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Localization of Allatostatin-Immunoreactive Material in the Central Nervous System, Stomatogastric Nervous System, and Gut of the Cockroach *Blattella germanica*

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Immunoreactivity against peptides of the allatostatin family having a typical YXFGL-NH_2 C-terminus has been localized in different areas of the central nervous system, stomatogastric nervous system and gut of the cockroach *Blattella germanica*. In the protocerebrum, the most characteristic immunoreactive perikarya are situated in the lateral and median neurosecretory cell groups. Immunoreactive median neurosecretory cells send their axons around the circumesophageal connectives to form arborizations in the anterior neuropil of the tritocerebrum. A group of cells in the lateral aspect of the tritocerebrum project to the antennal lobes in the deutocerebrum, where immunoreactive arborizations can be seen in the periphery of individual glomeruli. Nerve terminals were shown in the corpora allata. These terminals come from perikarya situated in the lateral neurosecretory cells in the pars lateralis and in the subesophageal ganglion. Immunoreactive axons from median neurosecretory cells and from cells positioned in the anteriormost part of the tritocerebrum enter together in the stomatogastric nervous system and innervate foregut and midgut, especially the crop and the valve between the crop and the midgut. The hindgut is innervated by neurons whose perikarya are located in the last abdominal ganglion. Besides immunoreactivity in neurons, allatostatin-immunoreactive material is present in endocrine cells distributed within the whole midgut epithelium. Possible functions for these peptides according to their localization are discussed. Arch. Insect Biochem. Physiol. 37:269–282, 1998. **© 1998 Wiley-Liss, Inc.**

Key words: Allatostatin; *Blattella germanica;* immunocytochemistry; peptide

Abbreviations used: CA, corpora allata; CC, corpora cardiaca; FITC, fluorescein isothiocyanate; JH, juvenile hormone; PAP, peroxidase-antiperoxidase

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INTRODUCTION

Allatostatins are neuropeptides with a typical YXFGL-NH_2 C-terminus that were initially identified by their inhibitory activity on juvenile hormone (JH) production in the cockroach *Diploptera punctata* [Pratt et al., 1989; Woodhead et al., 1989]. Until now, peptides structurally related to these allatostatins have been identified in cockroaches [*D. punctata:* Pratt et al., 1989, 1991; Woodhead et al., 1989, 1994; *Periplaneta americana:* Weaver et al., 1994; *Blattella germanica:* Bellés et al., 1994], crickets [*Gryllus bimaculatus:* Lorenz et al., 1995], flies [*Calliphora vomitoria:* Duve et al., 1993, 1994, 1996], locusts [*Schistocerca gregaria:* Veelaert et al., 1996a,b], and moths [*Cydia pomonella:* Duve et al., 1997; *Helicoverpa armigera:* Duve et al., unpublished data]. Furthermore, a cDNA encoding a prepro-hormone of this allatostatin family has been cloned and sequenced in the cockroaches *D. punctata* [Donly et al., 1993] and *P. americana* [Ding et al., 1995] and the locust *S. gregaria* [Vanden Broeck et al., 1996]. In the blowflies *C. vomitoria* and *Lucilia cuprina,* the gene encoding for homologous Leu-callatostatins [Duve and Thorpe, 1994] has been also cloned and characterized [East et al., 1996].

In addition to the identification of peptide sequences, the occurrence of allatostatins has been suggested by immunocytochemical techniques in different insects [Duve and Thorpe, 1994; Duve et al., 1995, 1997; Neuhäuser et al., 1994; Stay et al., 1992, 1994; Ude and Agricola, 1995; Veelaert et al., 1995; Yoon and Stay, 1995; Yu et al., 1995]. These studies showed allatostatin-like material to be widespread in neurons and endocrine cells, as well as in the corpora allata (CA) of certain insects.

The peptides identified in *C. vomitoria* were inactive as inhibitors of CA activity in the blowfly, whereas they showed a potent antimyotropic action on gut motility [Duve et al., 1993, 1996]. In fact, allatostatins have been shown to inhibit gut motility in blowflies, cockroaches, and moths [Duve and Thorpe, 1994; Duve et al., 1995, 1997; Lange et al., 1993, 1995] and peristaltic movements of the oviduct of locusts [Vanden Boeck et al., 1996; Veelaert et al., 1996a]. Furthermore, studies reporting the occurrence of allatostatins in the hemolymph of *D. punctata* [Yu et al., 1993] suggest that these peptides act as "classic" hormones in peripheral tissues. In connection with this, it is worth mentioning the inhibitory effects of allatostatins on vitellogenin production by the fat body of *B. germanica* described by Martín et al. [1996]. Taken together, the available data suggest that YXFGL-NH_2 allatostatins may have multiple functions, which can vary from species to species.

In the cockroach *B. germanica* four allatostatins have been identified [Bellés et al., 1994], and the question arises as to their biological role. Inhibition of JH and vitellogenin production [Bellés et al., 1994; Martín et al., 1996] have been reported, but the above antecedents suggest that other functions may exist. Within this context, we carried out the present immunocytochemical study, which describes the anatomical distribution of allatostatins in *B. germanica*, with the aim of shedding new light on other possible functions of these peptides and facilitating further functional studies in this cockroach.

MATERIAL AND METHODS

Insects and Tissue Preparation

Virgin adult females of *B. germanica* were from a colony reared at 30°C and 60–70% relative humidity and fed on a Panlab dog chow and water. For paraffin sections, tissues were fixed in aqueous Bouin's fluid, and for whole mounts in 2% paraformaldehyde in phosphate buffer, at pH 7.2.

Immunocytochemistry

The antiserum used in the present study was raised against the peptide Leu-callatostatin 3 (ANRYGFGL-NH_2) from the blowfly *C. vomitoria* [Duve et al., 1993]. The method for production of the antiserum was described by Duve and Thorpe [1994]. In the C-terminal sequence characteristic of the allatostatin family (YXFGL-NH_2), Leu-callatostatin 3 has Gly in the fourth position from the C-terminus. In the cockroach *B. germanica,* four peptides belonging to the allatostatin family have been purified using the same antiserum [Bellés et al., 1994]; one peptide (BLAST-4) also has a Gly residue in that position. Since *B. germanica* allatostatins are structurally similar to Leu-callatostatin-3 and the other members of the allatostatin family, we assume that the immunoreactivity observed in *B. germanica* tissues using this antiserum is generally representative of sites of allatostatin occurrence.

For every tissue and immunocytochemical technique, at least 15 individuals were used. The peroxidase-antiperoxidase (PAP) method for par-

affin sections was that of Sternberger [1974], as modified by Duve and Thorpe [1994]. Brains were dissected, fixed, embedded in paraffin, and cut in 8-μm-thick sections. Mapping of axon pathways was established by examining series of consecutive sections. The primary antiserum was used at a concentration of 1:1,000. The second, link antibody comprised swine anti-rabbit immunoglobulins (1:20) (DAKO, Denmark) and the third antibody was a rabbit PAP (1:50) (DAKO). Immunostaining was achieved by using diaminobenzidine (100 mg/200 ml Tris-buffer, pH 7.6) in the presence of H_2O_2. For whole mounts, the immunofluorescence method described by Tsang and Orchard [1991] was followed. It uses the primary antiserum at 1:1,000 and, as the second antibody, an FITC (fluorescein isothiocyanate)-conjugated swine anti-rabbit immunoglobulin preparation (DAKO) at 1:20. Using both techniques, incubation with the primary antisera was at 4°C for 20 h. For control experiments, consecutive sections were incubated with primary antiserum and with the primary antiserum preabsorbed overnight at 4°C with 20 nmol/ml Leu-callatostatin 3.

RESULTS

Brain, Subesophageal Ganglion, and Retrocerebral Complex

Immunoreactivity was observed in perikarya throughout several characteristic regions of the brain and subesophageal ganglion, all of them positioned symmetrically about the sagittal plane. In the protocerebrum, certain of the lateral neurosecretory cells of the pars lateralis show immunoreactivity (Fig. 2A–C) and project toward the retrocerebral complex. Among the median neurosecretory cells in the pars intercerebralis, only two pairs of cells, localized in a posterior dorsomedial position near the surface of the brain, show immunoreactivity (Figs. 1A, 3B). The neurites from their perikarya project anterior and immediately prior and dorsal to the central body. They subsequently bifurcate, the shorter branches forming a dendritic tree (Fig. 3C,G), whereas the major branches project anterior dorsally to the central body, then change direction and descend through the circumesophageal connectives (Fig. 3D–F) to the anterior neuropil of the tritocerebrum, where they form arborizations (Fig. 3G–I). In addition, some of these axons may also project to the connective leading to the frontal ganglion (Fig. 3J) and either terminate here or project further posterior along the esophageal nerve (see also Fig. 5A). No immunoreactive perikarya were observed in the frontal ganglion.

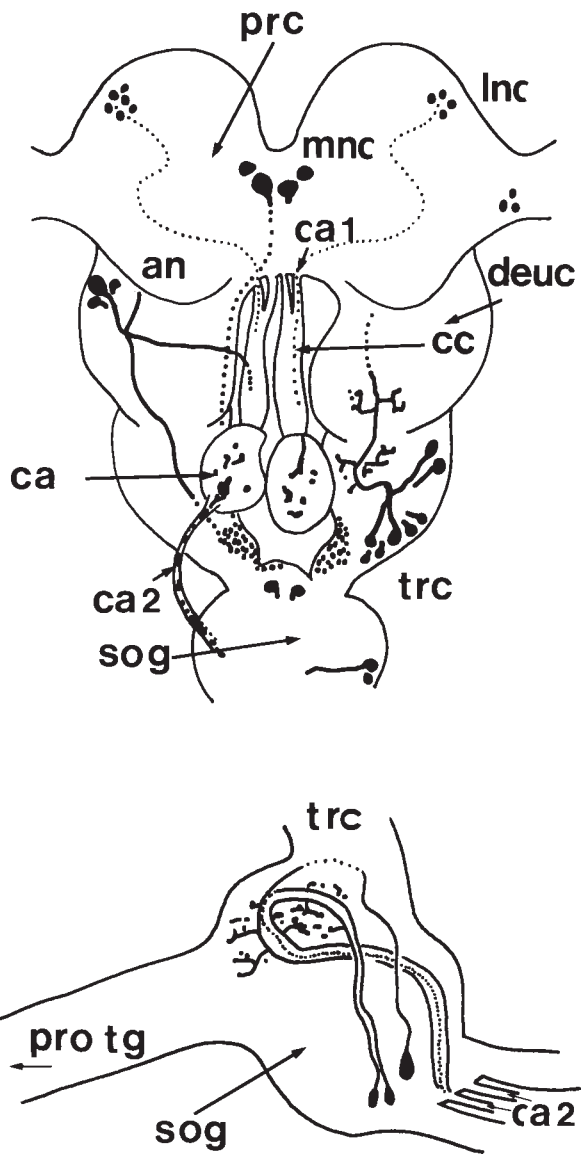


Fig. 1. **Top:** Drawing of brain (anterior frontal view) and retrocerebral complex folded forward, showing the position of the immunoreactive neurons described in this study. **Bottom:** Drawing of subesophageal ganglion (lateral view, anterior right) showing immunoreactive neurons and projections. an, antennal lobe; ca, corpora allata; cal, nervi corporis allati-1; ca2, nervi corporis allati-2; cc, corpora cardiaca; deuc, deutocerebrum; lnc, lateral neuroscretory cells; mnc, median neurosecretory cells; prc, protocerebrum; pro tg, prothoracic ganglion; sog, subesophageal ganglion; trc, tritocerebrum.

In the lateral aspect of the tritocerebrum, a characteristic group of 6–7 immunoreactive perikarya (Fig. 4F–H) project their neurites dorsally into the tritocerebrum neuropil, where they join together and project in an axonal bundle further

dorsal into the deutocerebrum. Some of these axons can be followed in the antennal lobes (Fig. 4C,D), where immunoreactive arborizations can be observed in the periphery of individual glomeruli (Fig. 4B). Also in the tritocerebrum, 6–8 immunoreactive neurons grouped in a tight cluster in the most anterior area (Fig. 5G,H) show their dendritic tree in the ventral medial neuropil, whereas their axons innervate the frontal ganglion.

The subesophageal ganglion also shows some immunoreactive perikarya and axon tracts (Fig. 1B). Axons from two pairs of immunoreactive

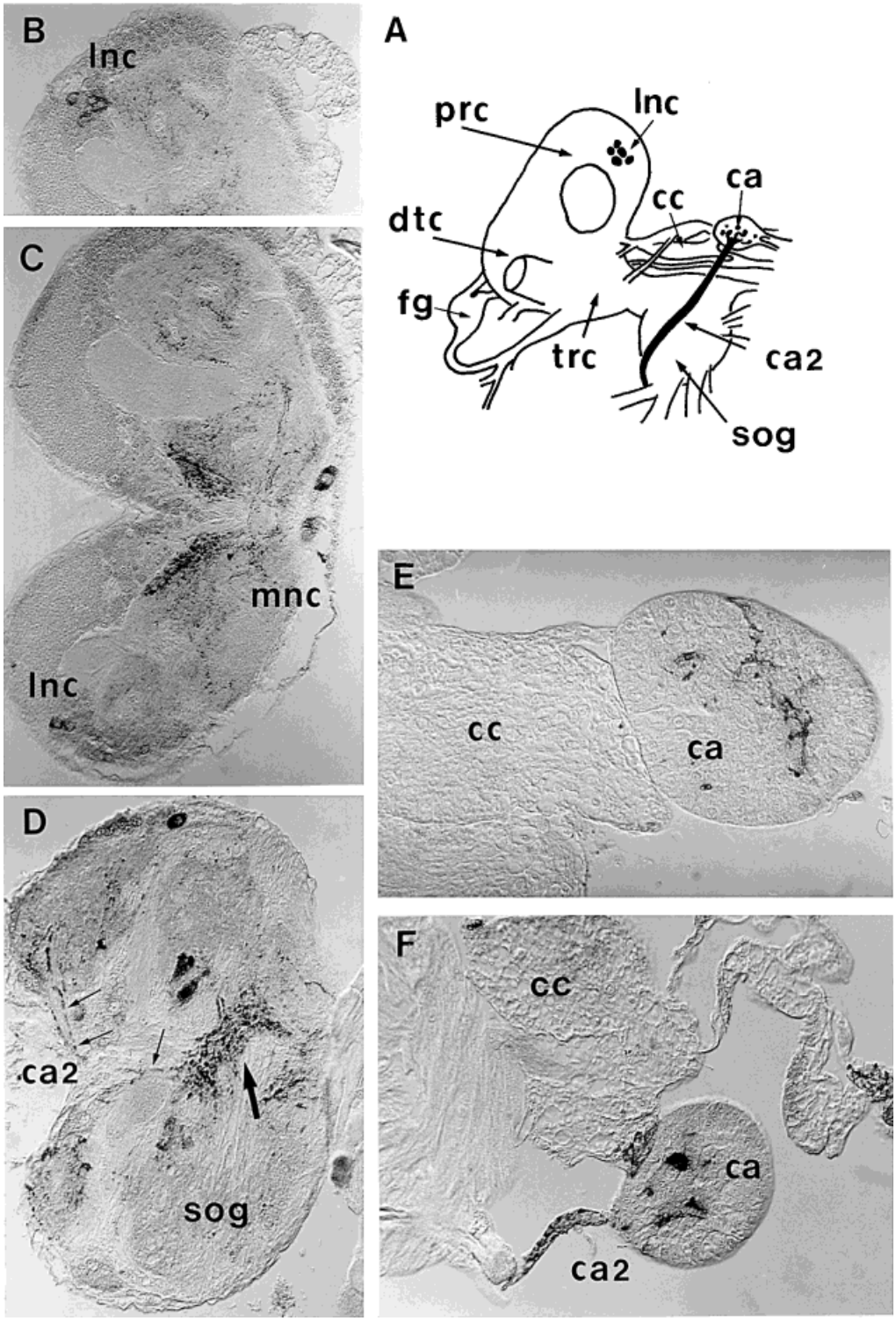


Fig. 2.

perikarya in the lateral surface of the ganglion in either side of the sagittal plane (Fig. 2D) can be followed projecting medially to turn dorsally, where they give rise to numerous immunoreactive arborizations. Then, they turn anterior ventrally to project into the nervi corporis cardiaci-2 and finally inervate CA (Fig. 2F). Two anteriorly positioned neurons also join the above described axonal projection to the CA. Only a small amount of immunoreactivity was observed in the corpora cardiaca (CC). This immunoreactive material had the appearance of belonging to nerve terminals.

Stomatogastric Nervous System and Gut

Foregut and midgut. The ganglia and main nerves of the stomatogastric nervous system are represented in Figure 6A and the perikarya from which the immunoreactive material originates in Figure 5. All these perikarya appear to be localized in the brain, and not to either the hypocerebral or ingluvial ganglia. From the frontal ganglion, the esophageal nerve containing immunoreactive material projects posteriorly through the hypocerebral ganglion into the foregut, where it divides into the two gastric nerves (Fig. 6A,B), which run laterally to the valve between the crop and the midgut. Along the crop, numerous small axonal branches are observed covering its whole surface (Fig. 6C,D); around the valve, the gastric nerves innervate the valve musculature extensively (Fig. 6E). From the valve and ceca, the gastric nerves continue posteriorly into the midgut, where they bifurcate several times and leave the posterior part of the midgut (Fig. 6G). Besides immunoreactive neurons innervating the midgut, numerous immunoreactive endocrine cells are distributed evenly over the whole midgut epithelium (Fig. 6F,G).

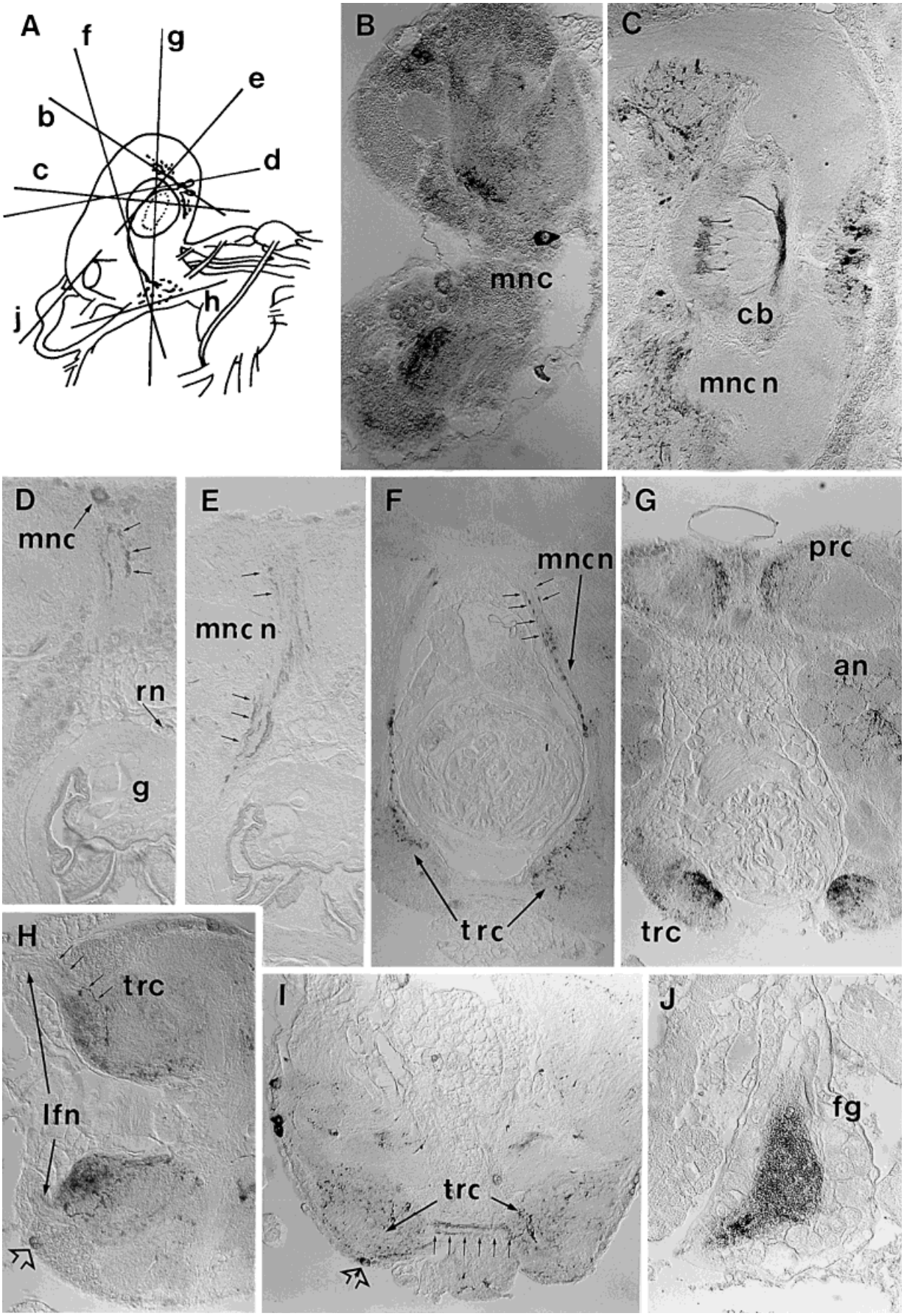
Hindgut. The hindgut is innervated by neurons having their perikarya in the last abdominal ganglion. At least one cell positioned lateroposteriorly in the ganglion projects its major axon contralaterally into the cercal nerve (nerve 11, Fig. 6J). Shortly after branching from the cercal nerve, the proctodeal nerve divides into an anterior and a posterior branch, both of them showing immunoreactivity (Fig. 6H,I). Branches of these nerves reach the midgut surface. In addition to the main innervation of the proctodeum, a bilaterally symmetrical complex of small peripheral nerves associated with the proctodeal nerves is observed (Fig. 6J,K).

Ventral Nerve Cord

Immunoreactivity observed in the subesophageal ganglion, the first ganglion in the ventral nerve cord, and its relationship with the CA, has been described above. In addition, a characteristic small group of perikarya positioned lateroposteriorly in the metathoracic ganglion has been traced in whole mounts (Fig. 6M–O), with their neurites projecting into the neuropil, where the axon bifurcates having its dendritic tree in

Fig. 2. Drawing and paraffin sections of the brain and retrocerebral complex of *B. germanica* immunostained using the peroxidase-antiperoxidase technique with an antiserum directed to the C-terminal sequence of the octapeptide Leucallatostatin 3 (ANRYGFGL-NH_2). **A:** Drawing of the brain and retrocerebral complex (sagittal view, anterior left) showing the innervation of corpora allata (ca) by nervi corporis allati-2 (ca2), originating from perikarya in the subesophageal ganglion (sog). **B,C:** Horizontal sections (B: anterior bottom; C: anterior left) through brain showing perikarya of lateral neurosecretory cells (lnc) in pars lateralis. ×270. **D:** Horizontal section (anterior left) through subesophageal ganglion showing one of six immunoreactive perikarya (top), the neurites of which project centrally, giving rise to dendritic trees (large arrow) with the main axon projecting into the ca2 (small arrow) (see Fig. 1 bottom). ×300. **E:** Longitudinal section of areas of the retrocerebral complex showing immunoreactivity in the corpus allatum (ca), but not in the corpus cardiacum (cc). ×650. **F:** Oblique section through cc and ca, showing immunoreactivity in ca and ca2. ×500. dtc, deutocerebrum; fg, frontal ganglion; mnc, median neurosecretory cells; prc, protocerebrum; trc, tritocerebrum.

Fig. 3. Drawing and sections of the brain of *B. germanica*. Capital figure letters correspond to the plane studied indicated with lower cases in A. **A:** Drawing of the brain in sagittal view showing immunoreactive median neurosecretory cells and their axonal projections traversing to innervate the tritocerebrum. **B:** Horizontal section through the dorsal part of the protocerebrum with one of the four median neurosecretory cells (mnc) showing immunoreactivity. **C:** Horizontal section through the fan-shaped part of the central body (cb). mnc n, median neurosecretory cells nerve. ×270. **D–G:** Sections from a frontal posterior view showing the major axonal projections traversing through the protocerebrum into the tritocerebrum by the circumesophageal connectives, with strongly immunoreactive arborizations in the anterior ventral tritocerebrum neuropil. ×270. **H:** Horizontal section through the tritocerebrum showing immunoreactive axons in the projection into the frontal ganglion (arrows). Open arrow, two immunoreactive cells as demonstrated in Figure 5G and H. ×630. **I:** Frontal section through the tritocerebrum showing immunoreactive arborizations in the neuropil. Contralateral immunoreactive axons indicated by small arrows, somata unknown. Open arrow as in H. ×380. **J:** Horizontal section through the frontal ganglion (fg) showing highly immunoreactive arborizations in the neuropil, but no somata. ×405. an, antennal lobe; g, gut; lfn, labrofrontal nerve; mnc n, median neurosecretory cells nerve; prc, protocerebrum; rn, recurrent nerve; trc, tritocerebrum.



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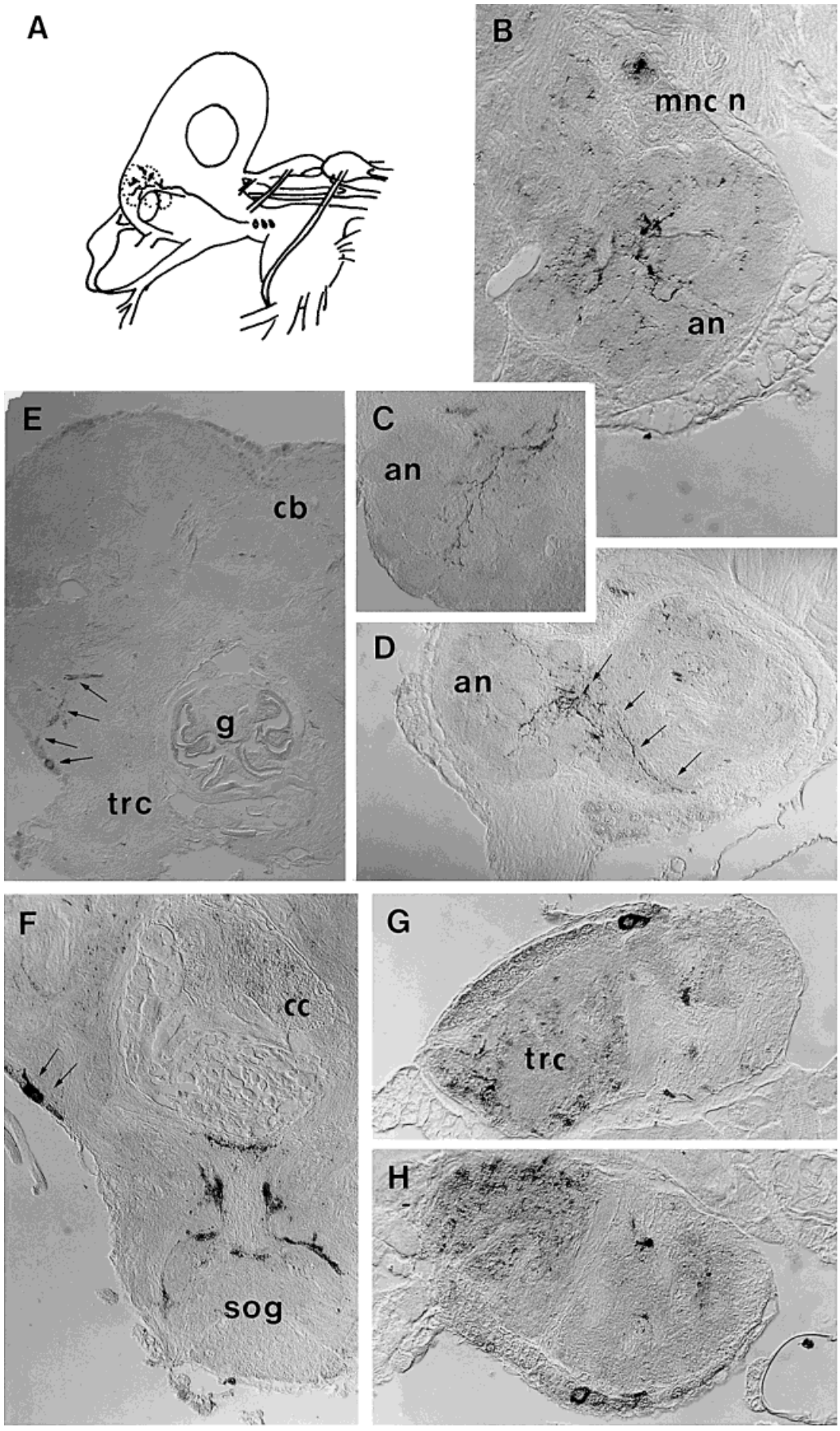

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the contralateral part of the ganglion and their major axon projecting anteriorly into the mesothoracic and prothoracic ganglia. In each ganglion, numerous small branches contribute to a highly immunoreactive neuropil. There are some other immunoreactive neurons in the nerve cord, but other approaches are necessary to characterize their axonal projections with certainty.

DISCUSSION

This study describes the distribution of immunoreactivity to antisera raised against Leucallatostatin 3 (ANRYGFGL-NH_2) in brain, ventral nerve cord, stomatogastric nervous system, and gut of the adult female cockroach *B. germanica*.

The presence of allatostatin immunoreactivity in a neurohemal area in the internal side of the tritocerebrum (Fig. 3G–I), coming from four immunoreactive median neurosecretory cells in the pars intercerebralis, indicates the possibility that allatostatins may be released into the hemolymph and act as circulating hormones in different tissues. In the cockroach *D. punctata* also four strongly immunoreactive cells have been detected in the pars intercerebralis. By contrast, axons from these cells terminate within the protocerebrum, in areas where lateral cells also form arborizations [Stay et al., 1992]. These immunoreactive group of cells has also been reported in *P. americana* [Schildberger and Agricola, 1992].

Allatostatin-immunoreactive material has been found in some cells in the lateral aspect of the tritocerebrum, which send their axons to the antennal lobes (Fig. 4B–D). Allatostatin immunoreactivity in the glomeruli of the antennal lobes was also shown in *D. punctata* [Stay et al., 1992]. The occurrence of immunoreactivity in this area suggests a possible role for allatostatins in olfactory processes. In a similar way, the presence of allatostatin immunoreactivity in some neuron arborizations in the neuropil of the pars intercerebralis (Fig. 3C,G) in the vicinity of the central body, or in interneurons connecting the thoracic ganglia (Fig. 6M–O) suggests a role for these peptides as neurotransmitter/neuromodulator.

Concerning the retrocerebral complex, Stay et al. [1992] reported extensive allatostatin immunoreactivity in arborizations within the CC of *D. punctata*. In the present study, only small amounts of immunoreactivity have been observed in these organs, suggesting that CC are not important neurohemal organs for the release of these peptides in *B. germanica*. On the other hand, the presence of allatostatin-like material in the CA (Fig. 2E,F) is in agreement with the role of allatostatins as inhibitors of JH production in *B. germanica* [Bellés et al., 1994]. Studies carried out in different species showed that in cockroaches and crickets (in which allatostatins inhibit JH production) immunoreactivity can be observed in CA and in the perikarya of the neurosecretory cells that project to them [Stay et al., 1992; Schildberger and Agricola, 1992; Neuhäuser et al., 1994], whereas the CA of flies do not show any allatostatin-immunoreactive material [Duve and Thorpe, 1994; Yoon and Stay, 1995]. In the blowfly *C. vomitoria* callatostatins are unable to inhibit JH bisepoxide synthesis by its CA [Duve et al., 1993]. In the locusts *S. gregaria* and *Locusta migratoria,* despite a report of the presence of immunoreactivity to an antiserum raised against allatostatin-5 of *D. punctata* in the CA [Veelaert et al., 1995], when allatostatins were tested in bioassay for JH synthesis, they did not show any inhibitory effect [Veelaert et al., 1995, 1996a].

Allatostatin immunoreactivity has also been

Fig. 4. Drawing and horizontal sections of the brain of *B. germanica*. **A:** Drawing of the brain and subesophageal ganglion in sagittal view showing the position of 6–7 cells sited on either side in the ventrolateral surface of the tritocerebrum and their axons projecting dorsally. At least some of these cells innervate the antennal lobes, whereas others innervate the tritocerebrum (see Fig. 1, top). **B–D:** Illustration in horizontal sections of the innervation of the antennal lobe (an), with immunoreactive arborizations in the periphery of each of the glomeruli. ×540. **C,D:** Axons and varicosities traversing into the lobes (arrows). ×380. **E:** Posterior frontal aspect of the brain showing one of the perikarya giving rise to the innervation of the antennal lobes (arrows). ×270. **F:** Tritocerebrum with immunoreactive perikarya (arrows). ×360. **G,H:** Horizontal (anterior left) sections through the tritocerebrum showing the lateral position of perikarya and arborizations in the neuropil. ×460. cb, central body; cc, corpus cardiacum; g, gut; mnc n, median neurosecretory cells nerve; sog, subesophageal ganglion; trc, tritocerebrum.

Fig. 5. Drawing and sections of the brain and frontal ganglion of *B. germanica*. **A:** Drawing of brain and part of the stomatogastric nervous system showing immunoreactive somata in the tritocerebrum and median neurosecretory cells (mnc) with axons projecting into the labrofrontal nerve (lfn), the frontal ganglion (fg) and the recurrent nerve (rn). **B–D:** Horizontal consecutive sections showing immunoreactive axons in the anterior part of the esophageal nerve (oe n) containing immunoreactive axons. B: ×530; C,D: ×380. **E–H:** Immunoreactive axons and arborization in frontal ganglion **(E)** and the axons originating in the tritocerebrum **(F–H)** (large arrows indicate somata and small arrows indicate axons). ×380. an, antennal lobe; fc, frontal connective; trc, tritocerebrum.

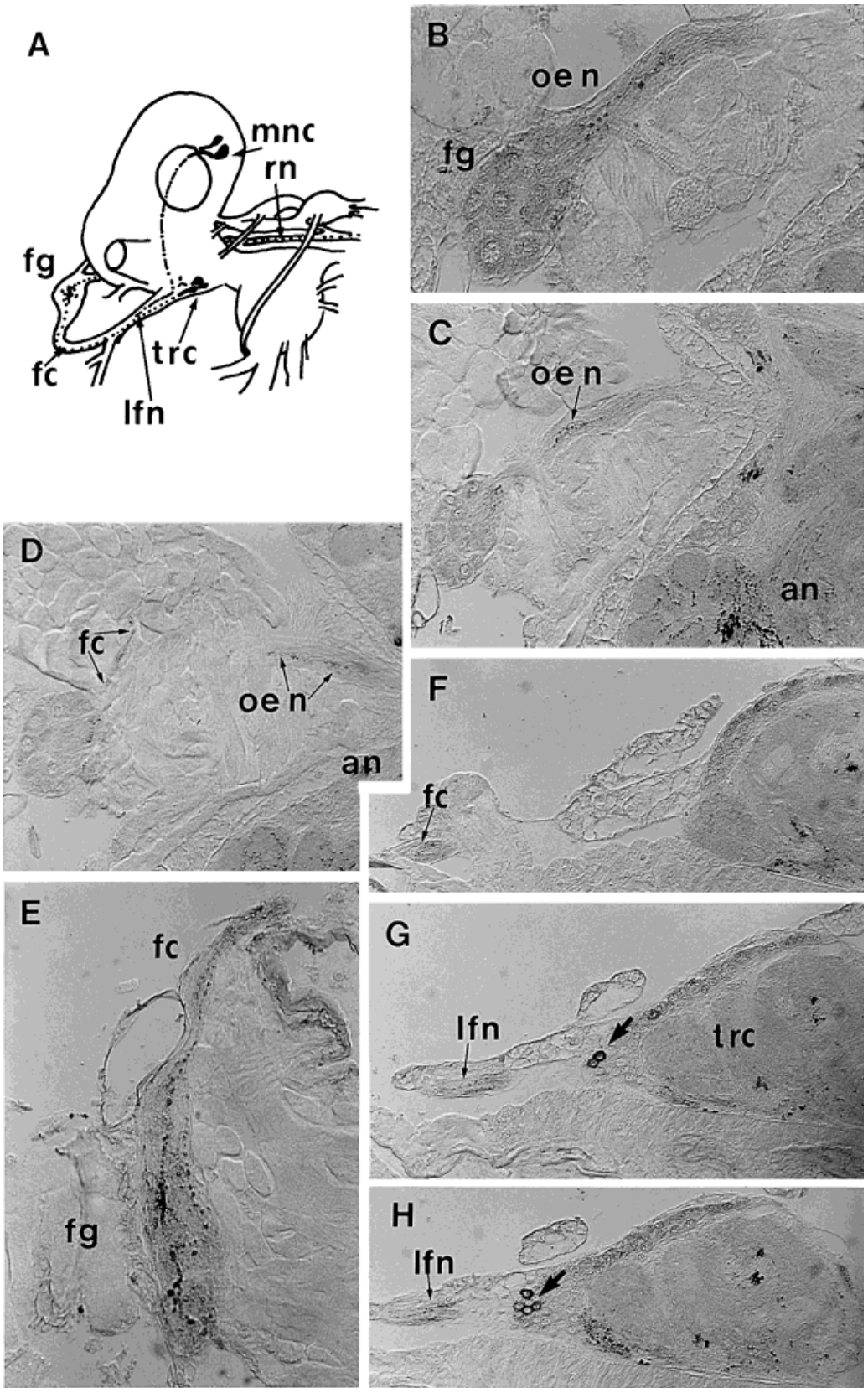
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Fig. 5.

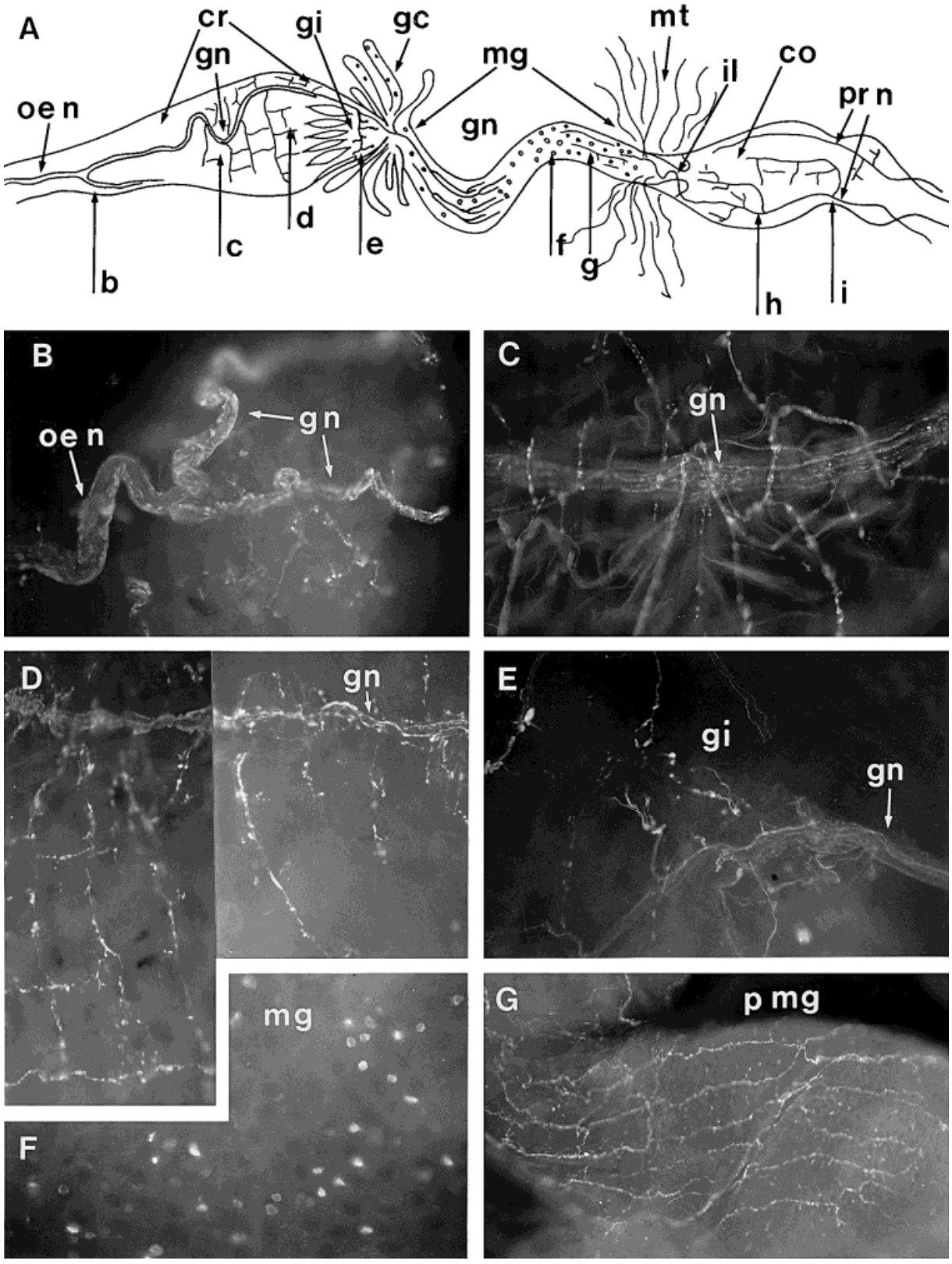

Fig. 6A-G (legend on page 280).

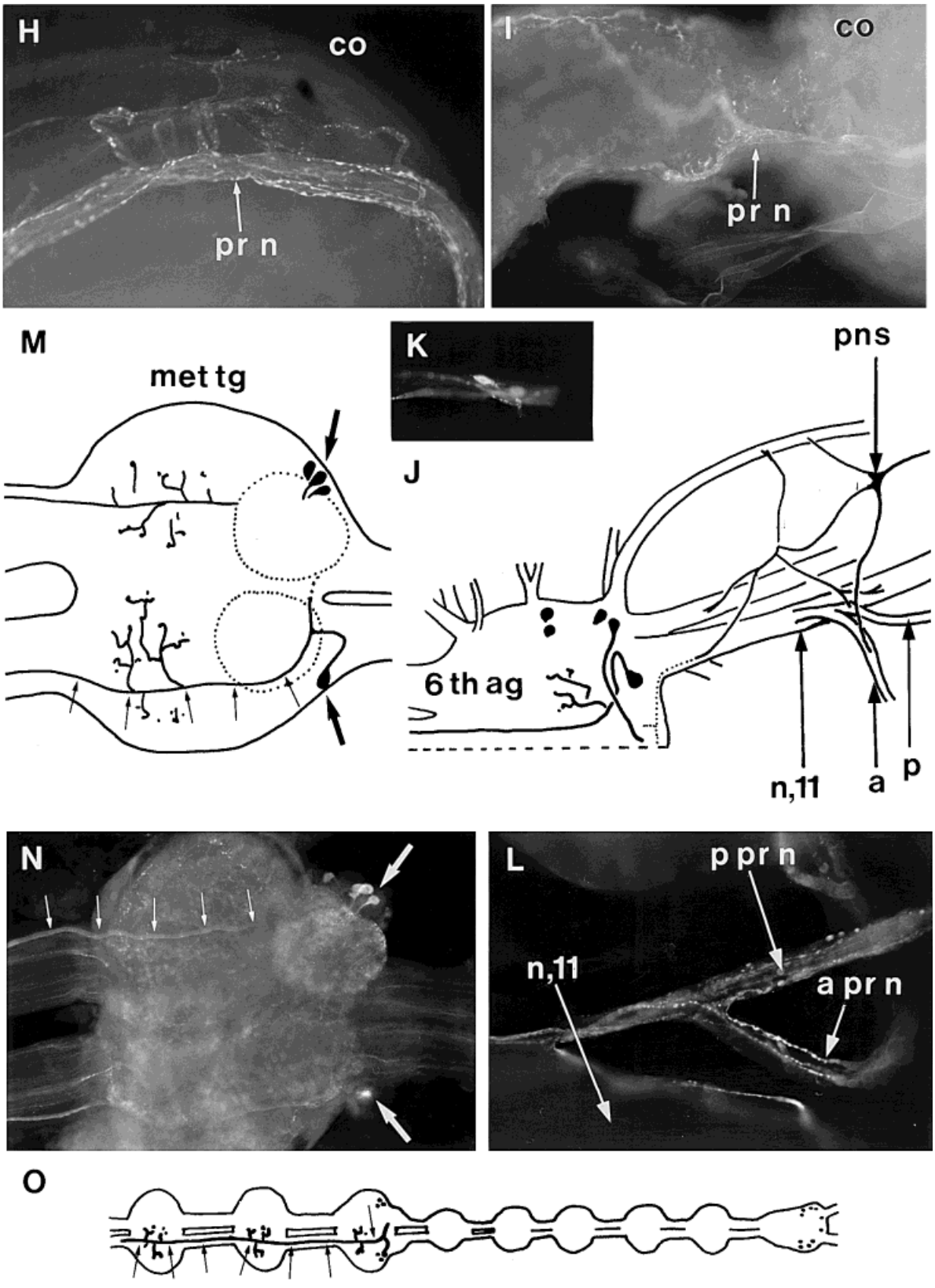

Fig. 6H-O (legend on following page 280).

localized in certain neurons innervating gut musculature. Axons from the four immunoreactive median neurosecretory cells join together with some axons from two clusters of immunoreactive cells in either side of the anterior tritocerebrum (Fig. 5A). These axons lead into the frontal ganglion (Fig. 5B–H) and continue along the stomatogastric nervous system to innervate the foregut and midgut (Fig. 6A–G). Neurons having their perikarya in the last abdominal ganglion (Fig. 6H–J,L) innervate the hindgut via the proctodeal nerves. A similar pattern of immunoreactive cells and axons innervating the gut has been reported in the cockroaches *D. punctata* [Lange et al., 1993] and *Leucophaea maderae* [Duve et al., 1995]. In the moth *C. pomonella* allatostatin-immunoreactive neurons also innervate the gut from the last abdominal ganglion and from the brain, but some immunoreactive perikarya have been detected in the frontal ganglion [Duve et al., 1997]. In *C. vomitoria*, extensive allatostatin-immunoreactive material in the rectal pouch and the ileum has been reported, but neither the foregut nor the midgut show evidence of immunoreactivity [Duve and Thorpe, 1994]. In *Drosophila melanogaster,* allatostatin immunoreactivity is present in axons having their perikarya in abdominal neuromeres and innervating the hindgut and posterior midgut [Yoon and Stay, 1995].

In cockroaches, blowflies, and moths, allatostatins inhibit the contractions of some areas of the gut [Duve and Thorpe, 1994; Duve et al., 1995, 1996, 1997; Lange et al., 1995]. In particular, Leucallatostatin 3 inhibits the contraction of the foregut, but not of the hindgut, in *L. maderae* [Duve et al., 1995] and in *D. punctata* all 13 allatostatins encoded by the cDNA [Donly et al., 1993] are able to inhibit myogenic and proctolin-induced contractions of the hindgut, but do not show any activity on the contraction of the oviduct muscle [Lange et al., 1995]. By contrast, schistostatins, peptides belonging to the allatostatin family identified in the locust *S. gregaria,* inhibit peristaltic movements of the lateral oviduct in this locust [Vanden Broeck et al., 1996; Veelaert et al., 1996a,b].

The presence of allatostatin immunoreactivity in midgut endocrine cells (Fig. 6F,G) has been reported in other cockroaches [Duve et al., 1995; Yu et al., 1995], flies [Duve and Thorpe, 1994; Yoon and Stay, 1995], and moths [Duve et al., 1997]. The function of these cells remains unknown, although a role in detection of the nutrient content of the gut for modulating gut motility or enzyme secretion has been postulated [Duve and Thorpe, 1994; Duve et al., 1995].

Summarizing the data for *B. germanica*, the distribution of allatostatin-immunoreactive material in the pathways leading to the CA is compatible with the inhibitory role of these peptides on JH synthesis as reported by Bellés et al. [1994]. Furthermore, the occurrence of allatostatins in some neurohaemal areas, for example in the anterior neuropile of the tritocerebrum, accounts for a release of allatostatins into the hemolymph and for a truly hormonal action of these peptides. The inhibition of vitellogenin release in the fat body [Martín et al., 1996] may be a reflection of that action. Conversely, immunoreactivity mapped in integrative areas of the nervous system suggests other functions, like neurotransmitter/neuromodulatory properties, or roles related with the modulation of sensory processes. Immunoreactivity associated with the gut points to functions related to gut motility, as it has been reported in

Fig. 6. Drawings and whole mounts of the gut and the ventral nerve cord of *B. germanica.* **A:** Drawing of wholemount preparations of the dorsolateral view of foregut, midgut, and hindgut. Drawing shows the innervation by the stomatogastric nervous system of the foregut and midgut and the main innervation of the hindgut by the proctodeal nerve. The lower cases appearing below the drawing, with arrows to particular parts of the gut, indicate the position from where the picture was taken (indicated by capital letters). **B–E:** Dorsal views of the gastric nerves. **B:** Shortly after the esophagus, the esophageal nerve (oe n) divides into the two gastric nerves (gn). Notably, there is no immunostaining of the ingluvial ganglion. ×115. **C,D:** Prolific network of immunoreactive axons from the gastric nerves of the posterior region of the crop. ×115. **E:** Innervation of the gizzard (gi) by multiple branches of the gastric nerves ×115. **F:** Immunoreactive endocrine cells of the posterior part of the midgut. These cells are distributed over the entire midgut epithelium. ×240. **G:** Branches of the gastric nerves showing immunoreactivity on the surface of the posterior midgut (p mg). ×100. **H,I:** The hindgut is innervated bilaterally by the proctodeal nerves (pr n), dorsomedially directed branches of the cercal nerve. H: ×375; I: ×100. **J:** Shortly after branching from the cercal nerve (n,11), the proctodeal nerve (pr n) divides into the anterior (a) and posterior (p) proctodeal nerves, and both nerves innervate muscles of the hindgut. ×115. **K:** Peripheral neurosecretory cells containing immunoreactivity in association with the proctodeal nerve. ×115. **L:** Illustration of the point of the origin of the anterior and posterior proctodeal nerves. ×115. **M–O:** Demonstrate a group of immunoreactive neurons in the metathoracic ganglion. Their axons can be seen to project into the posterior neuropil, where they bifurcate with the main axon followed anteriorly into the meso and prothoracic ganglia. In both ganglia they branch out to ramify in the neuropil. ×115. a pr n, anterior proctodeal nerve; ag, abdominal ganglion; co, colon; cr, crop; gc, gastric cecum; il, ileum; mg, midgut; mt, Malpighian tubules; pns, peripheral neurosecretory system; p pr n, posterior proctodeal nerve.

other species, whereas that observed in the stomatogastric nervous system may suggest functions involved in the transduction of signals concerning the nutritional status to the CNS and retrocerebral complex.

It is known that *B. germanica* cannot produce enough JH for oogenesis in conditions of starvation or insufficient protein nourishment [Piulachs, 1988; Schal et al., 1993; Osorio et al., 1998], which may suggest the occurrence of hormonal systems informing the nervous system about the nutritional state of the animal. The precise endocrine link between the digestive and the nervous system has proved an elusive problem [see Wheeler, 1996, for review]. The present anatomical studies point to the allatostatins as possible mediators of this regulatory pathway.

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