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Review article

Neuropeptides in the insect brain: a review

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A major factor in the recognition of the diverse roles of neuropeptides in the nervous system was the development of radioimmunoassays and immunohistochemistry (see Guillemin 1978; Hökfelt et al. 1980; Krieger 1983). It became clear that not only neurosecretory cells, but also neurons use neuropeptides as messengers, and the impact of immunohistochemistry on our understanding of the chemical heterogeneity of neurons and the complexity of chemical signalling has been great. Since the late 1970s, when about 20 neuropeptides were known in mammals, considerable effort has been directed at why the central nervous system operates with so many neuropeptides, in addition to the fast neurotransmitters and the monoamines. Consequently, a large number of additional neuropeptides have been sequenced, and their distribution patterns, often colocalized with other neuroactive compounds, have been found to be extremely complex. Also, in the last 20 years much has been learned about the diverse roles of neuropeptides, especially how they regulate neurotransmission (Lehmann 1990; Krause et al. 1990; Cooper et al. 1991; Hökfelt 1991).

Many important achievements in neuropeptide biology have been made with invertebrates as experimental animals. Invertebrates offer unique opportunities to study neuropeptides at the single cell level due to the presence of large identifiable neurons that are embedded in comparatively simple circuits or that have targets accessible for analysis of peptide action (Scheller et al. 1984; O'Shea and Schaffer 1985; O'Shea et al. 1985; Tublitz et al. 1986, 1991; Scharrer 1987; Scheller and Kirk 1987; Kobayashi and Suzuki 1992). As will be shown here, insects, such as locusts, cockroaches, moths and flies, have served as handy preparations in neuropeptide research.

The first insect neuropeptides to be isolated in pure form and sequenced from insects were proctolin (Starrat and Brown 1975) and adipokinetic hormone (AKH; Stone et al. 1976). Over the last 15 years many more insect neuropeptides have been sequenced (see Holman et al. 1990b, 1991; De Loof and Schoofs 1990; Hagedorn et al. 1990; Konopinska et al. 1992; Menn et al. 1991; Kobayashi and Suzuki 1992). A number of these were, as in the vertebrates, isolated with the use of bioassays so that some information on the biological action of these insect neuropeptides was available from the outset, and often their first determined functions were as neurohormones. Also in insects neuropeptides are present in interneurons and motor neurons, in addition to neurosecretory cells, and certain neuropeptides may function both as hormones and neurotransmitters or neuromodulators (O'Shea and Schaffer 1985; Raabe 1989; Menn et al. 1991).

A detailed map of peptide-containing neurons and their pathways is useful as a starting point when searching for sites of release and action for subsequent determination of functional roles of different peptide messengers. Immunohistochemistry of neuropeptides in insects was initially performed with heterologous antisera, raised against mammalian peptides (Kramer et al. 1977; Remy et al. 1977; El Sahly et al. 1980). Not until the early 1980s were the neuronal distribution patterns of the first insect neuropeptides, proctolin (Eckert et al. 1981; Bishop et al. 1981) and AKH (Schooneveld et al. 1983), mapped. A very large number of papers have now been published on the distribution of immunoreactive insect peptides and material recognized by heterologous antisera to vertebrate and other invertebrate neuropeptides (see De Loof 1987; Raabe 1989; Nässel 1991), and a complete account is not given here. Instead, this review outlines the distribution in the insect brain

Abbreviations: *A* alanine; *C* cysteine; *D* aspartate; *E* glutamate; *F* phenylalanine; *G* glycine; *H* histidine; *I* isoleucine; *K* lysine; *L* leucine; *M* methionine; *N* asparagine; *P* proline; *Q* glutamine; *R* arginine; *S* serine; *T* threonine; *V* valine; *W* tryptophan; *Y* tyrosine

of a selection of neuropeptides, in particular ones that have been sequenced from insects, to illuminate principles of organization of putative peptidergic pathways. Due to space limitations the neuropeptide distribution patterns are illustrated mainly from dipteran insects, but data are given also for other insect species. The justification for the general title of this review is that most of the data on neuropeptide biology summarized here is from studies of insects other than dipterans.

It is clear that many peptidergic hormones or neurohormones have defined actions in insects, including important effects on behavior regulation (see O'Shea and Schaffer 1985; Tublitz et al. 1986, 1991; Raabe 1989; Holman et al. 1990b; Menn et al. 1991), but what are the functions of neuropeptides in interneurons of insects?

Chemistry of neuropeptides and problems with their detection

To understand the problems in localizing neuropeptides to specific neurons with immunocytochemistry, it is important to consider how neuropeptides are synthesized and processed by neurons and neurosecretory cells. The known neuropeptides range in size from 3 amino acid residues (thyrotropin-releasing hormone) to more than 50, e.g., insulin. Neuropeptides are generated from larger precursor proteins, in the size range of 90–250 amino acids. Biosynthesis of these precursors requires gene activation, DNA transcription, and RNA translation as for regular protein synthesis (see Eipper et al. 1986; Sossin et al. 1989; Dores et al. 1990). Using recombinant DNA techniques the search for genes encoding for neuropeptide precursors has been quite successful, and amino acid sequences of many prohormones and the neuropeptides possibly generated from them have been deduced, including several insect prohormones: AKH, bombyxin (PTTH), eclosion hormone, FMRFamide-related peptides, and pheromone biosynthesis-activating neuropeptide (see Nambu et al. 1988; Nichols et al. 1988; Schneider and Taghert 1988; Horodyski et al. 1989; Iwami et al. 1989; Hekimi et al. 1991; Davis et al. 1992).

The precursors may contain multiple copies of a given neuropeptide sequence, as for example the precursors of the FMRFamide-related peptides (Schaefer et al. 1983; Nambu et al. 1988; Schneider and Taghert 1988). The biologically active peptide products of the prohormones are produced by a few different mechanisms. One is posttranslational processing by means of enzymatic processes within the secretory pathway: cleavage into smaller peptides, e.g., at pairs of basic amino acids, amidation at the carboxy terminus, glycosylation of asparagine residues, sulfation of tyrosine residues, and pyroglutamate formation by cyclization of glutamine (Eipper et al. 1986; Sossin et al. 1989; Dores et al. 1990). The biosynthesis of peptide precursors can involve two mRNAs as seen for the locust AKHs (Hekimi et al. 1991; O'Shea and Rayne 1992) where two mRNAs are translated into two prohormones (proAKH-I and proAKH-II), which are oxidized into three dimeric precursors. These in turn are processed into monomeric AKHs and dimeric AKH-precursor-related peptides (APRPs1–3). In some cases the different peptide products of a given precursor can be demonstrated immunocytochemically in the same neurons. Tissue-specific posttranslational prohormone processing, however, also occurs. This may be by differential endoproteolytic cleavage of the peptide products, as well as by other modifications listed above.

Another major mechanism by which different peptide products arise is by alternate splicing of the primary transcript, leading to tissue-specific expression of neuropeptides. One example is the splicing of the tachykinin message. In the rat central nervous system (CNS) the RNA splicing gives rise to an mRNA encoding for substance P, whereas the predominant splicing pattern in the gut gives rise to mRNA encoding both for substance P and substance K (Krause et al. 1990). An interesting example of alternate RNA splicing has been demonstrated for the FMRFamide gene transcript in the snail *Lymnaea stagnalis* (Saunders et al. 1992). This differential splicing differs from that in the vertebrate systems in that the processing is cell specific rather than just tissue specific. So far alternate splicing of neuropeptide transcripts has not been demonstrated for insects.

One important consideration when attempting to determine the cellular localization of neuropeptides is that colocalized peptides may be differentially distributed within the processes of a single neuron (or neurosecretory cell). In *Aplysia* it has been shown that the peptide products of the precursor of egg-laying hormone, the bag cell peptides and egg-laying hormone, are sorted and transported to different processes of the same cell (Sossin et al. 1989).

A recent discovery, which may have wide implications for our understanding of neuropeptide synthesis, is that in the rat vasopressin and oxytocin mRNAs can be transported from the hypothalamic perikarya to the axon terminals in the posterior pituitary (Mohr et al. 1991). This raises the possibility that protein synthesis occurs in axon terminals, although the components of a protein synthesis machinery have not yet been observed in axons.

The neuronal expression of precursors and different stages of processed neuropeptides (which may be tissue or neuron specific), the production of related peptides from the same precursor, as well as the existence of many neuropeptides from different precursors sharing amino acid sequences, may cause problems in interpretation of immunocytochemical results.

The problem of antiserum specificity for neuropeptide identification is well recognized (Van Leuwen 1986; Veenstra 1988), but actually many of the immunocytochemical studies on the mammalian nervous system have to a large extent been confirmed by in situ hybridization histochemistry with nucleotide probes to peptide precursors (Hökfelt 1991). However, when using antisera to heterologous neuropeptides, which was commonly done in insects earlier, problems of interpretations are acute (see Thorpe and Duve 1987; De Loof 1988; Veenstra 1988). Another drawback with immunocytochemistry is that although the morphology of the peptide-containing neurons often can be displayed in great detail, some parts of the neurons, e.g., dendrites, may remain unlabeled in insects, as has been shown by combining immunostaining with intracellular dye injection (Thompson et al. 1991).

Neuropeptides in insects: a general introduction

The total number of different insect neuropeptide *sequences* exceeds 100, including all species variants, and continues to grow. These peptides can be arranged in about 20 groups, some of which have been referred to as families (see Tables 1 and 2). The groups are based on homologies in amino acid sequence. Some peptides, like proctolin and CCAP, are identical in all species analyzed (and they are thus orphan peptides). For other peptides, like the FMRFamide- and AKH-related peptides, there are numerous members in the respective families (even within a species several members have been identified). Many of these neuropeptides, which for instance may have different N-terminus extensions and a conserved C-terminus, display cross-activities in different bioassays (Kuniyoshi et al. 1992; Duve et al. 1992).

In insects a variety of hormonal peptide functions are known in diapause, reproduction, development, osmoregulation, metabolism, pigment synthesis and color change, as well as in regulation of skeletal, heart, and visceral muscle (Raabe 1989; Menn et al. 1991). Addi-

Table 1. Representatives of the main groups of insect neuropeptides. *NC*, neurons; *NSC*, neurosecretory cells; *PT*, peripheral targets innervated; *CCI*, corpora cardiaca intrinsic cells; *PC*, secretory cells in intestine or other tissues. The insect neuropeptide terminology is according to Raina and Gäde (1989) and the neuropeptide abbreviations are: *AKH*, adipokinetic hormone; *AT*, allatotropin; *CCAP*, crustacean cardioactive peptide; *CCK*, cholecystokinin; *CRF*, corticotropin-releasing factor; *DH*, diapause hormone; *DP*, diuretic peptide; *EH*, eclosion hormone; *MIP*, myoinhibitory peptide; *MRCH*, melanization and reddish coloration hormone; *MT*, myotropin; *PBAN*, pheromone biosynthesis activating neuropeptide; *PTTH*, prothoracicotropic hormone; *RPCH*, red pigment concentrating hormone

Group (family)[a]	Examples of members	Related peptides	Immunocytochemistry
Adipokinetic and hypotrehalosaemic peptides			
AKH/RPCH family	AKH I–II	glucagon, corazonin	NC, NSC, CCI
Myotropins			
Proctolin	proctolin	–	NC, NSC, PT
Insect kinins	leucokinins I–VIII	–	NC, NSC, PT
Insect tachykinins	locustatachykinins I–IV	tachykinins	NC, PC, PT
Pyrokinins/myotropins	leucopyrokinin	MRCH, PBAN. LomMT	NC, NSC
Sulfakinins	drosulfakinins I–II	gastrin/CCK, FaRPs	NC, NSC
FMRFamide-related	calliFMRFamide	sulfakinins	NC, NSC, PT, PC
Cardioactive peptides	Lom-CAP, Mas CAP_{2a}	crustacean cardioactive peptide	NC, NSC, PT
Myoinhibitory peptide	Lom-MIP	*Aplysia* APGWamide	–
Accessory gland myotropin	Lom-AG-MT I–II	–	NC, NSC, PC
Corazonin-related	corazonin	hypertrehalosemic hormone	NC, NSC
Diuretic hormones			
Vasopressinlike	F1, F2	vasopressin, conopressin	NC/NSC
Diuretic hormones	Mas-DP	urotensin I, CRF	NSC
Other groups			
Pigment-dispersing peptides	PDF, PDH	–	NC, NSC, PT
PBAN/MRCH	Hez-PBAN	pyrokinins, Lom MT	NC, NSC
Diapause hormone	Bom-DP	PBAN/MRCH, pyrokinins	–
Neuroparsins	neuroparsins I–III	neurophysins	NSC
Eclosion hormones	Mas-EH	–	NSC
PTTHs	Bombyxin (4K PTTH)	insulin	NSC
	22K PTTH[b]	–	NSC
Allatotropins	Mas-AT	–	–
Allatostatins	allatostatins A1–4	Met-enkephalinRGL	NC, NSC, PT

[a] See text for original references to neuropeptides and Table 2 for sequences. The immunocytochemical findings have been discussed with references in the text

[b] The 22K PTTH is not insulinlike

tionally, some neuropeptides have been shown to be important in the regulation of specific behaviors, e.g., eclosion hormone and the cardioacceleratory peptides of the moth *Manduca sexta* (Tublitz et al. 1986, 1991). Neuropeptides are also known to be released as cotransmitters, modulating fast transmission at neuromuscular junctions (O'Shea and Schaffer 1985; Evans and Myers 1986). The actions of a given neuropeptide may occur at several different sites and at different levels, including CNS circuits, peripheral synapses, and at the peripheral targets such as muscles and glands (see Scheller et al. 1984; Scheller and Kirk 1987; O'Shea and Schaffer 1985; Raabe 1989; Tublitz et al. 1991). This is reflected in the morphology of peptidergic neurons. Single peptidergic neurons in insects have been found with varicose processes both in central neuropil and in neurohemal release sites (Remy and Girardie 1980; Nässel et al. 1988a, b; Lundquist and Nässel 1990; Thompson et al. 1991).

How do neuropeptides regulate behavior? The production of behavioral sequences is accomplished by temporal and spatial coordination of many neuronal circuits. Each of the circuits controlling behavior employs sets of sensory neurons, interneurons, and motor neurons. Since behaviors can be generated with a number of variants and some behaviors share elements, it is not surprising that multiple neural networks share neural elements. Furthermore, single networks can be modulated to produce different behaviors (Harris-Warrick and Marder 1991). In the crustacean stomatogastric ganglion the reconfiguration of neural circuits to produce different behaviors results from the actions of neuropeptides and monoamines. A large number of behavioral circuits can be established from a single anatomically defined network, and neural elements can switch between networks. The neuromodulators accomplish this by altering the intrinsic properties or the response properties (synaptic efficacy) of the neural components of the network as well as their target muscles (see Kravitz 1988; Harris-Warrick and Marder 1991).

An example of modulatory peptide actions on behavior in insects is provided by the cardioacceleratory peptides (CAPs) of *Manduca sexta*. The CAPs are known to modulate four different behaviors during defined peri-

Table 2. Amino acid sequences of insect neuropeptides representing major peptide groups[a]

Neuropeptides	Abbreviation	Species	Sequence
AKH-like	Lom-AKH-I	*Locusta*	pQLNFTPNWGT-NH_2
	Drm-AKH	*Drosophila*	pQLTFSPDW-NH_2
Corazonin		*Periplaneta*	pQTFQYSRGWTN-NH_2
Proctolin		*Periplaneta*	RYLPT
Kinins	Lem-K-I	*Leucophaea*	DPAFNSWG-NH_2
	Lom-K-I	*Locusta*	AFSSWG-NH_2
Tachykininlike	Lom-TK-I	*Locusta*	GPSGFYGVR-NH_2
Pyrokinins	Lem-PK	*Leucophaea*	pQTSFTPRL-NH_2
Myotropins	Lom-MT-I	*Locusta*	GAVPAAQFSPRL-NH_2
PBANs	Hez-PBAN	*Heliothis*	LSDDMPATPADQEMYRQDPEQIDSRTKYFSPRL-NH_2
Diapause hormone	Bom-DH	*Bombyx*	TDMKDESDRGAHSERGALCFGPRL-NH_2
Accessory gland myotropin	Lom-AG-MT-I	*Locusta*	GFKNVALSTARGF-NH_2
Sulfakinins	Lem-SK-I	*Leucophaea*	EQFEDY(SO_3)GHMRF-NH_2
	Drm-SK-I	*Drosophila*	FDDY(SO_3)GHMRF-NH_2
FMRFamide-like	Aea-HP-I	*Aedes*	TRF-NH_2
	Aea-HP-II[b]	*Aedes*	pQRP*hP*SLKTRF-NH_2
	CalliFMRFamide I	*Calliphora*	TPQQDFMRF-NH_2
	DrosoFMRFamide I	*Drosophila*	DPKQDFMRF-NH_2
	Lem-MS-I	*Leucophaea*	pQDVDHVFLRF-NH_2
Cardioactive peptides	Lom-CAP	*Locusta*	PFCNAFTGC-NH_2
Myoinhibitory	Lom-MIP	*Locusta*	AWQDLNAGW-NH_2
Vasopressinlike	F1	*Locusta*	CLITNCPRG-NH_2
	F2	*Locusta*	CLITNCPRG-NH_2  CLITNCPRG-NH_2
Diuretic hormones	Mas-DP	*Manduca*	RPMSLSIDLPMSVLRQKLSLEKERKVHALRAAANRN-FLNDI-NH_2
	FMRF-gene derived[c]	*Drosophila*	QAEQLPPEGSYAGSDELEGMAKRAAMDRY-NH_2
Pigment-dispersing factors	Rom-PDF	*Romalea*	NSEIINSLLGLPKLLNDA-NH_2
Neuroparsins	NP(compound γ)	*Locusta*	NPISRSCEGANCVVDLTRCEYGDVTDFFGRKVCAKGP-GDKCGGPYELHGKCGVGMDCRCGLCSGCSLH-NLQCFFFEGGLPSSC
Eclosion hormones	Mas-EH	*Manduca*	NPAIATGYDPMEICIENCAQCKKMLGAWFEGPLCAES-CIKFKGKLIPECEDFASIAPFLNKL
PTTHs[d]	Bom-PTTH-II	*Bombyx*	A-chain GIVDECCLRPCSVDVLLSYC
	(Bombyxin)	–	B-chain pQQPQAVHTYCGRHLARTLADLCWEAGVD
Allatotropins	Mas-AT	*Manduca*	GFKNVEMMTARGF-NH_2
Allatostatins	Dip-ASB2	*Diploptera*	AYSYVSEYKRLPVYNFGL-NH_2
	Mas-AS	*Manduca*	pQVRFRQCYFNPISCF

[a] Only one or a few representatives of each group are given to save space. References to literature are given in text
[b] The *hP* residue is 4-hydroxyproline
[c] Amino acid sequence deduced from cDNA sequence of *Drosophila* FMRFamide precursor; no bioactivity has been determined
[d] The "big" (22K) PTTH is a homodimer of a 109 amino acid peptide. Sequence is given by Nagasawa (1992)

ods of the insect's development. Two modulatory actions are on ingestion of nutrition, and the others are regulation of heart activity during wing inflation and during flight (Tublitz et al. 1991). Some neuropeptides are known to directly trigger a specific behavior. One example in insects is eclosion hormone, which initiates eclosion behavior (and all ecdysis) during development of the moth *Manduca* (see Tublitz et al. 1986).

How do neuropeptides modulate synaptic transmission? A few examples are given here from noninsect preparations since they are quite illustrative. Neuropeptides are often colocalized with fast neurotransmitters or monoamines (Lundberg and Hökfelt 1983; Adams and O'Shea 1983; Bartfai et al. 1988; Weiss et al. 1992). In *Aplysia*, it has been shown at the single neuron level, that different colocalized messengers are released during different frequencies of firing. In the terminal of motor neuron B15, acetylcholine alone is released during low frequency firing, whereas acetylcholine and the small cardioactive peptides (SCPs) are released at higher fre-

quencies (Whim and Lloyd 1989). The released neuropeptides can act pre- or postsynaptically, often on receptors coupled to second messenger systems, to modulate the synaptic transmission of a fast neurotransmitter. As an example, the B15 motor neuron of *Aplysia*, mentioned above, contains acetylcholine, the two SCPs, and a third neuropeptide, buccalin (Cropper et al. 1988). The SCPs act postsynaptically on the muscle to potentiate the action of acetylcholine, whereas buccalin acts presynaptically to inhibit acetylcholine release.

One example of complex interactions of coexisting peptides and fast neurotransmitters, which highlights the various levels at which substances may act, is seen in the synapses of the primary sensory afferents in the dorsal horn of the rat spinal cord (Hökfelt 1991). A scheme of events leading to full potentiation of the response has been suggested from experimental work. The sensory afferents can release colocalized glutamate, substance P, and calcitonin gene-related peptide (CGRP), each of which has excitatory effects on the postsynaptic neuron. Each of these compounds have postsynaptic receptors; additionally substance P and CGRP have presynaptic receptors. CGRP potentiates the release of substance P and glutamate via the presynaptic receptors. Substance P presynaptically potentiates release of glutamate and may also enhance the excitatory postsynaptic response to glutamate (sensitization of glutamate receptors). Furthermore, CGRP is known to inhibit an endopeptidase that cleaves substance P, thus keeping the extracellular level of substance P high.

Distribution and morphology of peptide-containing neurons in the insect brain

In the following the distribution of a number of neuropeptides will be described with emphasis on neuropeptides that have been isolated and sequenced in at least one insect species. First a brief outline of the brain organization is given.

Organization of the insect central nervous system (see Strausfeld 1976). The insect brain contains around 10^5–10^6 neurons depending on the species. It consists of three main divisions: the protocerebrum, deutocerebrum, and tritocerebrum (Figs. 1, 2). In each of these divisions different neuropil regions are located. Some are highly structured and are termed glomerular neuropils; others, called nonglomerular neuropils, lack obvious geometrical architecture. In the protocerebrum a multitude of higher sensory centers associated with vision are located together with centers processing multimodal information (e.g., the mushroom bodies and the different neuropil regions of the central complex; Fig. 1). The superior protocerebrum, with the pars intercerebralis, contains different sets of neurosecretory cells supplying neurohemal organs in the corpora cardiaca and corpora allata, which are located in the head or in some insects in the neck or prothorax, Fig. 2a). Additional neurosecretory cell groups in other locations also supply these neurohemal organs. The deutocerebrum contains antennal centers, notably the well-ordered antennal olfactory glomeruli. In the tritocerebrum, neurosecretory neurons and neurons associated with control of feeding and foregut activity can be found. The tritocerebrum is also connected to the frontal ganglion and the retrocerebral glandular complex. The optic lobes flanking the protocerebrum are composed of the three to four most well-ordered neuropils in the brain. Via connectives the brain is connected to the subesophageal ganglion and the thoracic and abdominal ganglia (often referred to as the ventral cord; Fig. 2a). For details of brain organization refer to Strausfeld (1976). The distribution and functions of non-peptide transmitters and modulators in insects are summarized in some recent reviews (Nässel 1987, 1988, 1991; Restifo and White 1990; Buchner 1991).

Proctolin

Proctolin, the first sequenced insect neuropeptide, was isolated from the cockroach *Periplaneta americana* (Starrat and Brown 1975). It is an unblocked and nonamidated myoactive peptide with the sequence RYLPT. In the three arthropod species for which proctolin has been sequenced, the cockroach *Periplaneta americana*, the crab *Carcinus maenas*, and the horseshoe crab *Limulus polyphemus*, the amino acid sequence is identical (Starrat and Brown 1975; Stangier et al. 1986; Groome et al. 1990), and no related peptides have been found. Proctolin is one of the most extensively studied invertebrate neuropeptides, and comprehensive reviews have been written, which cover aspects of distribution, modes of action, and pharmacology (O'Shea et al. 1985; Orchard et al. 1989). The action of proctolin has been studied mainly on skeletal, heart, and visceral muscles in arthropods, and in crustaceans proctolin has been shown to modulate central pattern-generating networks that control feeding and ventilatory behaviors (see Orchard et al. 1989; Harris-Warrick and Marder 1991).

In insects proctolin has been demonstrated in motor neurons, interneurons and neurosecretory cells (Bishop and O'Shea 1982; Veenstra et al. 1985; Nässel and O'Shea 1987; Davis et al. 1989; Homberg et al. 1991a). In the lobster there is evidence that dendrites of sensory neurons, the stretch receptors of the oval organ, contain proctolin and release this peptide upon stretching (Pasztor et al. 1988).

In the cockroach brain about 40 proctolinlike immunoreactive (PLI) cell bodies have been found (Bishop and O'Shea 1982). Most of these are located in the tritocerebrum (two pairs of clusters), and only three pairs of PLI cell bodies can be detected in the protocerebrum (Bishop and O'Shea 1982). A similar number and distribution of PLI cell bodies has been seen in the Colorado potato beetle, *Leptinotarsa decemlineata* (Veenstra et al. 1985).

In the blowfly brain about 80–90 PLI cell bodies have been labeled (Nässel and O'Shea 1987; Fig. 1b). Additionally about 100 PLI cell bodies are associated with each lobula of the optic lobe. Also in the blowfly brain, the PLI cell bodies are distributed in the proto- and

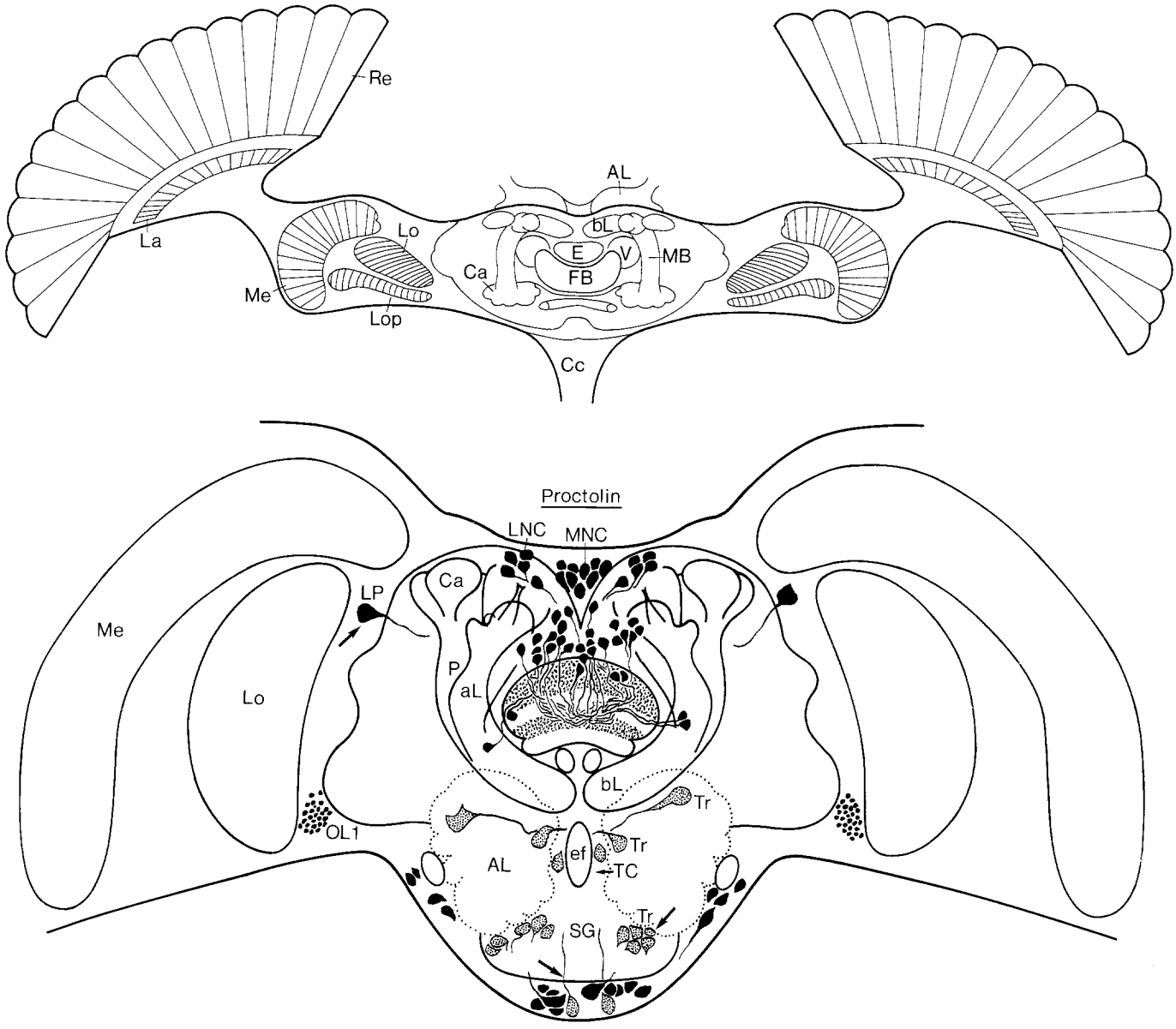


Fig. 1. Schematic diagrams of the blowfly brain. The *top drawing* shows a compressed horizontal view and the bottom one a frontal view. In the *bottom diagram* the proctolin-immunoreactive neurons of *Calliphora* are shown. The posteriorly located cell bodies are *black*, whereas the anterior ones are *stippled*. Note innervation of two layers of the fan-shaped body (other neuropil innervation is not shown here). The cell bodies indicated by *arrows* also react with AKH antiserum. *AL* Antennal lobe; *aL* alpha lobe; *bL* beta lobe; *Ca* calyx of mushroom body (MB); *Cc* cervical connective; *E* ellipsoid body; *ef* esophageal foramen; *FB* fan-shaped body; *La* lamina; *LNC* lateral neurosecretory cells; *Lo* lobula; *Lop* lobula plate; *Me* medulla; *MNC* median neurosecretory cells; *P* peduncle; *Re* retina; *SG* subesophageal ganglion; *TC* tritocerebrum; all other abbreviations denote neuron types. The proctolin immunoreactivity is redrawn from Nässel and O'Shea (1987)

tritocerebrum, as well as in the subesophageal ganglion. The protocerebral PLI neurons are located in several different clusters. Two of these constitute the neurosecretory cells of the median (MNC) and lateral (LNC) neurosecretory cell groups. A cluster of about 30 smaller cell bodies innervates two distinct layers of the fan-shaped body of the central complex (Fig. 1 b).

In the insect brain, proctolin may act as a neuromodulator in central synaptic neuropil (Nässel and O'Shea 1987) and may also be a neurohormone. In the moth *Manduca sexta*, cerebral neurosecretory cells containing PLI material have axon terminals that arborize in the corpora allata (Homberg et al. 1991 a), and in the Colorado potato beetle and several moths, PLI terminals are found in the corpora cardiaca (Veenstra et al. 1985; Davis et al. 1989).

Adipokinetic hormone

Adipokinetic hormone (AKH) was first isolated and sequenced (pQLNFTPNWGTamide) from the corpora cardiaca of the locust *Locusta migratoria* by Stone et al. (1976). Now we know that the adipokinetic hormones

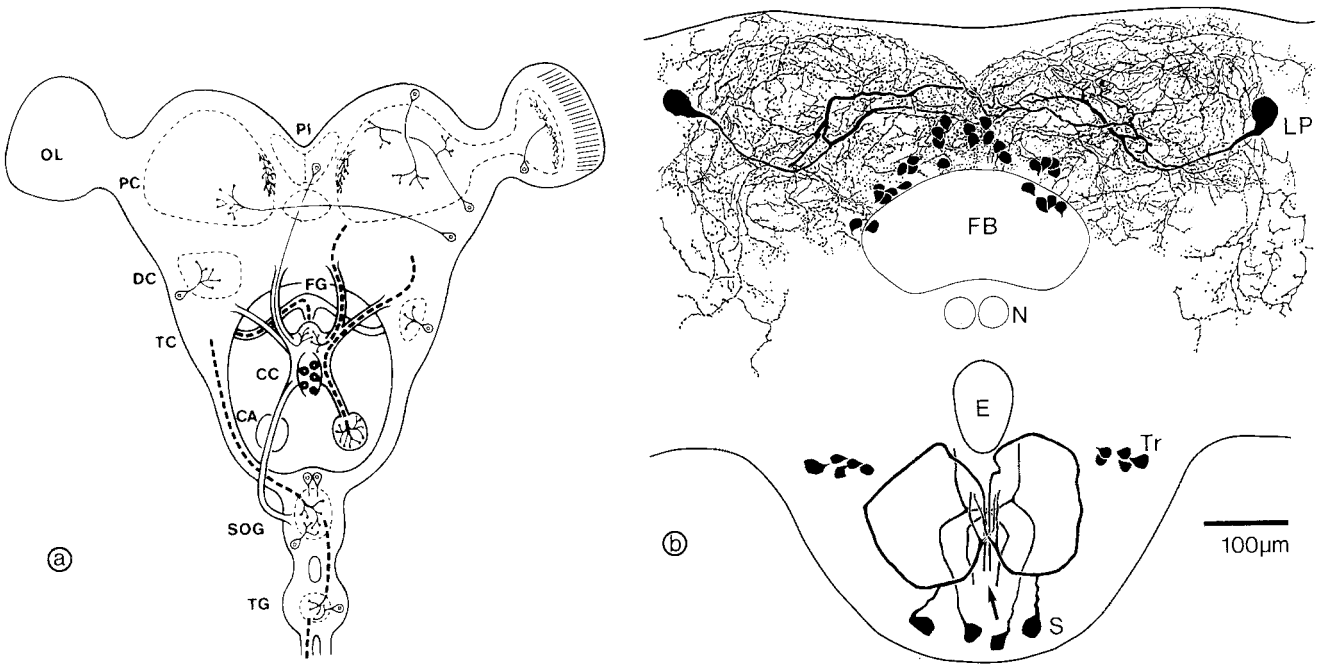


Fig. 2. Distribution of AKH-like immunoreactivity (AKHLI) in the insect brain. **a** A generalized diagram of AKH-immunoreactive neurons in brain, subesophageal (*SOG*) and thoracic (*TG*) ganglia and glandular cells in corpora cardiaca (*CC*). AKHLI neurons are seen in proto- (*PC*), deuto- (*DC*) and tritocerebrum (*TC*). *Dashed lines* indicate neurosecretory pathways. *FG* Frontal ganglion; *OL* optic lobe; *PI* pars intercerebralis. Slightly altered after Schooneveld et al. (1985) with permission from H. Schooneveld. **b** AKHLI neurons in the blowfly brain (*Calliphora*). The two large protocerebral neurons (*LP*) are traced in detail. The four subesophageal neurons (*S*) appear to have axons (*arrow*) descending to the thoracic ganglia. One set of small cells supplies processes to fan-shaped body (*FB*). *Tr* Tritocerebral neurons; *E* esophageal foramen; *N* noduli

constitute a large family of insect and crustacean neuropeptides, many of which bear other names, such as red pigment concentrating hormone (RPCH), hypertrehalosemic hormone, periplanetins CCI-II, MI, and MII, and neurohormone D (see Gäde 1990; Schaffer et al. 1990). So far about 20 members of the AKH/RPCH family have been sequenced from about seven insect orders (examples in Table 2). Commonly they are amidated and pyroglutamate-blocked octa- or decapeptides. In several species two forms of peptides have been isolated. As outlined in the introduction, the locust AKH precursor protein undergoes complex processing to yield active products (Hekimi et al. 1991; O'Shea and Rayne 1992). The prohormones, proAKH-I and proAKH-II, are formed from two different mRNAs. By oxidation three dimeric precursors arise, which are processed into monomeric AKH-I and AKH-II and three dimeric peptides (APRP1–3) whose functions are unknown. Many functions of the AKHs are, however, known, some of which are species specific – mobilization of lipids or carbohydrates, acceleration of heart beat, myoactivity, and inhibition of fatty acid and protein synthesis (Orchard 1987; Gäde 1990). The chief source of AKHs appears to be the glandular cells of the corpora cardiaca, and many of the listed functions for AKHs are probably mediated by hormonal action via the circulation.

AKH-like immunoreactivity (AKHLI) is found in the glandular cells of the locust corpora cardiaca as well as in neurosecretory cells and neurons in the brain and subesophageal ganglion of locusts (Fig. 2a) and many other insect species (Schooneveld et al. 1983, 1985). The neurons and neurosecretory cells can, however, be revealed only with an antiserum directed against the C-terminus of locust AKH-I (Schooneveld et al. 1983), whereas the glandular cells in corpora cardiaca can be labeled with both C- and N-terminus-specific antisera (Schooneveld et al. 1986). Hence, the exact chemical structure of the AKHLI material in the brain is not known, but extracts of locust brain contain small amounts of AKH-I-like material as determined by radioimmunoassay and bioassay (Moshitzky et al. 1987).

In the blowfly brain about 50 AKHLI neurons (labeled only with C-terminus directed antiserum) are distributed in the protocerebrum, tritocerebrum, and subesophageal ganglion (Fig. 2b). Additionally there are a few hundred neurons in the left and right medulla of the optic lobe (D. Nässel, unpublished). Two prominent AKHLI neurons have their cell bodies in the dorsolateral protocerebrum and extensive arborizations covering most of the superior protocerebrum (Fig. 2b). Another set of about 30 protocerebral neurons innervate the fan-shaped body of the central complex. Some of the subesophageal AKHLI neurons appear to be descending neurons with axons reaching the thoracic ganglia. The large dorsolateral protocerebral neurons and one pair of ventral subesophageal ones contain both AKHLI and proctolin-immunoreactive material (Fig. 1). Many AKHLI neurons also react with an antiserum against the mammalian peptide galanin message-associated peptide (Lundquist et al. 1992). At present there are no clues to central functions of AKH-like peptide. In the crustacean abdominal ganglia the AKH relative, RPCH, mod-

ulates neurons of pattern generator circuits controlling the swimmeret rhythm (Sherff and Mulloney 1991).

FMRFamide-related peptides (FaRPs)

The FMRFamide-related peptides (FaRPs) constitute a large family of neuropeptides with representatives throughout the Metazoa (Greenberg et al. 1988; Price and Greenberg 1977, 1989; Holman et al. 1990b; Walker 1992). Commonly the FaRPs have the C-terminus tetrapeptides -FMRFamide or -FLRFamide (Table 2), but a more liberal classification is sometimes also used (see Greenberg et al. 1988; Price and Greenberg 1989). Cloning of cDNAs encoding FaRP precursors of several invertebrate species, including insects, have added substantial numbers of new members of this family (e.g., Schaefer et al. 1983; Nambu et al. 1988; Nichols et al. 1988; Schneider and Taghert 1988; Darmer et al. 1991). The FaRPs are by far the best studied of the invertebrate neuropeptides. Not only do they exist in several molecular forms in a given species, they also have very complex distribution patterns in a variety of neurons, neurosecretory cell systems, and glandular cells and display multiple physiological actions in different tissues (Price and Greenberg 1989; Kobayashi and Muneoka 1989; Walker 1992). At least four FaRP receptor types have been indicated in molluscs. These are associated with slow potassium increase, fast potassium increase, sodium conductance increase, and potassium conductance decrease (via a second messenger), respectively (reviewed by Walker 1992).

FaRPs have been isolated and sequenced from a number of insects: *Leucophaea*, *Manduca*, *Schistocerca*, *Aedes*, and *Calliphora* (Holman et al. 1986; Matsumoto et al. 1989; Robb et al. 1989; Kingan et al. 1990; Duve et al. 1992). Some of these are shown in Table 2. Using extracts of blowfly thoracico-abdominal ganglia, Duve et al. (1992) isolated 13 FaRPs with the C-terminus sequence-DFMRFamide. The most abundant of the blowfly ventral cord FaRPs is CalliFMRFamide 5, which has the sequence APGQDFMRFamide. In *Drosophila* the sequence DPKQDFMRFamide is encoded in five copies on the FaRP precursor, and has been isolated from head extracts (Nambu et al 1988). In *Drosophila* a cDNA for another FaRP precursor has been cloned, which contains two drosulfakinins, sulfated peptides with the carboxyl terminus sequence -HMRFamide (Nichols et al. 1988). Of the 13 nonsulfated *Drosophila* FaRPs (deduced from cDNA sequence) and 13 isolated CalliFMRFamides, only one is identical: a heptapeptide (CalliFMRFamide 11) with the sequence PDNFMRFamide. When comparing two species of *Drosphila*, Taghert and Schneider (1990) found that their precursors of FaRPs differed. In *D. melanogaster* 13 FaRPs were deduced, in *D. virilis* only 10. Of these only 5 are identical in the two species. This illustrates the remarkable diversity in the FaRPs. Is the molecular diversity matched by a similar functional diversity (receptor multiplicity) as suggested by Payza (1987)? For the insect FaRPs only limited evidence is available so far. In an assay for fluid secretion from blowfly salivary glands, only CalliFMRFamides 1–3 can induce salivation at concentrations of 0.1–1.0 nM, indicating that their N-terminus extensions may increase their potency or be crucial for specific activity (Duve et al. 1992). Similar structure activity effects have been demonstrated on other target organs in insects (Cuthbert and Evans 1989) and in molluscs (Payza 1987). The *Drosophila* FaRP precursor also encodes a 29 amino acid peptide sequence (Table 2) with homologies to the mammalian corticotropin-releasing factor and *Manduca* diuretic hormone (Schneider and Taghert 1988; Kay et al. 1991).

Before turning to the distribution of FaRPs in the insect brain it should be noted that a number of other neuropeptides display small sequence similarities to the FaRPs. Peptides of the neuropeptide Y family (NPY, PYY, pancreatic polypeptides or PPs) have often been discussed in relation to work on FaRPs in invertebrates. The primary structures of the 36 amino acid NPY peptides are not strikingly similar to the FaRPs. Their carboxy terminus -TRTRFamide (reptilian PP) or-TRQRYamide (of NPYs) may, however, crossreact with FMRFamide antisera, or C-teminus-specific PP antisera may react with FaRPs. Thus, NPY and PP immunoreactivity has been dismissed as crossreactivity with FaRPs. There is, however, some recent evidence that some invertebrate groups synthesize peptides related to NPY/PP. In two platyhelminth worms and the molluscs *Helix* and *Aplysia*, peptides with 39–40 amino acid residues have been sequenced that in the 20 residues of the carboxy-terminus display striking homologies to PPs of lower vertebrates (Halton et al. 1992; Rajpara et al. 1992). These neuropeptides, termed neuropeptide F (plus species name), may represent the invertebrate NPY/PP homologs, and they clearly exist in parallel with the FaRPs in *Helix* (Halton et al. 1992). Some evidence for a PP-related peptide has accumulated also for the blowfly *Calliphora*, where a peptide was isolated that has 36 residues in proportions roughly similar to the vertebrate PPs (Thorpe and Duve 1987). In *Drosophila* a receptor protein was recently cloned with similarities to the NPY receptor (Li et al. 1992) adding further credibility to the presence of NPY/PP-like molecules in addition to the FaRPs.

A number of insect species have been investigated in some detail with respect to the distribution of immunoreactive FaRPs in the brain: Colorado potato beetle *Leptinotarsa* (Veenstra and Schooneveld 1984), *Drosophila* (White et al. 1986), the blowfly *Calliphora* (Nässel et al. 1988b), the moth *Manduca* (Homberg et al. 1990), and the honey bee *Apis* (Schürmann and Erber 1990). In these species the distribution of FMRFamide-like immunoreactivity (FLI) in neurons and neurosecretory cells is extensive; very large numbers of neurons of many different main types can be demonstrated. Furthermore smaller or larger subpopulations of the FLI neurons and neurosecretory cells in the insect nervous system also react with other antisera, notably antisera raised against PP, NPY, molluscan small cardioactive peptide (SCP_B), gastrin/CCK, Met-enkephalin-RF, and substance P (Veenstra and Schooneveld 1984; Homberg et al. 1990;

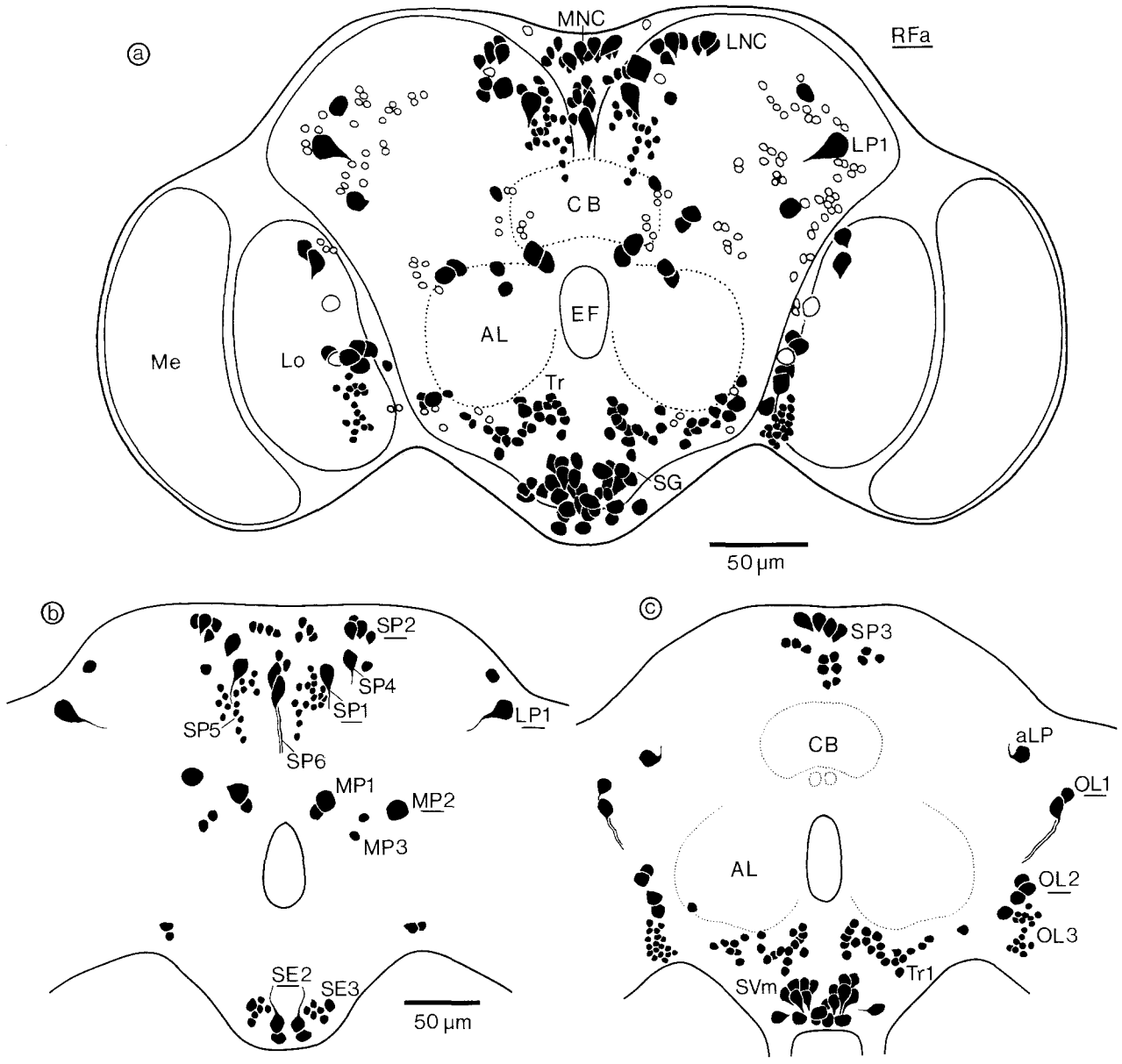
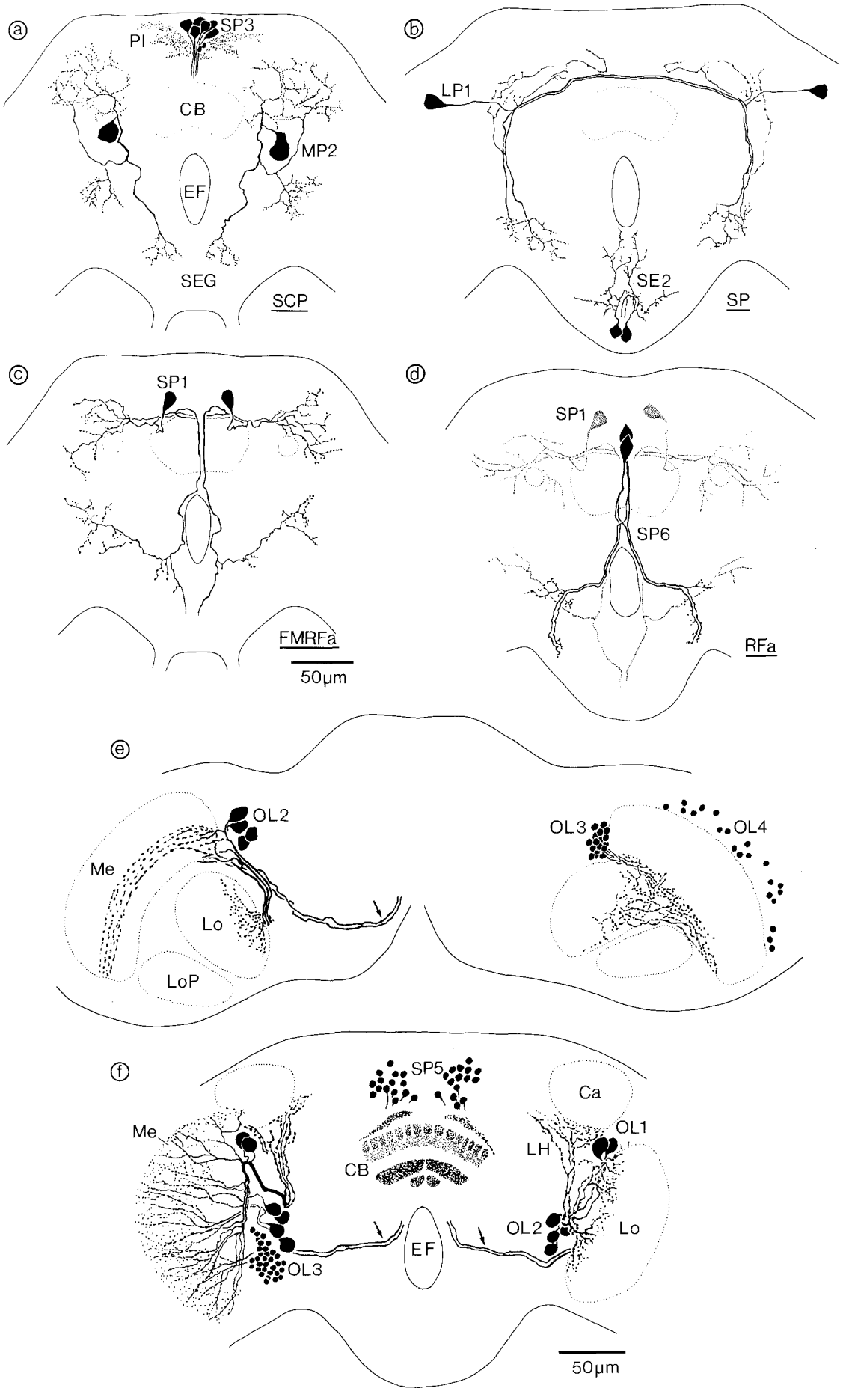


Fig. 3. Neurons in the *Drosophila* brain reacting with RFamide antiserum (frontal views). **a** Total number of intensely labeled neurons in compressed view. Abbreviations as in Fig. 1 b and c. Posterior and anterior cell bodies, respectively. Some identifiable neurons or clusters are indicated with the terminology used by White et al. (1986). The neurons with underlined labels are the ones expressing the FMRFamide precursor transcript as seen by in situ hybridization (O'Brien et al. 1991). The SP6 neurons are among the identifiable neurons that label only with RFamide antiserum

Lundquist and Nässel 1990). Does this mean that multiple peptides are colocalized in different FLI neurons or does it reflect crossreactivity of the different antisera with different FaRPs? In the *Drosophila* central nervous system FaRP-expressing cells have been mapped with a combination of antisera against the sequence FMRFamide and antisera to some portions of the *Drosophila* FMRFamide precursor as well as by in situ hybridization (Chin et al. 1990; O'Brien et al. 1991). It is thus possible to determine roughly which of the FLI neurons that express peptide derived from the FMRFamide precursor (see Fig. 3).

In *Drosophila* a total of about 240 FLI cell bodies have been found in the proto-, deuto-, and tritocerebrum and the subesophageal ganglion with antisera to FMRFamide (Figs. 3, 5). Using an antiserum against RFamide a few more neurons could be labeled (Fig. 3). Many of the FLI neurons can be identified individually or as small clusters from specimen to specimen (see Figs. 3, 4). The FMRFamide gene transcript has been revealed by in situ hybridization in many of the identifiable neurons (O'Brien et al. 1991) as indicated in Fig. 3 b, c. A number of the FLI neurons can be resolved in some detail both in the brain and the optic lobes. Many of these are large ipsi- or bilateral interneurons, including descending neurons; others are local interneurons in the optic lobe or central complex (Fig. 4). The immunolabeling of subpopulations of FLI neurons with differ-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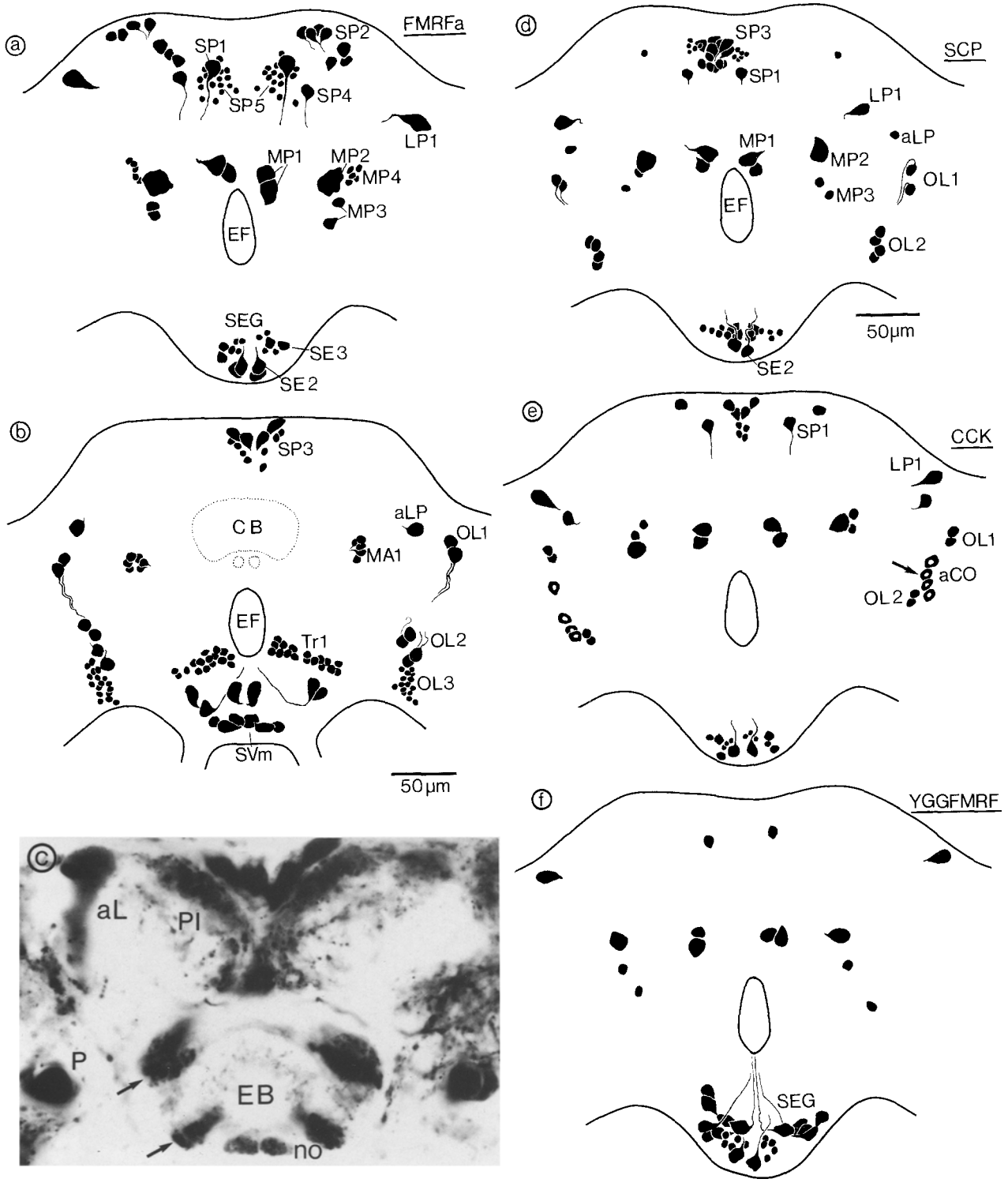
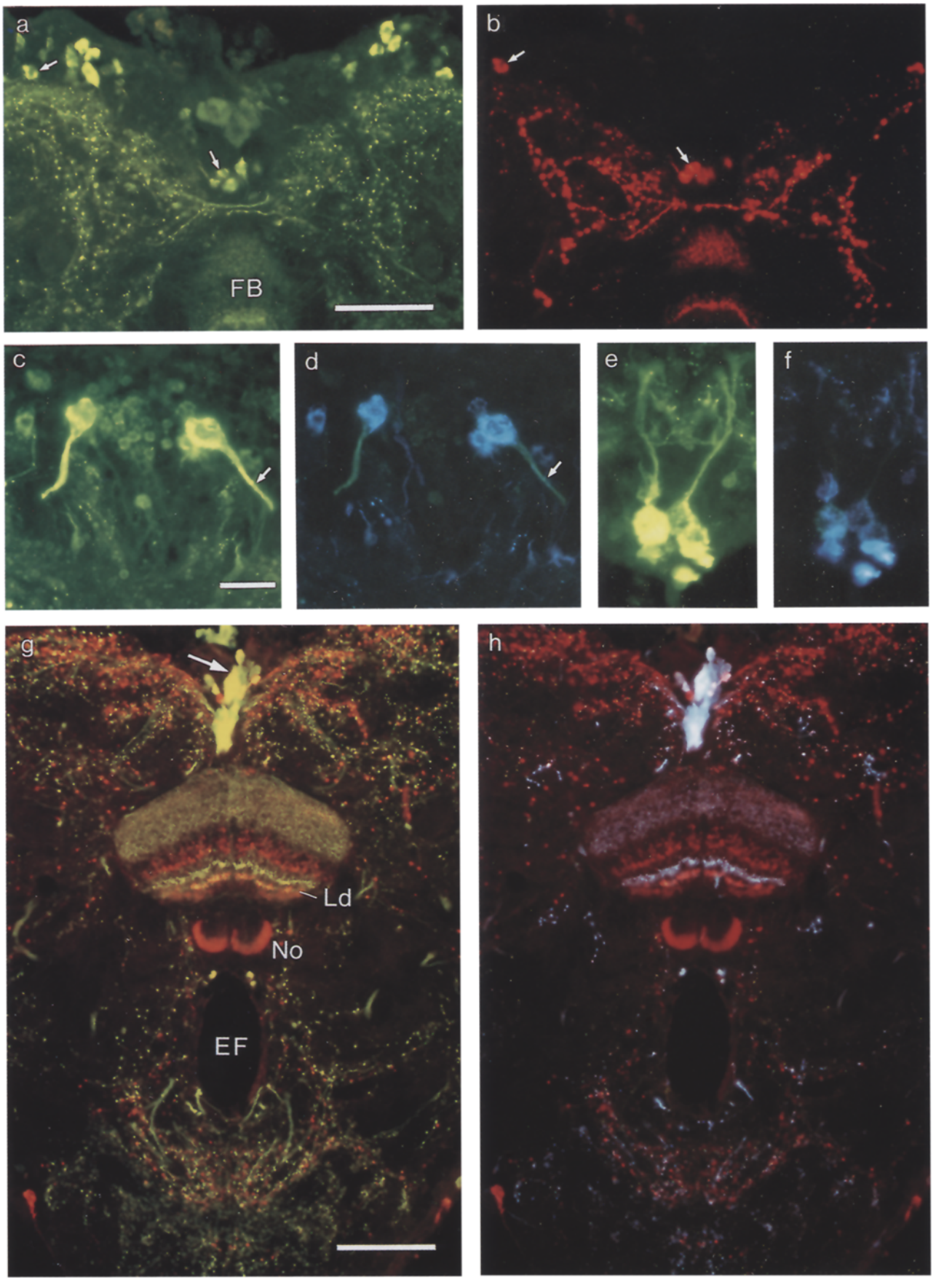


Fig. 5. Immunoreactive FaRPs in the *Drosophila* brain revealed with different antisera. Neuron terminology as in Fig. 3a, b. Anti-FMRFamide (FLI neurons) in posterior and anterior portion of brain, respectively. **c** Micrograph of FLI processes in the central part of the brain. FLI material is seen in α-lobe (*aL*), peduncle (*P*), pars intercerebralis (*PI*), ellipsoid body (*EB*), noduli (*no*), and two layers of fan-shaped body (*arrows*). SP3 cell bodies are seen at top. **d** Anti-SCP_B reveals a smaller subpopulation of the FLI neurons. **e** CCK antiserum labels a subpopulation of the FLI neurons, but also some additional neurons termed *aCO* (*arrow*). **f** Met-enkephalin-RF antiserum reveals another subpopulation of FLI neurons. As can be seen in this figure many identifiable neurons are labeled with all of the antisera used here

Fig. 4. Distribution of immunoreactive FaRPs in frontal sections (except **e**) of the *Drosophila* brain. **a–d** Identifiable neurons of the brain and subesophageal ganglion (neuron terminology as in Fig. 3). Of these only the SP6 neurons in **d** cannot be labeled with FMRFamide antiserum. The substance P-immunoreactive neurons (*LP1* and *SE2*) in **b** are redrawn from Nässel et al. (1990). *SE2* are descending neurons with processes to thoracic ganglia. **e**, **f** FMRFamide-immunoreactive neurons in the optic lobe of *Drosophila*. In **e** a horizontal section is shown, whereas **f** is a frontal view. *OL4* are small neurons whose projections could not be resolved. The axons indicated by *arrows* in **f** are derived from cell bodies in dorsal protocerebrum. *Ca* Calyx of mushroom body; *CB* central complex; *LH* lateral horn; *PI* pars intercerebralis; *SEG* subesophageal ganglion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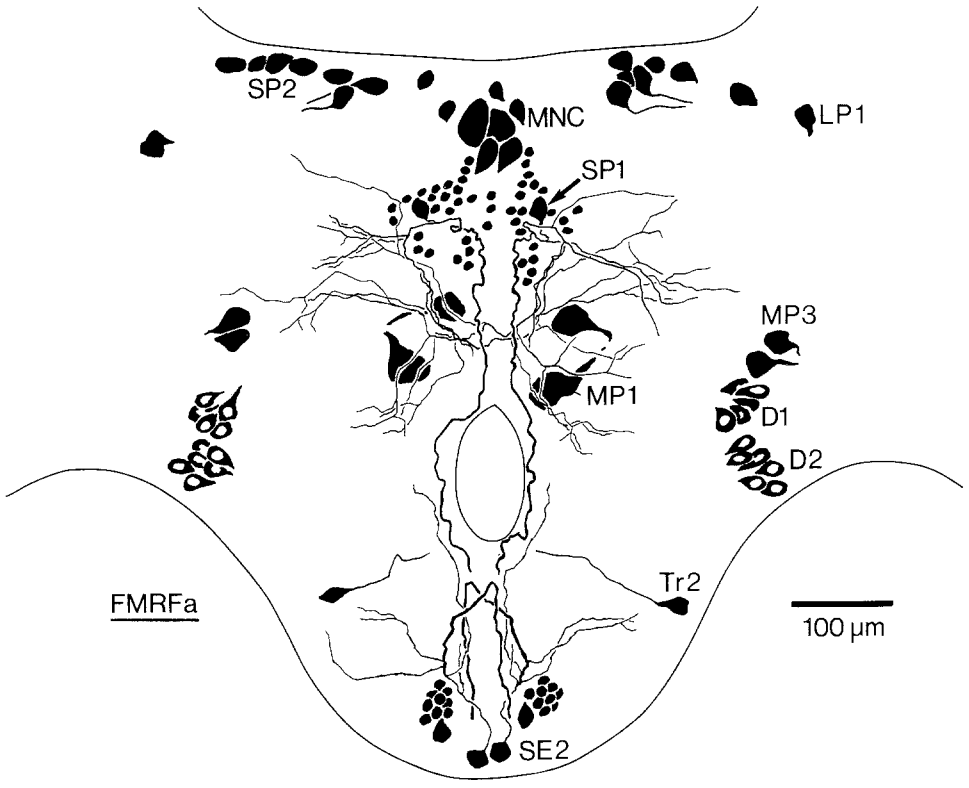


Fig. 7. FLI neurons in the blowfly brain (frontal compressed view). Many anterior and some posterior cell bodies have not been traced here to simplify the diagram. Two sets of neurons were traced in detail (*SP1* and *SE2*). The *SE2* neurons are subesophageal descending neurons, and the *SP1* may be protocerebral descending neurons (their axons could not be traced past the subesophageal ganglion). Note that several neurons appear to be homologous to *Drosophila* FLI neurons (*LP1*, *SP1*, *SP2*, *MP1*, *MP3*, *SE2*)

ent antisera is shown in Fig. 5. Progressively smaller subpopulations of FLI neurons are obtained with different antisera in the following order: RFamide > FMRFamide > SCP > gastrin/CCK > YGGFMRF > substance P (Nässel et al. 1991; unpublished results). Only the gastrin/CCK antisera labeled neurons in addition to the ones overlapping with the RFamide-immunoreactive ones (Fig. 5e).

Fig. 6. Fluorescence micrographs of double and triple labeling of frontal sections of the blowfly brain with antiserum against FMRFamide and other neuropeptide antisera. **a, b** On the same section binding of rabbit FMRFamide antiserum detected with FITC-labeled secondary antiserum (*yellow green*) and mouse SCP_B antiserum detected with TRITC labeled secondary antiserum (*red*). Note that some cells (*arrows*) react with both antisera, wheras some display only FMRFamide immunoreactivity. Also some subsets of the fibers react with both antisera. *FB* Fan-shaped body. *Scale bar* **a, b**: 100 µm. **c, d** Protocerebral cell bodies labeled with anti-FMRFamide (*yellow green*) and SCP_B (*blue*; AMCA). Note that fewer cells are labeled in **d** and that the neurites (*arrows*) contain only FLI material. *Scale bar* **c, f:** 25 µm. **e, f** Subesophageal cell bodies labeled as in **c** and **d**. Same *scale* as **c** and **d**. **g, h** Triple labeling of the same section with guinea pig antiserum to FMRFamide (*yellow green*), mouse anti-SCP_B (*blue*) and rabbit anti-leucokinin I (*red*). **g** Is a double exposure with FITC and rhodamine filter sets. The leucokinin immunoreactivity (LKIR) is not colocalized with FMRFamide immunoreactivity. **h** Is a double exposure with the AMCA and rhodamine filter sets. No colocalization between SCP_B and LKI immunoreactivities. In both micrographs the differential distribution of the two antigens in the lower division (*Ld*) of the fan-shaped body is seen. The noduli (*No*) contain only LKIR material. The cell bodies in median neurosecretory cell group (*arrow*) contain both FMRFamide and SCP_B immunoreactivity. *EF* Esophageal foramen. *Scale bar* **g, h:** 100 µm

In the blowfly brain double labeling or labeling of adjacent sections with the different antisera listed above has been used to establish the patterns of colocalization of immunoreaction (Fig. 6), and confirms the proposed *Drosophila* colocalization pattern. Several of the neuron types resolved in detail in *Drosophila* can also be resolved in the blowfly brain, and many identifiable cells and cell clusters appear homologous in the two species (Fig. 7). In the blowfly brain it is possible to obtain better details of arborizations of FLI neurons in different neuropil regions due to the larger size (Fig. 8). It is clear that the distribution of FLI processes is extremely complex. The labeling patterns seen with the different antisera listed above may represent crossreactivities with the different forms of FaRPs known in *Drosophila* and *Calliphora*. The FaRPs are variable in the N-terminus, and the different antisera used may have different affinities for the different N-terminus extensions. Thus, the YGGFMRF and SCP_B antisera may well recognize endogenous FaRPs in the flies. The gastrin/CCK antisera may also contain antibodies that identify sulfated residues and hence recognize the drosulfakinins as well as the nonsulfated FaRPs. The polyclonal substance P antiserum, however, may recognize colocalized tachykinin-like peptide in a subpopulation of the FMRFamide-immunoreactive neurons (Nässel et al. 1992b). The differential distribution of CalliFMRFamides 1–13 may be hard to investigate, but possibly some clues can be obtained by testing the synthetic blowfly FaRPs in RIA or ELISA against the different antisera that label subpopulations of the FLI neurons. It is for instance known that the SCP_B antiserum can bind to some N-terminus extended forms of FMRFamides, like lobster

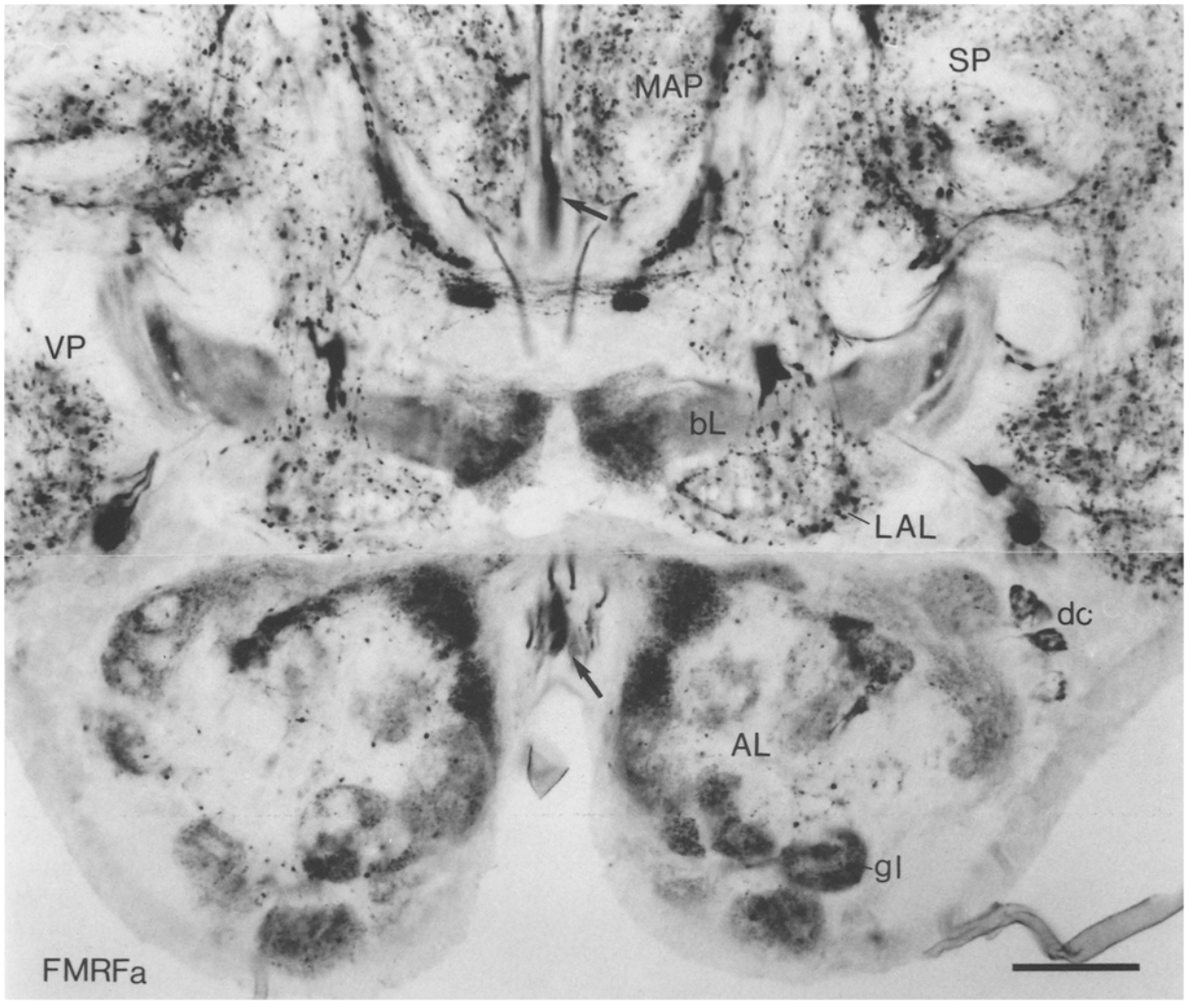


Fig. 8. Micrograph of a cryostat section through an anterior portion of the ventral brain of *Calliphora* labeled with an FMRFamide antiserum. The extensive FLI labeling of fibers in both glomerular and nonglomerular neuropil is seen. Note labeled glomeruli (*gl*) in antennal lobe (*AL*). FLI fibers are seen in beta lobes (*bL*) of mushroom body, lateral accessory lobes (*LAL*), median anterior protocerebrum (*MAP*), superior (*SP*) and ventral (*VP*) protocerebrum. *dc* Deutocerebral FLI cell bodies. *Scale bar:* 50 μm

TNRNFLRFamide (Arbiser and Beltz 1991). Of course, the most direct approach would be to raise antisera to the specific N-termini of the different FaRPs.

Peptides remotely related to tachykinins (including locustatachykinins and leucokinins)

The tachykinins constitute a large family of peptides most of which have been isolated from lower vertebrates and mammals; its most famous member is substance P (Erspamer 1981; Helke et al. 1990; Krause et al. 1990). The peptides of the tachykinin family are recognized by the C-terminus core -FXGLMamide (where X is F, V, Y, or I). Immunocytochemistry with heterologous antisera had quite early indicated the presence of tachykinin-related peptides in insects (El-Sahly et al. 1980), but not until much later were the first insect neuropeptides with sequence homology to vertebrate tachykinins isolated: the four so-called locustatachykinins (LomTK I–IV) isolated from extracts of the brain and corpora cardiaca of the locust *Locusta migratoria* (Schoofs et al. 1990a, b). The sequence of LomTK I is G**PSGFYG**V-Ramide (Schoofs et al. 1990a) (the bold letters denote homologies to physalaemin, a vertebrate tachykinin).

Another set of insect neuropeptides that constitute a family of insect kinins, distinct from the tachykinins, were discovered first in the cockroach *Leucophaea* and then in a cricket and a locust species (Holman et al. 1990a, b; Schoofs et al. 1992a). Leucokinins have myotropic action on visceral muscle and effects on ion transport in the Malpighian tubules (Holman et al. 1990a, b). The octapeptides leucokinin I–VIII (LK I–VIII) were originally considered remotely related to the vertebrate tachykinins by virtue of their analogous C-terminus core- FXSWGamide, i.e., four preserved and one variable residue (Nachman et al. 1990). Here the two types of peptides will, however, be treated as kinins (LK I–

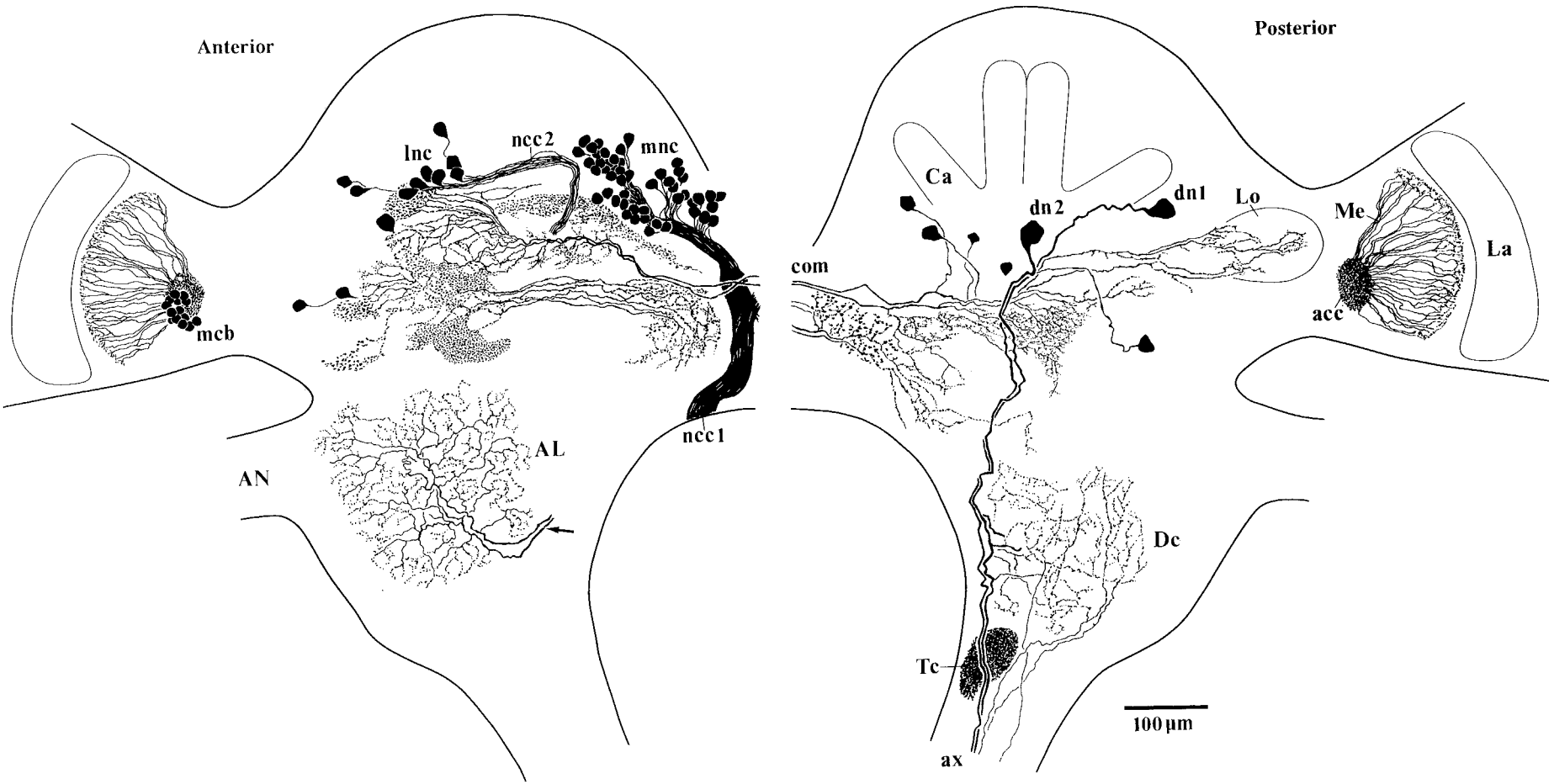


Fig. 9. Tracing of leucokinin-immunoreactive (LKIR) neurons in the brain of the cockroach *Leucophaea maderae*. *Left side* shows anterior neurons, *right side* posterior ones. Neurosecretory cells of the lateral (*lnc*) and median (*mnc*) neurosecretory cell groups send axons to the corpora cardiaca nerves (*ncc1, ncc2*). Local LKIR neurons (*mcb*) connect the medulla (*Me*) to the accessory medulla (*acc*). Descending neurons (*dn1, dn2*) arborize in antennal lobe (*AL*) and posterior deutocerebrum (*Dc*) before their axons (*ax*) project throughout the ventral cord. *AN* Antennal nerve; *Ca* calyx; *com* commissural fibers; *La* lamina; *Lo* lobula; *Tc* tritocerebral neuropil. This figure is slightly altered from Nässel et al. (1992a) with permission from Wiley Liss

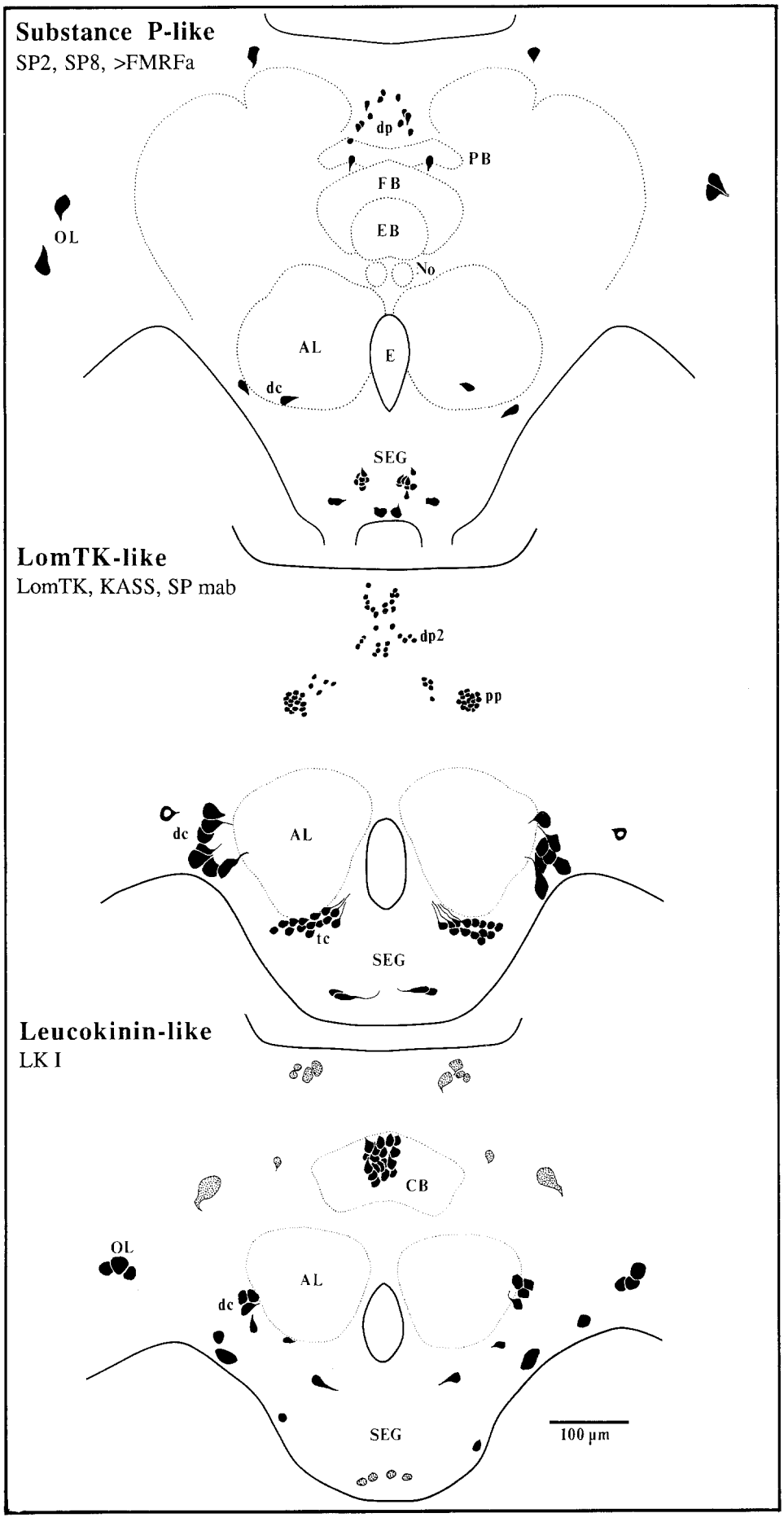


Fig. 10. Blowfly brain labeled with antisera against substance P, locustatachykinin I, kassinin, and leucokinin I. The substance P-immunoreactive neurons (*top panel*) are a subpopulation of the FMRFamide-immunoreactive neurons like in *Drosophila*. The locustatachykinin-immunoreactive neurons (*middle panel*) can also be labeled with a kassinin antiserum and a monoclonal substance P antiserum. The leucokinin-immunoreactive neurons (*bottom panel*) could not be labeled with any other antiserum. Note that these three neuron populations do not overlap. The lower case abbreviations indicate neuron types. Other abbreviations: *AL* antennal lobe; *E* esophageal foramen; *EB* ellipsoid body; *FB* fan-shaped body; *No* noduli; *OL* optic lobe neurons; *PB* protocerebral bridge; *SEG* subesophageal ganglion. Altered from Nässel et al. (1991, 1992c)

VIII, achetakinins I–V and locustakinin I) and tachykinins (LomTK I–IV).

Antisera raised in our laboratory against LK I (DPAFNSWGamide) and LomTK I have recently been applied to nervous tissue of a range of insect species (Nässel and Lundquist 1991; Cantera et al. 1992; Nässel et al. 1992a, b; Nässel 1993). The different antisera recognize two separate populations of neurons, leucokinin-immunoreactive (LKIR) and locustatachykinin-immunoreactive (LTKIR) ones, in each tested species. In the brain of *Leucophaea* we found about 160 LKIR neuronal cell bodies all located in the protocerebrum and optic lobes (Nässel et al. 1992a). Both interneurons and neurosecretory cells are labeled, and many glomerular and nonglomerular neuropil regions are innervated as shown in Fig. 9. About 40 LTKIR neurons are distributed in the proto-, deuto- and tritocerebrum of the same species. These are mainly interneurons, except a few small cells in the lateral neurosecretory group. The distribution of LKIR and LTKIR processes overlaps to some extent, but the morphology and projections of the individual neurons differ between the two types of immunoreactive peptides (unpublished observations).

In the blowfly brain about 60 LKIR and 160 LTKIR neurons have been found, both distributed in proto-, deuto-, and tritocerebrum (Fig. 10; see also Fig. 6g, h). Of the LKIR neurons only neurosecretory cells and two protocerebral descending neurons appear similar to the LKIR cockroach neurons, and most of the LTKIR neurons also appear to be of different types in the two species. We found that the LTKIR neurons also react with an antiserum against the vertebrate tachykinin kassinin (Nässel et al. 1992b; C.T. Lundquist and D. Nässel, in prep.). A third population of neurons, a subpopulation of the FLI neurons, can be labeled with two polyclonal substance P antisera in *Calliphora* (Fig. 10) and *Drosophila* (Fig. 4b; Nässel et al. 1990, 1992b). Both kinins and tachykinins seem to act as neuromodulators, and at least the kinins may have important roles as neurohormones.

In *Drosophila* two different G-protein-coupled tachykinin receptors have been isolated by recombinant DNA technique, one of these responds best to locustatachykinin II when expressed in a cell line, the other has been tested only with vertebrate tachykinin ligands (Li et al. 1991; Monnier et al. 1992). From the original bioassays from which the leucokinins were isolated and from structure-activity analysis with leucokinin analogs, it would seem that specific receptors for leucokinins also exist, at least in the intestine (Nachman et al. 1990).

Pigment-dispersing factors (pigment-dispersing hormones)

A number of closely related octadecapeptides have been isolated from crustaceans and insects by their ability to disperse pigment in chromatophores of fiddler crabs (Rao et al. 1985, 1991). These peptides have been named pigment-dispersing hormones (PDH) in crustaceans and pigment-dispersing factors (PDF) in insects. The PDF of the cockroach *Periplaneta* has the sequence NSELINSLLGLPKVLNDAamide (Mohrherr et al. 1991) and those isolated from a grasshopper (Table 2) and a cricket species are very similar (Rao et al. 1991). In head extracts of the blowflies *Calliphora* and *Phormia*, PDF activity can be demonstrated in the crab bioassay and ELISA and has been purified and partially characterized (Nässel et al. 1993). In *Calliphora* the peptide is clearly a PDH family member.

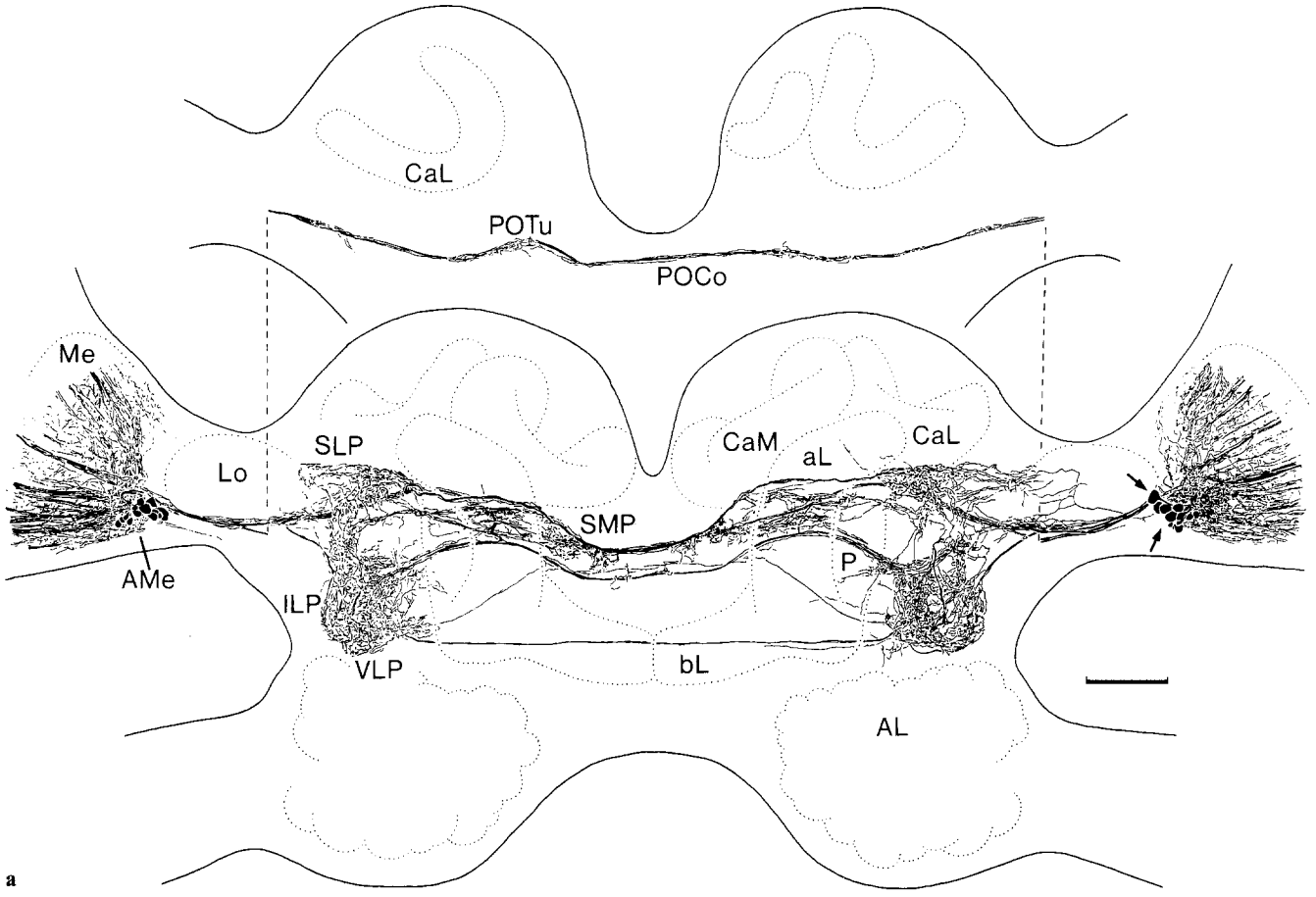
Antiserum raised against the β-PDH of the fiddler crab *Uca pugilator* has been used for localization of PDH-like immunoreactive (PDHLI) neurons in a variety of insects including grasshoppers, crickets, locusts, cockroaches, a phasmid, blowflies, and fruitflies (Homberg et al. 1991b; Nässel et al. 1991, 1993). One interesting finding that emerged from these studies is that the most prominent sets of PDHLI neurons are associated with the visual system and they are fairly well preserved morphologically between species (Fig. 11a, b). PDHLI cell bodies at the base of medulla form widely arborizing processes in the medulla, lamina, and several regions of the midbrain. In all orthopterans, except crickets, and in blowflies and *Drosophila* these neurons form strong bilateral connections (Nässel et al. 1991, 1993). The morphology and projections of the bilateral PDHLI neurons make them suitable candidates for being incorporated into a bilaterally synchronized circadian pacemaker system possibly connected to a system of extraocular photoreceptors (Homberg et al. 1991b; Nässel 1991; Nässel et al. 1991).

There are small sets of additional PDHLI neurons in some insect species, e.g., neurons supplying the locust tritocerebrum (Homberg et al. 1991b) and PDHLI neurosecretory cells in the median neurosecretory cell group (MNC) of the cricket *Acheta* and the moth *Manduca* (Homberg et al. 1991a, b) and lateral neurosecretory cell group (LNC) of the blowfly (Nässel et al. 1993).

Peptides of the PDF type may thus be involved in regulatory functions related to the visual system, possibly associated with a circadian pacemaker system. Compared to many of the other insect neuropeptides that are presented here, brain PDFs seem to have rather limited sites of action. Furthermore, the peptide may have a neurohormonal role, but not in control of pigment dispersion since this phenomenon is not known in the studied insects.

Vasopressinlike peptide (locust F2 peptide)

Rémy and Girardie (1980) described two neurons in the subesophageal ganglion of the locust *Locusta migratoria* that react with antisera against vasopressin and neurophysin. These neurons have axonal projections throughout the nervous system, including the brain and optic lobes as well as plexuses of immunoreactive terminals in peripheral nerve roots. Later two locust neuropeptides, F1 and F2 (Table 2), were isolated by monitoring fractions in radioimmunoassay with arginine vasopressin antiserum (Proux et al. 1987). F1 is a monomer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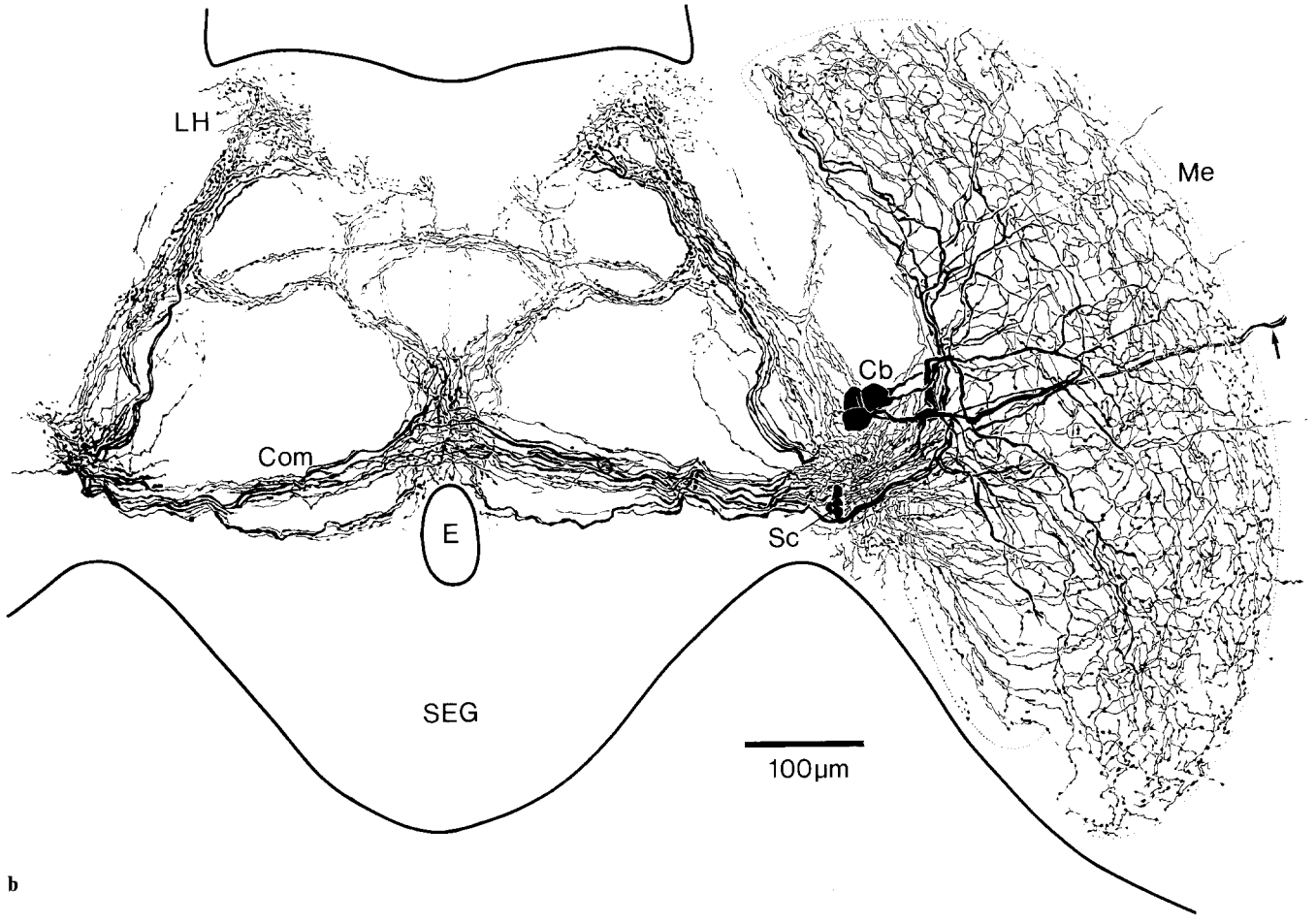
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b

with the sequence CLITNCPRGamide, and F2, which is the biologically active compound, is an antiparallel dimer of F1. Proux et al. (1987) showed that F2 has diuretic activity in the locust. Recently a detailed investigation was made of the arginine vasopressinlike immunoreactive (VPLI) neurons of *Locusta migratoria*, with immunocytochemistry combined with intracellular recordings and staining (Thompson et al. 1991; Thompson and Bacon 1991). Intracellular recordings from these neurons indicate a photosensory input: the spiking activity in the VPLI neurons is greater in the dark than in light (Thompson and Bacon 1991). It appears that the inputs to the VPLI neurons in the subesophageal ganglion are derived from descending neurons of the protocerebrum. Either these descending neurons are photosensory themselves or they have inputs from extraocular photoreceptors. Thompson and Bacon (1991) suggest that the VPLI neurons with their inputs from extraocular photoreceptors are integrated into a circadian control system, where possibly F2 or VPLI material is released in a circadian fashion. Recently Coast et al. (1992) found that synthetic F2 does not have any effect on fluid secretion or cyclic AMP levels of the locust Malpighian tubules (in contrast to locust diuretic peptide, Locusta-DP). These authors also dispute hormonal release of F2 into the circulation. Coast et al. (1992) suggest that all release is within the CNS, and that the varicose VPLI fibers in the nerve roots, which earlier were proposed to be in neurohemal release areas, are inside the neural sheath. In fact, in another locust species, *Schistocerca gregaria*, no VPLI fibers at all are found in peripheral nerve roots (Evans and Cournil 1990).

In the blowfly we have so far not been able to label any neurons in the subesophageal ganglion (or any other ganglion) with different arginine vasopressin antisera. When an antiserum against lysine vasopressin (LVP) that labels the VPLI cells in the locust subesophageal ganglion and additional cells elsewhere (Thompson et al. 1991) is applied to the blowfly, neurons can be labeled only in the abdominal ganglia (Nässel et al. 1989). It would thus appear that the blowfly uses a peptide structurally different from F1/F2 and arginine vasopressin, if indeed this system is present at all. Davis and Hildebrand (1992) have shown that the same LVP antiserum labels neurons in the brain and ventral ganglia of cockroaches and mantids, and in *Leucophaea* the neurons labeled with this LVP antiserum are a major subpopulation of the leucokinin-immunoreactive neurons (Nässel et al. 1992 a; Fig. 9).

Fig. 11. Frontal reconstructions of pigment-dispersing hormonelike immunoreactive (PDHLI) neurons in the brains of the cockroach *Periplaneta americana* (**a**) and the blowfly *Phormia terraenovae* (**b**). **a** The PDHLI neurons have cell bodies (*arrows*) near the medulla (*Me*) and innervate the medulla, accessory medulla (*AMe*) and protocerebral neuropil (*ILP, SLP, VLP, SMP, POTu*). Other abbreviations: *AL* antennal lobe; *aL, bL, CaL, CaM*; *P* portions of mushroom body; *Lo* lobula; *POCo* posterior commissure. *Scale bar:* 200 μm. Figure from Homberg et al. (1991) with permission from U. Homberg and Springer Verlag. **b** In the blowfly similar PDHLI neurons were resolved. Their cell bodies (*Cb, Sc*) are at the medullar base and their processes run in a posterior commissure (*Com*) and have collaterals to the lateral horn (*LH*) and arborizations in the medulla (*Me*), lamina and lobula (not shown here). Altered from Nässel et al. (1991, 1992c)

Other sequenced insect neuropeptides

The insect neuropeptides below are treated more briefly either because they have not been mapped in the brain in full detail yet or because their distribution in the brain is very restricted.

Allatostatins and allatotropins. Five peptides, allatostatins A1–4 and B2, that inhibit juvenile hormone synthesis have been isolated from cockroach brain-retrocerebral complexes (Pratt et al. 1991); the sequence of allatostatin A2 is APSGAQRLYGFGLamide. Another allatostatin has been isolated from the moth *Manduca* (Kramer et al. 1991) with a different sequence given in Table 2. Antiserum against the cockroach allatostatin A1 has been used to map immunoreactive neurons in the cricket and cockroach brains (Schildberger and Agricola 1992). Neurosecretory cells (LNC) with axons to corpora allata have been seen as well as neurons innervating the antennal lobes, central complex and nonglomerular protocerebral neuropil. The allatostatin antiserum thus labels more than neurosecretory cells, and allatostatin-related peptide(s) may have additional roles as neuroregulators in the CNS.

A peptide stimulating secretion of juvenile hormone from corpora allata of *Manduca* has also been isolated and sequenced (Kataoka et al. 1989). This peptide (sequence in Table 2), termed allatotropin, has not yet been localized by immunocytochemistry.

Crustacean cardioactive peptide (CCAP). A cardioactive peptide with the structure PFCNAFTGCamide, called CCAP, has been sequenced both from a crab and the locust *Locusta migratoria* (Stangier et al. 1989). Interestingly, it turned out that one of the *Manduca* cardioacceleratory peptides (CAP_{2a}) has an amino acid sequence identical to the crab and locust CCAP (Loi et al. 1992). The distribution of CAPs in the abdominal ganglia of larval *Manduca* has been studied with immunocytochemistry using antibodies to purified CAPs (Tublitz et al. 1992). In the ventral cord of the locust an antiserum against CCAP labels prominent efferent and intersegmental neuronal systems (Dircksen et al. 1991). So far data on the distribution of CCAP-immunoreactive neurons in the brain has been published only for the meal beetle *Tenebrio molitor* (Breidbach and Dircksen 1991). Ten neurons of the lateral neurosecretory cell clusters with processes in superior median protocerebrum and tritocerebrum display immunoreactivity.

Corazonin. Another cardioactive peptide, corazonin, with the sequence pQTFQYSRGWTNamide has been isolated from the cockroach *Periplaneta* (Veenstra 1989). Its distribution has been published for the cockroach brain (Predel et al. 1992), where neurosecretory cells with axons to the retrocerebral complex are immunore-

active. In the blowfly, neurosecretory cells of the LNC group and two protocerebral descending neurons have been detected with a corazonin antiserum (D. Nässel, R. Cantera and J.A. Veenstra, in prep.).

Locustamyotropin. An antiserum to the myotropic peptide locustamyotropin II (Lom MT II; EGNFTPRLamide) labels neurons and neurosecretory cells of the locust tritocerebrum and processes in proto- and tritocerebrum, frontal ganglion, corpora cardiaca, and corpora allata (Schoofs et al. 1992b). This antiserum may also recognize locustapyrokinin-, PBAN- and MRCH-like peptides since these peptides share the C-terminus sequence -PRLamide (see Tables 1 and 2). A locust myoinhibitory peptide with a sequence related to the mollusc peptide APGW has been isolated but not mapped by immunocytochemistry (Schoofs et al. 1991).

Pheromone biosynthesis activating neuropeptide (PBAN). A peptide controlling pheromone production in the pheromone glands was first isolated from the moth *Heliothis zea* (Raina et al. 1989; Table 2). PBAN-immunoreactive neurons have been described recently in the CNS of the moth *Helicoverpa zea* (Kingan et al. 1992). Some of these are neurosecretory cells of the subesophageal ganglion with terminals in the corpora cardiaca; others are neurons with release terminals within the subesophageal and body ganglia.

Locust accessory gland myotropin. This myotropin Lom-AG-MT I (Tables 1, 2) is actually the only insect peptide isolated from a nonneural tissue (male accessory reproductive gland) with a distribution also in the brain (Paemen et al. 1991, 1992). The distribution of immunoreactive cell bodies in proto- and deutocerebrum as well as the optic lobes and fibers in many neuropil regions throughout the brain indicate an important regulatory role of this peptide (with no sequence resemblance to any known neuropeptide).

Other insect peptides. Diuretic hormone, eclosion hormone, prothoracicotropic hormone (PTTH), and bombyxin (see Schooley et al. 1991; Nagasawa 1992; Table 2) have been detected only in neurosecretory cells of the moths *Manduca sexta* and *Bombyx mori* (Copenhaver and Truman 1986; Mizoguchi et al. 1987; Kono et al. 1990; Veenstra and Hagedorn 1991; Nagasawa 1992). The neuroparsins A and B (Table 2) from *Locusta*, with some sequence homologies to human neurophysin (Girardie et al. 1989; Hietter et al. 1991), and with possible roles in regulation of fluid reabsorption in the rectum and increase of lipid and trehalose levels in the hemolymph, can also be found only in neurosecretory cells of insects (Girardie et al. 1989; Tamarelle and Girardie 1989).

Opioid-related peptides in insects?

Opioid-related neuropeptides have not yet been isolated and sequenced from insect tissue, but probably will be in the near future, since pentapeptides identical to Met- and Leu-enkephalin (YGGFM and YGGFL, respectively) have been isolated from the mollusc *Mytilus* (Leung and Stefano 1984) and the crab *Carcinus maenas* (Lüschen et al. 1991). There is no lack of indications that peptides closely related to or identical to Met- and Leu-enkephalin are used as messengers in the insect nervous system. Radioimmunoassay and immunocytochemistry have demonstrated enkephalinlike material in locust, cockroach, and fly tissue (Pagés et al. 1983; Davenport and Evans 1986; Duve and Thorpe 1988). High-affinity binding sites for Met-enkephalin analogs can be shown in the brain and midgut of the cockroach *Leucophaea maderae*, and in the same insect, administration of a Met-enkephalin analog results in a decrease of locomotor activity, whereas a Leu-enkephalin analog and dynorphin enhance locomotor activity (Stefano and Scharrer 1981; Ford et al. 1986; Scharrer et al. 1988).

In the cockroach brain, Met-enkephalinlike immunoreactivity (M-ENK-LI) has been seen in median neurosecretory cells with axons to corpora cardiaca and in neurons of the tritocerebrum (Verhaert and De Loof 1985). In the blowfly Leu-enkephalinlike immunoreactivity (L-ENK-LI) and M-ENK-LI have been found to be distributed in distinctly different sets of neurons (Duve and Thorpe 1988). M-ENK-LI is seen in a rather small number of protocerebral and subesophageal neurons, and L-ENK-LI can be demonstrated in a few median neurosecretory cells and protecerebral and subesophageal cells. Also, distinct fiber labeling has been shown in tritocerebral neuropil and one layer of the fan-shaped body. Enkephalinlike peptide(s) may have both central neuromodulatory and peripheral neurohormonal role(s) in insects.

Recently it has also been shown that a Met-enkephalin analog induces enhancement of adherence and migration of hemocytes in *Leucophaea* and the clam *Mytilus*, indicating a cytokine function of this opioid peptide (see Scharrer 1991). The hemocytes appear to possess high-affinity opioid receptors and like in the mammals (Johnson et al. 1992; Plata-Salamán 1991), there may be a link between the neuroendocrine and "immune" systems in insects.

Is there a myomodulinlike peptide in insects?

Myomodulin A with the sequence PMSMLRLamide has been isolated from the accessory radula closer (ARC) buccal muscle of the marine mollusc *Aplysia californica* (Cropper et al. 1987). This peptide is released from a cholinergic motor neuron, B16, that innervates the ARC muscle, and its action is to potentiate the muscle's response to acetylcholine during consummatory biting (Cropper et al. 1987). Except for the immunocytochemistry presented here and by Nässel et al. (1992c) there is no evidence for the presence of a myomodulinlike peptide in insects.

In the brain of the cockroach, *Leucophaea*, there are about 120 MMLI cell bodies distributed in the proto-, deuto-, and tritocerebrum, but not in the optic lobes (Fig. 12A). Immunoreactive fibers innervate many neuropil regions of the brain, including the calyces of the

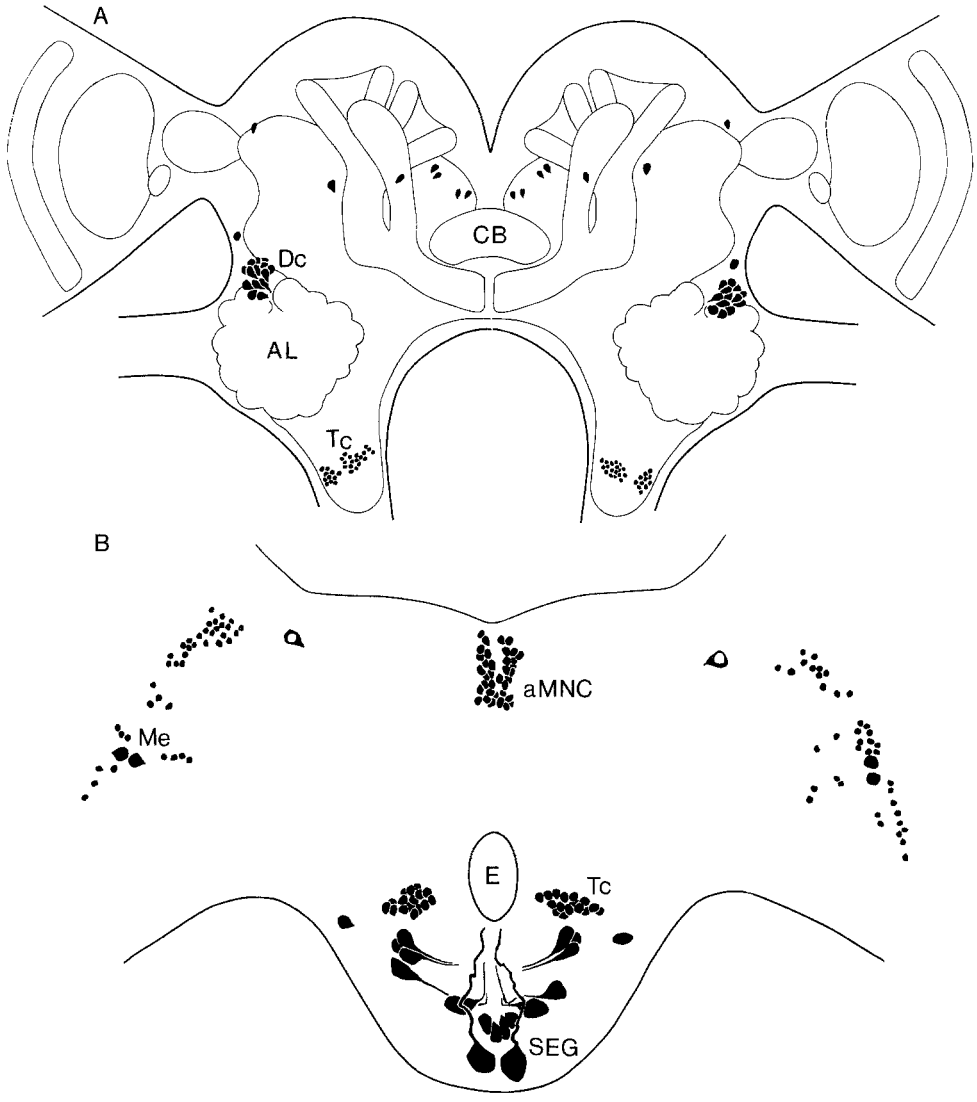


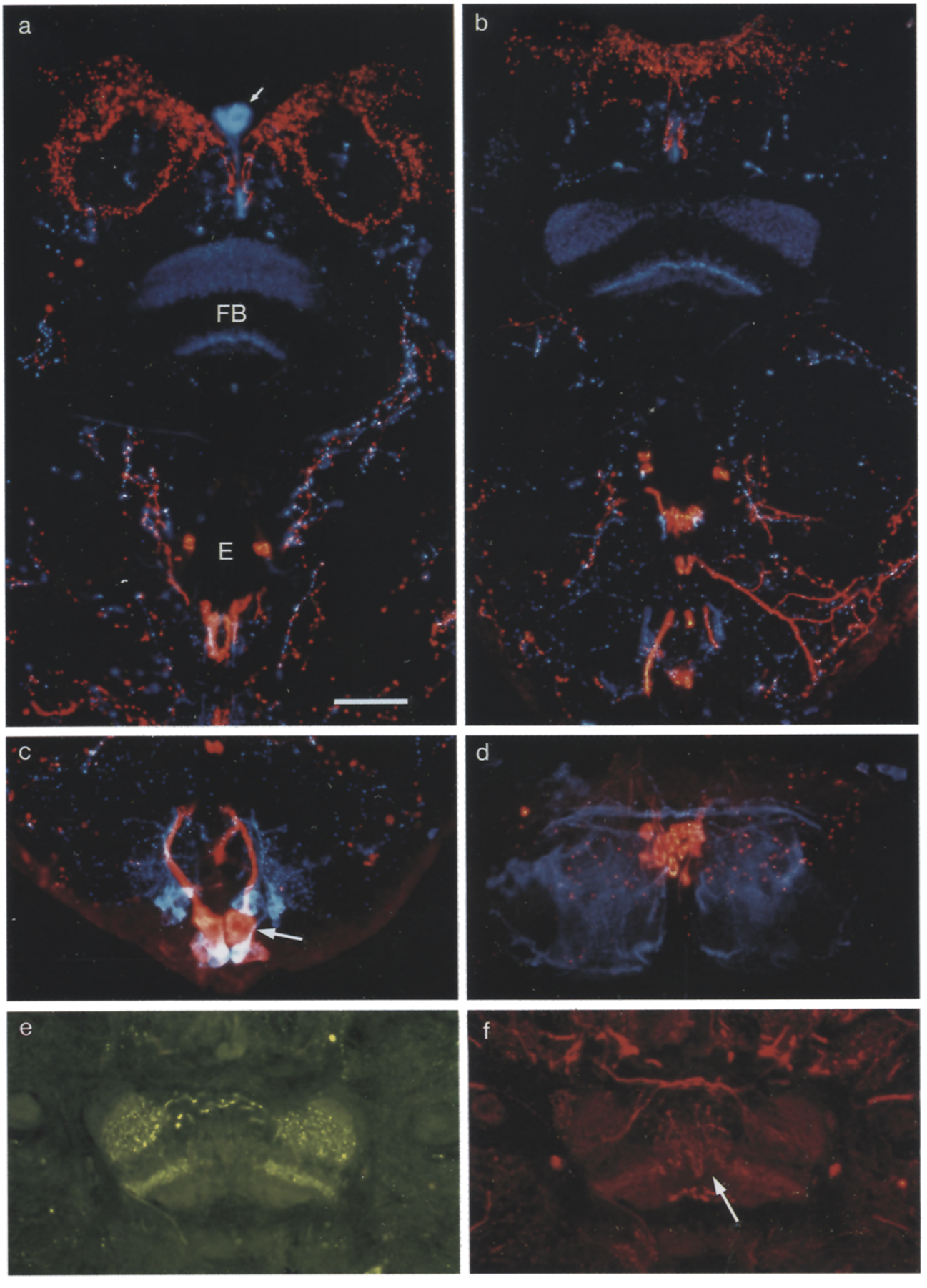
Fig. 12. Myomodulin-like immunoreactive (MMLI) neurons in the cockroach (**A**) and blowfly brains (**B**) in frontal views. Only the cell bodies are shown here (for fiber staining, see Fig. 13). Major differences between the species is that deutocerebral cells (*Dc*) are seen only in the cockroach, whereas only in the blowfly neurosecretory cells (*aMNC*) and optic lobe neurons (*Me*) were detected. Possibly homologous cells are seen in the tritocerebrum (*TC*). From Nässel et al. (1992c)

mushroom bodies, the fan-shaped body, and the antennal lobes. The MMLI neurons are efferents of the tritocerebrum and interneurons in other regions. As a comparison the blowfly brain contains about 140 MMLI cell bodies, distributed in the optic lobe, proto- and tritocerebrum, but not in the deutocerebrum (Figs. 12B, 13a–c). Additionally there are about 18 large MMLI cell bodies in the subesophageal ganglion. The MMLI neurons in *Phormia* are efferents of the tritocerebrum, neurosecretory cells of the anterior portion of the median neurosecretory cell group, and interneurons in the midbrain and optic lobe, but not in the mushroom bodies and fan-shaped body (Fig. 13a, b). It is thus clear that the only candidates for homologous neurons displaying MMLI in the blowfly and cockroach brain are the tritocerebral neurons. Double labeling experiments have shown that only a single pair of MMLI neurons also display FMRFamide and SCP_B-like immunoreactivity (Fig. 13c). This unique distribution indicates that the native MMLI peptide has a specific function in a set of neurons where so far no other neuropeptide or monoamine has been demonstrated (except in the FLI subesophageal giant neurons).

Are there neuropeptides related to galanin and galanin-message associated peptide in insects?

Galanin (GAL), which consists of 29 amino acid residues, has been isolated from pig small intestine but has a widespread distribution in the mammalian central and peripheral nervous system and has multiple functions (Rökaeus 1987). On the GAL precursor (preproGAL) a second peptide consisting of 59 residues named GMAP has been found (Rökaeus and Brownstein 1986). The sequences of GAL and GMAP display no homologies to known insect peptides.

In the blowfly central nervous system both GAL- and GMAP-like immunoreactive (GALI and GMAPLI) neurons and neurosecretory cells can be found (Lundquist et al. 1991, 1992). The distribution of GALI fibers in the fan-shaped body is shown in Fig. 13e. If the fly peptides were derived from a common precursor, as in mammals, one would expect GALI and GMAPLI material to be colocalized in neurons of the fly brain. Indeed, double labeling experiments have demonstrated that about half of the GALI neurons also react with GMAP antiserum (Lundquist et al. 1992). There are, however,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neurons reacting only with GMAP antiserum or GAL antiserum. It is not clear whether this is due to the fact that the two immunoreactive peptides in flies are derived from two different precursors (unlike mammals), or if differential gene splicing, differential posttranslational processing, or neuropeptide turnover causes differential expression of immunoreactive material in the neurons.

We have demonstrated ^{125}I-GAL binding sites in the blowfly brain by autoradiography (Johard et al. 1992). Specific binding of porcine ^{125}I-GAL1–29 (the holopeptide) as well as its C-terminus fragment ^{125}I-GAL15–29 can be seen.The highest binding intensity is obtained with the C-terminus fragment. Specific binding sites are seen in the fan-shaped body, the antennal lobes, the medulla of the optic lobe, pars intercerebralis, and the subesophageal ganglion. All binding sites, except the antennal lobes, correspond well to the neuropil regions with dense innervation by GALI fiber terminals (Lundquist et al. 1991; Johard et al. 1992).

Concluding remarks

Immunocytochemistry is clearly not sufficient to obtain an understanding of neuropeptide function, but there are cases where the distribution of immunoreactive neuropeptide in insects may reflect a possible function or at least a distinct target for peptide release. Obvious examples are when neuropeptides are expressed in efferent neurons, for instance proctolin in efferents to the hindgut (see Eckert et al. 1981) and to skeletal muscles (O'Shea and Schaffer 1985), where modulatory actions on muscle activity can be demonstrated (O'Shea and Schaffer 1985).

Fig. 13. Fluorescence micrographs of double labeling of frontal sections of the blowfly brain. **a, b** Double exposure of double labeling with rabbit anti-myomodulin (TRITC, *red*) and mouse anti-SCP_B (AMCA, *blue*). In these two adjacent sections the two antisera each recognize a unique set of neural processes. **a** Dense myomodulinlike immunoreactivity (MMLI) is seen in varicose fibers in pars intercerebralis of dorsomedial protocerebrum (*top of* micrograph) whereas SCP_B immunoreactivity (SCLI) is seen in two layers of the fan-shaped body (*FB*). *E* Esophageal foramen. In **b** arborizing MMLI fibers (red) are seen in the subesophageal ganglion (*lower part* of micrograph). *Scale bar* **a, b:** 50 μm. **c** MMLI and SCLI material (labeling as in **a** and **b**) in the subesophageal ganglion. In one pair of large cells (*arrow*) MMLI and SCLI material is colocalized. *Scale* as in **a**. **d–f** These figures illustrate that in the blowfly brain monoamines and neuropeptides are not colocalized. **d** Double exposure of double labeling with rabbit antiserum against leucokinin I (TRITC, *red*) and mouse anti-tyrosin hydroxylase (AMCA, *blue*). The frontalmost part of protocerebrum is shown with a group of leucokinin-immunoreactive (LKIR) neurosecretory cells. Note the partial overlap of LKIR varicosities (*red*) and tyrosin hydroxylase immunoreactive processes in neuropil. *Scale* as in **a**. **e, f** Double labeling of the same section of the central complex with anti-galanin (FITC, *yellow green*) and anti-tyrosin hydroxylase (TRITC, *red*). The two antigens are distributed in separate neuron populations, but with partly overlapping fields of innervation in the fan-shaped body, bot not in ellipsoid body (*arrow*). *Scale* as in **a**

It is rare to see insect neuropeptides with a distribution indicating a single or a few defined functions. Two examples are the PDHLI visual interneurons in dipteran and orthopteran insects (Homberg et al. 1991; Nässel et al. 1991), and neurokinin A-like immunoreactive neurons restricted to the visual system of the cockroach *Leucophaea* (Nässel 1991; Nässel et al. 1992b). These systems might lend themselves to studies of neuropeptide action by a combination of pharmacology and intracellular recordings. Some of the peptide neurohormones, like PTTH, eclosion hormone, and diuretic hormone, appear to be restricted to single neurosecretory systems (see Tublitz et al. 1986; Raabe 1989; Veenstra and Hagedorn 1991), which may limit the search for functions to hormonal actions.

Many neuropeptides, however, have extensive distributions in large populations of neurons in the brain and appear to have multiple functions in the organism. Examples are the FaRPs and leucokininlike peptides, which exist in many forms, are distributed in diverse classes of neurons, and may act on multiple types of receptors, similar to for instance neuropeptide Y and tachykinins in mammals (Lehmann 1990; Helke et al. 1990; Krause et al. 1990). Here the distributed actions of individual peptide species may be hard to get a handle on due to the small size of the insect brain.

A few aspects of peptide distribution patterns may be worthwhile summarizing. Some specific neuropil regions appear especially rich in innervation by neurons expressing different neuropeptides. In the blowfly the fan-shaped body of the central complex is innervated by different neurons reacting with antisera against FaRPs, proctolin, AKH, leucokinins, locustatachykinins, Leu-enkephalin, galanin, and GMAP. The neuropil in the superior protocerebrum associated with pars intercerebralis and the basal portion of the medulla in the optic lobe are other regions with about the same number of different immunoreactive neuropeptides. On the other hand there are some neuropils in which so far only one neuropeptide has been detected, e.g., the ellipsoid body of the central complex, the different compartments of the mushroom bodies, and the lamina of the optic lobes. This differential distribution may imply that peptidergic modulatory actions are more important and complex in some centers than in others, or alternatively that many neuropeptides have escaped detection in the listed neuropils since antisera against them are not yet available.

Very few suggestions about functions of peptidergic systems in the CNS centers are available. Is it even justified to say that there are peptidergic systems within the insect brain? Is it possible that peptides are always released with other primary messengers and have modulatory actions on synaptic transmission only? Two different peptidergic systems have been proposed in the insect CNS: the PDHLI neurons associated with the accessory medulla and the subesophageal vasopressin immunoreactive neurons have both been implicated in circadian regulatory functions (Homberg et al. 1991b; Thompson and Bacon 1991; Stengl and Homberg 1992). Both systems are amenable to experimental studies, and it may turn out that neuropeptides can be mediators of syn-

chronization of circadian activity as has been seen in the rat suprachiasmatic nucleus (see Albers et al. 1991).

There is ample evidence for colocalized peptides in insect neurons (see section on FaRPs and Figs. 5, 6). Surprisingly few examples of colocalization of neuropeptides and monoamines and other neurotransmitters have, however, been published for insects, although studies of mammalian systems provide numerous cases of coexistence (Lundberg and Hökfelt 1983; Bartfai et al. 1988). A few examples in insects are: coexistence of FMRFamide-related peptides and GABA and serotonin (Homberg et al. 1990), α-endorphin and dopamine (Takeda et al. 1986), and proctolin and glutamate (Adams and O'Shea 1983). Superposition of peptidergic and monoaminergic processes in neuropil (Fig. 13d–f) is on the other hand fairly common.

One of the most urgent aspects of neuropeptide biology to pursue in insects is the spectrum of actions of neuropeptides in the central and peripheral nervous system. The rate by which new peptides are sequenced and subsequently mapped by immunocytochemistry is by no means matched by attempts to delineate their functions. It is obvious that the fastest route to understanding the cellular and molecular mechanisms of peptide action is to select accessible neuron-target systems, such as glands and muscles (or studying hormonal actions). Consequently this is where most progress has been made (see O'Shea and Schaffer 1985; O'Shea et al. 1985; Evans and Myers 1986; Tublitz et al. 1986, 1991; Cuthbert and Evans 1989; Raabe 1989; Walker 1992). To understand the diversity of actions of different neuropeptides, however, it is crucial also to include central circuits. The shortage of peptide ligands (of peptide and nonpeptide nature) that can traverse the blood-brain barrier commonly precludes simple systemic delivery of substances for behavioral analysis. However, in a simple behavior, such as the proboscis extension reflex of the honeybee, the antagonistic modulatory actions of FMRFamide and CCK8 on the gustatory and olfactory responses can be analyzed after injection of the peptides into the mushroom bodies (Erber et al. 1991). In small simple ganglia such as the crustacean stomatogastric ganglion, with less well-developed blood brain barriers, modulatory actions of neuropeptides have been studied extensively (Harris-Warrick and Marder 1991).

Another rather uncharted domain in insect neuropeptide biology is the characterization of peptide receptors and associated second messenger systems, although quite some progress has been made in this area for acetylcholine, amino acid transmitters, and monoamines (see Restifo and White 1990; Buchner 1991). One neuropeptide Y- and two tachykininlike receptors have been cloned from *Drosophila*. One of the tachykinin receptors (NKD), when expressed in mouse NIH-3T3 cells, induces increases in inositoltriphosphate (IP_3) synthesis in response to tachykinins, notably locustatachykinin II (Monnier et al. 1992). When the other receptor (DTKR) is expressed in *Xenopus* oocytes, substance P evokes inward chloride currents, a response that can be blocked by pertussis toxin indicating that the DTKR is a G-protein coupled receptor and can activate the phospholipase C pathway (Li et al. 1991).

With the advances of molecular genetics in *Drosophila* (see Buchner 1991) it is to be expected that we will see rapid progress in understanding of neuropeptide receptors and second messenger pathways. Some work on neuropeptides and their receptors indicates, that this class of bioactive substances also has roles during embryonic development (Hökfelt 1991; Li et al. 1991, 1992; Monnier 1992) and as cytokines in the immune system (Scharrer 1991; Johnson et al. 1992), fields that still are in their infancy in insects.

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Note added in proof. After this manuscript was sent to press I became aware of a number of recent publications that seem appropriate to mention. Among some novel FMRFamide-related (FaRP) peptides recently isolated from *Drosophila* (Nichols 1992a, b), one peptide has a sequence, TDVDHFLRFamide, that is not encoded on the two known *Drosophila* precursors of FaRPs. This raises the possibility that a third gene exists that encodes for FaRPs in this organism (see Nichols 1992b). To the list of peptide families in Tables 1 and 2 a new one can be added after the isolation of a locust ion-transport peptide (Scg-ITP) that resembles the crustacean moult-inhibiting hormone, vitellogenesis-inhibiting hormone and hyperglycaemic hormones (Audsley et al. 1992). The *Schistocerca gregaria* peptide has not been fully sequenced but 31 of estimated 65 amino acids have been identified. The isolation and primary structure of the cardioactive peptide of *Manduca*, identical to CCAP, has been described in more detail by Cheung et al. (1992). A few interesting reviews on insect neuropeptides have appeared recently (Masler et al. 1993; Raina and Menn 1993).

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