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Development of Nerve Cells in Hydrozoan Planulae: II. Examination of Sensory Cell Differentiation Using Electron Microscopy and Immunocytochemistry

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Abstract. The development of sensory cells in hydrozoan planulae of *Halocordyle disticha* was examined using transmission electron microscopy and light immunocytochemistry. Sensory cells arise in the anterior end of the planula in the ectoderm at 24 hours postfertilization. These cells extend from the free surface of the planula to a ganglionic plexus located just above the mesoglea. The cytomorphosis of sensory cells is characterized by the appearance of a single apical cilium, microtubules, mitochondria, one to several Golgi complexes, electron-dense droplets, dense-cored vesicles, and neurites. The basal end of the sensory cell forms one to several processes (neurites) which contribute to the ganglionic plexus. Apical specialization of the sensory cell precedes basal differentiation. Sensory cells increase in number as planulae develop and many become organized into clusters of 3–6 cells distributed along the entire length of the planula. Within some of these clusters, two morphological types of sensory cells are discernible: light sensory cells and dark sensory cells. Light sensory cells outnumber the dark sensory cells and are the first sensory cells to appear at 24 hours postfertilization. Use of immunocytochemical techniques on wholemounts and paraffin-embedded sections of planulae demonstrates the presence of FMRFamide-like immunoactivity associated with some of the sensory cells. Such FMRFamide-like expression is first detected at 24 hours postfertilization in the anterior ectoderm of the planula. By 96 hours postfertilization, the spatial distribution of FMRFamide-like positive sensory cells is such that many are found in clusters along the entire anterior-posterior axis of the planula. There is, however, an abundance of FMRFamide-like positive cells in the anterior region of the planula just prior to metamorphosis. The apices and cell bodies of the sensory cells exhibit intense immunostaining, whereas the basal processes stain faintly. This study identifies neuropeptide-like substances in nerve cells of cnidarian larvae and demonstrates a developmental correlation between the time of appearance of the synthetic machinery of sensory cells with the pattern of expression of the FMRFamide-like peptide.

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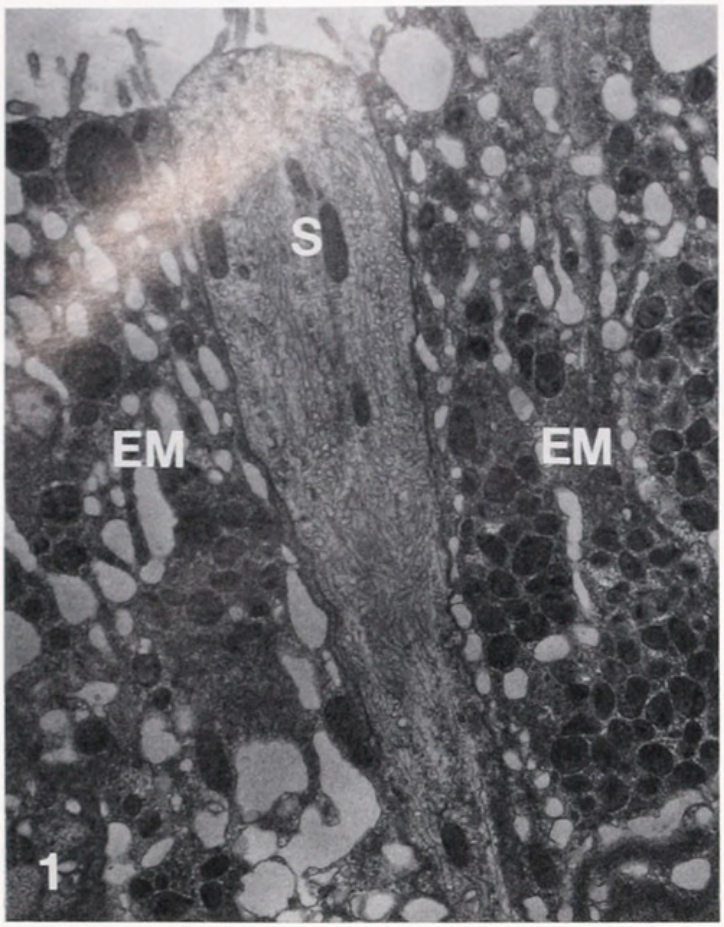
Introduction

Early light microscopists defined two types of nerve cells in cnidarians: sensory cells and ganglionic cells (Burnett and Diehl, 1964). Sensory cells are oriented perpendicular to the mesoglea with their apical ends contacting the outer free surface of the animal and their basal ends drawn out into processes. Ganglionic cells exhibit round perikarya and lie in the basal part of the ectoderm with their axes oriented parallel to the mesoglea. Furthermore, Westfall and associates have demonstrated that, in hydra, many types of sensory cells and ganglionic cells exist which can be classified as unipolar, bipolar, or multipolar depending on the number of processes extending from the perikaryon (Yu *et al.*, 1985).

Chemical synapses with electron-dense and dense-cored vesicles have been observed in the nervous systems of adult hydrozoans, scyphozoans, and anthozoans (Horridge and Mackay, 1962; Lentz and Barrnett, 1965; Jha and Mackie, 1967; Davis *et al.*, 1968; Westfall, 1970, 1973; Westfall *et al.*, 1971; Stokes, 1974; Peteya, 1975; Yamasu and Yoshida, 1976; Singla, 1978; Spencer, 1979). However, only recently has a specific peptide been identified in adult cnidarians that might be acting as a neurotransmitter (Grimmelikhuijzen and Graff, 1986; Grimmelikhuijzen and Groeger, 1987). Electron-dense droplets and dense-cored vesicles also have been identified in planular nervous systems (Martin, 1988), and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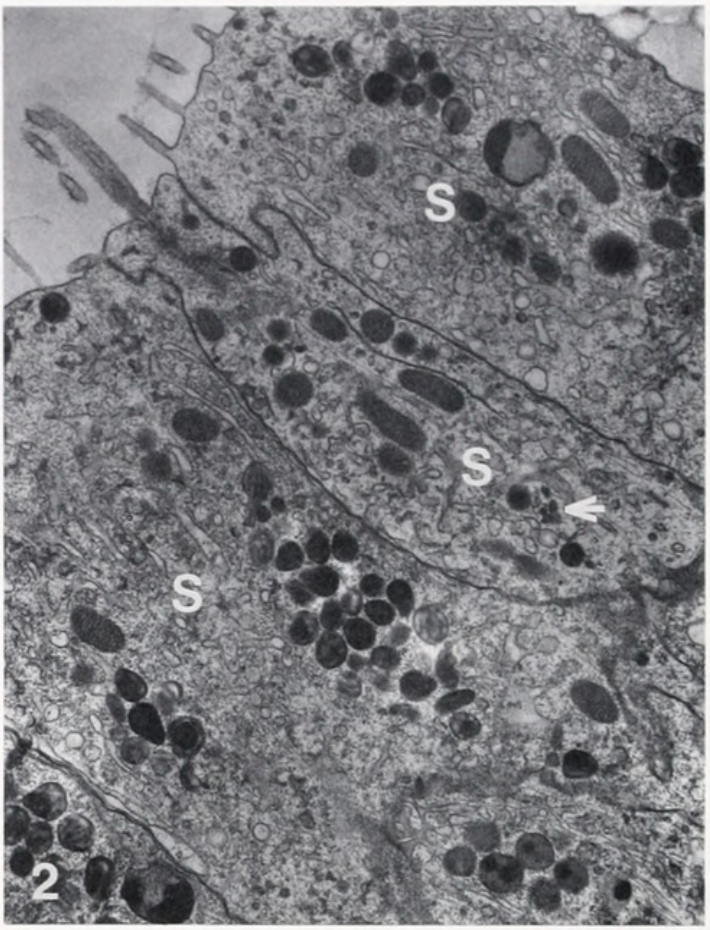
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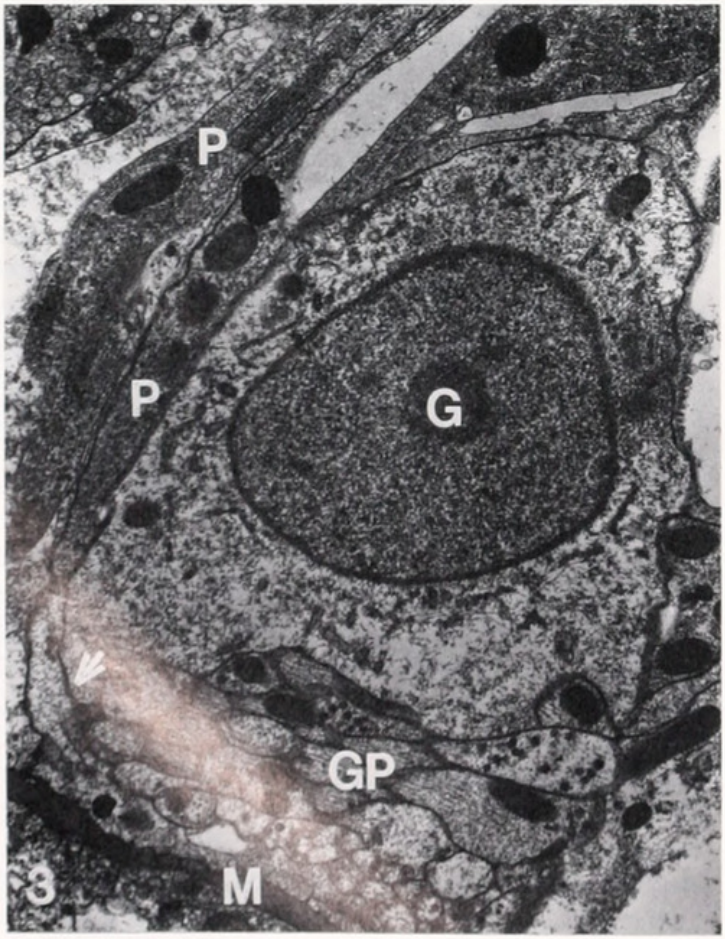
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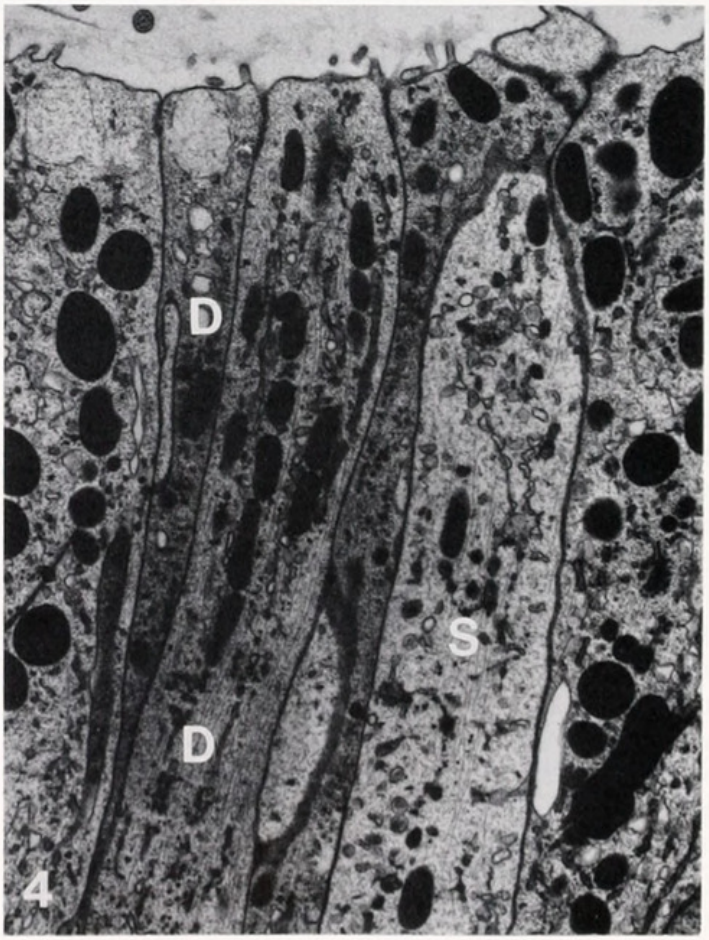
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Kolberg and Martin (1988) have demonstrated catecholamines in association with planular nerves. Furthermore, they provide evidence that such catecholamines may be functioning as neurotransmitters, neurohormones, or neuromodulators during embryogenesis (Kolberg and Martin, 1988).

A reagent (anti-FMRFamide) is available which will stain cells containing peptides ending in -Arg-Phe-NH_2. The work of Grimmelikhuijzen and associates suggests that, when this antiserum is applied to cnidarians, the peptides bound to it are likely to be related to pGlu-Gly-Arg-Phe-amide (PQGRFa) which is present in large amounts in nervous systems of adult anthozoans and probably also in scyphozoans and hydrozoans (Grimmelikhuijzen and Graff, 1986; Grimmelikhuijzen and Groeger, 1987). The question is: how early in development, and in what cells, is the gene for this peptide (or peptide family) expressed? The planula larva is a good system in which to examine this problem because the number of cell types in the larva is small, their arrangement is simple, and neither the variety nor the arrangement are very far from those of the adult (Martin and Thomas, 1980; Martin *et al.*, 1983; Thomas *et al.*, 1987; Martin, 1988).

In this study, the development of the planula of the marine hydrozoan *Halocordyle disticha* was followed with transmission electron microscopy to determine when sensory nerve cells appeared and when the synthetic machinery of these cells appeared. Different aged planulae were exposed to FMRFamide antiserum, and the pattern of expression of the FMRFamide-like peptide was correlated with the electron microscopic findings.

Materials and Methods

Mature colonies of *Halocordyle disticha* were collected from wharf pilings in Morehead City, North Carolina. Fronds from male and female colonies were placed together in large finger bowls of filtered seawater. The bowls were placed in the dark at 6:00 pm, and at 9:00 pm, early cleavage embryos were collected, placed in small finger bowls of seawater, and reared at 23°C.

Eight-hour embryos, as well as 10-, 16-, 24-, 48-, 72-, 96-, and 120-hour planulae, were prepared for transmission electron microscopy. The animals were fixed for 1 hour in 2.5% glutaraldehyde, pH 7.4, in 0.2 *M* phosphate buffer. They were postfixed for 1 hour in 2% osmium tetroxide, pH 7.2, in 1.25% sodium bicarbonate. The specimens were dehydrated in an ethanol series, infiltrated, and embedded in Spurr's embedding medium. Serial thin-sections were cut with a Porter-Blum MT-2B ultramicrotome, placed on 150-mesh copper grids, and stained with 3.5% uranyl acetate in ethanol followed by lead hydroxide. The grids were examined and photographed with a Hitachi H-600 transmission electron microscope. Thick plastic sections were also cut, placed on subbed glass slides, and stained with methylene blue-azure II.

To better visualize the basal processes of sensory cells, early cleavage embryos were cultured in seawater containing 0.01 *M* hydroxyurea until they reached the mature planula stage (Martin, 1985, 1986). These treated larvae were then prepared for transmission electron microscopy. Embryos reared continuously in hydroxyurea contain reduced numbers of ganglionic cells and slightly fewer ganglionic neurites, yet possess the same number of sensory cells as do comparable controls (Martin, 1985, 1986, pers. obs.). The sensory cells of hydroxyurea-grown planulae are morphologically identical to those of comparable controls, and their basal processes are more easily traceable due to the reduced size of the ganglionic plexus (Martin, 1985, 1986, pers. obs.).

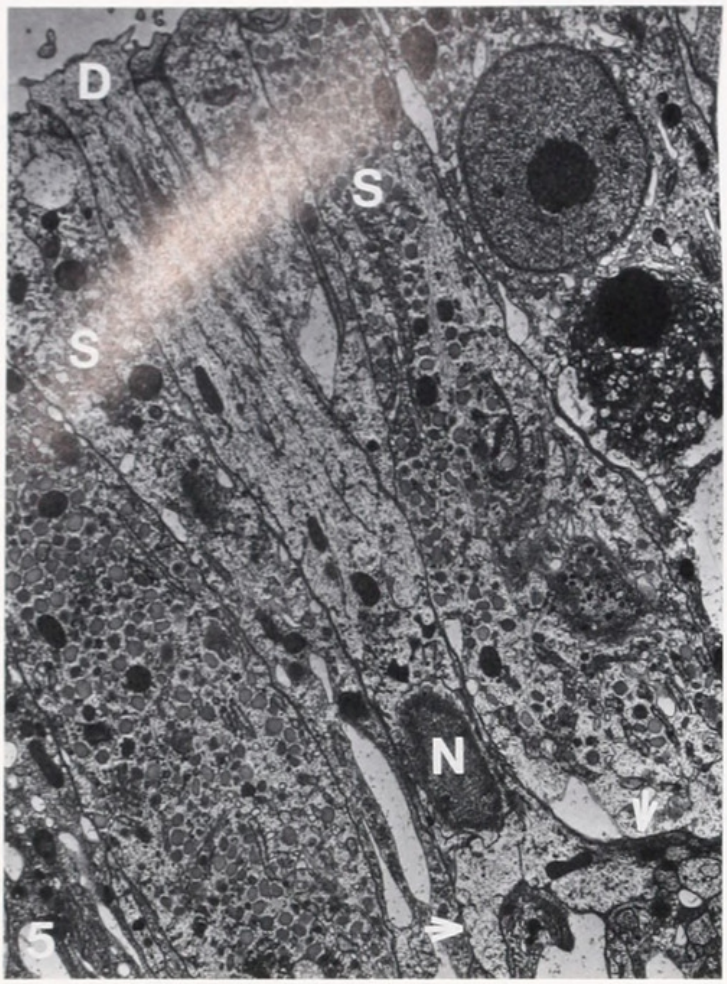
Planulae of eight different ages, wholemounts and paraffin sections, were tested for their ability to bind a rabbit antiserum raised to FMRFamide (Immuno Nuclear Corporation). The planular ages included 10-, 16-, 24-, 36-, 48-, 72-, 96-, and 120-hour planulae. To visualize

Figure 1. Single light sensory cell (S) in the anterior ectodermal region of a 24 hour planula. The cytoplasm is filled with microtubules, Golgi cisternae, some rough endoplasmic reticulum, and a few mitochondria. EM, epitheliomuscle cell. ×8400.

Figure 2. Cluster of three light sensory cells (S) in the mid ectodermal region of a 72 hour planula. Each cell possesses an apical cilium and a cytoplasm rich in microtubules, Golgi cisternae, electron-dense droplets (arrow), and dense cored vesicles. ×11,200.

Figure 3. Ganglionic nerve cell (G) and ganglionic plexus (GP) at the base of the ectoderm in a 72 hour planula. Basal neurite extensions (P) of light sensory cells (arrow) and dark sensory cells (not visible here) project into and help constitute the ganglionic plexus. Neurites of the plexus contain microtubules, mitochondria, electron-dense droplets, and dense-cored vesicles. M, mesoglea. ×10,000.

Figure 4. Cluster of light (S) and dark (D) sensory cells in the ectoderm of a mature hydroxyurea-grown planula. From the early cleavage stage, animals were continuously cultured in 0.01 *M* hydroxyurea in seawater. All cells in treated planulae are morphologically identical to those in control planulae. Photographs of treated embryos (Figs. 4, 6, 9) were included because excellent planes of section illustrating clustering of sensory cells and sensory cell processes were obtained from these embryos. ×7000.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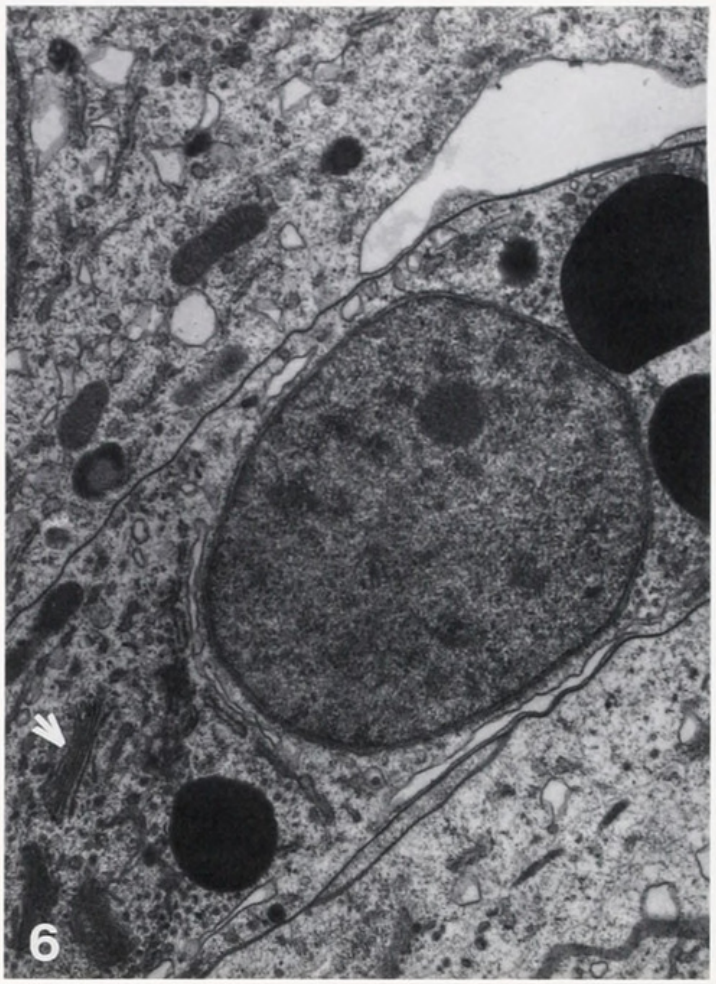
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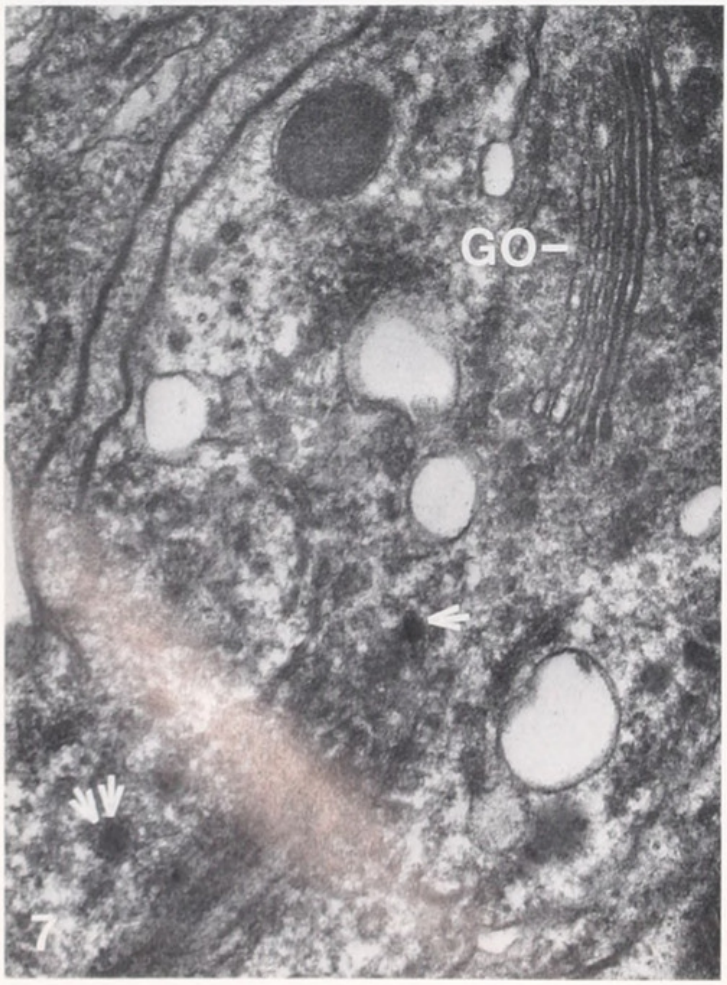
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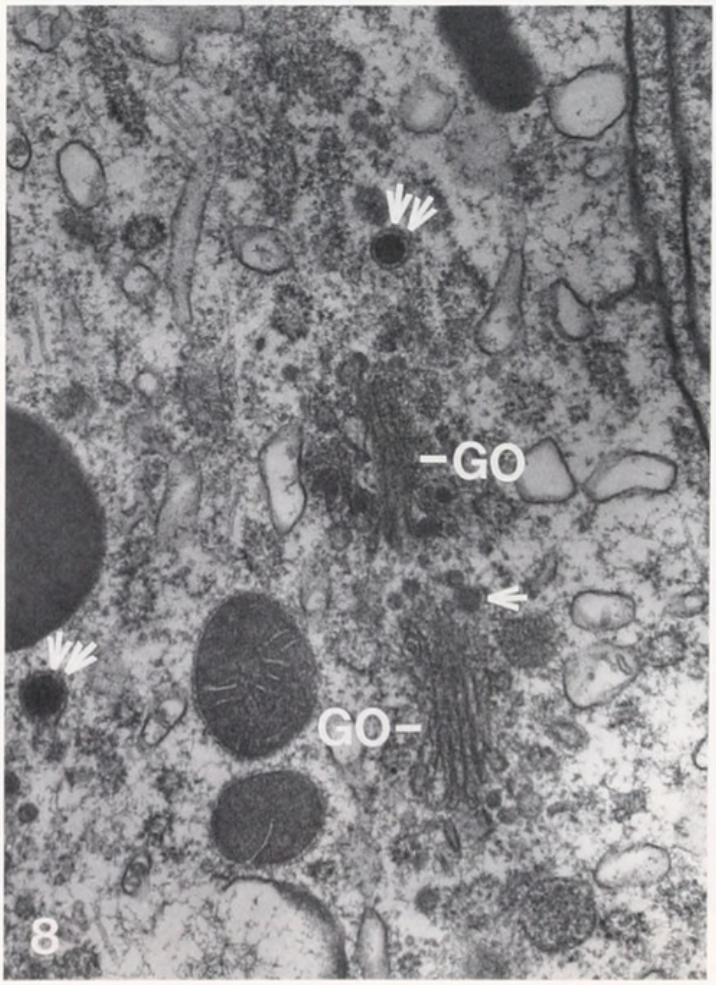
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the binding of FMRFamide antiserum on wholemounts of planulae, the procedure presented by Koizumi and Bode (1986) was followed with some modifications. Planulae were fixed for 1 hour in 10% formalin in seawater. After fixation, the animals were washed 3 times, for 15 minutes each, in 10 m*M* phosphate-buffered saline (PBS, pH 7.2). Incubation with the FMRFamide antiserum was for 18 hours, with the primary antibody diluted 1:200 with 10 m*M* PBS, pH 7.2, containing 2% neonatal calf serum (Irvine Scientific), 0.3% Triton X-100, and 0.1% sodium azide. The incubation was carried out with the planulae in lid-covered 96 well tissue culture plates that were resting on a rotating shaker platform set at 60 rpm. After the first incubation period, the primary antibody was pulled off with a pipette, and the animals were washed for three 15-minute changes in 10 m*M* PBS, pH 7.2. Incubation with the second antibody was for 1 hour in fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobins (U. S. Biochemical Corporation) diluted 1:120 in 10 m*M* PBS, pH 7.2, containing 10% fetal calf serum, 0.3% Triton X-100, and 0.1% sodium azide. The second incubation was also in 96 well plates rotated at 60 rpm. After the second incubation, the animals were washed 3 times, for 15 minutes each, in fresh 10 m*M* PBS, pH 7.2. Wholemount preparations were examined for fluorescently labelled cells with a Zeiss microscope equipped with epifluorescence.

To visualize binding of FMRFamide antiserum to paraffin sections of planulae, the following procedure was followed. Samples fixed in formalin were dehydrated through an alcohol series, infiltrated and embedded in paraffin, and serially sectioned at 8 μm. Approximately nine sections were mounted in the center of a single glass slide, three rows one above the other, and each row containing three sections. The slides were rehydrated to distilled water, and the sections were surrounded by an outer ring of vacuum grease (the grease ring was just to the outside of the sections). The grease was applied in a moist chamber to prevent the sections from drying.

The protocol for indirect immunofluorescence for paraffin sections was identical to that described for wholemounts. The FMRFamide antiserum was placed in the grease-created well thus immersing the sections. Such slides were placed in a lid-covered moist chamber and rotated at 40–60 rpm for 18 hours. PBS rinses and incubation in the second antibody were also carried out in the moist chamber. After incubation, the grease was carefully removed from the slides, and the sections were covered with mineral oil and examined for fluorescently labeled cells. Some of the paraffin sections were subsequently stained with azure B after their initial examination for immunofluorescence.

For wholemounts and paraffin sections, the binding specificity of the FMRFamide antiserum was determined by preincubating a 1:200 dilution of the antiserum with either 1 or 10 μg/ml synthetic FMRFamide (Peninsula Lab) for 24 hours at 4°C before using it to stain the samples.

Results

Sensory cells begin to arise in the ectoderm of the planula at 24 hours postfertilization (Fig. 1). They first appear as single cells scattered in the anterior region of the planula. As development progresses, these cells increase in number and become distributed along the entire length of the planula, many arranged in clusters of 3–6 cells (Fig. 2). Sensory cells are columnar and extend from the free surface of the planula to a ganglionic plexus located just above the mesoglea (Fig. 3). Sensory cells are characterized by an apical cilium, a medially to basally located nucleus, and small basal neurite extensions which project into and help constitute the ganglionic plexus.

Two morphological types of sensory cells are identifiable at the fine-structural level: a light sensory cell and a dark sensory cell (Fig. 4); the light sensory cell has a more electron-lucent cytoplasm than does the dark sensory cell. The light and dark sensory cells can be distinguished on the basis of their distribution and time of appearance, their cytology, and their neurite processes.

First, the light cells are in the vast majority, and they appear first in planulae that are only 24 hours old (Fig. 1). I have, as yet, only seen dark sensory cells in the most

Figure 5. A cluster of sensory cells containing one dark sensory cell (D) and several light sensory cells (S) in the anterior ectoderm of a 72 hour planula. The dark sensory cell has a cytoplasm rich in microtubules, mitochondria aligned in rows between the microtubules, electron-dense droplets, and dense-cored vesicles. The nucleus (N) of the dark sensory cell is mid to basally located, and the basal extension is bipolar (arrows). ×5000.

Figure 6. Medially located nucleus of a dark sensory cell. A single Golgi complex (arrow) is found in close association with the nucleus, as are numerous granules and vesicles. ×11,900.

Figure 7. Cytoplasm of a dark sensory cell in a maturing planula. Electron-dense droplets (single arrow) and dense-cored vesicles (double arrows) are abundant in the Golgi region (GO) of the cell. ×41,000.

Figure 8. Cytoplasm of a light sensory cell in a mature planula. Multiple Golgi complexes (GO) appear throughout the apical cytoplasm, as do electron-dense droplets (arrow) and dense-cored vesicles (double arrows). ×41,000.

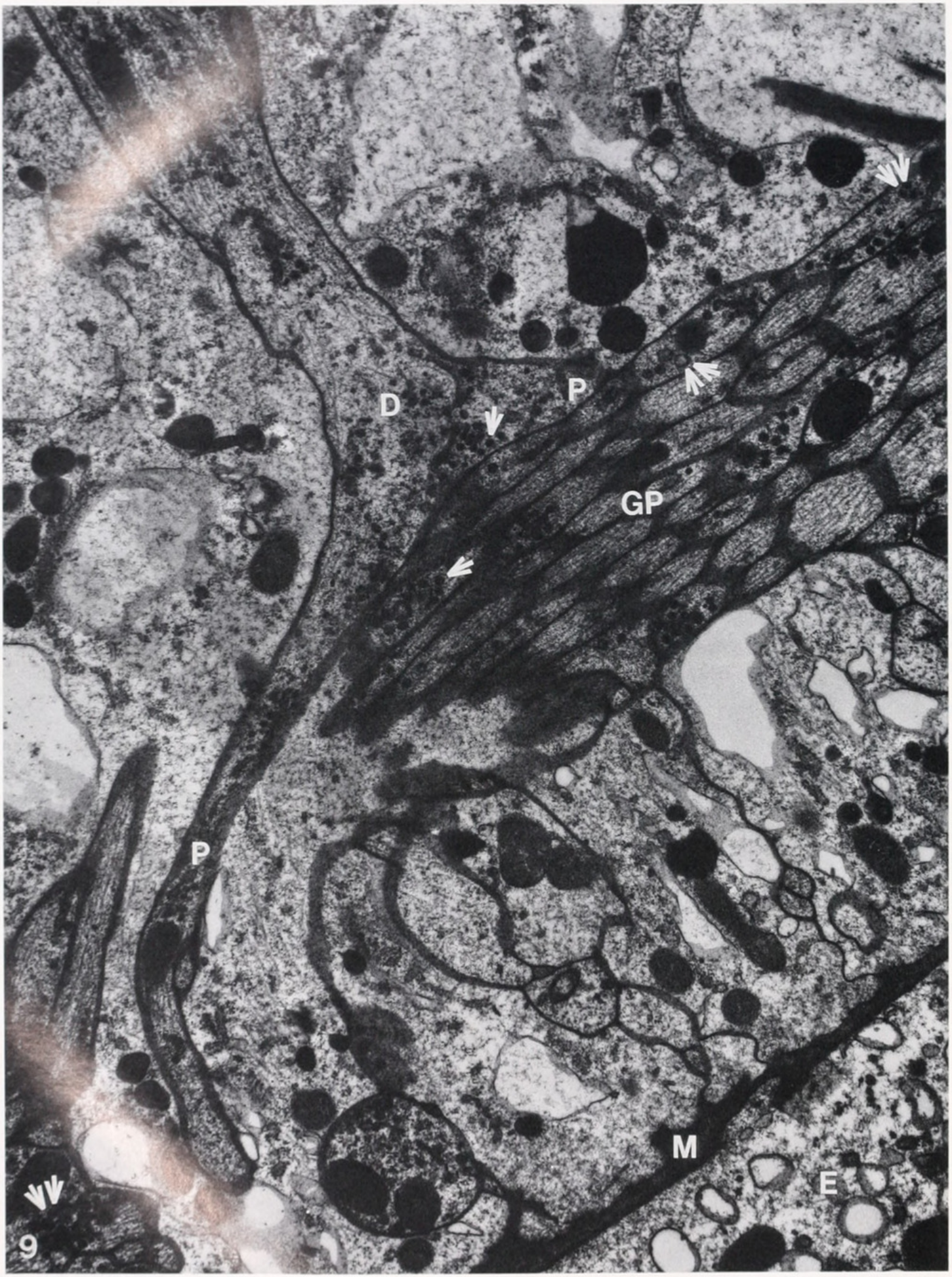


Figure 9. Basal region of a dark sensory cell (D) in a mature hydroxyurea-grown planula. The base of the cell forms two processes (P) which contribute to the ganglionic plexus (GP). Mitochondria, microtubules, electron-dense droplets (single arrow), and dense-cored vesicles (double arrows) fill the sensory neurites and are abundant in the other neurites of the ganglionic plexus. E, endoderm; M, mesoglea. ×19,200.

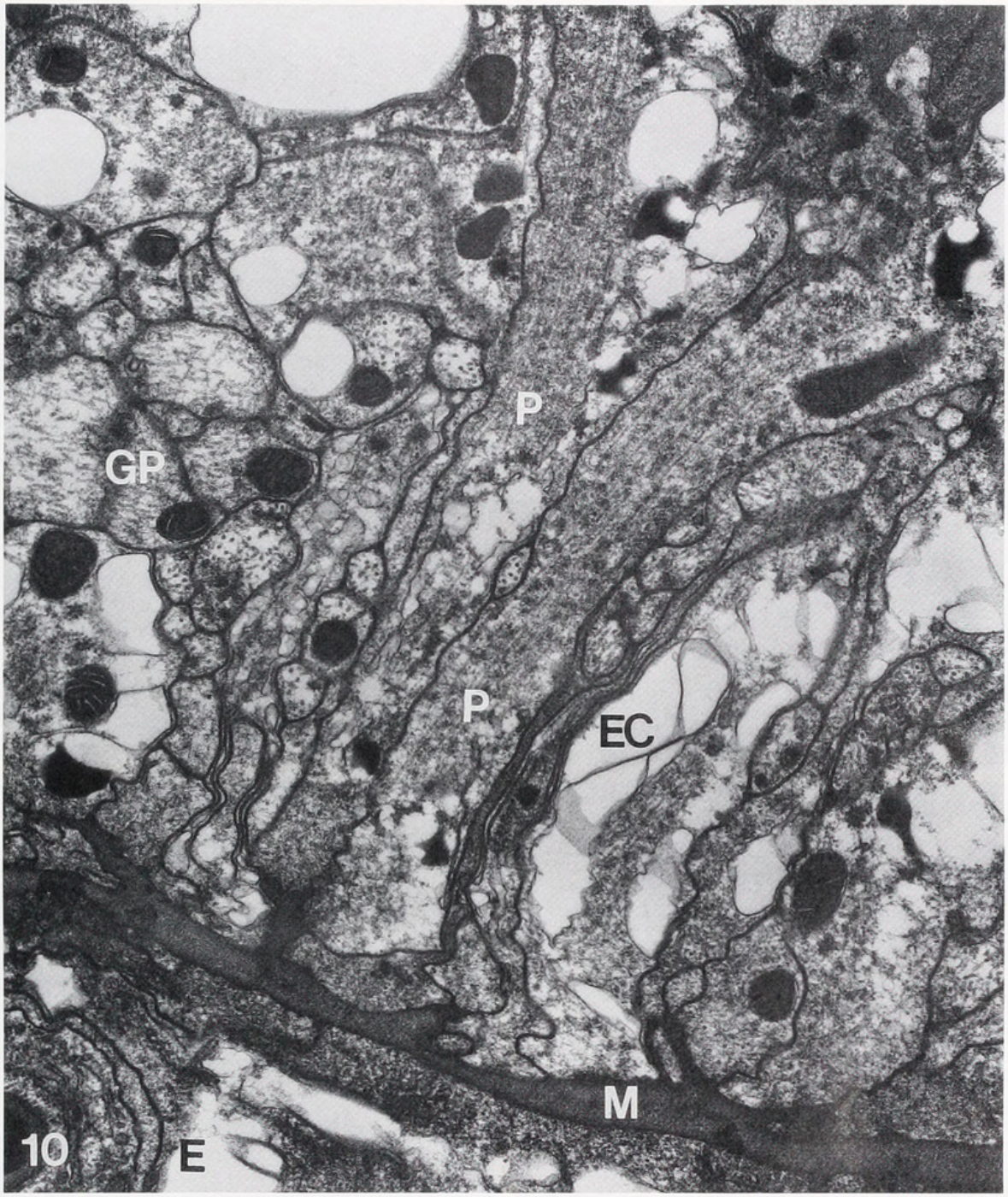
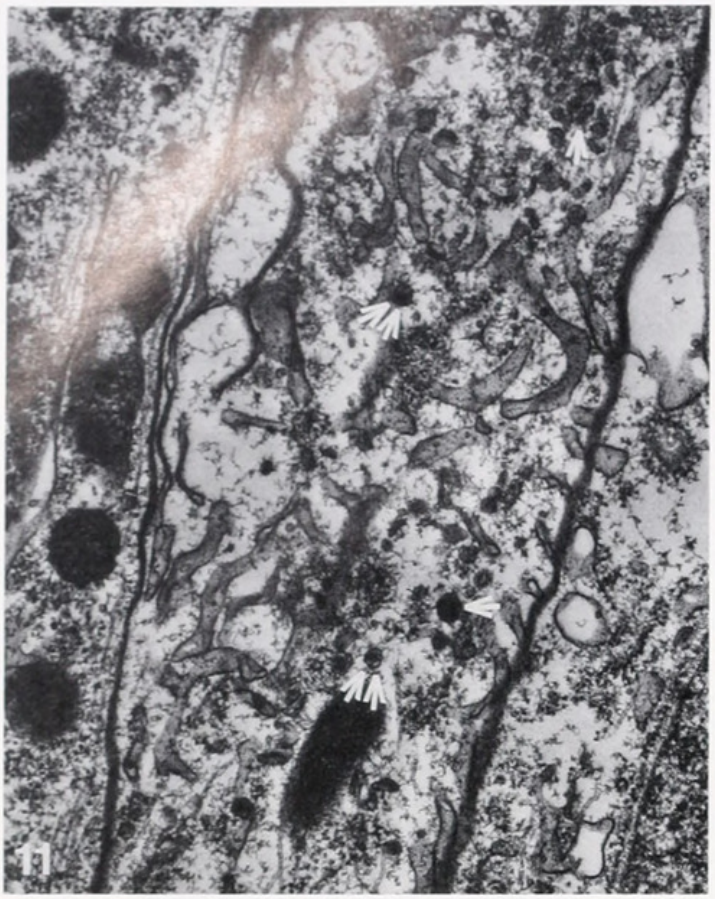


Figure 10. Basal regions of light sensory cells in a maturing planula. Basal processes (P) from two light sensory cells extend into the ganglionic plexus (GP). Numerous microtubules occupy the cytoplasm of these processes, however, electron-dense droplets and dense-cored vesicles have not yet appeared. The appearance of these droplets and vesicles in the basal extensions constitutes the last phase of sensory cell differentiation. E, endoderm; EC, ectoderm; M, mesoglea. ×19,000.

mature planulae (36–96 hours postfertilization depending on temperature: just prior to attachment). Furthermore, the dark sensory cells do not appear in all sensory cell clusters (Fig. 2), and when they are present, they generally occur singly or in pairs (Fig. 5). Light sensory cells may occur singly along the length of the mature planula, but dark sensory cells have only been seen among the clusters.

The cytoplasm of the dark sensory cells contains numerous bundles of microtubules, rows of mitochondria dispersed in between the microtubule bundles, generally a single Golgi complex in close proximity to the nucleus, and electron-dense, non-membrane bound droplets and dense-cored, membrane-bound vesicles (Figs. 4–7). The cytoplasm of light sensory cells also contains numerous bundles of microtubules, many Golgi complexes in the



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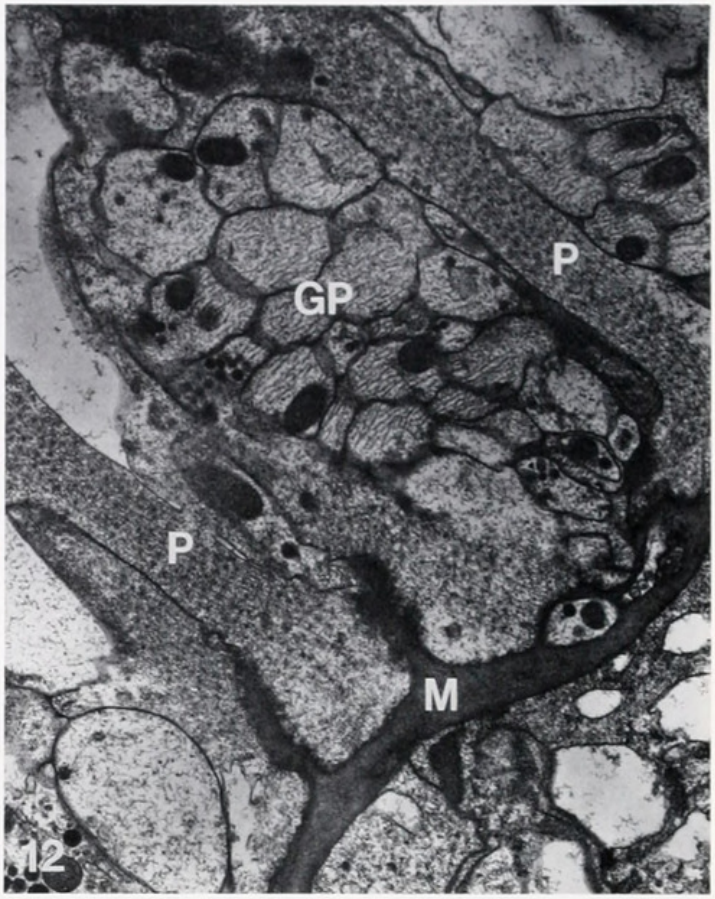

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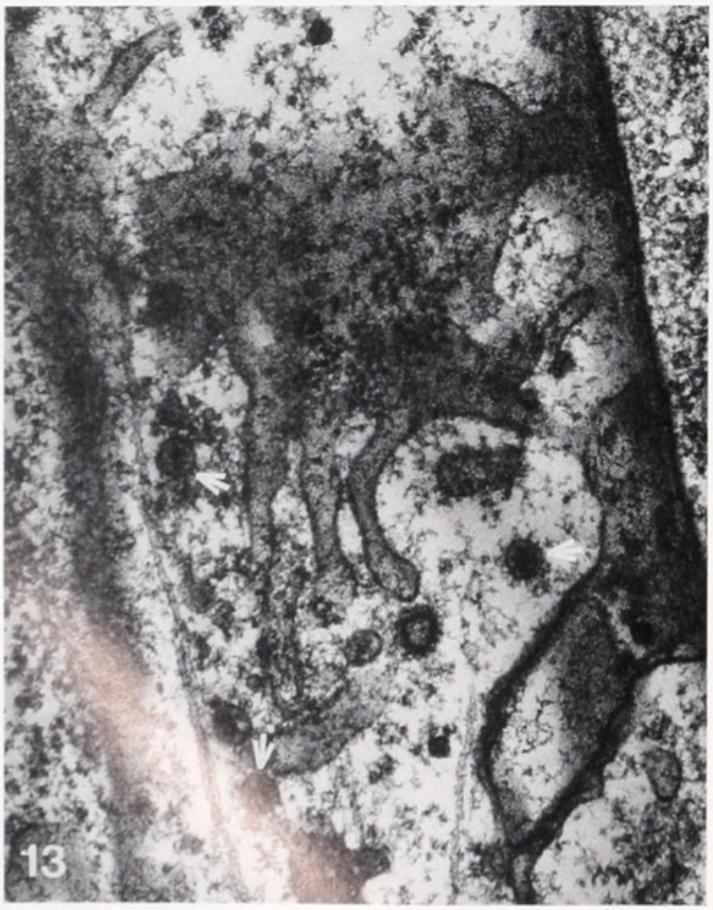

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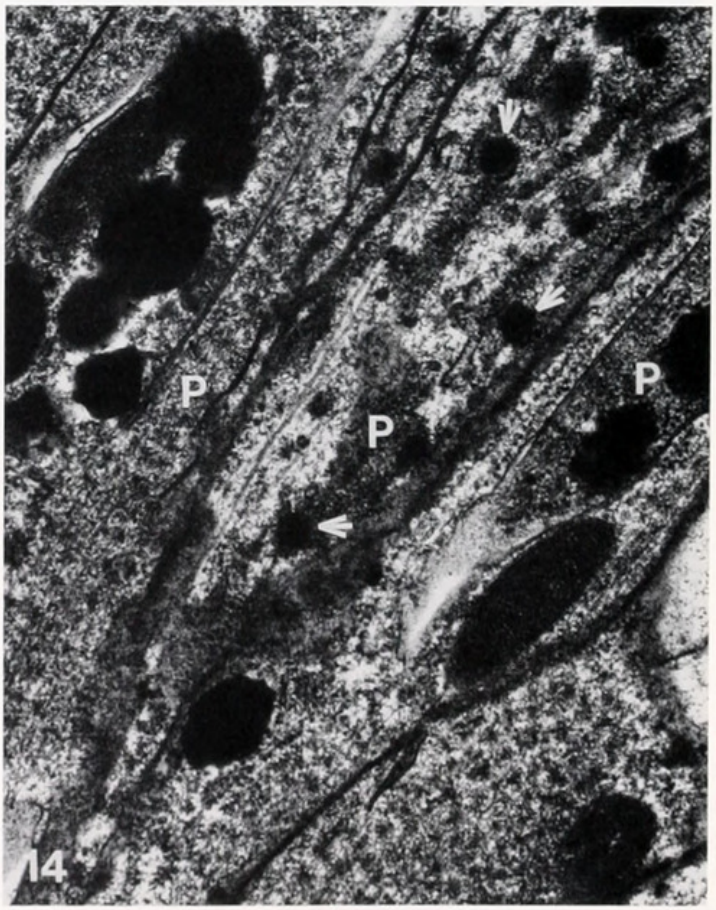

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upper apical regions, and electron-dense, non-membrane bound droplets and dense-cored, membrane-bound vesicles (Figs. 1, 2, 4, and 8). However, there are fewer mitochondria than in dark sensory cells, and the mitochondria are not arranged in the distinct rows that characterize the dark cells (Fig. 4).

The basal extensions of dark sensory cells contain numerous mitochondria, microtubules, electron-dense droplets and dense-cored vesicles and often bifurcate to form two neurites that project into the ganglionic plexus (Fig. 9). In contrast, the basal processes of light sensory cells have not been seen to bifurcate, and they appear to contain fewer droplets and vesicles than do the processes of dark sensory cells (Fig. 10). Thus, the dark sensory cells appear to be bipolar, whereas the light cells may be considered as unipolar.

During the development of both types of sensory cells, the apical region of each cell becomes specialized before the basal region (Figs. 7, 8, 11–13). One or several Golgi complexes, depending on the type of sensory cell, form early in close association with the nucleus. Droplets and vesicles soon appear within the region of the Golgi (Figs. 7, 8, 13). Concurrent with the appearance of the Golgi, mitochondria and microtubules fill the apical cytoplasm. Next, the basal regions of the cells become specialized to form neurites. Mitochondria, microtubules, droplets, and vesicles appear within the forming basal neurites (Figs. 9, 14).

FMRFamide-like immunoactivity is observed in paraffin sections and wholemounts of planulae of *Halocordyle disticha* (Figs. 15, 17–26). Such immunoactivity is first detected in single cells in the ectoderm at 24 hours postfertilization in the anterior region of the planula (Fig. 15). Before 24 hours animals lack immunostaining (Fig. 16). As the planulae mature, the immunostaining increases and appears scattered along the planular anterior-posterior axis (Figs. 17–22). The appearance and distribution of the FMRFamide-like positive cells corresponds to the appearance and distribution of some of the sensory cells as viewed by transmission electron microscopy. As planulae age, some of the positively staining cells appear in clusters (Figs. 18–22) and display the characteristic morphology of sensory cells: columnar cells in the ectoderm with tiny tortuous processes that project toward the mesoglea. An examination of the FMRFamide-like positive cells in paraffin sections confirms that they are sensory cells (Fig. 28). When such sections are subsequently stained with azure B, the immunopositive cells stain faintly as they lack apical granules. The only other columnar cells in the ectoderm, glandular and epitheliomuscle cells, possess numerous large apical granules; such granules stain darkly. Thus, epitheliomuscle cells and glandular cells stain darker with azure B than do the sensory cells. Furthermore, light azure B-staining sensory cells are first detected at 24 hours postfertilization, whereas dark azure B-staining cells are visible shortly after gastrulation (10–12 hours postfertilization). No distinction between dark and light sensory cells, as seen via transmission electron microscopy, is possible at the light microscopic level.

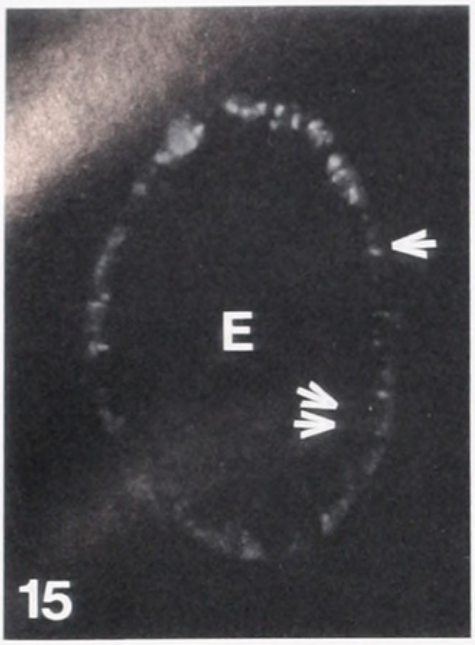
Examination of paraffin sections from different aged planulae and from different axial regions of the planula illustrates the abundance and distribution of immunopositive cells with respect to axial location and developmental time. All FMRFamide-like expression, with the exception of a few scattered fluorescent dots in the endoderm, is confined to the planular ectoderm (Figs. 15, 17–23). Developmental expression of the FMRFamide-like peptide by sensory cells is such that it is first detected in the upper apical two-thirds of the cell and last, if at all, in the basal region (Figs. 15, 17–22). The upper portion of the cell exhibits brilliant immunostaining while the basal processes stain weakly. Hence, the first immunopositive cells to appear at 24 hours exhibit intense fluorescence in their apical regions and little or no staining in their basal ends (Fig. 15). Examination of different axial regions of mature planulae indicates that FMRFamide-like immunopositive cells are found along the entire planular axis by 48–72 hours postfertilization (Figs. 18–22). However, there does appear to be more immunopositive cells at the anterior end of the planula than at the posterior end (Figs. 20–22). Just prior to metamor-

Figure 11. Apical region of a differentiating light sensory cell in a 72 hour planula. Apical differentiation of sensory cells precedes basal differentiation as Golgi complexes, microtubules, electron-dense droplets (single arrow), and dense-cored vesicles (double arrows) fill the apical cytoplasm. Electron-dense droplets, dense-cored vesicles, microtubules, and mitochondria form later in the basal processes. ×32,000.

Figure 12. Immature basal extensions of light sensory cells. The processes (P) are not yet filled with electron-dense droplets, dense-cored vesicles, or mitochondria. These neurites extend into a well-formed ganglionic plexus (GP). M, mesoglea. ×15,000.

Figure 13. Golgi region in the apex of a light sensory cell. Electron-dense droplets and dense-cored vesicles (arrows) are found in the area of the Golgi. ×63,000.

Figure 14. Differentiating basal processes (P) of light sensory cells. These processes become filled with mitochondria, electron-dense droplets (arrows), and dense-cored vesicles (not visible). Compare this micrograph with Figure 12 illustrating immature basal processes of light sensory cells. ×31,000.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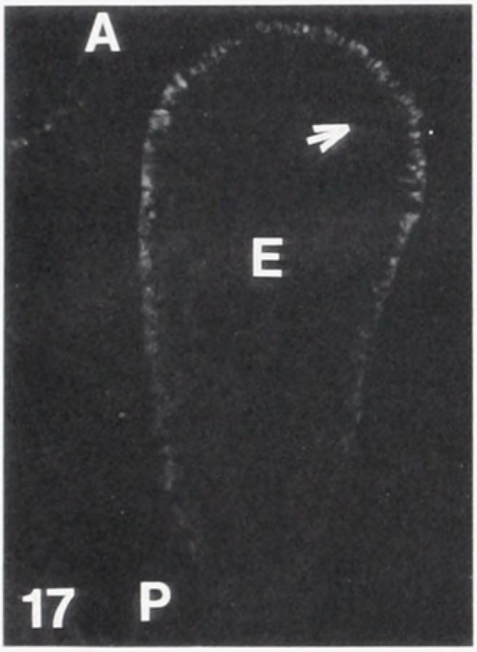
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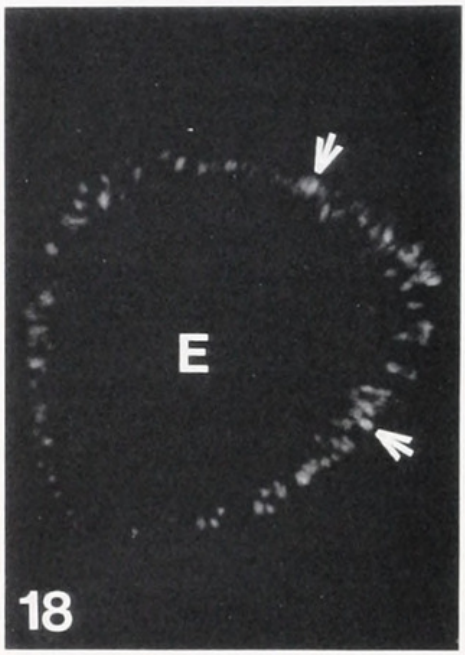
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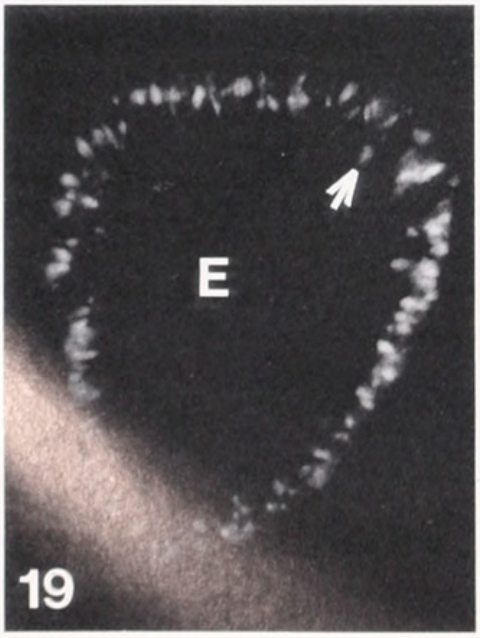
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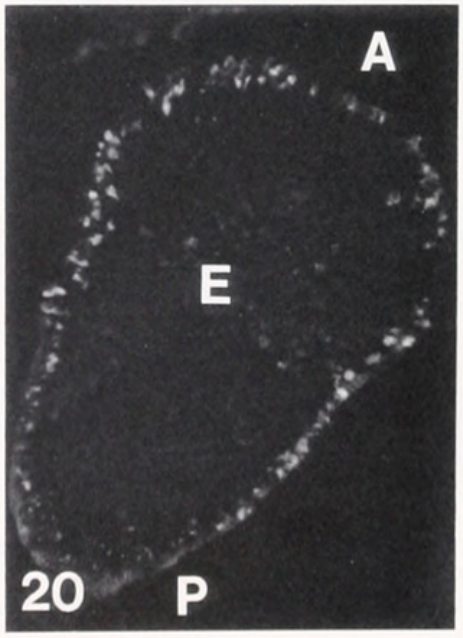
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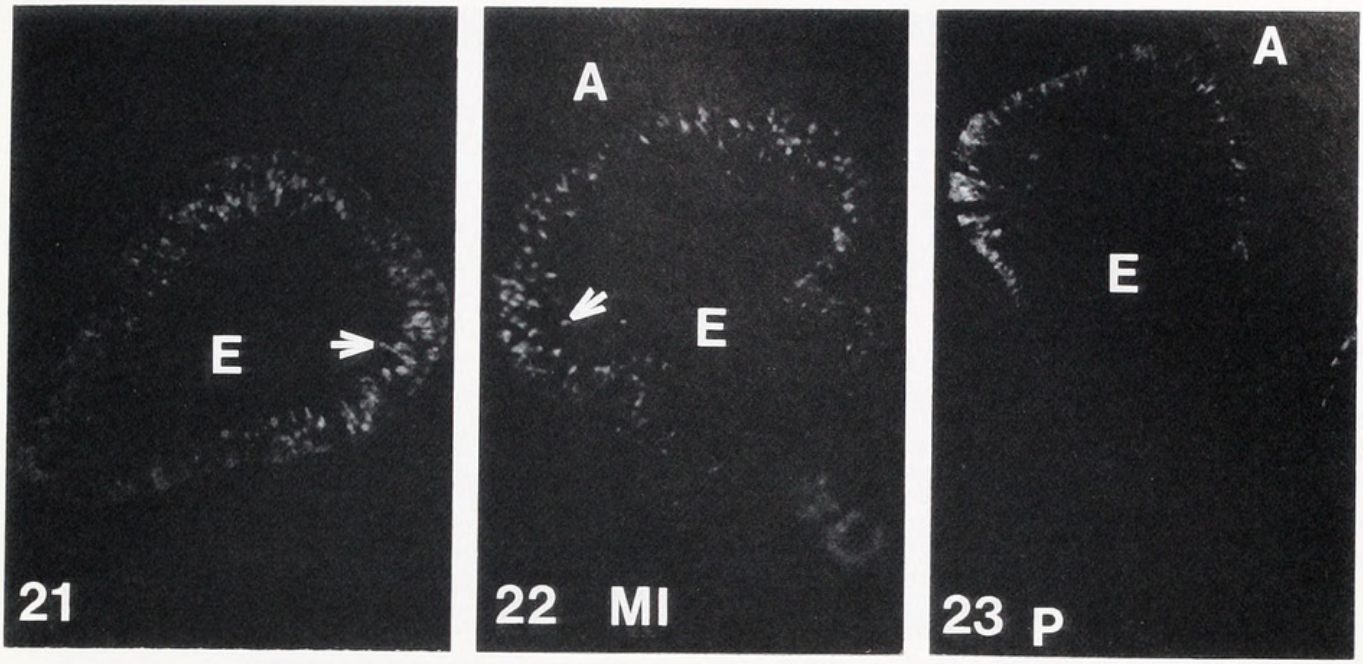

Figure 21. Slightly oblique paraffin section through the anterior region of a young 96 hour planula. Basal processes (arrow) of some sensory cells exhibit weak FMRFamide-like activity. E, endoderm. ×150.

Figure 22. Longitudinal paraffin section of a young 96 hour planula. Immunopositive cells are abundant in the anterior region (A) and a few are visible along the mid-sides (MI) of the animal. The arrow denotes the location of the mesoglea. E, endoderm. ×220.

Figure 23. Longitudinal paraffin section of a mature 120 hour planula just prior to metamorphosis. Immunopositive cells are present in the anterior (A) region of the planula, however, they are absent from the more posterior (P) regions of the animal. E, endoderm. ×100.

Figure 15. Paraffin section through the anterior region of a 24 hour planula. A few FMRFamide-positive cells first appear in the anterior region of the planula at this stage of development. These cells probably correspond to some of the first sensory cells seen via transmission electron microscopy. The FMRFamide-like peptide is first expressed in the apices of these cells (as indicated here) and only later in the mid to basal regions of the cells. Expression of the peptide-like material at this time is confined to a few ectodermal cells, as the endoderm (E) lacks immunostaining. The single arrow denotes the outer edge of the ectoderm, whereas the double arrows indicate the mesoglea. ×230.

Figure 16. Paraffin section through the anterior region of a 16 hour planula. These embryos do not express the FMRFamide-like peptide, as indicated by their lack of immunostaining. The arrow indicates the outer margin of the ectoderm. E, endoderm. ×250.

Figure 17. Longitudinal paraffin section of a 36 hour planula. As development proceeds, the number of cells expressing the FMRFamide-like peptide increases, as demonstrated by the larger number of positive-staining cells at 36 hours compared to 24 hours (Fig. 15). By 36 hours, immunopositive cells are visible in the anterior region (A) of the planula and also along the sides of the planula. For the most part, the immunostaining at this stage is strongest in the apical regions of cells. The arrow indicates the location of the mesoglea. E, endoderm; P, posterior. ×150.

Figure 18. Paraffin cross section through the mid region of a 72 hour planula. The number of immunopositive cells has increased by 72 hours, and many of these cells associate to form intense immunopositive clusters (arrows) along the length of the planula. E, endoderm. ×200.

Figure 19. Oblique paraffin section of a 72 hour planula. Single immunopositive cells and clusters of immunopositive cells are visible in the planular ectoderm. By 72 hours, in those cells that express the FMRFamide-like peptide, the immunostaining is not confined solely to the apices of the cells but has extended to include the mid regions of the cells, and in some cases the basal regions of the cells (arrow). Faint staining of basal processes is seen in the anterior region of the planula and decreases towards the posterior end of the planula. E, endoderm. ×220.

Figure 20. Oblique paraffin section of a 72 hour planula. Immunopositive sensory cells are found in the ectoderm along the entire anterior-posterior axis of the planula. There appears to be more immunopositive cells in the anterior region (A) of the planula than in the more posterior regions (P). A few immunopositive small cells are visible in the anterior endoderm (E) at this stage, and these cells represent a subpopulation of interstitial cells differentiating along the ganglionic cell line. ×200.

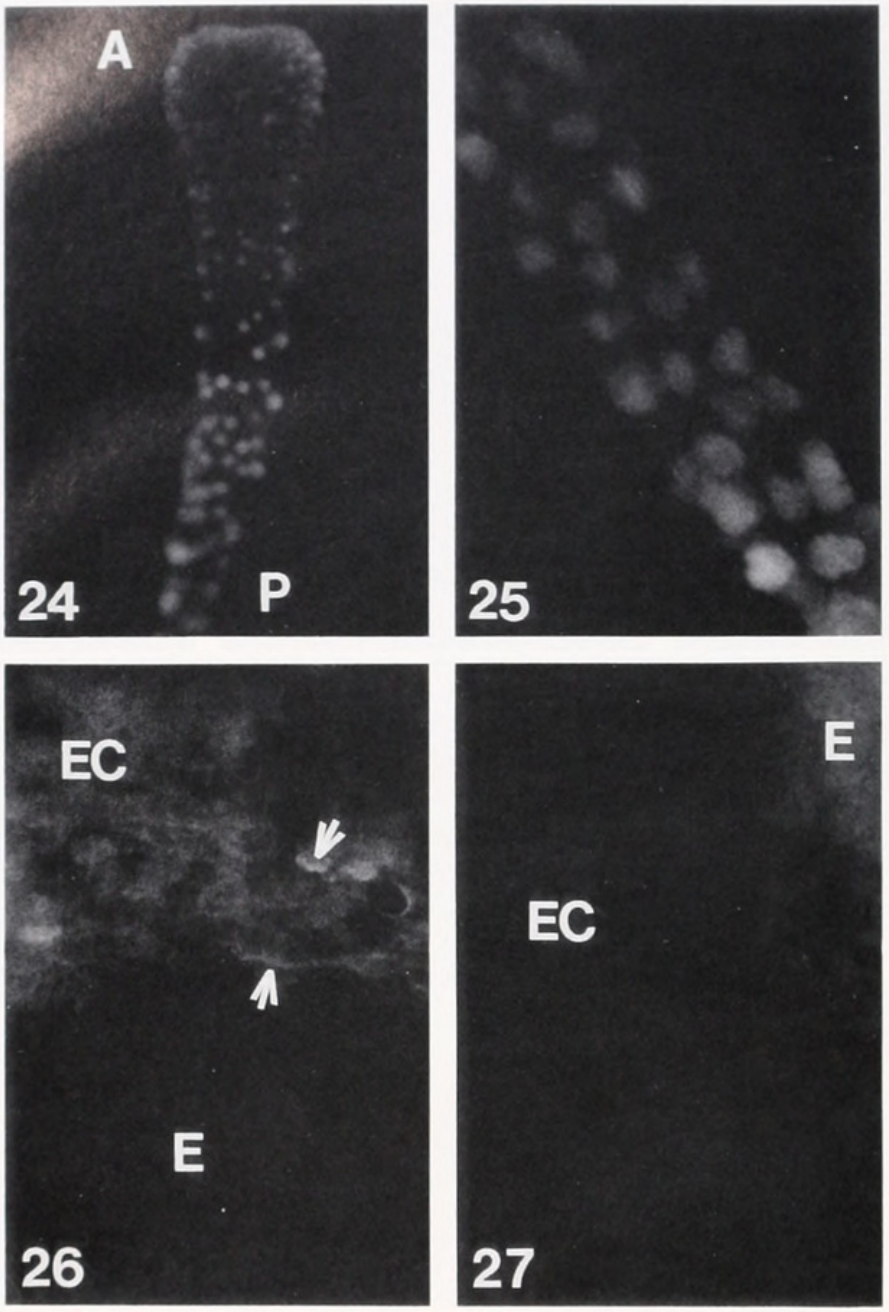

Figure 24. Distribution of clusters of immunopositive cells in a wholemount of a 96 hour planula. These cells are found in the anterior (A), middle, and posterior (P) regions of the planula. ×80.

Figure 25. Wholemount of the mid to posterior region of a 96 hour planula showing the distribution of FMRFamide-like positive clusters of cells. ×90.

Figure 26. Wholemount of a mature planula showing the anterior region. Processes (arrows) stain faintly and are located in the ectodermal region of the ganglionic plexus and also just above the plexus. E, endoderm; EC, ectoderm. ×250.

Figure 27. Wholemount of a mature planula showing the posterior region. There are no positive staining processes detected in this area. E, endoderm; EC, ectoderm. ×250.

phosis, a large number of immunopositive cells are detected in the extreme anterior region of the planula (Fig. 23). Basal processes of sensory cells located in the anterior region of the planula stain more intensely than do those of sensory cells distributed in the mid to posterior region of the animal (Figs. 21–23).

The spatial distribution of cells expressing the FMRFamide-like peptide is easily visualized using wholemounts of planulae (Figs. 24–27). In mature planulae (96 h) clusters of immunopositive cells are visible as large dots scattered along the length of the animal. A few of these FMRFamide-like positive clusters first appear in the anterior region of the late 24 hour planula and later in the mid to posterior regions of the mature planula. In wholemounts, the basal processes of the sensory cells are very difficult to visualize as they are tiny and stain only

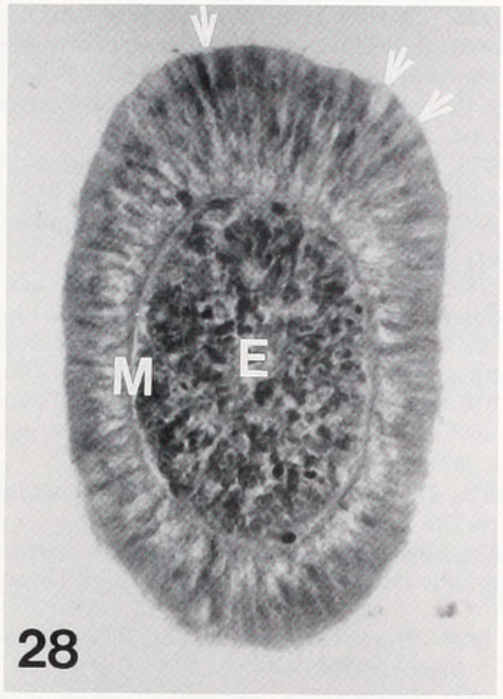

Figure 28. Paraffin section through the anterior region of a maturing planula stained with azure B. Light staining sensory cells (arrows) are found among darker staining epitheliomuscle cells and glandular cells. Dark staining cells outnumber the light staining cells. When processed for immunocytochemistry the light cells exhibit a positive response for FMRFamide-like activity. E, endoderm; M, mesoglea. ×250.

faintly (Fig. 26). Visualization of weak immunopositive basal processes is more obvious in paraffin sections (Fig. 21).

Wholemounts and paraffin sections of planulae stained with FMRFamide antiserum preabsorbed with 10 μg/ml synthetic FMRFamide do not exhibit any immunopositive staining. Furthermore, treatment of samples with antiserum preabsorbed with 1 μg/ml FMRFamide results in very dim staining of cells.

Discussion

Using immunocytochemistry and radioimmunoassays, Grimmelikhuijzen and associates identified substances resembling vertebrate or invertebrate neuropeptides in the nervous systems of adult cnidarians (Grimmelikhuijzen and Graff, 1985, 1986; Grimmelikhuijzen and Groeger, 1987). The most common neuropeptides seen were those related to the molluscan neuropeptide Phe-Met-Arg-Phe-amide (FMRFamide). When anti-FMRFamide was applied to adult cnidarians, cells containing peptides ending in -Arg-Phe-NH_2 bound the antiserum. Recently, Grimmelikhuijzen and co-workers isolated and sequenced a specific neuropeptide, pGlu-Gly-Arg-Phe-amide (antho-RFamide), from sea anemones and pennatulids (Grimmelikhuijzen and Graff, 1985, 1986; Grimmelikhuijzen and Groeger, 1987).

In this study hydrozoan planulae of different developmental ages were tested for their ability to bind a rabbit antiserum raised to FMRFamide to determine if peptides ending in -Arg-Phe-NH_2 were present in larval cnidarians and, if so, to determine when in development the gene for such a peptide (or peptide family) was expressed. Planulae of *Halocordyle disticha* exhibited a positive staining response when exposed to anti-FMRFamide, indicating that peptides ending in -Arg-Phe-NH_2 are present in cnidarian larvae. The expression of the FMRFamide-like peptide was first observed at 24 hours postfertilization in the ectoderm in association with some of the sensory cells. As planulae matured, the number of immunopositive sensory cells increased, and such cells were seen along the entire length of the planula. Just prior to metamorphosis a large number of FMRFamide-positive cells appeared in the anterior ectoderm (attachment end), suggesting the involvement of the FMRFamide-like peptide in planular attachment or metamorphosis.

The time of appearance of the synthetic machinery of planular sensory cells, and the pattern of appearance of FMRFamide-like immunofluorescence, appear to be correlated. Ultrastructural examination of planulae reveals that the planular nervous system begins to form in the ectoderm at 24 hours in development. A few sensory cells are found in the anterior end of the planula at this time, and as planulae age, the number of sensory cells increase. Furthermore, as sensory cells differentiate, their apical regions become specialized before their basal regions. One of the first differentiative events detected in the sensory cell is the formation of one to several Golgi complexes in close association with the nucleus. Shortly after the formation of the Golgi complexes, electron-dense droplets and dense-cored vesicles appear in the apical cytoplasm, and only later in the forming basal neurites. Individual immunopositive cells first appear at 24 hours in the anterior ectoderm of the planula and, most probably, correspond to the first sensory cells seen via transmission electron microscopy. As development progresses, many of these immunopositive cells become organized into clusters along the length of the planula. Furthermore, such immunopositive cells exhibit brilliant immunofluorescence first in their apical regions, and only later do they show a dim staining in their basal regions. Such a spatial and temporal staining response in the immunopositive cells is expected in view of the ultrastructural findings concerning time of appearance and location of the Golgi complexes, droplets, and vesicles.

The presence of peptides in the nervous systems of planulae suggest that: (1) peptides may play crucial roles in the development of these larvae; and (2) peptides may be important for metamorphosis. In either case, the sim-

ple nervous system of the planula provides an excellent system with which to analyze neuropeptide action on developmental processes. Also, because the planula can be easily visualized, maintained, and acquired throughout the entire metamorphic process, it offers a unique developmental model for examining the temporal appearance of new neuropeptides and for analyzing possible switches that may occur in neuropeptide phenotype (*i.e.*, plasticity of neuropeptide expression) as an animal passes from the embryonic condition to the adult state.

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