J. Exp. Biol. (1972), 56, 695–702
With 5 text-figures
Printed in Great Britain

ELECTROTONIC CONDUCTION IN THE OPTIC NERVES OF PLANORBID SNAILS

BY MARION L. PATTON AND STANLEY B. KATER
Department of Zoology, University of Iowa, Iowa City, Iowa 52240

(*Received* 12 *November* 1971)

INTRODUCTION

While action potentials are the usual means of transmission in axons, recent work has established that decrementally conducted potentials are also utilized. Decremental conduction has been demonstrated to be the exclusive mode of transmission in certain sensory axons of vertebrates (Werblin & Dowling, 1969; Kaneko, 1967, 1970) and invertebrates (Gwilliam, 1965, Ripley, Bush & Roberts, 1968; Ioannides & Walcott, 1971). Although decremental conduction had long been suspected in gastropod nerves, there was no compelling evidence for it (Bullock & Horridge, 1965). Recently, however, a DC response has been recorded from the optic nerve of *Helix* (Gillary, 1970). Since the axons of the primary receptors of the gastropod eye usually leave via the optic nerve and pass directly to the central nervous system (Smith, 1906; Clark, 1963; Eakin & Brandenburger, 1967; Newell & Newell, 1968; Jacklet, 1969), the DC shift observed by Gillary could be the decrementally conducted receptor potential as well as a manifestation of an active process. These alternatives might be distinguished by recordings obtained under experimental conditions that block active processes. Employing this approach, the present communication provides evidence for decremental conduction of receptor potentials along the length of the axons of the optic nerves of planorbid snails.

MATERIALS AND METHODS

Laboratory-reared *Helisoma trivolvis* and albino *Planorbis corneus* (shell diameters of 5–10 mm) were examined. Experiments were performed at room temperature (20–23 °C) with a variation of less than ± 0·5 °C in any single experiment. Dissection procedures and Ringer's solution were the same as those used by Kater, Heyer & Hegmann (1971). In some experiments the preparation was treated with a 0·5% procaine hydrochloride (Sigma Chem. Co.) solution made up in physiological saline.

Standard histological techniques were used to examine the anatomy of the eye. Paraffin sections 20 μm thick were stained with hematoxylin and phloxine and examined with both bright field and Nomarski optics.

Conventional electrophysiological techniques were employed. Recording from the optic nerve was made with a glass suction electrode. The eye and its surrounding tissue was excised and pinned, anterior-side down, to a surface of clear encapsulating resin (Sylgard, 184) through which the light was directed onto the eye. To record the ERG, the eye and the surrounding tissues were dissected from the animal and pinned, with the anterior side of the eye oriented upwards, in a dish of saline. The tissue overlying the eye was removed and a broken-off glass micro-electrode filled with 2 M potassium

acetate, or a suction electrode filled with Ringer's solution, was pressed against the eye. A pass band of DC – 500 Hz was used for most recordings. The stimulus light for these experiments was either a Bausch and Lomb monochromator or a heat-filtered, tungsten-filament, microscope lamp. In the figures a positive deflexion is upwards.

To establish the spectral sensitivity of the eye, the preparation was dark-adapted for 30 min, then stimulated with a series of 20 msec light flashes at 60 sec intervals. Pilot experiments had shown that the preparations recovered from the light adaptation produced by a 20 msec flash within this time period. After every six light flashes a standard test flash was given. This test flash was at maximal intensity at 550 nm. If the response to the test flash deviated more than 20% from average, that series of six responses was discarded. The light intensities used in these experiments were adjusted with a photocell calibrated by the National Bureau of Standards.

I. ANATOMY

The eye is embedded in the tissue at the base of the tentacle (Fig. 1) and is surrounded by a perioptic sinus similar to that of *Littorina littorea* (Newell, 1965). The lens appears to be homogeneous and the retina is cup-shaped. Between a retinal pigment layer and the lens are structures which may be the distal ends of the photoreceptor cells (possibly the 'rods' of Smith, 1906, and of Arey, 1916, or the microvilli seen on these 'rods' by Eakin and Brandenburger, 1967, and by Newell and Newell, 1968). The optic nerve of snails with a shell diameter of 5–10 mm is less than 1·5 mm long.

II. ELECTROPHYSIOLOGY OF THE EYE

A. *Optic nerve*

Fig. 2 shows an extracellular recording of the response of the optic nerve. The response in both species is a DC shift with a series of oscillations superimposed upon it. All activity lasts as long as the light flash (even if the flash is 10 sec long) then slowly decays. With a 20 msec flash the DC shift lasts about 6 sec. Both the oscillations and the DC wave are large initially, then decline to a steady state. (Qualitatively similar results were obtained with pass bands as wide as DC to 10 kHz.) The magnitude of the DC shift increases with stimulus intensity and, to a lesser extent, with stimulus duration (Fig. 2). Responses like those in Fig. 2 were obtained with light levels as low as 100 lux (the absolute light levels of Fig. 2 were not recorded). The DC wave is not a physical artifact for the following reasons. It could not be recorded from any other nerve; it disappeared after several hours or when the preparation dried out; it showed light and dark adaptation; its latency increased from 340 to 1000 msec when the temperature was lowered from 24 to 17 °C. The wave is not an artifact of the glass suction electrode because it was recorded with hook electrodes and with a paraffin-tipped glass suction electrode.

To demonstrate that the slow wave is not an artifact of summing action potentials, we used the local anaesthetic, procaine, which abolishes regenerative phenomena (e.g. Katz, 1950). Application of a 0·5% solution reversibly abolished the oscillatory waves in 10 min but did not affect the DC wave, even 30 min after application (Fig. 3). The procaine solution reversibly abolished action potentials in the osphradial and the anterior facial nerves within 10 min of application (Fig. 3).

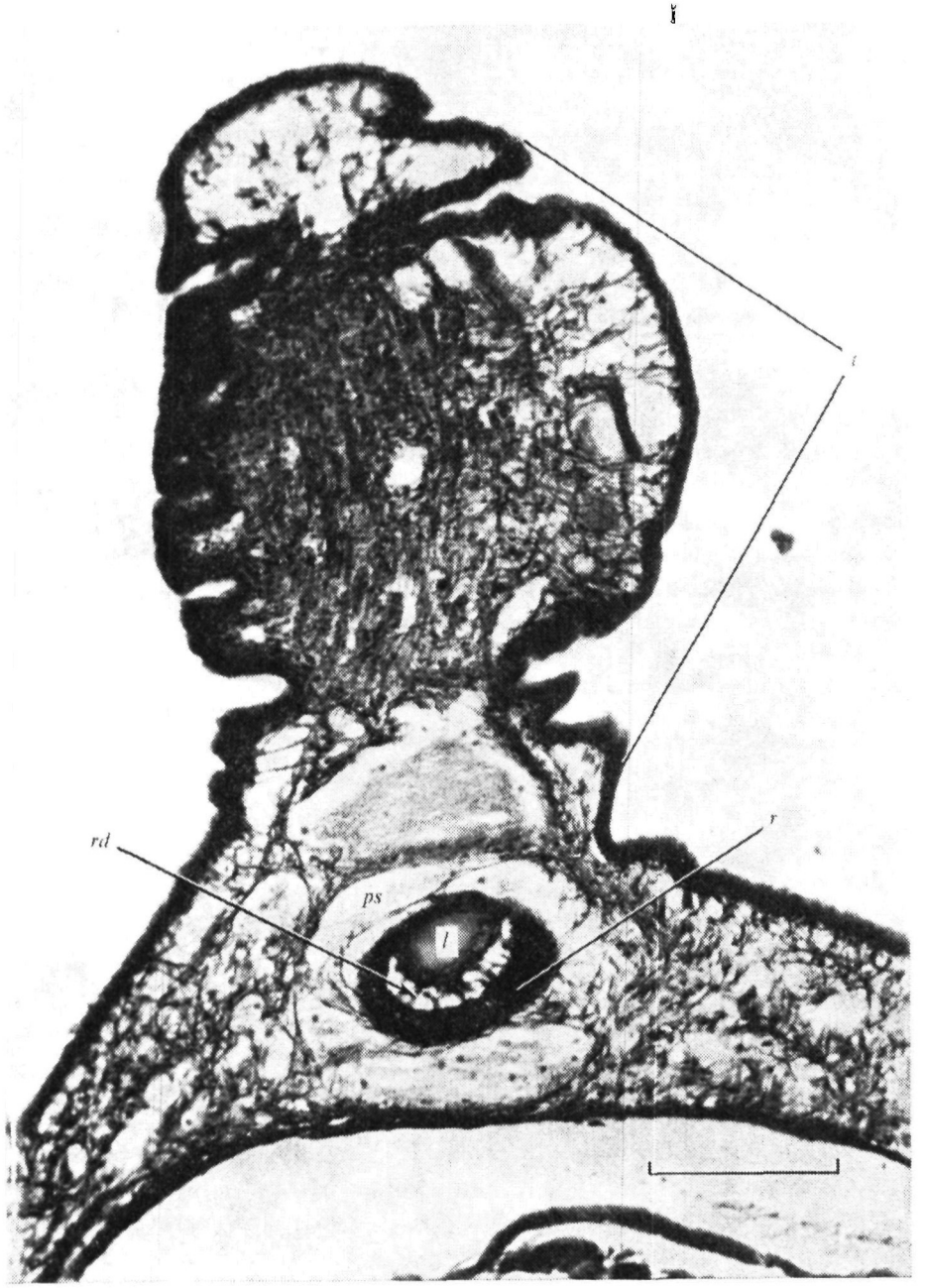

Fig. 1. Frontal section of eye and tentacle. *t*, tentacle; *r*, retina; *l*, lens; *ps*, perioptic sinus, *rd*, rod. Calibration, 1 mm.

B. *Electroretinogram*

A typical ERG (Fig. 4, inset) has the appearance of two superimposed waves: a long, slow surface-negative wave and (usually) a short, sharp surface-positive wave that has a longer latency but a shorter duration than the negative wave. In one preparation

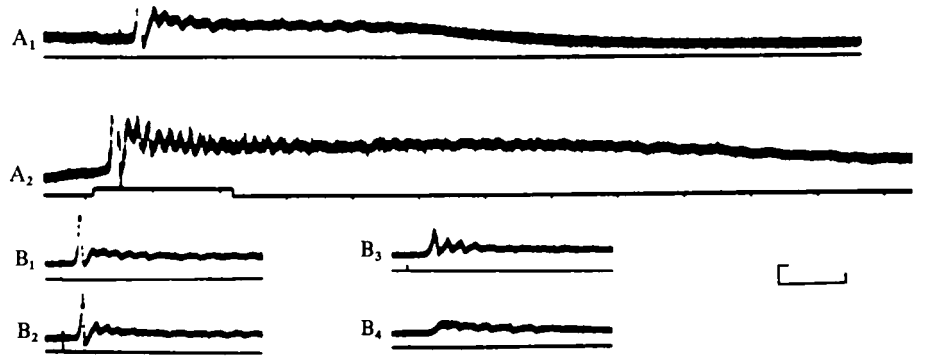

Fig. 2. Effect of stimulus duration (A) and stimulus intensity (B) on the optic nerve response in *P. corneus*. Stimulus duration is 20 msec in A_1, 2000 msec in A_2 and 20 msec in B. Density of attenuating filters, in log units, is 0 in B_1, 0·37 in B_2, 0·92 in B_3, 1·54 in B_4, and 0 in A. Vertical calibration is 40 μV in A, 100 μV in B. Horizontal calibration is 1000 msec. Dark adaptation, 30 min.

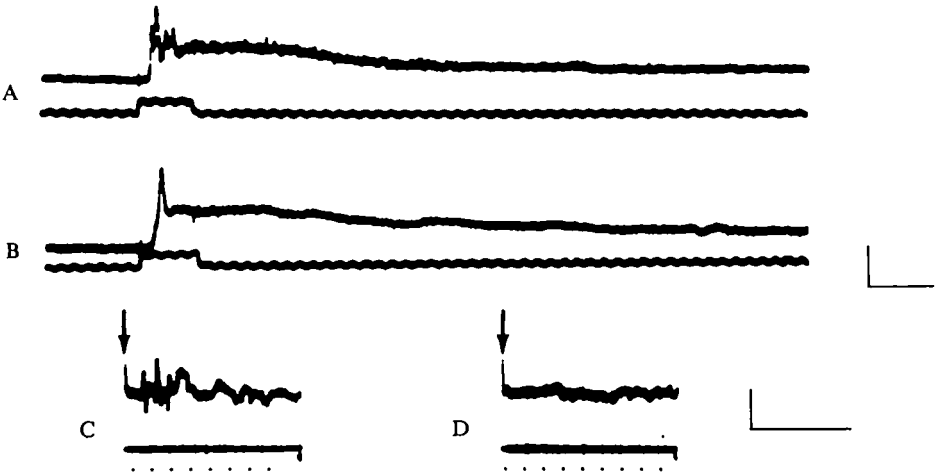

Fig. 3. Effects of procaine on the optic nerve (A, B) and the anterior facial nerve (C, D) of *P. corneus*. A and C are the control responses, B and D the responses after the application of 0·5 % procaine. Upper trace, nerve response; lower trace, stimulus monitor. The stimulus was light for the optic nerve, touch for the anterior facial nerve. Stimulus artifact in C and D indicated by an arrow. Vertical calibration, 100 μV in A and B; 200 μV in C and D. Horizontal calibration, 500 msec in A and B, 50 msec in C and D.

a decrease in temperature produced a reversible increase in the latency from 340 msec at 20 °C to 600 msec at 12 °C. The latency is comparable to that observed in *Aplysia*, 200 msec at 20 °C (Jacklet, 1969) and in *Helix*, 500–1250 msec at 19 °C (Gillary, 1970). The duration of the negative wave, with a 20 msec light flash, was about 7 sec. This is quite similar to the time course of the slow, procaine-insensitive wave of the nerve response (Fig. 4).

III. SPECTRAL SENSITIVITY

Fig. 5 shows the spectral sensitivities of the surface-negative wave of the ERG and of the initial transient of the optic nerve response. The response magnitude at each wavelength was obtained by averaging the responses to a given range of intensities. Within experiments, this range was identical at each wavelength. However, a different

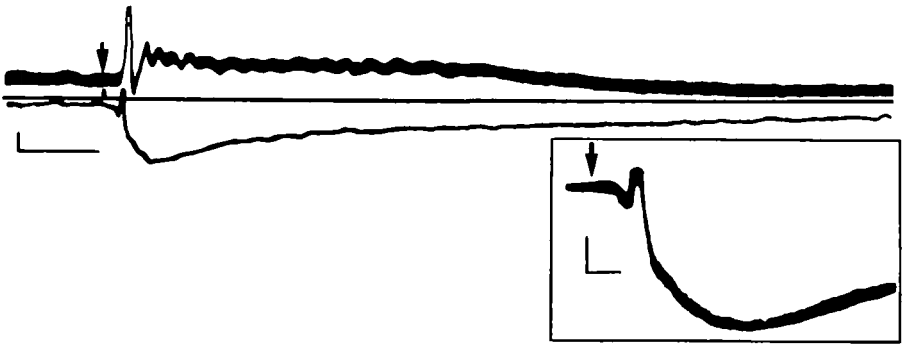

Fig. 4. ERG compared with nerve response in *P. corneus*. Top record, tracing of nerve response; middle record, tracing of stimulus monitor; bottom record, tracing of ERG. Inset, a photograph of the ERG. All three records taken from different preparations. The stimulus, indicated by the arrows, was 20 msec long. Vertical calibration, 40 μV for the nerve response; 100 μV for the ERG; horizontal calibration, 1000 msec for both. Inset, vertical calibration, 200 μV; horizontal calibration, 200 msec. Dark adaptation is 30 min.

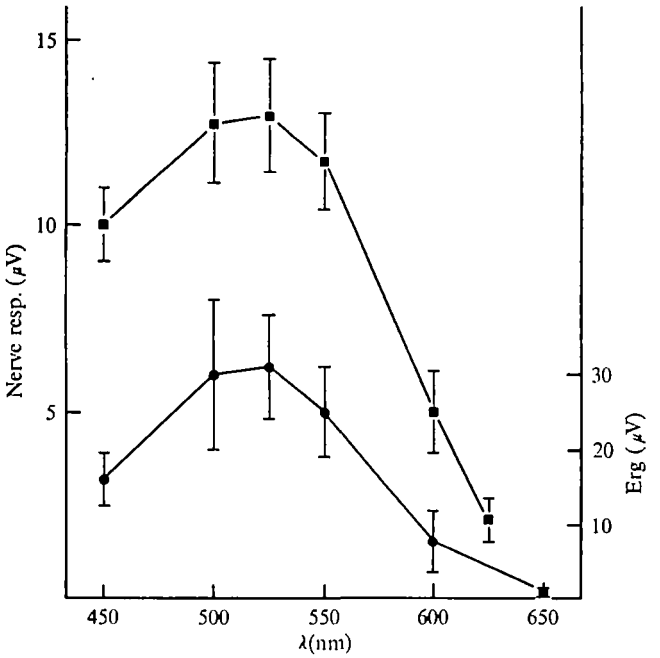

Fig. 5. Spectral sensitivity of the ERG and the nerve response in *P. corneus*. ■, Mean amplitude of the initial peak of the optic nerve response to 20 msec light flashes of equal intensities. ●, Mean amplitude of the surface-negative wave of the ERG response to 20 msec light flashes of equal intensities. Vertical bars indicate ± one standard error.

range of intensities was used for the ERG and the nerve response. Stimulus intensities were well below saturation in all cases. The curves obtained are quite similar and resemble the spectral sensitivity curve of the ERG of *Aplysia* (Waser, 1968). They are very broad and show little sensitivity to red light, but the responses are nearly as strong at 450 nm as at 550 nm (Fig. 5).

DISCUSSION

The DC potential recorded from the nerve (Figs. 2 and 3) is not affected by procaine. Procaine has been shown (e.g. Katz, 1950) to abolish regenerative potentials, both local and propagated, without affecting the generator potential. Since procaine abolished both the oscillations which accompany the DC shift in the optic nerve and the action potentials of the osphradial and anterior facial nerves, it is unlikely that its lack of effect on the DC shift was due to a failure to penetrate. The simplest interpretation of the DC shift is that it represents decrementally conducted receptor potentials. It is possible, however, that this wave is produced by a change in the activity of an ion pump, or is a local regenerative event which is inside the eye and shielded from the procaine by the optic capsule.

The procaine-sensitive oscillations superimposed on the slow wave could be due to regenerative events not shielded by the optic capsule. The regular oscillations seen in some records could be produced by synchronous bursts of spikes like the bursts seen in the eye of the eel (Adrian & Matthews, 1928). Since both the slow wave and the oscillations were recorded at the junction of the optic nerve and the cerebral ganglion, it is possible that both regenerative and electrotonic conduction is used to transmit information from the snail's eye to the snail's brain. This would be an unusual situation. Although the existence of sensory nerves that conduct information with *either* the regenerative process (Matthews, 1931; Katz, 1950) or by decremental conduction of a receptor potential (Gwilliam, 1965; Bush & Roberts, 1968; Ripley *et al.* 1968; Ionannides & Walcott, 1971) is well documented, there is little evidence for dual mechanisms. Combined electrotonic and regenerative conduction may be important in the eyes of at least two other gastropods. Gillary (1970) recorded a DC shift with superimposed compound action potentials from the optic nerve of *Helix*. (However, as mentioned above, the DC shift might have been due to an active process.) Also, 10% of the cells impaled in the eye of *Hermissenda* responded to light with graded potentials only, and the rest fired spikes (Dennis, 1967). Though the electrode may have damaged the spike-generating locus in some of the non-spiking cells, their large numbers encourage the speculation that some of these cells may normally transmit information without spiking.

The negative wave of the ERG (Fig. 4) is very similar to the ERGs recorded from the eyes of *Helix* (Gillary, 1970) and *Aplysia* (Jacklet, 1969). The structure of the eyes of planorbid snails is quite similar to that of *Helix* (Eakin & Brandenburger, 1967) and *Aplysia* (Jacklet, 1969). These similarities suggest strongly that the negative wave of the planorbid *ERG* is also like these ERGs in that it is produced by the summed receptor potential (Gillary, 1970; Jacklet, 1969).

The ERG and the nerve response are very much the same in both *Planorbis* and *Helisoma*. However, the brief, positive-going component of the ERG of *Planorbis* is missing in *Helisoma*. Likewise, the nerve response of *Helisoma* lacks the large initial positive transient seen in *Planorbis*. Furthermore, spike-like activity (10 msec duration), not seen in *Planorbis*, is present in *Helisoma*. The presence of the action potentials in *Helisoma* is in agreement with the idea that both electrotonic and regenerative conduction may be used to transmit information in the optic nerves of planorbids.

Three different kinds of evidence suggest that the negative wave of the ERG and the

DC response of the nerve have a common source. Fig. 5 shows that they have parallel spectral sensitivities. The latency of the nerve response is slightly greater than that of the ERG and the time courses are similar (Fig. 4). Taken together, these data suggest that the ERG and the nerve response mirror the same event, which is possibly the receptor potential.

SUMMARY

1. The conduction of information from the eyes of two basommatophoran snails has been investigated.

2. In the optic nerves of *Helisoma trivolvis* and *Planorbis corneus* a flash of light to the eye produces a procaine-insensitive DC potential that is graded with stimulus intensity and lasts as long as the stimulus.

3. The similar spectral sensitivities, time courses and latencies of the ERG and the slow wave of the optic nerve suggest that the DC response is a decrementally conducted receptor potential.

4. Superimposed on the DC shift are procaine-sensitive oscillations.

5. The oscillations may be either local or propagated regenerative events.

We thank Drs M. F. Land and H. L. Gillary for their criticism of earlier forms of this manuscript. This research was supported by PHS Grant NS09696-01 (awarded to S.B.K.) and, in part, by University Development Grant GV2591 from NSF.

REFERENCES

AREY, L. B. (1916). The influence of light and temperature upon the migration of retinal pigments of *Planorbis trivolvis*. *J. comp. Neurol.* **26**, 359–389.

ADRIAN, E. D. & MATTHEWS, R. (1928). Action of light on the eye. III. The interaction of retinal neurons. *J. Physiol.* **65**, 273–98.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous Systems of Invertebrates*, p. 1306. San Francisco and London: W. H. Freeman and Co.

BUSH, B. M. H. & ROBERTS, A. (1968). Resistance reflexes from a crab muscle receptor without impulses. *Nature, Lond.* **218**, 1171–3.

CLARK, A. W. (1963). Fine structure of two invertebrate photoreceptor cells. *J. cell Biol.* **19**, 14A.

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

EAKIN, R. M. & BRANDENBURGER, J. L. (1967). Differentiation in the eye of a pulmonate snail, *Helix aspersa*. *J. Ultrastruct. Res.* **18**, 391–421.

GILLARY, H. L. (1970). Electrical responses from the eye of *Helix* to photic stimulation and simultaneous electrical stimulation of the optic nerve. *Vision Res.* **10**, 977–91.

GWILLIAM, G. F. (1965). The mechanism of the shadow reflex in cirripedia. II. Photoreceptor cell response, second order responses and motor cell output. *Biol. Bull. mar. biol. Lab. Woods Hole* **129**, 244–56.

IOANNIDES, A. C. & WALCOTT, B. (1971). Graded illumination potentials from retinula cell axons in the bug, *Lethocerus*. *Z. vergl. Physiol.* **71**, 315–25.

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

KANEKO, A. & HASHIMOTO, H. (1967). Recording site of the single cone response determined by an electrode marking technique. *Vision Res.* **7**, 847–51.

KANEKO, A. (1970). Physiological and morphological identification of horizontal, bipolar and amacrine cells in the goldfish retina. *J. Physiol.* **207**, 623–33.

KATER, S. B., HEYER, C. & HEGMANN, J. P. (1971). Neuromuscular transmission in the gastropod mollusc, *Helisoma trivolvis*: Identification of motoneurons. *Z. vergl. Physiol.* **74**, 127–139.

KATZ, B. (1950). Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. *J. Physiol.* **111**, 261–82.

MATTHEWS, B. H. C. (1931). The response of a single end organ. *J. Physiol.* **71**, 64–110.

NEWELL, G. E. (1965). The eye of *Littorina littorea*. *Proc. zool. Soc. Lond.* **144**, 75–86.

NEWELL, P. F. & NEWELL, G. E. (1968). The eye of the slug, *Agriolimax reticularis* (Müll.). *Symp. zool. Soc. Lond.* **23**, 97–111.

RIPLEY, S. H., BUSH, B. M. H. & ROBERTS, A. (1968). Crab muscle receptor which responds without impulses. *Nature, Lond.* **218**, 1170–1.

SMITH, G. (1906). The eyes of certain pulmonate gastropods, with special reference to the neurofibrillae of *Limax maximus*. *Bull. Harvard Museum. Comp. Zool.* **48**, 233–83.

WASER, P. M. (1968). The spectral sensitivity of the eye of *Aplysia californica*. *Comp. Biochem. Physiol.* **27**, 339–47.

WERBLIN, F. S. & DOWLING, J. E. (1969). Organisation of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**, 339–54.