Brain Research, 130 (1977) 556–563

Golgi EM evidence for visual information channelling in the crayfish lamina ganglionaris*

DICK R. NÄSSEL and TALBOT H. WATERMAN

Department of Zoology, University of Lund, Lund (Sweden) and Department of Biology, Yale University, New Haven, Conn. 06520 (U.S.A.)

(Accepted April 6th, 1977)

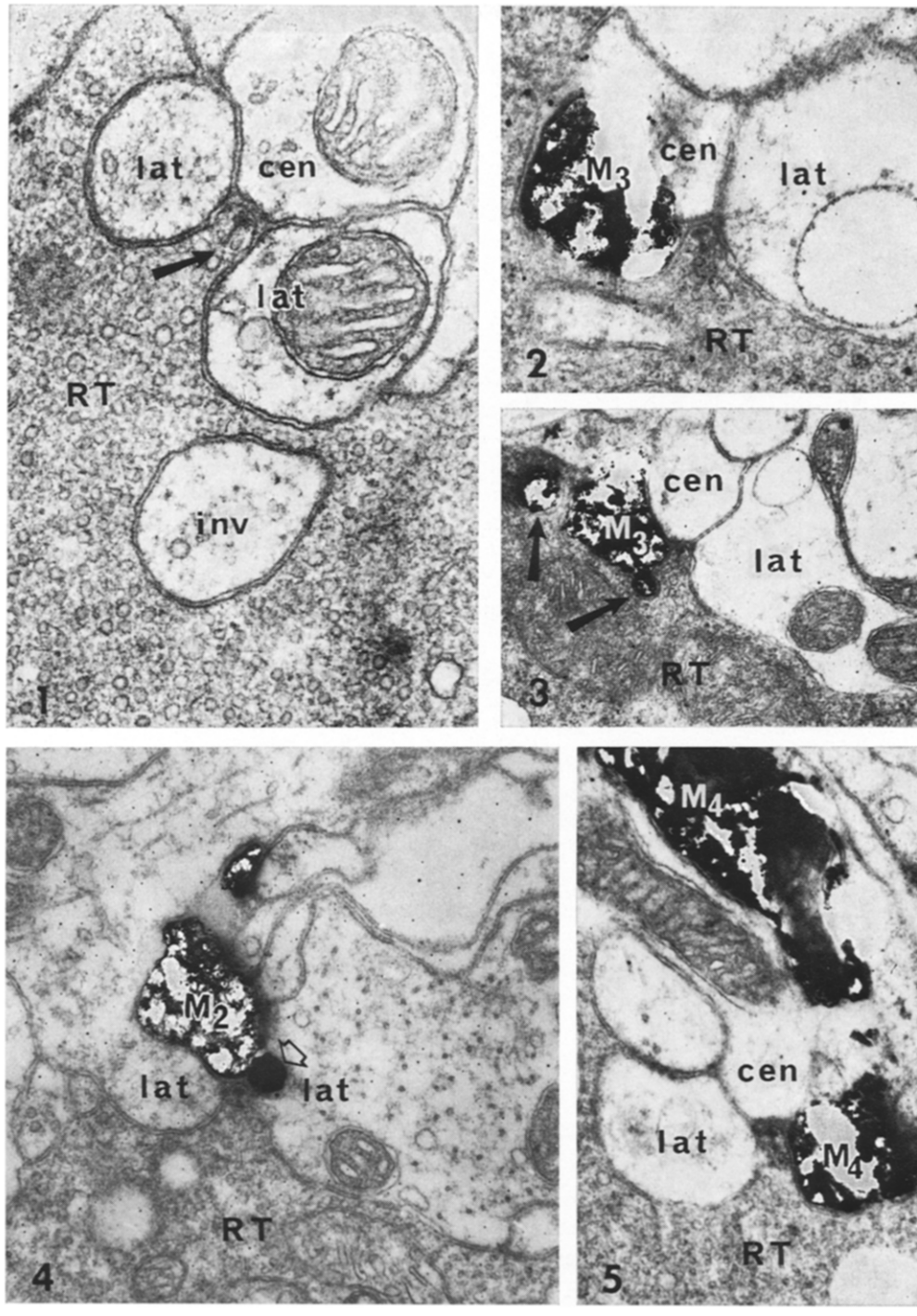
Five types of monopolar neurones (M_1–M_5) occur in the most peripheral optic ganglion (lamina ganglionaris [LG]) of the crayfish. These all have lateral postsynaptic spines in the LG plexiform layer and axons terminating in the next optic ganglion, the medulla externa[17]. The nuclei of the monopolar cells are distally located and their synaptic connections occur in the LG 'cartridges' (classically referred to in crustaceans as *neurommatidia*[1,7,19,21,23]) one of which underlies each ommatidium. Every cartridge comprises 4–5 monopolar neurones and seven retinular cell terminals (RTs of R_1–R_7) derived from three adjacent retinulas[15].

Four RTs end in the distal laminar layer (epl_1) and three in the proximal layer (epl_2)[6,15]. The axon of R_8 has been identified in the LG but passes through it without synapsing to terminate in the medulla externa, the next more proximal optic ganglion[15]. With light micoscopy (LM) three monopolar neurone types have previously been identified in each cartridge (M_2–M_4)[17]. At present the synaptology of M_1 is uncertain but M_5 appears to be involved in intercartridge connections since it is present in only a fraction, perhaps between 1/10 and 1/5, of the cartridges.

To identify the synaptic input of such monopolar neurones is tedious with conventional EM due to repeated irregular branching of the axons. In the present study neurones identified with LM in Golgi preparations have been reimbedded and further analyzed in EM. Of particular interest are the three monopolar neurones identifiable in each cartridge. With LM they seem to be postsynaptic to the photoreceptors: M_2 in both laminar layers, and M_3 in epl_1, and M_4 in epl_2[15,17]. This is striking since the RTs of epl_1 and epl_2 might represent two functional classes of photoreceptors establishing two orthogonal channels involved in polarization sensitivity (PS)[3,24,27]. If so, M_3 and M_4 would be like the two polarization sensitive units (P) in the visual information channelling model proposed earlier[25,26]. But the synaptic confirmation of such a possibility requires the EM evidence presented here.

The animal used in this study was the crayfish *Pacifastacus leniusculus* (Dana)

* Send reprint requests to D. R. Nässel, 604 Kline Biology Tower, Department of Biology, Yale University, New Haven, Conn. 06520, U.S.A.



Figs. 1–5. Synaptic triads formed between retinular cell terminals and interneurones in the plexiform layer in crayfish lamina ganglionaris. Note that each triad comprises RT, M_2, M_3 or M_4, plus an unidentified lateral element. Fig. 1: general pattern showing presynaptic bar in cross-section (arrow), three postsynaptic elements and an invaginating knob from one of these. × 58,800; Fig. 2: one type (M_3) of monopolar cell shown by Golgi silver deposit to form a lateral element of the postsynaptic triad. × 38,300; Fig. 3: similar preparation in which the invaginating knobs (arrows) are shown to be part of a postsynaptic spine of M_3. × 23,700; Fig. 4: Golgi silver deposit marks another type (M_2) of monopolar cell which is central in every postsynaptic triad. Open arrow marks uranyl acetate deposit on lateral element. × 29,600; Fig. 5: similar preparation demonstrating a third type (M_4) of monopolar cell which like M_3 is always lateral in the triads. × 29,600. lat, cen: lateral and central postsynaptic units of the triad. M_2–M_4: three distinct types of monopolar interneurones. RT: retinular cell terminal (presynaptic element of the triads).

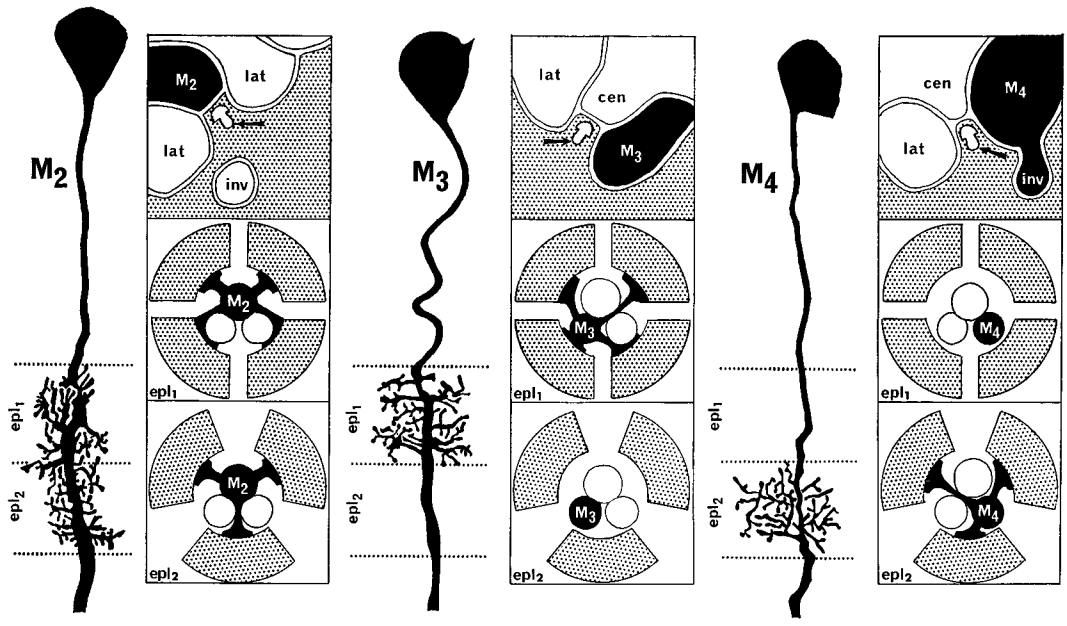


Fig. 6. Golgi tracings and connectivity diagrams of three types of monopolar neurones (M_2–M_4) in the lamina ganglionaris. To the right of each silhouette the diagrams show the participation of that cell type in the postsynaptic triads (top) and the corresponding distributions of these contacts in the distal epl_1 (middle) and proximal epl_2 plexiform layers (bottom). The shaded areas of all diagrams indicate the presynaptic terminals of the 7 regular retinular cells (RT) and the black areas the components of the monopolar cell in question. cen = central triad element, lat = lateral triad component, inv = invaginating knobs of lateral postsynaptic components. See Figs. 1–5 for EMs of synaptic details.

obtained from Simontorp Aquatic Breeding Laboratories, Blentarp, Sweden. Sections with well-impregnated neurones from Golgi-rapid and osmic acid prefixed Golgi–Colonnier methods[17] were selected and re-embedded in Spurr's medium[20]. Thin serial sections were cut on an LKB Ultratome and analyzed with a Zeiss EM 10 electron microscope.

The axonal terminals of R_1–R_7 are characterized by presynaptic vesicles and by electron dense presynaptic bars which appear mushroom-shaped in cross-section and protrude in localized evaginations under the central element of the postsynaptic triads (Figs. 1–5). The presence of such triple synapses in this location has been reported previously[6,15,16]. Note that two or more triads may be fused and share certain elements. Each RT comprises many (perhaps up to 20) contacts with each of its specific second order neurones. For a given retinular cell the RTs occur either in epl_1 or epl_2.

Thus for R_1–R_7 four RTs are in the outer layer (epl_1) and three in the inner (epl_2). In the plexiform layers of LG every RT makes contact with two types of monopolar postsynaptic elements. One of these, M_2, synapses with 4 RTs in epl_1 and three RTs in epl_2 and hence with all 7 regular retinular axons (Fig. 6). In addition type M_3 cells also synapse with the 4 RTs in epl_1. Type M_4 cells synapse instead with the three RTs in epl_2.

The present results show that all presynaptic membrane areas of R_1–R_7 contact

two types of monopolar postsynaptic spines as specified as well as a third not yet identified postjunctional unit (Figs. 1–5). As viewed from the monopolar cells these synaptic patterns can be described as follows.

The axon of M_2 is centrally located in each cartridge and has radially arranged spines making contact with all of the numerous presynaptic areas of R_1–R_7. Within the triad of postsynaptic elements the M_2 spines are always the central ones as suspected previously[16]. Hence there is a non-selective 7:1 retinular cell convergence on this type of secondary neurone coupled with an approximately 1:20 multiplication of synaptic area per RT.

The M_3 axon is typically somewhat off-center in the cartridge and has radially arranged spines which contact the presynaptic membrane of 4 specific RTs only in the epl_1 region. Here its spines are always a lateral element of each triad otherwise comprising one M_2 spine and one spine of unknown cellular origin. In epl_2, M_3 axons are smooth and no diagnostic silver chromate deposits except in the axon are seen in this region using the Golgi EM technique. Thus M_3 is postsynaptic exclusively to the 4 regular retinular cells which converge on it.

Small knobs (0.16–0.50 μm in diameter) from the postsynaptic spines of M_3 arise in the contact area and invaginate the presynaptic terminal (Fig. 3). Knobs of similar appearance and M_3 origin can also be found invading the RTs in regions away from afferent synaptic sites. Hence the functional connotations of these invaginations are ambiguous.

The M_4 postsynaptic relations are essentially like those of M_3 except that their radial spines' contacts are in the epl_2 layer (Fig. 6). Only three receptor cell axons converge on this monopolar type, namely those three from R_1–R_7 which don't synapse with M_3 cells. Thus M_4 axons are somewhat eccentric in the cartridge, their postsynaptic contacts are lateral elements in the postsynaptic triads and they give rise to knobs invaginating the presynaptic cells. In the epl_1 region their axons are smooth and no synaptic contacts are made with the RTs. Thus M_4 is postsynaptic exclusively to the three regular retinular cells not transmitting to M_3. Hence M_3 and M_4 divide the regular retinular cell output into two mutually exclusive channels.

Present data do not permit identification of the third postsynaptic element present at all the RTs (Figs. 1–5). It is always lateral in location and, again like the M_3 and M_4 profiles, gives rise to knobs invaginating the photoreceptor cell terminals. There are at least 4 possible candidates for this universal third unit: (1) processes of type M_1 monopolar cells (2) processes of type M_5 monopolar cells (3) contacts from some kind of tangential LG neurone or (4) from an amacrine cell.

The present results establish by direct synaptic evidence the presence of three different visual information channels among the first order interneurones of the crayfish visual system (Fig. 6). One of these (M_2) integrates information from all regular retinular cells. Consequently it might be expected to show functional properties postulated for the intensity channel (I) of the earlier model[24]. Note that although three adjacent ommatidia contribute RTs to a single M_2 there is in this 3:1 partial retinular convergence one cell of each of the 7 kinds (R_1–R_7) present in every ommatidium.

This provides the neural prerequisite for some *neural* superposition (discovered

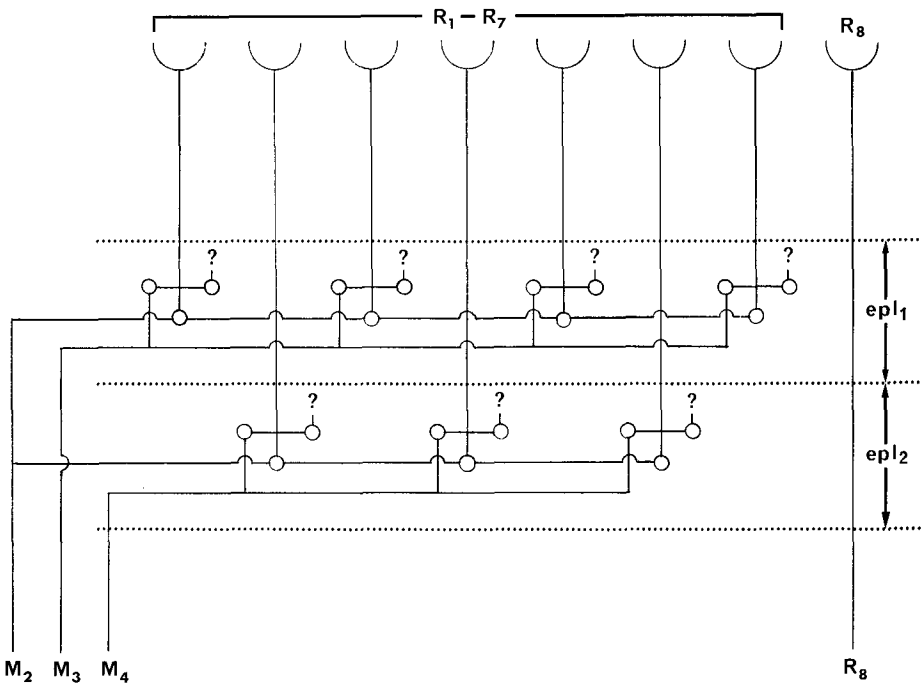


Fig. 7. Schematic channelling of initial visual information processing from one crayfish ommatidium revised from Waterman[24,26] to accommodate current information. Each synaptic triad of the seven RTs are shown as three small open circles. Like channel I of the original schema M_2 integrates input from all regular retinular cells (R_1–R_7). M_3 and M_4 divide the RTs into two classes where components correspond in number with the two P channels of the original. However, the mvl orientation and hence *e*-vector direction maximally absorbed has not yet been identified in the cells providing the M_3 and M_4 inputs. The identity of the third component of all postsynaptic triads (labelled with ?s) remains to be demonstrated. Thus we cannot currently specify distinctive connections for the wavelength discriminating channels known at the receptor cell level. R_8 does not synapse in the lamina ganglionaris but terminates instead in the medulla externa. epl_1, epl_2 and the dotted lines indicate layers of the lamina ganglionaris.

in dipterans by Kirschfeld[8]). But at present we have no reason to expect that the optical requirements for this type of information processing exist in a fused rhabdom like that of decapod crustaceans. *Optical* superposition by a new reflecting mechanism has in fact recently been reported in crayfish and in a deep water shrimp[13,22], but few details are available as yet.

The distribution of four and three RTs to M_3 and M_4 respectively is consistent with the model proposed earlier for two orthogonal PS channels arising from R_1–R_7 (Fig. 7). Yet specific cell identification is necessary to prove that the implied connectivity is actually present. We know that maximum absorption by rhabdomeres occurs parallel to the axes of their constituent mvl[3,28]. Consequently for a simple two-channel PS hypothesis R_1, R_4 and R_5 should synapse on M_4 while R_2, R_3, R_6 and R_7 should synapse on M_3. These connections remain to be shown.

Also the specific way in which PS at the receptor cell level is processed to permit polarotactic or other polarized light-related adaptive behavior remains to be clarified

even in the best known cases (e.g., *Apis*[31] and *Musca*[9]). To achieve such understanding requires not only completion of appropriate connectivity models to which the present report is a contribution but also an effective study of the higher order afferent responses in the visual system.

In decapods this has been hindered inter alia by previous failure to detect *e*-vector discrimination at the optic nerve level[29,30]. Now two independent research reports have shown that such differential responses do in fact occur not only in decapods but also in stomatopods[14,32,33]. The key to this advance appears to have been the dependence of the response on a moving *e*-vector (as do Boehm's brushes in the human eye[2]). This demonstration of PS in higher order visual interneurones should open the way both to determining the channelling mechanism here discussed and to developing a better understanding of the adaptive significance of PS to these animals in general.

Another important desideratum is the identity of the third profile in the universally present triads marking the first order synapses of the photoreceptor cells. We have reported here the identities of the three monopolar cell types occupying two of these three postsynaptic sites but have no reliable information on the cell type(s) which comprise the remaining one. In terms of earlier crayfish results two additional channels for wavelength discrimination need to be identified (Fig. 7). Violet- and yellow-sensitive retinular cells are known to be present[18,27].

Available evidence indicates that usually R_3 and R_4 are the violet receptors and R_1, R_2, R_5, R_6 and R_7 are predominantly the longer wavelength units[5]. If this λ discrimination is in fact made in LG the presently unidentified postsynaptic profiles must be somehow involved. Consequently attempts to analyze this relationship should be particularly interesting.

Meanwhile, however, R_8 has emerged in the crayfish as well as many other crustaceans as a retinular component of quite unknown but probably important function[4,10–12,15]. This four-lobed cell has its own rhabdomere lying distal to the main rhabdom as well as a proximal axon which extends through LG without synapsing and terminates in the medulla externa (Fig. 7). Whether this unit may be a short wavelength receptor or have a role in PS is purely speculative at the moment but identifying its functions is a considerable challenge. Also, the statistical basis for identifying R_3 and R_4 as the predominant location of violet receptors[5] should be reinforced or extended to explain the apparent variability in color receptor pattern (two C units in the earlier model[26]). Furthermore, their relation to R_8, not explicitly studied, now needs to be explored.

This research has been supported by the Swedish Natural Science Research Council Grant 2760-009 and U.S. National Institutes of Health Grant EY-00405.

The authors are grateful to Mrs. R. Wallén for technical assistance.

1 Bernard, R., Recherches sur la morphogénèse des yeux composés d'arthropodes, *Bull. Biol. France*, 23, Suppl. (1937) 1–162.
2 Boehm, G., Über ein neues entoptisches Phänomen im polarisierten Licht. 'Periphere' Polarisationsbüschel, *Acta Ophthalmol.*, 18 (1940) 143–169.
3 Eguchi, E. and Waterman, T. H., Cellular basis for polarized light perception in the spider crab, *Libinia*, *Z. Zellforsch.*, 84 (1968) 87–101.
4 Eguchi, E. and Waterman, T. H., Orthogonal microvillus pattern in the eighth rhabdomere of the rock crab *Grapsus*, *Z. Zellforsch.*, 137 (1973) 145–157.
5 Eguchi, E., Waterman, T. H. and Akiyama, J., Localization of the violet and yellow receptor cells in the crayfish retinula, *J. gen. Physiol.*, 62 (1973) 355–374.
6 Hafner, G. S., The ultrastructure of retinula cell endings in the compound eye of the crayfish, *J. Neurocytol.*, 3 (1974) 295–311.
7 Hanström, B., *Vergleichende Anatomie des Nervensystems der wirbellosen Tiere*, Springer Verlag, Berlin, 1928, 628 pp.
8 Kirschfeld, K., Die Projektion der optischen Umwelt auf das Raster der Rhabdomere im Komplexauge von *Musca*, *Exp. Brain Res.*, 3 (1967) 248–270.
9 Kirschfeld, K. and Franceschini, N., The possible role of photosensitive pigments within the membrane of photoreceptors, *Biophys. struct. Mech.*, (1977) In press.
10 Krebs, W., The fine structure of the retinula of the compound eye of *Astacus fluviatilis*, *Z. Zellforsch.*, 133 (1972) 399–414.
11 Kunze, P., Histologische Untersuchungen zum Bau des Auges von *Ocypode cursor* (Brachyura), *Z. Zellforsch.*, 82 (1967) 466–478.
12 Kunze, P., Die Orientierung der Retinulazellen im Auge von *Ocypode*, *Z. Zellforsch.*, 90 (1968) 454–462.
13 Land, M. R., Superposition images are formed by reflection in the eyes of some oceanic decapod crustacea, *Nature (Lond.)*, 263 (1976) 764–765.
14 Leggett, L. M. W., Polarized light-sensitive interneurones in a swimming crab, *Nature (Lond.)*, 262 (1976) 709–711.
15 Nässel, D. R., The retina and retinal projection on lamina ganglionaris of the crayfish *Pacifastacus leniusculus* (Dana), *J. comp. Neurol.*, 167 (1976) 341–360.
16 Nässel, D. R., The fine structure of photoreceptor terminals in the compound eye of *Pandalus* (Crustacea), *Acta Zool.*, 57 (1976) 153–160.
17 Nässel, D. R., Types and arrangement of neurons in the crayfish optic lamina, *Cell Tiss. Res.*, 179 (1977) 45–75.
18 Nosaki, H., Electrophysiological study of color encoding in the compound eye of crayfish *Procambarus clarkii*, *Z. vergl. Physiol.*, 64 (1969) 318–323.
19 Parker, G. H., The retina and optic ganglia in decapods, especially *Astacus*, *Mitteilung Zool. Station Neapel*, 12 (1895) 1–73.
20 Spurr, A. R., A low-viscosity epoxy resin embedding medium for electron microscopy, *J. Ultrastruct. Research*, 26 (1969) 31–43.
21 Viallanes, M. H., Contribution à l'histologie du systemè nerveux des invertébrès — La lame ganglionaire de la langouste, *Ann. Sci. nat. Ser.*, 7 (1892) 385–397.
22 Vogt, K., Optics of the crayfish eye, *Z. Naturforsch.*, 30 C (1975) 691.
23 Waterman, T. H., Light sensitivity and vision. In T. H. Waterman (Ed.), *The Physiology of Crustacea, Vol. II*, Academic Press, New York, 1961, pp. 1–64.
24 Waterman, T. H., Information channeling in the crustacean retina. In P. W. Nye (Ed.), *Proceedings of the Symposium on Information Processing in Sight Sensory Systems*, California Institute of Technology, Pasadena, 1966a, pp. 48–56.
25 Waterman, T. H., Polarotaxis and primary photoreceptor events in Crustacea. In C. G. Bernhard (Ed.), *The Functional Organization of the Compound Eye*, Pergamon Press, Oxford, 1966b, pp. 493–511.
26 Waterman, T. H., The optics of polarization sensitivity. In A. W. Snyder and R. Menzel (Eds.), *Photoreceptor Optics*, Springer-Verlag, Berlin, 1975, pp. 339–371.
27 Waterman, T. H. and Fernández, H. R., *E*-vector and wavelength discrimination by retinular cells of the crayfish *Procambarus*, *Z. vergl. Physiol.*, 68 (1970) 154–174.
28 Waterman, T. H., Fernández, H. R. and Goldsmith, T. H., Dichroism of photosensitive pigment in rhabdoms of the crayfish *Orconectes*, *J. gen. Physiol.*, 54 (1969) 415–432.

29 Waterman, T. H. and Wiersma, C. A. G., Electrical responses in decapod crustacean visual systems, *J. cell. comp. Physiol.*, 61 (1963) 1–16.
30 Waterman, T. H., Wiersma, C. A. G. and Bush, B. M. H., Afferent visual responses in the optic nerve of the crab *Podophthalmus*, *J. cell. comp. Physiol.*, 63 (1964) 135–156.
31 Wehner, R., Bernard, G. D. and Geiger, S., Twisted and non-twisted rhabdoms and their significance for polarization detection in bee, *J. comp. Physiol.*, 104 (1975) 225–245.
32 Yamaguchi, T., The mechanism of polarized light perception and its visual processing in the optic tract of crayfish, *Zool. Mag. (Japan)*, 76 (1967) 443 (Abstr., in Japanese).
33 Yamaguchi, T., Katagiri, Y. and Ochi, K., Polarized light responses from retinula cells and sustaining fibers of the mantis shrimp, *Biol. J. Okayama Univ.*, 17 (1976) 61–66.