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Histamine-like immunoreactivity in photoreceptors of the compound eyes and ocelli of the flies *Calliphora erythrocephala* and *Musca domestica*

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Summary. Antibodies to histamine were used for immunocytochemical studies of the visual system in the flies *Calliphora erythrocephala* and *Musca domestica*. Specific immunolabeling of photoreceptors was found both in the compound eyes and ocelli of both species. In the compound eyes histamine-like immunoreactivity (HA-IR) was found in all the short visual fibers (photoreceptors R1–6) and one type of long visual fiber (photoreceptor R8). In addition, the ocellar photoreceptors also show HA-IR. In view of earlier biochemical and pharmacological/physiological findings by Elias and Evans (1983) and Hardie (1987) it thus seems likely that histamine is a neurotransmitter in insect photoreceptors. Interestingly, the second type of long visual fiber (photoreceptor R7) has recently been found to be GABA-immunoreactive (Datum et al. 1986). The two types of long visual fibers may hence use different transmitters which act on different receptors of the postsynaptic neurons in the second visual neuropil, the medulla. In addition to the photoreceptors in the retina and ocelli, we found processes of HA-IR neurons in one of the optic lobe neuropils, the lobula. This finding indicates that histamine may also be a transmitter in certain interneurons in the visual system.

Key words: Insect visual system – Photoreceptors – Neurotransmitter – Histamine – Immunocytochemistry – *Calliphora erythrocephala, Musca domestica* (Insecta)

Recently, evidence has accumulated that histamine may be a neurotransmitter in photoreceptors of the insect compound eye. Large amounts of histamine have been measured in the retina and the first synaptic neuropil, the lamina, of the locust, the tobacco hornworm moth and the cockroach (Elias and Evans 1983). The same authors also demonstrated that histamine can be synthesized from histidine and inactivated by oxidation or N-acetylation, and that there are histamine receptors (^{3}H-mepyramine binding sites) in the visual system (Elias and Evans 1983; Elias et al. 1984; see also Maxwell et al. 1978). Later Hardie (1987) showed that the lamina neurons that are postsynaptic to the photoreceptors R1–R6 in the flies *Musca domestica* and *Calliphora erythrocephala* are activated by ionophoretically applied histamine. The responses of these large monopolar neurons (LMCs) to histamine are fast hyperpolarizations similar to those elicited by light. Histamine antagonists were found to block or reduce the responses to both histamine and light. It was also shown in pharmacological experiments that the histamine-sensitive receptors in the lamina of the fly may be of a novel type (Hardie 1987; in preparation). Other putative neurotransmitter substances either had no effect, caused depolarizations or slow hyperpolarizations of the LMCs (Hardie 1987). Hence, the available evidence so far favors histamine as a neurotransmitter in the photoreceptors of the insect compound eye.

There is, however, no convincing demonstration of the localization of histamine in the photoreceptor cells or their synaptic terminals. Uptake of ^{3}H-histamine failed to yield distinct labeling of the photoreceptor terminals in the lamina of the locust (Elias and Evans 1984), and no other reliable histochemical technique for the demonstration of histamine has been available for the insect visual system. Meanwhile, immunocytochemistry has indicated that one of the photoreceptor types (R7) in the blowfly may contain γ-aminobutyric acid (GABA) and the GABA-forming enzyme glutamic acid decarboxylase (GAD) (Datum et al. 1986). Antisera to certain other putative neurotransmitters (and neuropeptides) are known to label neurons in the insect visual system (reviewed by Nässel 1987a, b, 1988; Hardie 1988); however, with the exception of antisera to taurine (Schäfer et al. 1988) these fail to recognize antigens in the photoreceptors.

Recently, specific antisera against histamine have become available (Steinbusch and Mulder 1984; Panula et al. 1984, 1985; Håkanson et al. 1986) so that immunocytochemical localization of the amine is now possible. In the present account we describe the use of antibodies raised

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Abbreviations: *HA* histamine; *GABA* γ-amino butyric acid; *GAD* glutamic acid decarboxylase; *5-HT* 5-hydroxytryptamine (serotonin); *HA-IR* histamine-like immunoreactivity; *R1-R6* class of short-axoned photoreceptors; *R7* and *R8* long-axoned photoreceptors; *LMC* large monopolar neuron of lamina; *HSA* human serum albumin; *PBS* phosphate-buffered saline; *DEPC* diethylpyrocarbonate

against histamine to map immunoreactive cells in the visual system, including the ocellar system, of two species of flies *Musca domestica* and *Calliphora erythrocephala*. In both species the histamine antiserum labels both short (R1-R6) and long (R8) photoreceptors of the compound eye and the photoreceptors of the ocelli. With the present immunocytochemical demonstration of a histamine-like substance in the insect photoreceptors, the earlier suggestion of histamine as a neurotransmitter in the visual system (Elias and Evans 1983, 1984; Hardie 1987) receives further support. We also demonstrate that certain interneurons in the visual system and other parts of the brain show histamine-like immunoreactivity, suggesting that histamine may be a more widely distributed neurotransmitter or neuromodulator in the insect CNS.

Materials and methods

Animals. Blowflies, *Calliphora erythrocephala,* and house flies, *Musca domestica,* were taken from laboratory cultures kept under a 16D:8L cycle at 25 °C. Light-adapted adult (1–2 weeks posteclosion) male and female *Calliphora erythrocephala* and female *Musca domestica* were used.

Antisera. The histamine antiserum (code # 8431, Milab, Malmö, Sweden) was raised in rabbit using histamine dihydrochloride covalently bound to human serum albumin (HSA) as antigen (Håkanson et al. 1986). Carbodiimide [1-ethyl-3-(dimethylaminopropyl) carbodiimide] was used as a coupling agent. Antiserum 8431 stains endocrine cells and mast cells of the gastric mucosa in a wide spectrum of vertebrate species (Håkanson et al. 1986). The same authors tested the specificity of the antiserum in liquid phase cross absorption tests with histamine, L-histidine, tele-methyl histamine, β-alanyl-L-histidine, L-histidyl-L-leucine, thyrotropin-releasing hormone (pGlu-His-Pro-NH_2) and 5-hydroxytryptamine (5-HT), all at 10, 100 or 1000 μM. Only histamine, histamine conjugate and tele-methyl histamine abolished immunolabelling in mast cells and endocrine cells.

Immunocytochemistry. Two fixation methods employed for histamine immunocytochemistry gave reproducible results. One relied on fixation by immersion in paraformaldehyde solution and immunostaining of cryostat sections. The other method entailed freeze drying, vapor fixation with diethylpyrocarbonate (DEPC) and immunocytochemistry on paraffin sections.

(1) Liquid fixation. Tissue was fixed by immersion of opened heads in 4% paraformaldehyde in 0.1 M phosphate buffer at 4 °C for 4 h. After infiltration with 20% sucrose and freezing, cryostat sections (12 μm) were cut at −20 °C, collected on microscope slides and used for post-embedding immunocytochemistry.

(2) Freeze drying. Opened heads were frozen in a mixture of propane and propylene at the temperature of liquid nitrogen. The tissue was freeze dried and vapor fixed in DEPC for 3 h at 55 °C according to Pearse and Polak (1975). After paraffin embedding (under vacuum), serial sections were cut at 10 μm. The deparaffinized sections were processed for immunocytochemistry.

The sections from methods 1 and 2 were processed for immunocytochemistry as follows. The histamine antiserum was applied at a concentration of 1:300 or 1:600, diluted in 0.1 M phosphate-buffered saline (PBS) with 0.25% Triton X-100 and 0.25% bovine serum albumin. The incubation was for 20 h at room temperature. The peroxidase anti-peroxidase method was employed using unlabeled swine anti-rabbit immunoglobulins (DAKO, Copenhagen) at 1:50 and rabbit peroxidase anti-peroxidase complex (DAKO) at 1:50 (both applied for 1 h each) for second and third layers, respectively. The peroxidase reaction was run in 0.04% diaminobenzidine and 0.01% H_2O_2 in 0.05 M Tris-HCl buffer for 10 min. One set of sections was used for the indirect immunofluorescence method, employing fluorescein-conjugated secondary antiserum (FITC-conjugated swine anti-rabbit immunoglobulins; DAKO) at 1:30 in PBS.

The specificity of the histamine antiserum was tested on tissue sections after liquid-phase absorption. The diluted antiserum was pre-incubated with the histamine conjugate used for immunization. This histamine-HSA conjugate was used at a concentration of 100 μg/ml of diluted antiserum [it was estimated that 30–35 molecules of histamine are bound to each molecule of HSA (Håkanson et al. 1986)]. Further tests included running the immunocytochemistry after excluding the primary antiserum. Supression of possible endogenous peroxidase activity was accomplished with 0.03% H_2O_2 in methanol.

Results

The strongest histamine-like immunoreactivity (HA-IR) was seen in freeze-dried and vapor-fixed specimens. In the paraformaldehyde-fixed cryostat sections the immunostaining was distinct, but much weaker. Similar results were shown also for cells of the vertebrate gastric mucosa (Håkanson et al. 1986). No immunoreactivity could be detected in the visual system when the antiserum was pre-incubated with histamine-HSA conjugate. The following description pertains to both *Calliphora erythrocephala* and *Musca domestica* unless otherwise stated.

Strong HA-IR could be seen in the short visual fibers, photoreceptors R1-R6. It was ascertained from cross sections of the lamina that the ring of six photoreceptor terminals (R1-R6) was immunolabeled in each cartridge. The immunostaining was particularly intense in their terminations in the first synaptic neuropil of the optic lobes, the lamina (Figs. 1a, 2a–c). In the retina the immunostaining in the R1-R6 is weak and furthermore partly masked by the pigmentation of the photoreceptor cells. As seen in Fig. 1a and b the entire extent of the terminals within the lamina cartridges is immunolabeled. Also with the less sensitive immunofluorescence technique (FITC-conjugated secondary antiserum) the lamina terminations of R1–R6 were distinctly labeled (Fig. 1a).

Of the two types of long visual fibers, photoreceptor R7 and R8, only one type was immunolabeled with the histamine antiserum. Based on the proximal location of the cell bodies in the retina (Trujillo-Cenoz and Melamed 1966) and the depth and shape of the terminations in the second synaptic neuropil, the medulla (Strausfeld and Nässel 1981), these HA-IR photoreceptors can be identified as R8. In the retina the cell bodies of the R8 display HA-IR (Figs. 1a, 2c) and these photoreceptors are also strongly immunolabeled in their terminations as demonstrated both with immunoperoxidase (Fig. 2a, d–g) and immunofluorescence methods (Fig. 1b). The histamine antiserum revealed some interesting regional differences in the morphology of

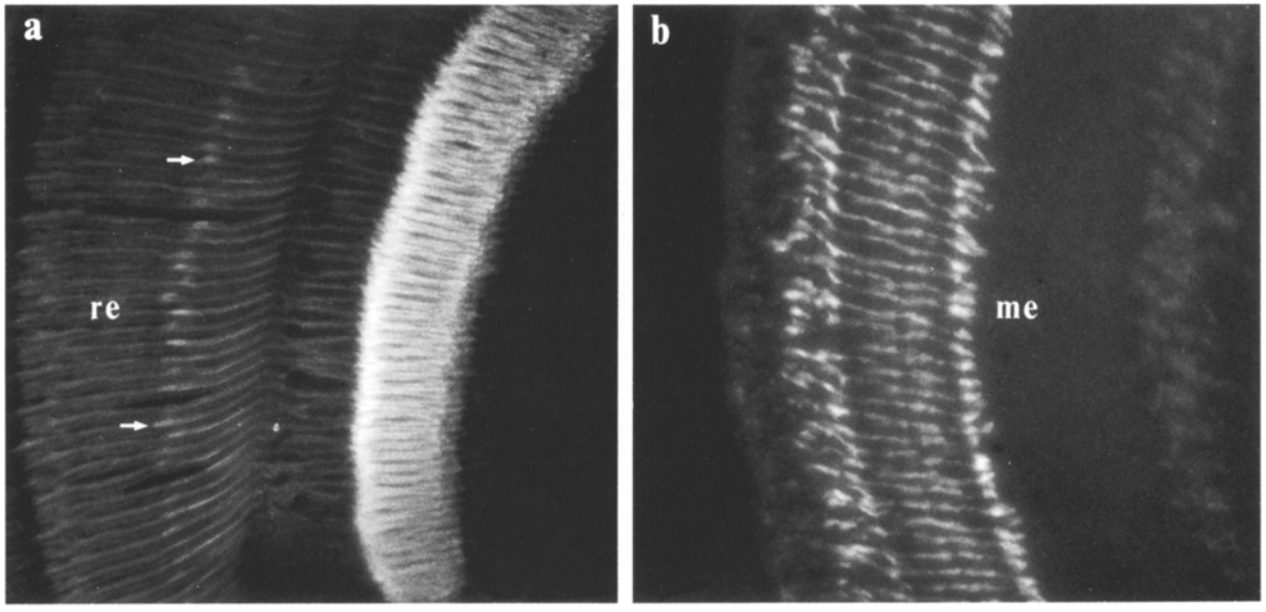


Fig. 1 a, b. Fluorescence micrographs of histamine-immunoreactive (HA-IR) photoreceptors in the compound eye of *Calliphora erythrocephala*. Polyclonal antiserum 8431; FITC-conjugated secondary antiserum. **a** HA-IR in R8 receptor cell bodies (*at arrows*) in the retina (*re*) and in R1–R6 terminations in the lamina. **b** HA-IR terminations of R8 photoreceptors in the medulla (*me*). a, b × 240

the R8 terminals in the medulla. The R8 terminals projecting from the dorsalmost portion of the visual field (approximately three rows of photoreceptors) have enlarged terminals (Fig. 2d) compared to the remainder of the the R8 population (see below, however). Comparison with cobalt mass filling of photoreceptors in *Calliphora erythrocephala* (Strausfeld and Wunderer 1985) leaves no doubt that these profiles correspond to the terminals of the specialized dorsal marginal R8 receptors. These are anatomically and functionally distinct from other R8 photoreceptors, and are believed to be involved in the detection of ultraviolet polarized light (Hardie 1984). A second set of morphologically specialized photoreceptor terminals is seen in males of *C. erythrocephala:* the R8 axons from the anteriormost portion of the visual field, which terminate in the posterior medulla, have enlarged terminals that extend laterally (Fig. 2e). These R8 axons are derived from the anteriorly situated binocular overlap zone of the retina, which is expanded in male flies (Hausen and Strausfeld 1980; Strausfeld 1980).

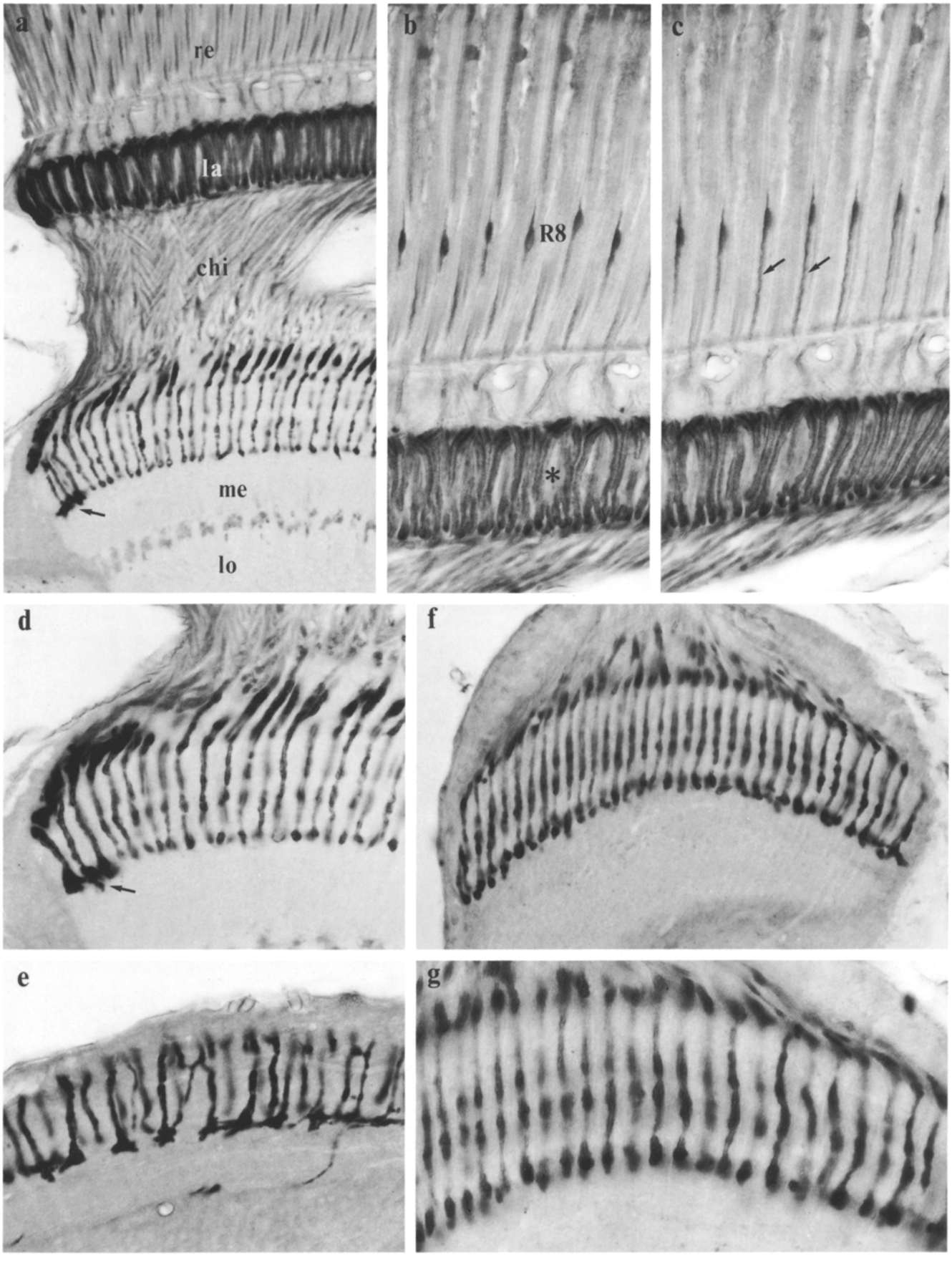
In addition to the long photoreceptor axons in the medulla, a set of four to five HA-IR axons was seen to run from the region of the posterior lamina, via the anterior-posterior optic chiasma to terminations at the base of the anterior medulla (Fig. 4a, b). Both in *M. domestica* and *C. erythrocephala* these terminations each form two to three stout lateral branches. The cell bodies of these axons have not yet been located.

In the ocelli the photoreceptors display strong HA-IR (Fig. 3). The immunoreactive photoreceptors from the three ocellar cups terminate in the common peripheral ocellar neuropil. As seen in Fig. 3., the receptors form no distinct layering or other morphological patterns. From light-microscopical immunocytochemistry it cannot be determined whether all the photoreceptors are immunoreactive. No immunoreactive fibers could be traced in the ocellar stalk connecting the peripheral ocellar neuropil to the brain. Hence, all HA-IR photoreceptors appear to end in the peripheral neuropil and no HA-IR interneurons invade the ocellar neuropil.

We were also able to demonstrate HA-IR neurons in the optic lobe and brain of both insect species. These neurons have immunoreactive processes in the lobula neuropil (Fig. 4c–e) and outside the optic lobes in the dorsal and lateral protocerebrum and in the suboesophageal ganglia (not shown). In the present report we ignore the HA-IR neurons outside the optic lobe. In the neuropil of the lobula two layers of fine varicose fibers display HA-IR (Fig. 4c–e). One thin layer is seen in the most distal portion of the neuropil, another broader layer is situated in the basal portion of the lobula. The cell bodies most likely to give rise to the HA-IR processes invading the lobula are those located in the latero-ventral part of the protocerebrum near the base of the lobula. The reason for the uncertainty is that the immunostaining is seen mainly in cell bodies and varicosities, and hence it is not possible to resolve the connecting fibers.

Discussion

We report here that the photoreceptors of the compound eye and ocelli of two fly species, *Calliphora erythrocephala* and *Musca domestica*, contain a substance recognized by an antiserum to histamine. The immunostaining is specific to the extent that it can be blocked by pre-absorption of the antibodies with the antigen (histamine-HSA conjugate). It has previously been demonstrated (i) that the same antiserum labels known histamine-containing cells, namely, mast cells and a population of endocrine cells in the gastric mucosa of different vertebrates, and (ii) that the reactivity of the antibodies can be blocked with histamine, histamine conjugated to HSA and tele-methyl histamine, but not by related compounds (Håkanson et al. 1986). Since direct biochemical evidence has already revealed the presence of unusually large quantities of endogenous histamine in the reti-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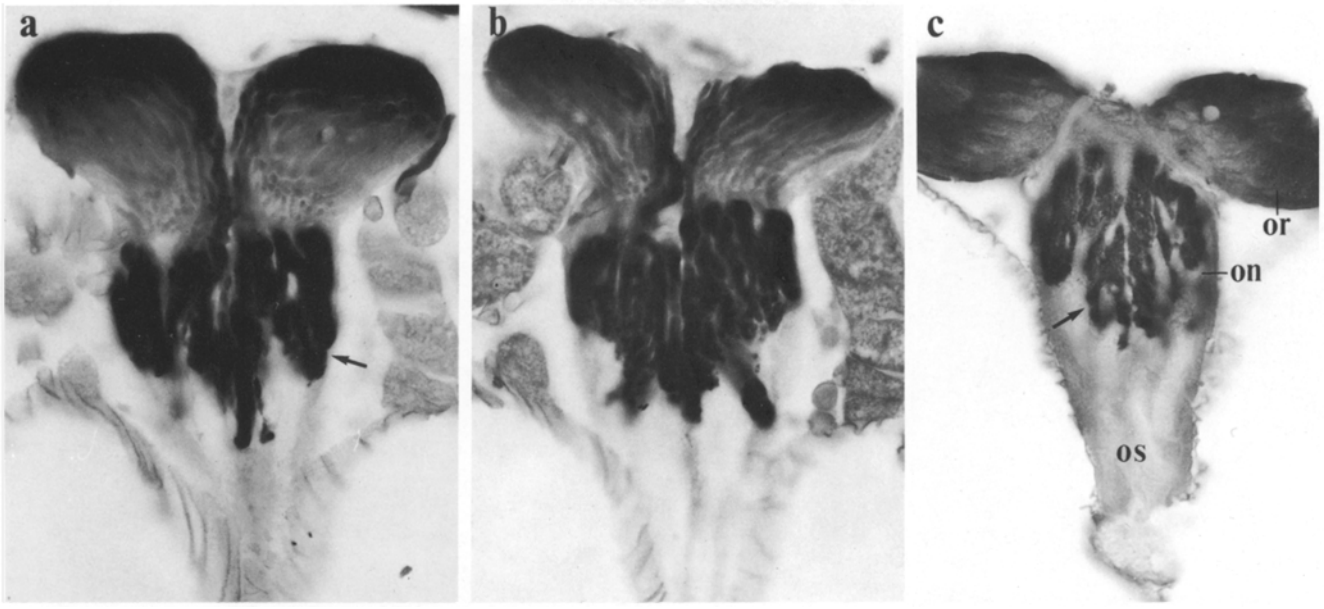

Fig. 3a–c. Histamine-immunoreactive photoreceptors in the ocelli of *C. erythrocephala* **(a, b)** and *M. domestica* **(c)**. Frontal sections, PAP method, antiserum 8431. In all cases the specimens are cut at the level of the two lateral ocelli. **a, b** Strong HA-IR is seen mainly in the photoreceptor terminations in the fused ocellar neuropil (*arrow*). **c** Also in *M. domestica* the HA-IR is mainly seen in the terminations; *or* ocellar receptor layer; *on* ocellar neuropil; *os* ocellar stalk (ocellar nerve). *Arrow* indicates HA-IR photoreceptor terminals. a–c ×380

na and lamina of a variety of insects (Elias and Evans 1983) it would thus appear very likely that the immunoreactive substance identified in photoreceptor terminals of the fly is indeed histamine.

There is, thus, now substantial evidence for several of the criteria necessary for establishing histamine as a major neurotransmitter in at least some insect photoreceptors, namely:

(1) The insect retina and lamina are capable of synthesizing histamine (Maxwell et al. 1978; Elias and Evans 1983).

(2) The retina and lamina contain endogenous histamine (Elias and Evans 1983) which, according to the present study, is located in the photoreceptor terminals.

(3) Metabolic pathways for inactivation of histamine have been demonstrated in the locust retina and optic lobe (Elias and Evans 1983).

(4) The action of photoreceptors R1–6 on their postsynaptic neurons (LMCs) is closely mimicked by ionophoretically applied histamine but not by any other transmitter candidate (Hardie 1987).

(5) Pharmacological agents that block the effects of light on LMCs also block the action of exogeneously applied histamine (Hardie 1987; in preparation).

The present findings also suggest that the photoreceptors of the ocelli contain histamine. Since Simmons and Hardie (1988) have shown, in the locust, that histamine mimicks the action of the natural transmitter on neurons postsynaptic to the ocellar photoreceptors, there is also here a strong case for postulating histamine as the photoreceptor neurotransmitter.

Several other substances have been postulated as neurotransmitters in compound eye and ocellar photoreceptors, but as discussed by Hardie (1987, 1988) the evidence is difficult to uphold. In the ocelli, for example, acetylcholine was postulated on the grounds that curare blocked the response to light (Klingman and Chappel 1978). However, when applied by local ionophoresis, histamine, but not acetylcholine, was found to mimic the action of the ocellar photoreceptor transmitter (Simmons and Hardie 1988). Furthermore, as is the case in the only other known arthropod histaminergic system, the lobster stomatogastric ganglion (Claiborne and Selverston 1983), curare was found to be an effective antagonist for histamine-induced responses (Simmons and Hardie 1988).

Despite extensive immunocytochemical investigations of insect optic lobes in recent years, the photoreceptors have,

Fig. 2a–g. Micrographs of histamine-immunoreactive photoreceptors in the compound eye of *Calliphora erythrocephala* **(a–e)** and *Musca domestica* **(f, g)**. Polyclonal antiserum 8431 against histamine, peroxidase anti-peroxidase method. All specimens were freeze dried and DEPC fixed. **a** Overview of part of the visual system (oblique frontal section) showing the receptor layer (*re*) and HA-IR terminals of the photoreceptors R1–6 ending in the lamina (*la*). Immunoreactive fibers of long visual fibers traverse the chiasma (*chi*) before terminating in the medulla (*me*). Note that the R8 axons of the dorsalmost rows of ommatidia have larger terminations (*arrow*). Below the medulla the lobula (*lo*) is seen. **b, c** Detail of receptor layer and lamina. The cell bodies (*R8*) and fibers (*arrows*) of the R8 receptors are HA-IR. In the lamina (*asterisk*) the HA-IR terminations of R1–R6 are arranged in columns (cartridges). **d** Detail of R8 terminations in dorsal portion of the medulla. Expanded terminals are indicated by *arrow*. **e** In the posteriormost part of the medulla, in this male fly, the R8 terminations are irregular in shape and often expanded laterally. This portion of the medulla corresponds to the anteriormost part of the visual field. **f, g** R8 terminations in the medulla of *M. domestica* in overview and at higher magnification. a ×240; b–f ×380; g ×600

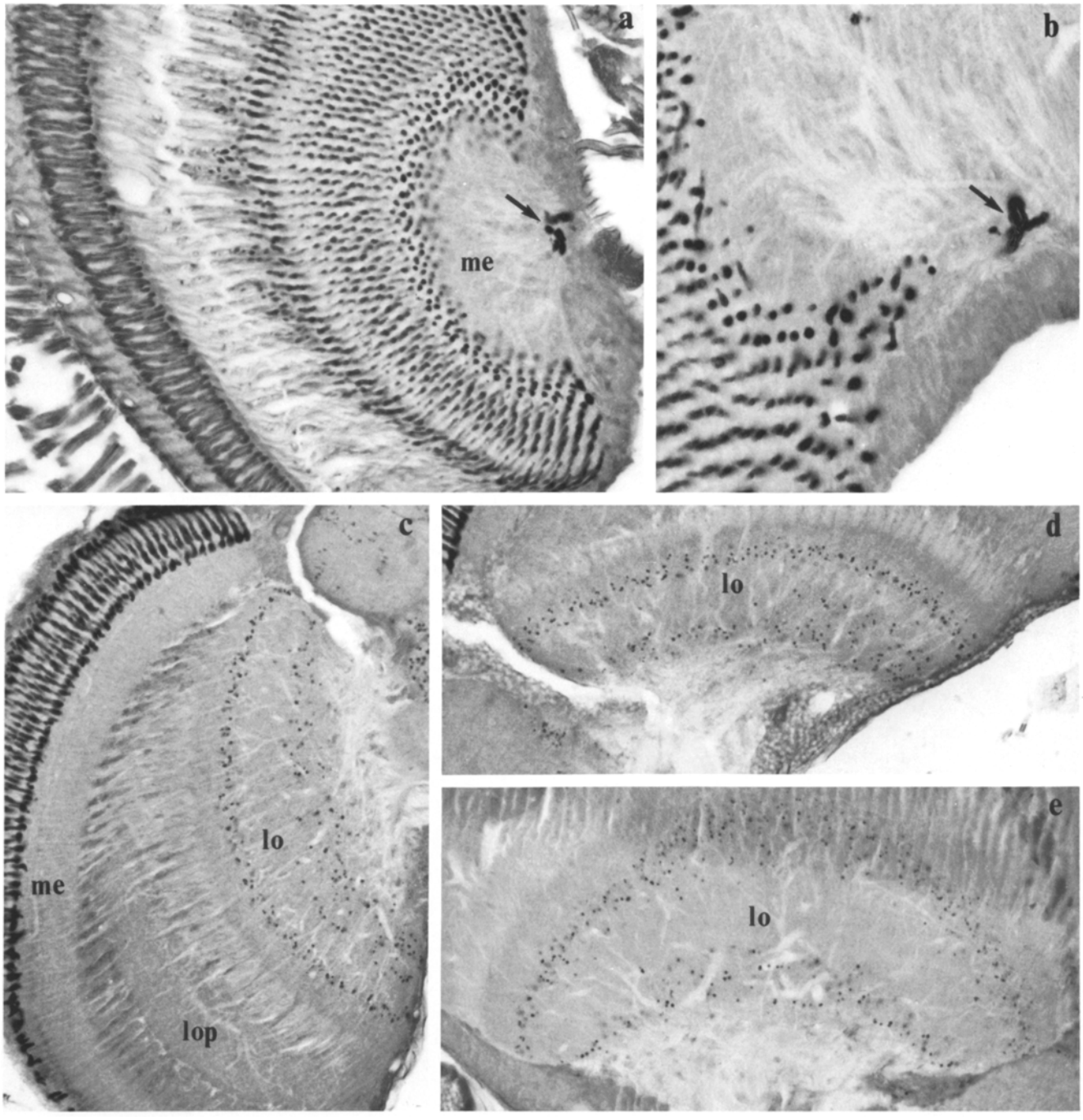

Fig. 4a–e. HA-IR in frontal sections of the compound eyes of *C. erythrocephala* **(b, e)** and *M. domestica* **(a, c, d)**. PAP method, antiserum 8431. **a, b** Anteriorly at the base of the medulla (*me*) terminations of four or five HA-IR axons (*arrows*) are found in both species. These may be derived from extraocular photoreceptors. **c–e** HA-IR varicose fibers in the lobula (*lo*) of the two species. Note that the varicosities are distributed in a distal and a proximal layer of the lobula neuropil. There are no HA-IR fibers in the lobula plate (*lop*), and apart from the R8 terminations the medulla (*me*) contains no HA-IR processes. a ×240; b ×380; c–e ×240

in general, failed to show immunolabelling with different transmitter-related antisera, including those raised against choline acetyltransferase (Buchner et al. 1986), glutamate (Schäfer 1987), serotonin (reviewed by Nässel 1987a), dopamine (Nässel, Elekes, Johansson and Steinbusch, in preparation), and against different neuropeptides (see Nässel 1988). One exception is anti-taurine, which was found to immunostain photoreceptors of both compound eyes and ocelli of bees (Schäfer et al. 1988). However, whilst the presence of taurine in insect retinae has been confirmed by biochemical studies in flies (Langer et al. 1976) and locust (Whitton et al. 1987), ionophoresis of taurine onto fly LMCs evoked no measurable response (Hardie 1987). Taurine is of widespread occurrence in nervous tissue (Oja and Kontro 1983), and some function other than that of a neurotransmitter may thus be suspected.

Another exception was provided by Datum et al. (1986), who found that the second type of long visual fiber (R7) in the fly showed both GABA- and GAD-like immunoreactivity, whilst R1–R6 and R8 did not. Although this finding

could not be confirmed in an independent study (Meyer et al. 1986), at least it indicates the presence of an immunoreactive substance unique to R7. Conversely, the present study clearly shows that R7 is not HA-IR, whilst R8 displays a strong immunoreaction. Although there is a minimal information on the synaptic connections of R7 and R8 with interneurons in the medulla, it is interesting that the functionally distinct R7 and R8 receptors (cf. Hardie 1985) may well use different neurotransmitters. For example, since R7 and R8 usually have different spectral sensitivities (Hardie 1985) one may speculate that their respective transmitters have antagonistic actions on hypothetical color opponency neurons in the medulla. In the dorsal margin of the eye, where again only R8 shows HA-IR (Fig. 2d), R7 and R8 have the same (ultraviolet) spectral sensitivities but orthogonally arranged preferred directions of polarization sensitivity (Hardie 1984). This arrangement can only be readily understood by assuming antagonistic actions of R7 and R8 on postsynaptic neurons, which would then act as very efficient polarization detectors (Hardie 1984; Strausfeld and Wunderer 1985).

One additional finding deserves special comment. A set of four or five HA-IR axons was found to terminate with short stout lateral processes in a distinct region below the medulla where also serotonin-immunoreactive (5-HT-IR) optic lobe neurons are known to have processes. In fact, in an earlier report terminals similar to the HA-IR ones were detected after anterograde tracing with horseradish peroxidase from lesions in the retina/lamina region and erroneously correlated with terminations of 5-HT-IR neurons connecting the lamina and the medulla (Nässel et al. 1985). It is not yet clear from where the four or five HA-IR axons terminating in the basal medulla are derived. One possibility is that they are the axons of extraretinal photoreceptors located in the vicinity of the lamina. This suggestion is partly based on the fact that these axons and their characteristic terminals also react with antibodies raised against mammalian retinal S-antigen (Nässel, unpublished). S-antigen immunoreactivity seems to be characteristic for photoreceptors since such immunoreactivity has been detected in photoreceptor cells of the retina and pineal organ of most vertebrates studied (Korf et al. 1985; van Veen et al. 1986), as well as in different types of photoreceptors in a variety of invertebrates (van Veen et al. 1986) including compound eyes and extraretinal photoreceptors in insects (Hagberg 1986; Nässel, unpublished). The four to five HA-IR neurons in the adult flies terminate in the same region as the axons of the stemmata (photoreceptors) in larval flies (see Meinertzhagen 1973), and it may be speculated that they in fact are axons of extraretinal photoreceptors derived from metamorphosing larval stemmata. So far, such extraretinal photoreceptors have not been localized in adult dipterous flies; but in, e.g., caddisflies and some beetles, larval stemmata survive metamorphosis and in the adults their photoreceptors terminate at a location analogous to that in the flies described here (Schultz et al. 1984; Hagberg 1986).

In the present study we also show that certain interneurons display HA-IR. Most of these are in brain centers and will be described elsewhere. In the optic lobes HA-IR interneurons are restricted to the lobula. It is rare in flies that a transmitter candidate is found only in one of the four columnar optic lobe neuropils (Nässel 1987a, b; 1988; Hardie 1988). So far, the only other case of chemically identified neurons being restricted only to the lobula is proctolin-immunoreactive neurons (Nässel and O'Shea 1987). Due to the incomplete immunolabeling of processes of HA-IR neurons it cannot be determined whether the HA-IR neurons in the lobula are local amacrines or projection neurons.

Histaminergic interneurons have been demonstrated in molluscs (Weinreich et al. 1975; Weinreich 1977; Ono and MacCaman 1980; Walker 1982), but only in *Aplysia* have all the classical criteria been satisfied indicating histamine as a true neurotransmitter in certain identified central neurons (reviewed by Walker 1982). There is also good evidence for histamine as a neurotransmitter or neuromodulator in the vertebrate brain (see Prell and Green 1986). Amongst arthropods, however, the only previous report on histaminergic neurotransmission is in the lobster stomatogastric ganglion (Claiborne and Selverston 1984). The probable role of histamine as a neurotransmitter in photoreceptors represents the first known example of a histaminergic system in insects. The high number of densely packed histaminergic photoreceptor cells in the retina/lamina combined with the concentration of histamine receptors in the lamina make this a very attractive system for further investigations of histaminergic neurotransmission. It will also be of interest to investigate whether histaminergic photoreceptors are present generally in insects as well as in other invertebrate groups and to study the role of histaminergic transmission in interneurons of the insect central nervous system.

References

Buchner E, Buchner S, Crawford G, Mason WT, Salvaterra PM, Sattelle DB (1986) Choline acetyltransferase-like immunoreactivity in the brain of *Drosophila melanogaster*. Cell Tissue Res 246:57–62

Claiborne BJ, Selverston AI (1984) Histamine as a neurotransmitter in the stomatogastric nervous system of the spiny lobster. J Neurosci 4:708–721

Datum K-H, Weiler R, Zettler F (1986) Immunocytochemical demonstration of γ-amino butyric acid and glutamid acid decarboxylase in R7 photoreceptors and C2 centrifugal fibers in the blowfly visual system. J Comp Physiol 159:241–249

Elias MS, Evans PD (1983) Histamine in the insect nervous system: distribution, synthesis and metabolism. J Neurochem 41:562–568

Elias MS, Evans PD (1984) Autoradiographic localization of ^{3}H-histamine accumulation by the visual system of the locust. Cell Tissue Res 238:105–112

Elias MS, Lummis SCR, Evans PD (1984) [^{3}H] mepyramine binding sites in the optic lobes of the locust: autoradiographic and pharmacological studies. Brain Res 294:359–362

Hagberg M (1986) Ultrastructure and central projections of extraocular photoreceptors in caddiesflies (Insecta: Trichoptera). Cell Tissue Res 245:643–648

Håkanson R, Böttcher G, Ekblad E, Panula P, Simonsson M, Dohlsten M, Hallberg T, Sundler F (1986) Histamine in endocrine cells in the stomach. A survey of several species using a panel of histamine antibodies. Histochemistry 86:5–17

Hardie RC (1979) Electrophysiological analysis of fly retina. I. Comparative properties of R1–6 and R7 and 8. J Comp Physiol 129:19–33

Hardie RC (1984) Properties of photoreceptors R7 and R8 in dorsal marginal ommatidia in the compound eyes of *Musca* and *Calliphora*. J Comp Physiol 154:157–165

Hardie RC (1985) Functional organization of the fly retina. In: Ottoson D (ed) Progr Sensory Physiol V. Springer, Berlin Heidelberg New York, pp 1–79

Hardie RC (1987) Is histamine a neurotransmitter in insect photoreceptors? J Comp Physiol 161:201–213

Hardie RC (1988) Neurotransmitters in the compound eye. In: Hardie RC, Stavenga DG (eds) Facets of Vision. Springer, Berlin Heidelberg New York (in press)

Hardie RC, Franceschini N, Ribi W, Kirschfeld K (1981) Distribution and properties of sex specific photoreceptors in the fly *Musca domestica*. J Comp Physiol 145:139–152

Hausen K, Strausfeld NJ (1980) Sexually dimorphic interneuron arrangements in the fly visual system. Proc R Soc Lond Biol 208:57–71

Klingman A, Chappel RL (1978) Feedback synaptic interaction in the dragonfly ocellar retina. J Gen Physiol 71:157–175

Korf H-W, Möller M, Gery I, Zigler JS, Klein DC (1985) Immunocytochemical demonstration of retinal S-antigen in the pineal organ of four mammalian species. Cell Tissue Res 239:81–85

Langer H, Lues I, Rivera ME (1976) Arginine phosphate in compound eyes. J Comp Physiol 107:179–184

Maxwell GD, Tait JF, Hildebrand JG (1978) Regional synthesis of neurotransmitter candidates in the CNS of the moth *Manduca sexta*. Comp Biochem Physiol 61C:109–119

Meinertzhagen IA (1973) Development of the compound eye and optic lobes of insects. In: Young D (ed) Developmental neurobiology of arthropods. University Press, Cambridge, pp 51–104

Meyer EP, Matute C, Streit P, Nässel DR (1986) Insect optic lobe neurons identifiable with monoclonal antibodies to GABA. Histochemistry 84:207–216

Nässel DR (1987a) Serotonin and serotonin-immunoreactive neurons in the insect nervous system. Progr Neurobiol 30:1–85

Nässel DR (1987b) Neuroactive substances in the insect CNS. In: Ali MA (ed) Nervous Systems of Invertebrates. Plenum, New York, pp 171–212

Nässel DR (1988) Immunocytochemistry of putative neuroactive substances in the insect brain. In: Salanki J, S-Rosza K (eds) Invertebrate neurobiology: transmitters, modulators and receptors. Akadémiai Kiadó, Budapest (in press)

Nässel DR, O'Shea M (1987) Proctolin-like immunoreactive neurons in the blowfly central nervous system. J Comp Neurol 265:437–454

Nässel DR, Meyer EP, Klemm N (1985) Mapping and ultrastructure of serotonin-immunoreactive neurons in the optic lobes of three insect species. J Comp Neurol 232:190–204

Oja SS, Kontro P (1983) Taurine. In: Lathja A (ed) Handbook of Neurochemistry 3:501–533

Ono JK, McCaman RE (1980) Identification of additional histaminergic neurons in *Aplysia*: improvement of single cell isolation techniques for in tandem physiological and chemical studies. Neuroscience 5:835–840

Panula P, Yang H-YT, Costa E (1984) Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci USA 81:2572–2576

Panula P, Kaartinen M, Mäcklin M, Costa E (1985) Histamine-containing neuronal and endocrine systems. J. Histochem Cytochem 33:933–941

Pearse AGE, Polak JM (1975) Bifunctional reagents as vapour-and liquid phase fixatives for immunohistochemistry. Histochem J 7:179–186

Prell GD, Green JP (1986) Histamine as a neuroregulator. Ann Rev Neurosci 9:209–254

Schäfer S (1987) Immunocytochemische Untersuchungen am Bienengehirn. Thesis, Freie Universität, Berlin

Schäfer S, Bicker G, Ottersen OP, Storm-Mathisen J (1988) Taurine-like immunoreactivity in the brain of the honey bee. J Comp Neurol 268:60–70

Schultz W-D, Schlüter U, Seifert G (1984) Extraocular photoreceptors in the brain of *Epilachna varivestris* (Coleopter, Coccinellidae). Cell Tissue Res 236:317–320

Simmons PJ, Hardie RC (1988) Evidence that histamine is a neurotransmitter of photoreceptors in the locust ocellus. J Exp Biol (in press)

Steinbusch HWM, Mulder AH (1984) Immunohistochemical localization of histamine in neurons and mast cells in the rat brain. In: Björklund A, Hökfelt T, Kuhar M (eds) Handbook of chemical neuroanatomy, Classical transmitters and transmitter receptors in the CNS. Elsevier, New York, Vol 3, part II, pp 126–148

Strausfeld NJ (1980) Male and female visual neurones in dipterous insects. Nature 283:381–383

Strausfeld NJ, Nässel DR (1980) Neuroarchitectures serving compound eyes of crustacea and insects. In: Autrum H (ed) Handbook of sensory physiology VII/6B. Springer, Berlin Heidelberg New York, pp 1–132

Strausfeld NJ, Wunderer H (1985) Optic lobe projections of marginal ommatidia in *Calliphora erythrocephala* specialized for detecting polarized light. Cell Tissue Res 242:163–178

Trujillo-Cenoz O, Melamed J (1966) Electron microscope observations on the peripheral and intermediate retinas of Dipterans. In: Bernhard CG (ed) The functional organization of the compound eye. Symp Wenner-Gren Center. Pergamon Press, London, pp 339–361

Veen T van, Elofsson R, Hartwig HG, Gery I, Mochizuki M, Cena V, Klein DC (1986) Retinal S-antigen: immunocytochemical and immunochemical studies on distribution in animal photoreceptors and pineal organs. Exp Biol 45:15–22

Walker RJ (1982) Current trends in invertebrate neuropharmacology. Verh Dtsch Zool Ges 1982:31–59

Weinreich D (1977) Synaptic responses mediated by identified histamine-containing neurons. Nature 267:854–856

Weinreich D, Weiner C, McCaman R (1975) Endogenous levels of histamine in single neurons isolated from CNS of *Aplysia californica*. Brain Res 84:341–345

Whitton PS, Strang RHC, Nicholson RA (1987) The distribution of taurine in the tissues of some species of insects. Insect Biochem 17:573–577

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