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The Accessory Photosensory Organ of the Terrestrial Slug, *Limax flavus* L. (Gastropoda, Pulmonata): Morphological and Electrophysiological Study

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ABSTRACT—The accessory photosensory organ of the slug *Limax flavus* L., accessory eye was studied morphologically and electrophysiologically. The accessory eye is situated in the protuberance of the eyeball, and is separated by the septal structure from the main eye having a cavity of its own. One type of receptor potential was recorded intracellularly from sensory cells in the main eye and accessory eye, and identified with sensory cells type I by intracellular staining. The peaks of the spectral sensitivity curves of type I sensory cells in both eyes were found at 460 nm.

INTRODUCTION

An accessory photosensory organ called accessory retina or accessory eye was first described in the last century [1] and came to be known as common in some terrestrial slugs [2, 3] and also in a certain snail *Achatina fulica* [4]. The accessory photosensory organ of *Achatina fulica* had structure complex including an accessory lens and the septal structure between the two eyes, to call it an accessory eye. The fine structure of the accessory retina in *Limax flavus* has been closely studied [3], but the relationship between the main and accessory retina such as the septal structure, their cavities and lenses are still not clear in the slug.

As for the function of the slug accessory retina, two hypotheses, that it serving as a light intensity meter [3] and that it may be an infrared light receptor [2], have been proposed on the basis of morphological and behavioral studies. In order to know the function of the snail accessory photosensory organ, electrophsiological study was carried out in *Achatina fulica* [5], and I obtained evidence of visible light reception by the accessory organ.

In this paper I consider the relationships between the main retina and the accessory retina in terrestrial slug, and report the evidence of visible light reception by the accessory organ in the terrestrial slug *Limax flavus* L.

MATERIALS AND METHODS

Animals and preparation for light and electron microscopy

Limax flavus slugs were collected in a suburb of Osaka and maintained in cyclic light (12L:12D) at room temperature. Tip of the optic tentacles were fixed in 2.5% glutaraldehyde containing 0.1 M phosphate buffer (pH 7.4). The eye and optic nerve were dissected free from the tentacular tissue, postfixed in 1% OsO_4 containing 0.1 M phosphate buffer (pH 7.4), dehydrated with an alcohol series and embedded in Spurr resin. Semithin sections were stained with toluidine blue and silver thin sections were contrasted with uranil acetate and lead citrate.

Intracellular recording and intracellular staining with HRP

The eye and optic nerve were dissected free

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from the tentacular tissue in snail Ringer's solution [6] and the preparation was mounted in an experiment chamber containing the Ringer's solution whose temperature was maintained at approximately 25°C. Stimulus light intensity was controlled with a neutral density circular wedge and neutral density filters. The maximum intensity of the stimulus light, indicated by 0 (−log I unit) in the figure, on the preparation was 6.5×10^{10} photons/cm^2. s with 5 nm bandpass. To obtain higher intensity in the near infrared region (600 to 800 nm), the slit bandpass was opened to 10 nm. These experimental arrangements for optical stimulation and electrical recording are reported previously [5].

After the specimens had been prepared, the eye was dark adapted for at least 30 min. Intracellular recordings were made with micropipettes filled witn 10% HRP (Sigma type VI) dissolved in 0.5 M KCl, 0.1 M Tris. buffer (pH 8.6). Electrode resistance was generally 40–80 MΩ. The microelectrode was inserted into the cells through the opening formed in the cornea by removing the main lens. Test flashes were of 1 sec duration, 3 (−log I) unit intensity and in steps of 50 nm from 400 nm to 800 nm in wavelength.

After recording electrical events, HRP was iontophoresed into cells, and HRP reaction was carried out following Graham-Karnovsky [7]. Details of these processes were also reported previously [5].

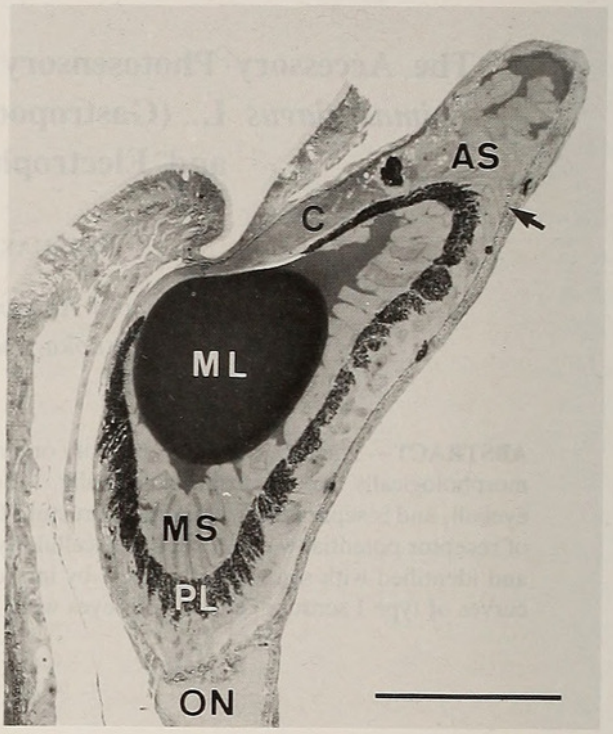


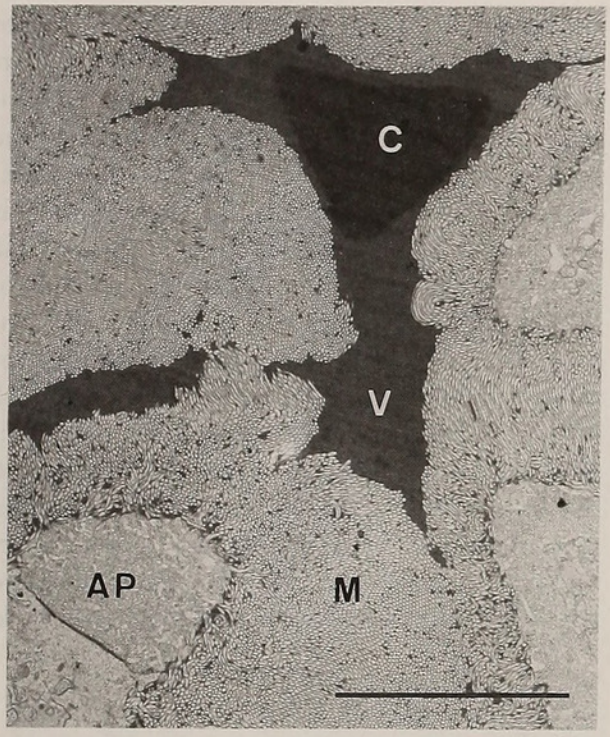
FIG. 1. Light micrograph of parasagittally sectioned eyeball. AS, sensory cells in accessory retina; C, cornea; ML, lens of main retina; MS, sensory cells in main retina; ON, optic nerve; PL, pigment layer; Arrow, indicating the location of septal structure. Calibration bar=100 μm.

RESULTS

Light and electron microscopic observation of the relationship between the two retinas

The eyeball of the slug *Limax flavus* was shaped like a twisted pear and the accessory retina was situated in the protuberance which corresponded to the corner of the cornea (Fig. 1). In the figure, some distortion may be caused in the main retina by dehydration because of its large cavity and lens. The cavity of the main retina was occupied by the main lens and vitreous body into which the apical projections of sensory cells were projected. The cavity of the accessory retina is also occupied by a vitreous body and apical projections of its sensory cells. Some cores of the vitreous body which would grow into an accessory lens were sometimes contained in it (Fig. 2). As can be seen in Figure 1, the two cavities, i.e. of the main retina and the accessory retina, were separated by the cornea and the main retina. In some rare cases, in about one in ten eyeballs, partial destruction or emaciation of the cornea resulted in continuation of the two cavities. Therefore it is concluded that the cavities are ordinarily separated. Moreover the two retinas were separated by a septal structure composed of elongated cells (arrow in Figs. 1 and 3). The elongated cells contained some bundles of filaments (arrow in Fig. 3).

It is said that the main retina of the *Limax* eye are composed of two types of sensory cells [8, 9]. One of the two types of sensory cells is named sensory cell type I and characterized by aggregation of so-called photic vesicles [10], large cell body and long apiclal projection in the cavity. The other type of sensory cell is named sensory cell



type II. In addition to the main retina [8], the accessory retina of *Limax flavus* is also composed of two types of sensory cell, type I and type II [3]. The cell somata of these sensory cells are recognizable in Figure 3.

Intracellular recording

Resting potentials of photosensory cells were 50-60 mV, while photoinsensitive cells were 70–90 mV. Only one type of receptor potentials were recorded stably from both the main and the accessory retina long enough that the spectral sensitivity curves could be constructed. The receptor potentials recorded from both retinas were similar

FIG. 2. Electron micrograph of core of vitreous body. AP, apical projection of type I sensory cell in the accessory retina; C, core of vitreous body; M, microvilli; V, vitreous body. Calibration bar = 10 μm.

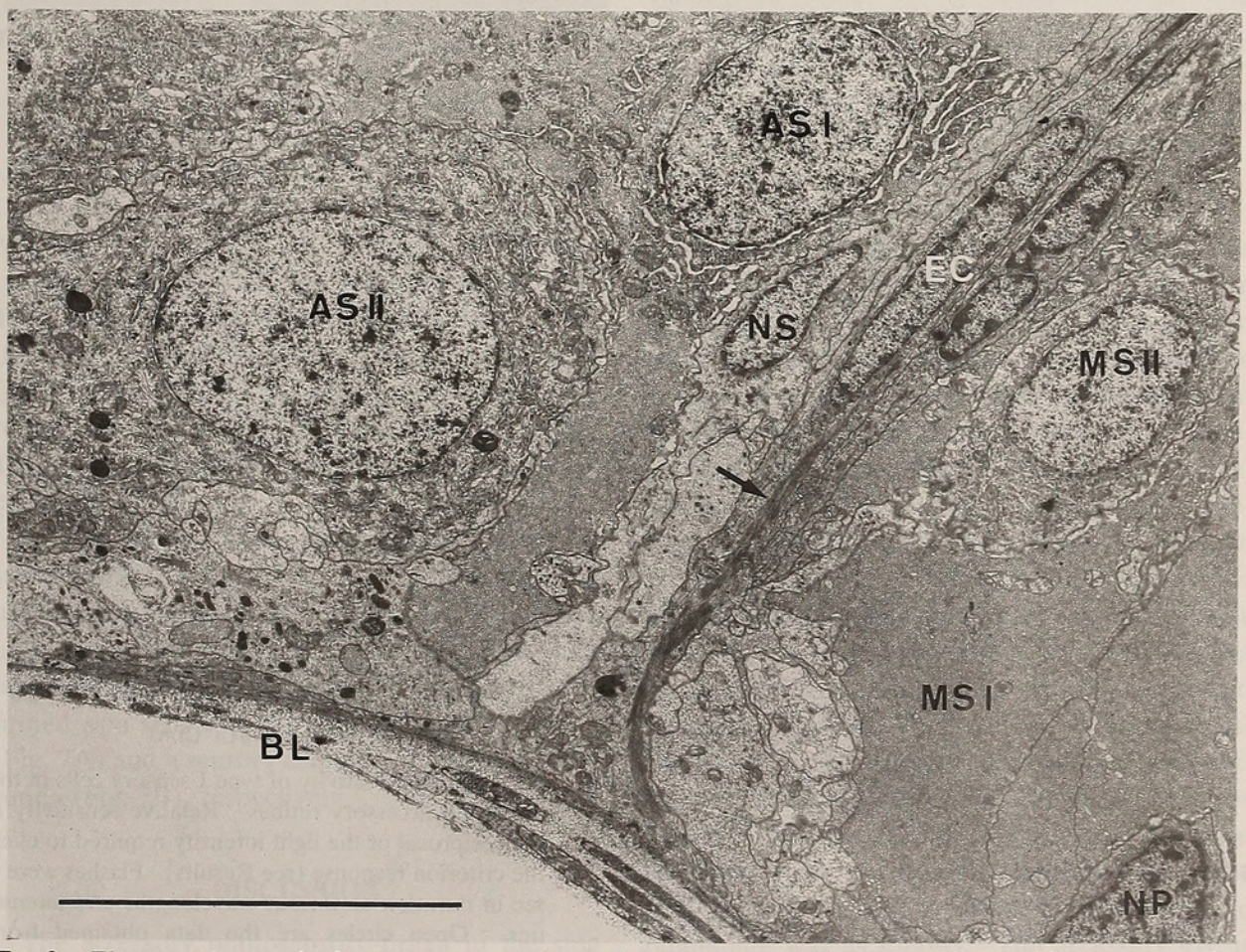


FIG. 3. Electron micrograph of septal structure between the two retinas. ASI, sensory cells type I in accessory retina; ASII, sensory cell type II in accessory retina; BL, basal lamina; EC, elongated cell; MSI, sensory cell type I in main retina; MSIII, sensory cell type II in main retina; NP, nucleus of pigment cell; NS, nucleus of supporting cell in the accessory retina; Arrow, bundles of filaments. Calibration bar = 10 μm.

graded depolarizations of as much as 40 mV, with no overshooting (Fig. 4). Therefore it was impossible to decide from which retina the responses were recorded by studying the waveform alone.

The amplitudes of these receptor potentials (from baseline to peak) were measured with stimulus lights of various wavelengths and intensities at intervals of 30 sec. Figure 5 shows the amplitude-intensity plots of the receptor potentials elicited by the stimulus light of 460 nm in wavelength. Figure 6 shows the spectral sensitivity curve for the sensory cells in both retinas. The sensitivity was the relative intensity required to elicit a criterion response. The criterion response was obtained as follows: As the stimulus intensity increased, the spectral response curve at a constant photons of stimulus light, whose peak was found at 460 nm, became flat. When the receptor potentials for similar amplitudes (within a deviation range of 5 mV) were recorded in the range over 100 nm (it was from 400 to 500 nm), their amplitude at 460 nm was taken as the standard. The criterion response was a half of the standard. For instance, the standard response of the sample used in Figure 5 fell on the asterisk in the figure. The standard

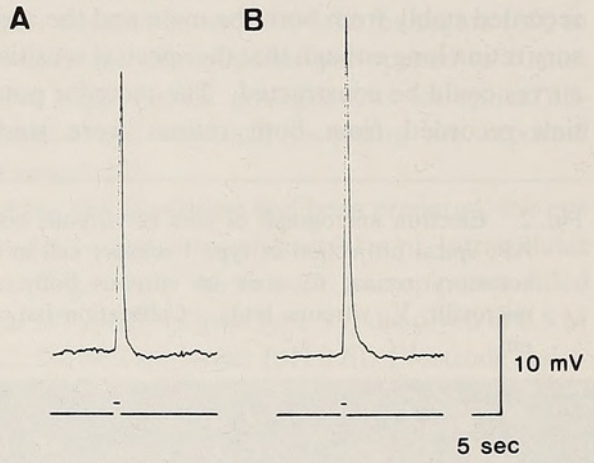


FIG. 4. Receptor potentials recorded from sensory cells in both retinas. A, receptor potential from a sensory cell in main retina. B, receptor potential from a sensory cell in accessory retina. Traces beneath the recording indicate the stimulus light of a 1 sec duration at 460 nm in wavelength and 2.14 (−log I) unit intensity. Difference in the amplitudes of receptor potentials is due to the recording condition. Ultrastructural features of sensory cell in A and B are shown in Fig. 7A and B.

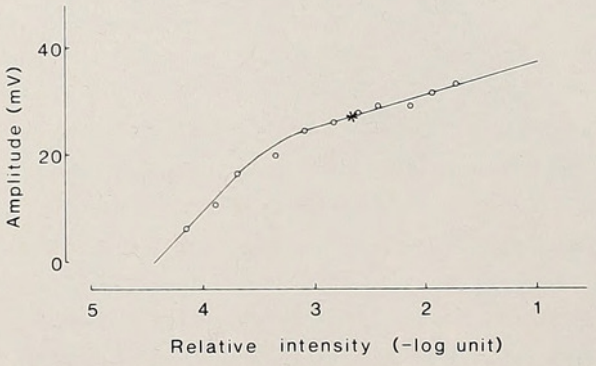


FIG. 5. Amplitude-log intensity curve for receptor potentials recorded from accessory retina. Flashes were 1 sec duration at 460 nm after the sensory cells were fully dark-adapted. All data were obtained from a single preparation. Asterisk indicates the standard response (see Results) of the preparation.

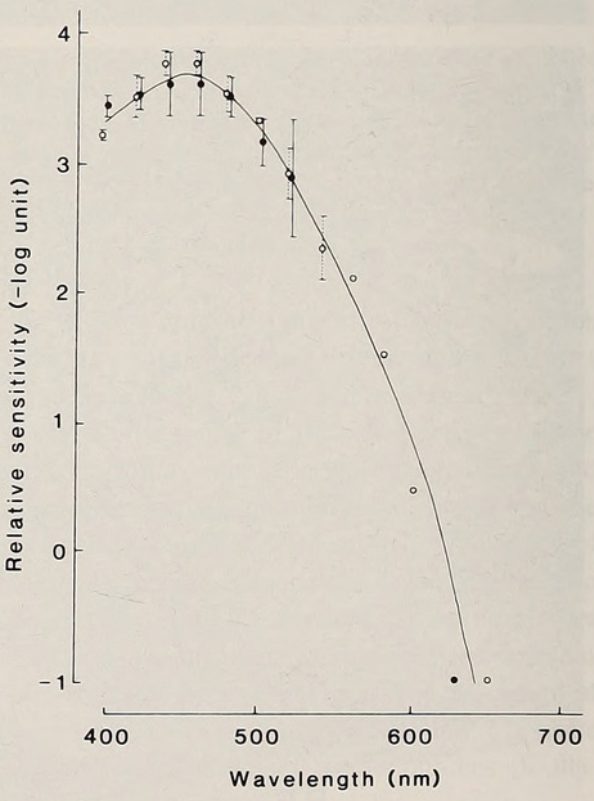


FIG. 6. Spectral sensitivity of type I sensory cells in the main and accessory retinas. Relative sensitivity is the reciprocal of the light intensity required to elicit the criterion response (see Results). Flashes were 1 sec in duration at various wavelengths and intensities. Open circles are the data obtained from accessory retina and filled circles are from main retina. These were obtained from each of 2 preparations of the main and accessory retina.

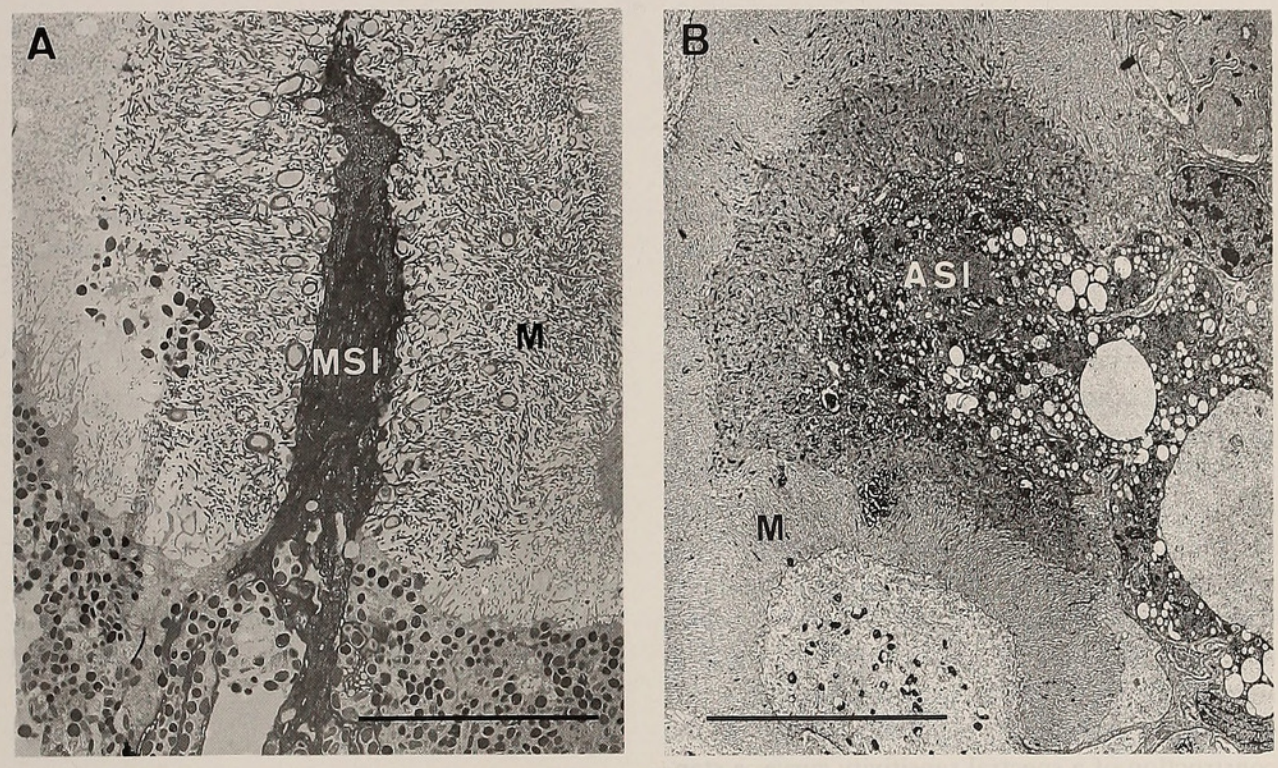


FIG. 7. Electron micrographs of sensory cell type I stained intracellularly with HRP. A, sensory cell type I in main retina. MSI, sensory cell type I in main retina; M, microvilli. B, sensory cell type I in accessory retina. ASI, sensory cell type I in accessory retina; M, microvilli. Receptor potential of each cell is shown in Fig. 4A and B. Calibration bar = 10 μm.

response was 28 mV at 2.62 (−log I) unit intensity. Therefore the criterion response was 14 mV. The sensory cells recorded in both retinas exhibit the same spectral sensitivity peaking at 460 nm. Open circles are data recorded from the accessory retina and filled circles are from the main retina. These were obtained from two preparations of each retina.

In no case was a receptor potential elicited by stimulus light having a wavelength greater than 700 nm, even if the stimulus intensity was -1 (−log I) unit. After the recording, the sensory cells were labeled intracellularly with HRP and were confirmed as a sensory cell type I in the main retina (Fig. 7A) and a sensory cell type I in the accessory retina (Fig. 7B), respectively.

DISCUSSION

The accessory photosensory organ in the slug *Limax maximus* was first described by Henchman [1] and he used two terms, accessory retina and accessory eye, for the organ in the report. But the following researchers hesitated to use the term accessory eye. For instance Smith [11] hesitated to use it because he could not find a septal structure between the two retinas in spite of the recognition of the discontinuity of the two cavities. In *Achatina fulica* these three items, accessory lens, septal structure and discontinuity of cavities, were stationary elements, which led me to call the organ an accessory eye [4]. This time I looked at the relationship of main retina to the accessory retina in *Limax flavus* and found a trace of lens and a septal structure as well as discontinuity of the two cavities. Concerning the accessory lens, the accessory retina in *Agriolimax reticulatus* was comparable to that of *Achatina fulica* (unpubli. data). It may be, therefore, preferable to revive the term accessory eye in slugs. I concluded that the situation and structure of the accessory eye in pulmonate was as follows. The accessory eye is situated in the corner of the cornea and discontinuous with the main eye. It is composed of two

types of sensory cells similar to those in the main eye and corneal cells or nonpigmented supporting cells.

The electrophysiological studies reported here showed that sensory cell type I in the accessory eye of the slug receives visible light similarly to the corresponding cell type in the main eye. In support of visible light reception by the accessory eye in *Limax flavus,* retinal pigments were detected with fluorescence microscopy [12]. Although the function of sensory cell type II in the accessory eye still remains unknown, structural similarity of sensory cell type II in the main and accessory eye may also exclude it from the candidate of infrared light receptor. The recording from the type II cells, anyhow, must be performed in the following study. The intensity-amplitude plots of the receptor potentials may present a higher part of the typical sigmoid curve [13]. Responses at lower light levels will be also the subjects of further studies. In the construction of a spectral sensitivity curve, a standard response was adopted in order to obtain a criterion response immediately and to reduce the effects of the intracellular-recording conditions on relative sensitivity in each cell. Although the standard response is a value used tentatively, it was reliable in comparing the relative sensitivity and providing the evidence of visible light reception by the accessory eye.

The work in this report showed that both sensory cells type I in the main and accessory eyes had a sensitivity peak at a wavelength of 460 nm, while the sensory cells type I in *Achatina fulica* had their peak at 480 nm [5] similarly to the peak of sensitivity in other gastropod eyes [14–18]. In another electrophysiological study of *Limax* main eye, the peak of spectral sensitivity at 460 nm was found in the light-adapted sample using spike discharge as the criterion response [6]. The report suggested that the sensitivity maximum for sensory cell type I is 480 nm and that for type II is 460 nm, which seems to be inconsistent with my data. The more close intracellular recording and HRP study will settle these inconsistency.

As one of the characteristics to advance speculation on the functions of the accessory eye, there is the difference in the location of the two eyes. The accessory eye is situated in the corner of the cornea. If the eye preparation is illuminated from a direction other than the pupillary opening of pigment layer in the main eye, the main eye may not perceive the illumination, even though the accessory eye perceives it well. Such a condition was expected to occur in partially retracted optic tentacles and the accessory eye would be working specifically in these conditions [2, 5]. Newell and Newell observed the behavior of looking around with partially retracted optic tentacles in *Agriolimax reticulatus*. In the partially retracted optic tentacles, the eyeball was rotated about a right angle and the pupillary opening of the main eye was masked by epidermal tissues, while the accessory eye might be exposed to environmental light as in the fully extended tentacles. Therefore, the accessory eye may function principally as a luminous intensity meter in the partially retracted tentacle.

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