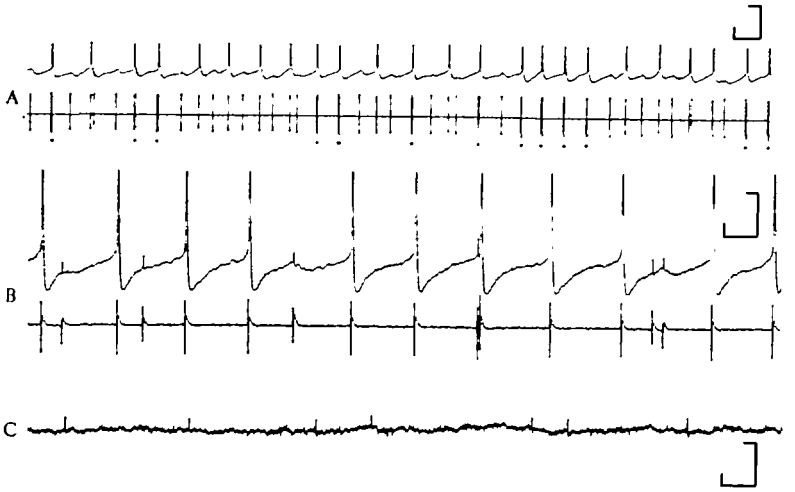
Two types of phenomena, each infrequently observed in separate receptors, suggested the occurrence of interactions between retinal cells. Two cells showed small irregular fluctuations in the resting membrane potential that were possibly indicative of synaptic activity (Text-fig. 6). These deflexions did not exceed 5 mV in amplitude and appeared to be entirely depolarizing in sign. As shown in Text-fig. 6, the fluctuations were enhanced during low-level illumination and absent during intense illumination. They were suppressed after the termination of illumination for a period of time which varied with the intensity. While this behaviour may reflect an intrinsic instability, perhaps due to injury or spontaneous activity in the rhabdomeres, it may also have a synaptic origin. Since spikes were most evident in all soma when these were depolarized, the increased amplitude of the fluctuations with weak-light stimulation could be accounted for by increased activity of presynaptic neurones. Similarly, since spike height was reduced or spikes were absent during strong depolarization,



Text-fig. 6. Fluctuations of the membrane potential in a receptor cell. A–D show the cell's behaviour when stimulated with light for 1 min, as indicated by the horizontal line. Intensity for A was $8{\cdot}2 \times 10^{-2}$ mW/cm^2; intensity for B, C and D was progressively attenuated in 1 log-unit steps. E is the same cell recorded in darkness. Calibration is 15 mV, 25 msec for A–D, 10 msec for E.

this could explain the disappearance of fluctuations at high stimulus intensities, assuming a site of initiation near the soma. Suppression after illumination could be due to repolarization inhibiting presynaptic discharge. Although the rarity of this phenomenon precludes a conclusive interpretation, the fluctuations are not likely to be due to a recording artifact because if this were so the activity would be unaffected by responses to light.

On two other occasions cells appeared to be coupled electrotonically. Text-fig. 7A illustrates the unstimulated activity of a receptor cell with an accompanying optic nerve record showing two extracellular spikes of slightly different amplitude. The smaller spike appears in near simultaneity with the spontaneous receptor spike and is assumed to be identical with it. The larger spike representing activity in a second receptor, is reflected in the intracellular trace by a small, rapid depolarization (barely visible in the illustration) followed by a hyperpolarization. The small depolarization is probably the consequence of electrotonic coupling between the two receptor cells. The coupling is apparently reciprocal and weakly excitatory since the units often fire sychronously. However, it is also evident that the hyperpolarization is inhibitory since, when not active synchronously, the cells tend to fire alternately with approximately equal intervals between spikes. Given that the two units have similar firing frequencies, the phasic behaviour can be explained as follows. If one cell fires near the peak of the other cell's pacemaking potential, the coupling depolarization will be sufficient to trigger the second cell and produce a synchronous discharge. But if the spike occurs earlier during the pacemaking ramp, the coupling potential may be insufficient to

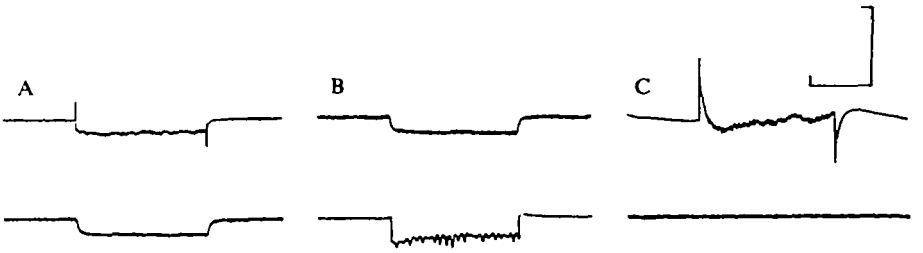


Text-fig. 7. Electrotonic and synaptic interactions between receptor cells. (A) Top trace is intracellular record from a receptor; bottom trace is extracellular record from the optic nerve. The smaller of the two nerve spikes is identical with the receptor spike; the larger is associated with a small coupling potential and a hyperpolarization in the receptor. Dots indicate synchronous or near synchronous discharge of the two units. Recorded in darkness. Calibration is 15 mV (receptor), 200 μV (Op N), 2 sec. (B) Different preparation. Top trace is intracellular record from a receptor; bottom trace is extracellular record from the optic nerve. Large nerve spike is identical with receptor spike; two other units with smaller nerve spikes are electrotonically coupled to the receptor and can exert an inhibitory effect on its discharge. Continuous illumination of $8{\cdot}2 \times 10^{-3}$ mW/cm^2. Calibration is 2 mV (receptor), 100 μV (Op N), 500 msec. (C) Same receptor as in (B) hyperpolarized by extrinsic current. Recorded in darkness. Calibration is 2 mV, 500 msec.

trigger the second cell, and in that case the hyperpolarization will delay the next spike by re-setting the pacemaker. Dennis (1967) has described similar interactions in *Hermissenda*.

The inhibitory effect is seen to better advantage in Text-fig. 7B, from a different preparation, at greater recorder sensitivity. In this case the intracellular trace shows that the spikes of two other cells are coupled. Each of the coupled spikes is identified by the size of the coupling potential and by the shape of the extracellular waveform recorded from the optic nerve. Two nearly synchronous discharges are shown in this figure, and it can be seen that when both of the non-impaled cells fire together near the middle of a pacemaking ramp of the impaled cell, arrival at spike threshold is significantly delayed. When the resting potential is increased by passing current through the recording electrode (Text-fig. 7C), the hyperpolarization which usually follows the coupling potential is now absent. This result is consistent with an interpretation of the hyperpolarizing events as inhibitory synaptic potentials.

The existence of low-resistance pathways between receptors was confirmed in the experiment illustrated in Text-fig. 8, where hyperpolarizing currents introduced into one cell reciprocally produced hyperpolarization in a second cell. When the recording electrodes was moved just outside the cell, spread of current was not detectable, but no index of coupling efficiency is available.



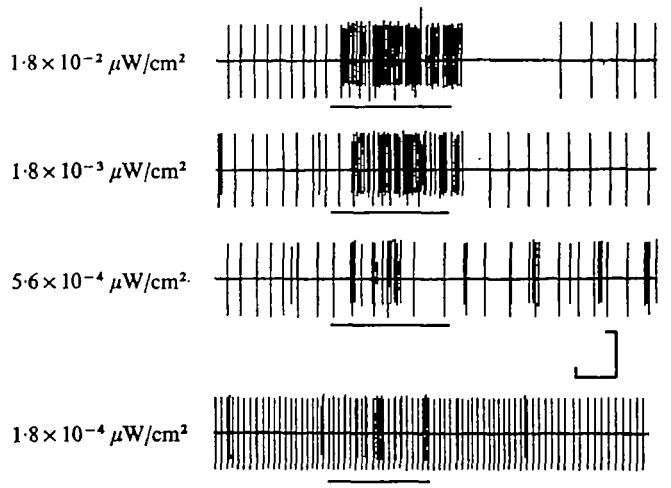
Text-fig. 8. Determination of low-resistance pathway between two receptor cells. Top and bottom traces are records from two different receptors. Hyperpolarizing current (2·1 nA) passed through top electrode in A, bottom electrode in B. There is no current spread in C, when bottom electrode is pulled just outside the cell and 3·1 nA are passed through top electrode. Bridge left unbalanced in each case. Calibration is 10 mV for stimulating electrode, 5 mV for passive electrode, 500 msec.

Functional capabilities of the eye

The animals used in these experiments were dredged from sandy ocean bottoms at depths ranging from 20 to 120 m. (Capture was reported at deeper levels in Los Angeles than in Puget Sound.) Since there is little light at these depths (see Discussion), it was of interest to assess the sensitivity of the *Tritonia* eye. Due to the fact that dark-adaptation significantly increases sensitivity, and because intracellular penetration of receptors requires prior illumination of the eye at high intensities for observation, sensitivity was best measured by looking at the output of the eye as represented by action potentials in the optic nerve. Text-fig. 9 shows that the optic nerve is tonically active in the dark and that the tonic activity increases as the available light increases. This was also observed in a preparation from which recordings were made throughout the night. Tonic activity was maintained for 8 h in absolute darkness and then increased in the morning when the dim light of the sunrise was permitted to enter the experimental chamber. Note in Text-fig. 9 that relatively strong illumination evokes a vigorous response which is followed by an interval of silence at the termination of the stimulus.

The preparation illustrated in Text-fig. 9 was the most sensitive observed in these experiments; it showed a response to light at $1{\cdot}8 \times 10^{-4}$ μW/cm^2, after 2·5 h of dark-adaptation. It must be recalled, however, that the tissue overlying the eye in an intact animal will filter the available light in the animal's natural environment. In order to evaluate the extent of this attenuation, a light source was directed through a small aperture and the amount of transmitted light was evaluated with a photometer when the hole was clear and when it was covered with a piece of integument excised from the area over an eye in a freshly sacrificed animal. Skin reduced the amount of light available at the photometer by a factor of $2{\cdot}3 \times 10^{-3}$ (average of five measurements from different preparations).

Behaviourally, it was observed that a dark-adapted animal is aroused by the onset of diffuse light. If the animal is at rest in the dark, the oral veil and the rhinophores extend at light onset and the animal engages in what appear to be searching movements. If the light is directed to only one anterior side, the animal turns toward that side while performing searching activities. The discrimination of visual form can probably be excluded for the *Tritonia* eye on the basis of its primitive structure and



Text-fig. 9. Extracellular, AC-coupled record of optic nerve activity in response to illumination of different intensities. Timing of the stimuli is given by the horizontal line. Calibration is 200 μV, 400 msec (top three traces), 10 sec (bottom trace).

the interference caused by overlying skin. However, single eyes of similar morphology belonging to the species *Hermissenda* have been shown to be sensitive to the position of a discrete spot of light and to the direction of movement of light spots (Dennis, 1967; Alkon & Fuortes, 1972).

DISCUSSION

It is of interest to compare the structure of the *Tritonia* eye with that of *Hermissenda crassicornis*, the other Pacific Ocean nudibranch that has been well studied (Dennis, 1967; Eakin, Westfall & Dennis, 1967; Stensaas, Stensaas & Trujillo-Cenoz, 1969; Alkon & Fuortes, 1972). The two species belong to separate superfamilies within the nudibranch order and differ considerably in morphology apart from the visual system (MacFarland, 1966). Yet the similarity of morphological detail in the two eyes, and the presence of an equal number of receptors (five) is striking, particularly when compared to a very different structure in an opisthobranch of the tectibranch order, *Aplysia californica* (Jacklet, 1969). The major difference between the visual apparatus of *Hermissenda* and *Tritonia* is that the former has an extremely short optic nerve (total length about 100 μm (reported in Alkon & Fuortes, 1972) that passes through an optic ganglion 5–10 μm from the eye before entering the cerebropleural ganglion, whereas *Tritonia* has an optic nerve several millimetres in length that connects directly with the cerebral ganglion via the sixth cerebral nerve.

The presence of multiple nucleoli in the large *Tritonia* photoreceptors is worth noting in light of the recent report by Lasek & Dower (1971) that giant ganglion cells in *Aplysia* contain an amount of nuclear DNA equal to many thousand times the haploid content. In *Tritonia*, as well as in *Aplysia* (Coggeshall, 1967), the number of nucleoli per nucleus varies with the size of the nucleus. Thus the present observations support the suggestion, first made by Coggeshall, that exceptionally large neuronal size is achieved by chromosomal replication not associated with cytokinesis.

In *Tritonia* all of the photoreceptors produce depolarizing responses to light over a wide range of intensities. This is in contrast to *Hermissenda*, where hyperpolarizing responses are seen in at least three of the five cells. These hyperpolarizing responses in *Hermissenda* have been attributed to inhibitory interactions between the photoreceptors (Dennis, 1967; Alkon & Fuortes, 1972). While inhibition has also been encountered in *Tritonia* and may contribute to the dual nature of photoreceptor generator potentials (Text-fig. 5), it was never observed to be more potent than excitation. Unless sampling was deficient, the results of the present study indicate a significant difference in the physiology of these eyes despite a marked similarity of structure.

The form of the generator potentials recorded from *Tritonia* and from *Hermissenda* also differ. While both responses show a deflexion on the rising phase of depolarization, the first component of the dual response rises more sharply than the second in *Tritonia*, whereas in *Hermissenda* the second component is the faster. Although potentially interesting, the difference in shape cannot be meaningfully interpreted because the significance of the dual response itself is not understood. Two component responses have also been reported for the eye of *Aplysia*, where the first component is the faster (Jacklet, 1969).

Returning to the question of interactions between *Tritonia* receptors, evidence for inhibitory and excitatory interactions has been presented. The inhibition is probably synaptic (Text-fig. 7); the excitation is electrotonic (Text-fig. 7) and only possibly synaptic (Text-fig. 6). The data suggest that the effect of these interactions on the response properties of the cells is minimal. Electrotonic coupling between cells may facilitate amplitude and uniformity of generator potentials in each of the receptors, but synaptic inhibition seems to be of little consequence. Despite the inhibitory interactions, diffuse light was always depolarizing and always resulted in at least a phasic increase in the number of optic nerve spikes, and usually a tonic increase, with units often responding that were silent in the dark. Thus it appears that the optic nerve signals the onset of light by increased spike activity in all receptor axons, and the spike response, as well as the receptor graded potential, has a simple monotonic relation to stimulus intensity (Text-fig. 9). In the following paper it is shown that spikes are often absent from the *Tritonia* optic nerve, but this is not likely to be due to synaptic inhibition since the nerve is frequently completely silent with no detected units active that could be responsible for the inhibition. Although not investigated in *Tritonia*, it is possible that receptor interactions play a role in directional sensitivity for moving lights, as has been demonstrated for *Hermissenda* (Alkon & Fuortes, 1972). But the absence of significant interactions in the *Tritonia* eye might also reflect a more limited functional capability compared to that of the *Hermissenda* eye.

The small amplitude and long duration of the recorded synaptic potentials suggest that they are produced at a location distant from the soma. If this were so, it may be that synaptic interaction is more common than it has been possible to determine through intrasomatic recording. But characteristics of the optic nerve activity still contradict a significant effect of the interactions on the output of the eye, at least when the eye is uniformly illuminated. Since receptor action potentials as seen in the soma were small or absent, and a loading effect of depolarization was observed, an axonal site for initiation of action potentials is also indicated. This hypothesis is confirmed in

the accompanying paper (Chase, 1974). It is therefore likely that the axon is the site of both spike initiation and synaptic interaction, as has been concluded for *Hermissenda* (Stensaas, Stensass & Trujillo-Cenoz, 1969; Alkon & Fuortes, 1972).

Reference has been made to the rather deep habitat which *Tritonia* occupies in the coastal waters. What might be the usefulness of photoreception for a relatively immobile bottom dweller at depths as great as 120 m? One possible function would be to regulate photoperiodic behaviour, which has not yet been determined for *Tritonia* but which has been reported for *Aplysia*, an intertidal tectibranch (Kupfermann, 1968). The intensity of sunlight reaching 100 m below the ocean surface in clear coastal waters can be estimated at 10^{-6} μW/cm^2 (Clarke & Denton, 1962). This corresponds to an intensity about five orders of magnitude less than that detectable by *Tritonia* according to the maximum sensitivity of the eye as measured in these experiments (including attenuation by the skin). But considering the margin of error inherent in all of the stated values, as well as the likelihood that under optimal conditions the eye is more sensitive than reported here (see the accompanying article), one cannot exclude the possibility that *Tritonia* uses the available sunlight to regulate behaviour. Studies are in progress to determine whether *Tritonia's* activity is in fact photoperiodic.

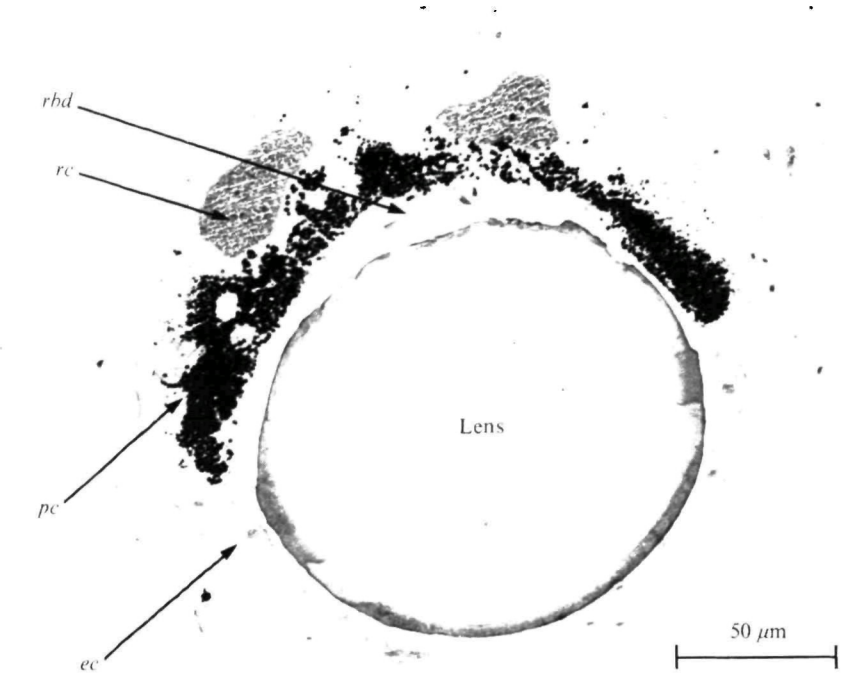
With respect to other possible functions for the *Tritonia* eye, it may perhaps be significant that all of the known food sources of *Tritonia diomedia* belong to the pennatulid order of coelenterates, e.g. *Acanthoptilum*, *Stylatula*, and *Virgullaria*, and members of this order are characteristically luminescent. Within this order an unidentified sea pansy is reported to emit $0{\cdot}8$–$7{\cdot}0 \times 10^{-10}$ μW/cm^2 at a distance of 1 m (Nichol, 1963), an intensity which *Tritonia* might be capable of detecting at distances of a few centimetres. Since the pennatulids flash only when stimulated electrically or mechanically, and since *Tritonia* forages with an extended and active oral veil which could cause a pennatulid to flash at close range, it is conceivable that *Tritonia* utilizes the phosphorescence of the pennatulids as an aid in the location or identification of its food. This speculation receives some support from the observation, noted above, that *Tritonia* will move its oral veil in the direction of a unilateral light source. Since food flashes could easily be distinguished from atmospheric illumination on the basis of temporal characteristics, both light sources might be effective stimuli for *Tritonia*.

SUMMARY

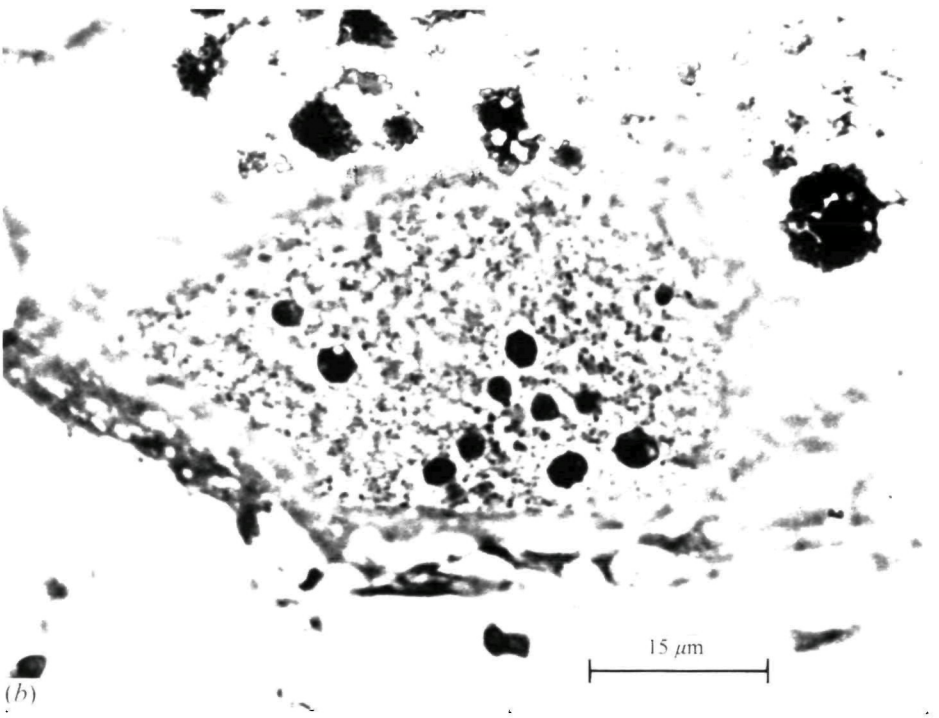
1. *Tritonia* has two eyes, each lying beneath the integument at the base of the rhinophore. Three cell types are present in the eye: large photoreceptors with nuclei up to 50 μm in diameter, pigment cells, and epithelial cells. A count of nuclei revealed a total of five receptors in each eye.

2. All receptors produced depolarizing generator potentials in the presence of light, regardless of intensity within the responsive range. Responses usually consisted of a fast early depolarization followed by a more slowly rising component. Spikes were uncommon in receptor somata. When present, spikes were superimposed on the slower phase of the response and measured no more than 12 mV. Depolarization was maintained at a steady level when illumination was sustained.

3. The receptors are coupled electrotonically, and synaptic inhibition was evident



(*a*)



(*b*)

in a few cells. The functional consequences of these interactions are considered to be minimal.

4. Morphologically, the eye of *Tritonia* is very similar to that of the nudibranch, *Hermissenda*. But there are significant differences in the electrophysiology of the two eyes, particularly with respect to cell interactions.

5. Although the maximum recorded sensitivity was about five orders of magnitude less than that considered to be necessary for the detection of sunlight in *Tritonia's* natural habitat, a visual role in the regulation of periodic behavioural patterns cannot be definitely excluded. The eye may also function in the location or identification of luminescent pennatulids, the major source of food for *Tritonia*.

This work was supported by grant A 6677 from the National Research Council of Canada. I wish to thank Lorraine Pawson for excellent technical assistance and Edward Moore for many helpful discussions. I also thank Geoffrey Manley, Edward Moore and Valerie Pasztor for criticism of the manuscript.

REFERENCES

ALKON, D. L. & FUORTES, M. G. F. (1972). Responses of photoreceptors in *Hermissenda*. *J. gen. Physiol.* **60**, 631–49.

CHASE, R. (1974). The initiation and conduction of action potentials in the optic nerve of *Tritonia*. *J. exp. Biol.* **60**, 721–34.

CLARKE, G. L. & DENTON, E. J. (1962). Light and animal life. In *The Sea; Ideas and Observations on Progress in the Study of the Seas* (ed. M. N. Hill), vol. 1, pp. 456–68. Interscience Publishers.

COGGESHALL, R. E. (1967). A light and electron microscope study of the abdominal ganglion of *Aplysia californica*. *J. Neurophysiol.* **30**, 1263–87.

DENNIS, M. J. (1967). Electrophysiology of the visual system in a nudibranch mollusc. *J. Neurophysiol.* **30**, 1439–65.

EAKIN, R. M., WESTFALL, J. A. & DENNIS, M. J. (1967). Fine structure of the eye of a nudibranch mollusc *Hermissenda crassicornis*. *J. Cell Sci.* **2**, 349–58.

JACKLET, J. W. (1969). Electrophysiological organization of the eye of *Aplysia*. *J. gen. Physiol.* **53**, 21–42.

KUPFERMANN, I. (1968). A circadian locomotor rhythm in *Aplysia californica*. *Physiol. Behav.* **3**, 179–81.

LASEK, R. J. & DOWER, W. J. (1971). *Aplysia californica*: Analysis of nuclear DNA in individual nuclei of giant neurons. *Science* **172**, 122, 278–80.

MACFARLAND, F. M. (1966). Studies of the opisthobranchiate mollusks of the pacific coast of North America. *Mem. Calif. Acad. Sci.* **6**, 1–546.

NICOL, J. A. C. (1963). Luminescence in animals. *Endeavour* **22**, 37–41.

STENSAAS, L. J., STENSAAS, S. S. & TRUJILLO-CENOZ, O. (1969). Some morphological aspects of the visual system of *Hermissenda crassicornis* (Mollusca: Nudibranchia). *J. Ultrastruct. Res.* **27**, 510–32.

WILLOWS, A. O. D., DORSETT, D. A. & HOYLE, G. (1973*a*). The neuronal basis of behaviour in *Tritonia*. I. Functional organization of the central nervous system. *J. Neurobiol.* **4**, 207–37.

WILLOWS, A. O. D., DORSETT, D. A. & HOYLE, G. (1973*b*). The neuronal basis of behaviour in *Tritonia*. III. The neuronal mechanism of a fixed action pattern. *J. Neurobiol.* **4**, 255–85.

EXPLANATION OF PLATE 1

(*a*) Anterior-posterior section of the eye. The lens occupies the anterior part. *ec*, Epithelial cell; *pc*, pigment cell; *rc*, nucleus of receptor cell; *rbd*, rhabdomeres. Stained with haematoxylin and eosin. 2 μm thick.

(*b*) Section through the nucleus of a receptor cell showing the presence of multiple nucleoli. Granular aggregates outside the nucleus are from a pigment cell. Stained with toluidine blue. 2 μm thick.