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Structure and function of antennal lobe neurons in the male turnip moth, *Agrotis segetum* (Lepidoptera: Noctuidae)

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Abstract Interneurons with dendritic branches in the antennal lobe of the male turnip moth, *Agrotis segetum* (Schiff., Lepidoptera: Noctuidae), were investigated with intracellular recording and staining methods. Seventeen projection neurons that transmit information from the antennal lobe to higher centers in the brain displayed dendritic arbors in the male specific macroglomerular complex (MGC) and responded to chemical components of the female sex pheromone used in species-specific sexual communication. Most of the projection neurons responded to several of the pheromone components tested, and a precise correlation between the location of the dendritic arborization and the physiological response could not be demonstrated. One MGC-projection neuron fit the definition of "blend specialist". It did not respond to the individual components of the behaviorally active pheromone blend, but showed a strong response to the components when combined in the species-specific blend. Some of the projection neurons also showed clear responses to phenylacetaldehyde, a flower-produced compound and/or to (*E*)-2-hexenal, a common green-leaf volatile. In eight neurons, the axonal projection could be followed to the calyces of the mushroom body, and subsequently to the inferior lateral protocerebrum.

Four local interneurons were characterized both morphologically and physiologically. Each neuron arborized extensively throughout the antennal lobe, and each responded to one or several of the pheromone compounds, and/or to one or both of the plant-produced compounds. One of the local interneurons responded exclusively to the pheromone blend, but not to the individual components.

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Abbreviations *AL* antennal lobe · *AN* antennal nerve
CB cell body · *E2H* (*E*)-2-hexenal
IACT inner antennocerebral tract
ILPR inferior lateral protocerebrum
LH lateral horn of the protocerebrum
LN local interneuron · *MB* mushroom body
MGC macroglomerular complex
OACT outer antennocerebral tract
PAA phenylacetaldehyde · *PN* projection interneuron
RN receptor neuron · *Z5-10:OAc* (Z)-5-decenyl acetate
Z5-10:OH (Z)-5-decenol
Z5-12:OAc (Z)-5-dodecenyl acetate
Z7-12:OAc (Z)-7-dodecenyl acetate
Z9-14:OAc (Z)-9-tetradecenyl acetate

Introduction

Like many insects, the male turnip moth uses olfactory cues to locate a suitable mate or a nectar-rich flower. When searching for a female, the male makes use of a chemical signal emitted by the female: a sex pheromone. In the case of the flower, the male follows odors emitted by the plant and the flower (Haynes et al. 1991). The olfactory sense in moths is, in general, very well developed, and in the male it is largely devoted to the detection of the female-emitted sex pheromone (Schneider 1992). The peripheral olfactory receptor neurons (RN) are located on the paired antennae. From its origin on the antenna, a RN sends its axonal projection directly into the brain, more specifically into the antennal lobe (AL) (Boeckh and Boeckh 1979; Hansson et al. 1992; Kootz and Schneider 1987; Christensen et al., unpublished). In the AL, the axon terminals of the RNs make synaptic contact with AL interneurons within specialized neuropil structures common to olfactory systems in animals – the olfactory glomeruli (Boeckh and Tolbert 1993; Tolbert and Hildebrand 1981). The moth AL typically houses

40–70 such glomeruli (e.g., Rospars 1983; Rospars and Hildebrand 1992). In the male, a portion of the AL neuropil is devoted to the macroglomerular complex (MGC), which resembled a group of enlarged and highly-structured glomeruli that is situated at the entrance of the antennal nerve (AN) into the AL (Bretschneider 1924; Koontz and Schneider 1987; Christensen and Hildebrand 1987). The MGC is typically not present in the female. In the large sphinx moth *Manduca sexta*, all pheromone specific RNs project to the MGC (Christensen et al., unpublished), while RNs for other odors like flower scents and green-leaf volatiles project to the sexually isomorphic "ordinary" glomeruli (Christensen et al., unpubl.). The RNs make direct synaptic connections mainly to local interneurons (LNs) neurons that restrict their arborizations to the AL, and fulfill several intra- and interglomerular functions (Christensen et al. 1993; Malun 1991a, b). Odor signals are transmitted from the AL through the projection neurons (PNs) that send axonal projections to higher centers in the ipsilateral protocerebrum. These areas include, but may not be restricted to, the calyces of the mushroom body (MB) and sites in the lateral protocerebrum (Christensen and Hildebrand 1987; Kanzaki et al. 1989; Hansson et al. 1991). The different morphological types of interneurons present in the AL of *M. sexta* have been reviewed in detail by Homberg et al. (1988).

The function of AL interneurons in male moths has been investigated in a number of studies (Boeckh and Boeckh 1979; Christensen and Hildebrand 1987; Christensen et al. 1991; Hansson et al. 1991; Kanzaki and Shibuya 1986; Kanzaki et al. 1989; Matsumoto and Hildebrand 1981; Olberg 1983). Neurons that respond specifically to pheromone compounds have been revealed in all of these studies. However, only in a few has a careful analysis of the AL arborization of PNs been combined with a functional investigation using the complete pheromone of the species (Christensen and Hildebrand 1987; Christensen et al. 1991; Hanssen et al. 1991; Kanzaki et al. 1989).

The pheromone communication system of the turnip moth is very well investigated (Arn et al. 1980; Hansson et al. 1990; Löfstedt et al. 1982, 1985). When a male *A. segetum* is exposed to an odor plume containing the synthetic conspecific sex pheromone in the wind tunnel, it responds with an upwind, anemotactic flight, often ending with a copulation attempt with the odor source. When field traps were baited with the synthetic female pheromone blend, a large number of males were caught (Löfstedt et al. 1985). A female *A. segetum* produces a large number of compounds in the pheromone gland (Arn et al. 1980; Löfstedt et al. 1982), but only four of these compounds are needed to elicit a full behavioral response from the male: Z5-10:OAc, Z5-12:OAc, Z7-12:OAc and Z9-14:OAc (Wu, Hansson and Löfstedt, pers. comm.). It has also been shown that the male attraction is deterred by Z5-10:OH (Löfstedt et al. 1985).

The turnip moth antenna is sexually dimorphic. The male antenna is pectinate, and contains around 50,000 long trichoid sensilla specialized for detection of the female pheromone. The female antenna, in contrast, is filiform and lacks the long sensilla characteristic of the male (Hallberg 1981). Electrophysiological investigations of the male antenna have shown that three physiological sensillum types are present. One type contains RNs for Z5-10:OAc, Z5-10:OH and Z5-12:OAc. A second type embraces a RN for Z7-12:OAc, and a third type houses a RN specific for the detection of Z9-14:OAc (Löfstedt et al. 1982; Hansson et al. 1990). The female shows no electrophysiological response whatsoever to her own pheromone (Hansson et al. 1989).

In an investigation of the AL projections of the different types of RNs in *A. segetum*, it was possible to show that different physiological types projected to exclusive subcompartments of the MGC, suggesting that each compartment was functionally unique (Hansson et al. 1992) (Fig. 2C). When pheromone specific PNs were characterized physiologically and morphologically in the sphinx moth, *M. sexta*, a similar pattern was observed, where the dendritic arborizations of neurons with different specificities invaded different parts of the MGC (Hansson et al. 1991).

We have applied intracellular recording and marking techniques to investigate the AL interneurons in male *A. segetum*. In this study, we describe the response characteristics of these interneurons to pheromone and plant-derived stimuli, as well as their neuronal morphology. We also try to correlate the structural and functional features of these neurons that are critical elements in the olfactory information-processing pathways in this insect.

Materials and methods

Male turnip moths, *A. segetum*, were obtained from a culture maintained at the Department of Ecology, Lund University for several years. The culture has periodically been replenished with field collected insects to avoid inbreeding effects. The larvae were fed a synthetic diet (modified from Hinks and Byers 1976) using potatoes instead of pea beans. The sexes were separated as pupae to avoid exposing males to female sex pheromone after emergence.

A male moth (1–3 days old) was restrained in a 1 ml plastic pipette tip (Finnpipette Oy). The moth was inserted from the wide end of the pipette tip, and pushed in so that the head emerged out of the narrow end, which had been cut to fit the size of the head. The head was immobilized with dental wax (Surgident, Miles Inc., USA). The head capsule was opened, and the mouth parts, muscle tissue, tracheae and the neural sheath were removed to expose the brain. In this way, the antennal lobes were exposed in an upward facing position. To prevent desiccation of the brain, the tip of a syringe delivering a saline solution (Christensen and Hildebrand 1987), was positioned inside the opened headcapsule. An Ag/AgCl wire (0.1 mm O.D.) was inserted into one of the optic lobes to serve as a ground connection. Finally, a glass pipette electrode was filled only in the tip with 4% Lucifer Yellow (dilithium salt in water) and then backfilled with 1 *M* LiCl. The pipette was pulled on a Sutter P-87 glass pipette puller, and had a tip diameter of ~0.1 μm.

When an intracellular contact had been established with a neuron, a series of stimuli was presented. The stimulus source was a Pasteur pipette containing a filter paper strip (5×15 mm) onto which the stimulus substance, dissolved in hexane or paraffin oil,

had been applied. A 2 ml airpuff of a 0.5 s duration was passed through the pipette by using a stimulation device (Syntech, Hilversum, The Netherlands). The airpuff containing the stimulus molecules was injected into a constant airflow, flushing over the ipsilateral antenna. This airflow was mechanically and charcoal filtered, and subsequently moistened before reaching the antenna. The stimuli consisted of the four sex-pheromone components needed to elicit a full behavioral sequence from the male (Z5-10:OAc, Z5-12:OAc, Z7-12:OAc, and Z9-14:OAc), a behavioral antagonist (Z5-10:OH), one flower-produced compound (phenylacetaldehyde, PAA), and a green leaf volatile ((*E*)-2-hexenal, E2H)

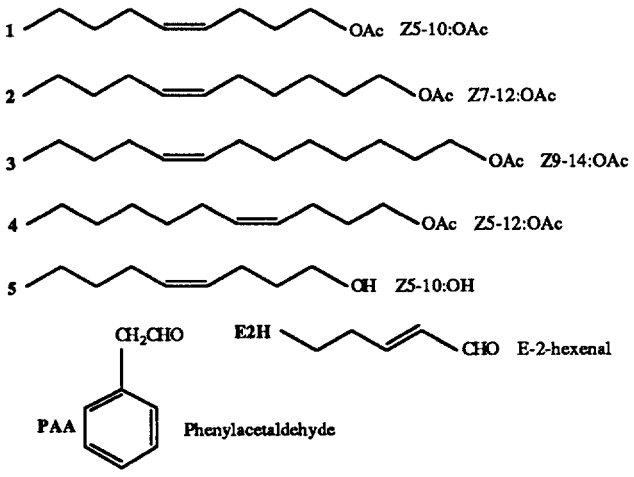


Fig. 1 Chemical structures of the seven stimuli used in this investigation. Compounds 1–4 are components of the female-emitted pheromone blend; compound 5 is a behavioral antagonist; E2H is a common green leaf volatile; and PAA is a flower-produced odorant

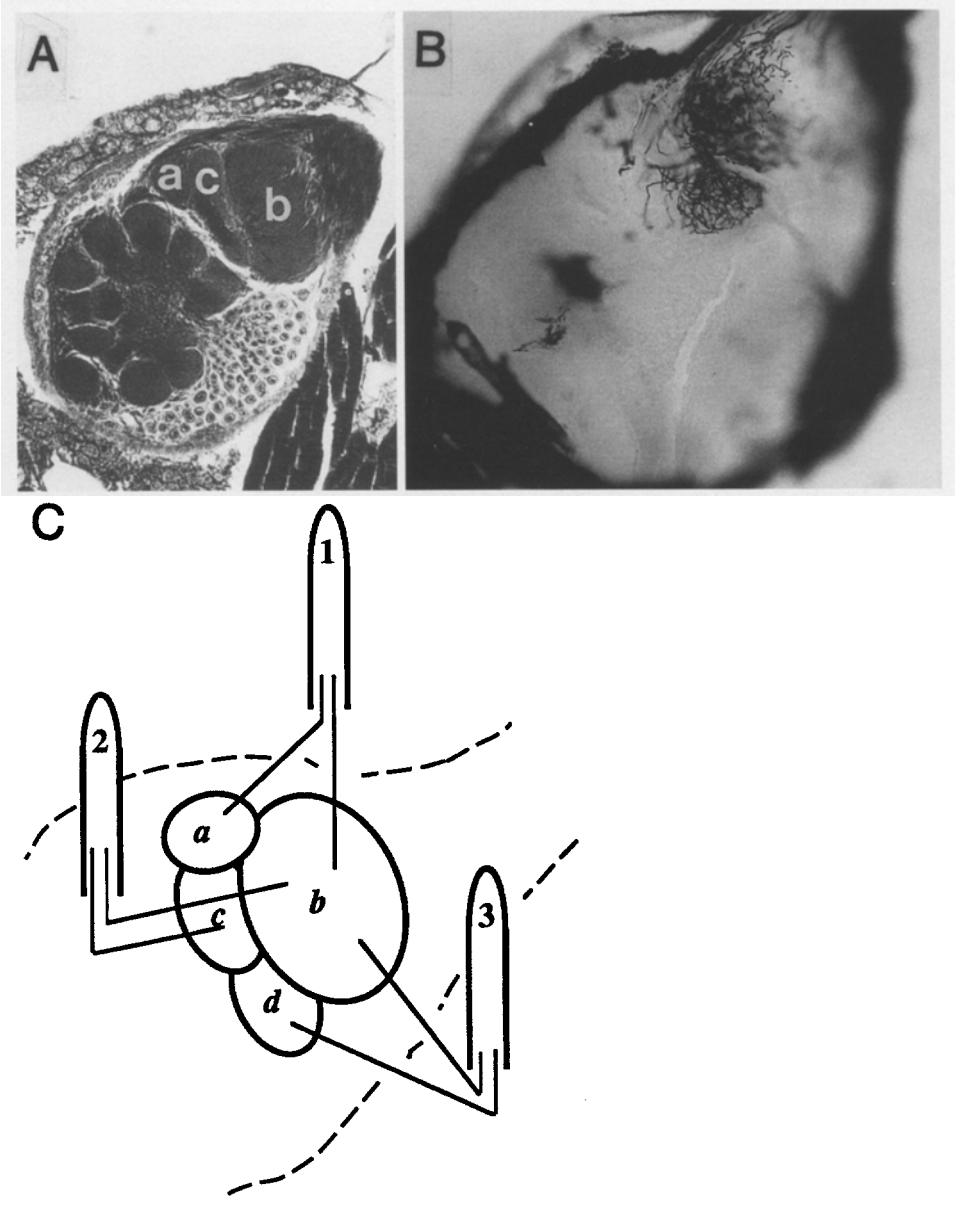


Fig. 2 A A section through the antennal lobe of a male *A. segetum* at a plane of sectioning showing three of the four MGC compartments. **B** A section of a Golgi stained male AL, with stained receptor neurons terminating in compartment *b* and *d*. **C** Schematic representation of the male MGC and the different RN projections according to Hansson et al. (1992). Sensillum type *1* sends one RN specifically tuned to Z5-10:OAc (component 1 in Fig. 1.) to MGC compartment *a*, and one RN tuned to Z5-12:OAc (4) or to the behavioral antagonist Z5-10:OH(5) to compartment *b*. From sensillum type *2*, one RN tuned to Z7-12:OAc (2) projects to compartment *c*, while a RN of unknown specificity terminates in compartment *b*. From sensillum type *3*, one RN projects to compartment *d* and one to compartment *b*. One of the RNs in sensillum type 3 is tuned to Z9-14:OAc (3), while the second RN is of unknown specificity. The AL outline is shown by the *hatched line*. The MGC compartment outlines are shown by *thick, unbroken lines*

(Fig. 1). The pheromone components and the behavioral antagonist were diluted in hexane, and applied at 0.1, 1 and 10 ng amounts on the filter paper. The plant-produced compounds were diluted in paraffin oil and applied at 1, 10 and 100 µg amounts.

The physiological response was amplified by an AxoClamp intracellular amplifier (Axon Instruments, CA, USA), and stored on a Vetter video recorder adapted for storage of neurophysiological signals (Vetter Instruments, PA, USA). The responses were visualized on a Tektronix Digital Storage oscilloscope and on a Gould electrostatic printer. After the physiological response of the neuron was registered, a hyperpolarizing current of 1 nA was passed through the electrode for approximately 10 min, to inject the Lucifer Yellow dye. The brain was then dissected out, and fixed in Carnoy's solution for 1 h. After fixation the brain was dehydrated, and cleared in methyl salicylate for observation. If a neuron was filled, the brain was embedded in Spurrs and sectioned on a LKB pyramitome at 10 µm sections. Each section was photographed on positive color film, and the slides produced were used for subsequent reconstruction of the neuron.

Fig. 3 A Reconstruction of a preparation in which two PNs of very similar morphologies were stained. In this and subsequent figures, the MGC compartments are outlined by the *thicker lines*, the antennal lobe by the *thinner line*, and the calyces of the ipsilateral MB by the *dashed line*. One of these neurons corresponds to the recording listed as PN no. 4 in Table 1. **B** Dendritic branches in the MGC. The arborizations are confined to compartments *b, c* and *d*. **C** Cell bodies and primary neurites. **D** Terminals in the calyces of the MB, and the axons leaving the MB (*arrow head*) towards the ILPR. **E** Terminals in the ILPR, and the axons leading to the MB from the AL (*arrow head*). *Scale bar*=100 µm

Results

Antennal lobe architecture

The paired ALs are the most prominent structures in the deutocerebrum in the brain. The AN, which carries the axons from RNs situated in the ipsilateral antenna, enters the AL antero-dorsally. The basic architecture of the AL

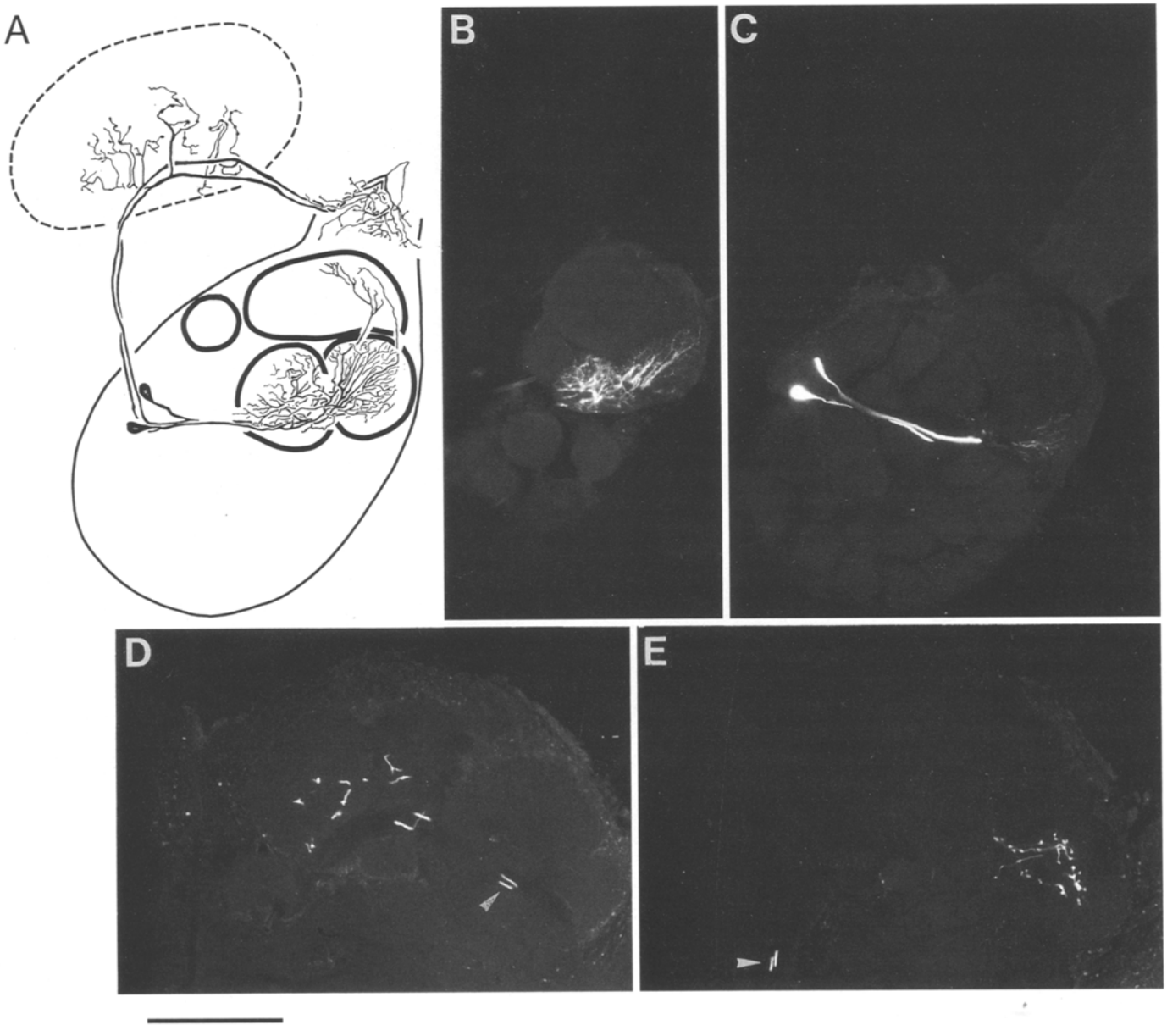


Table 1 Compilation of physiological responses, and morphological characteristics of all the antennal lobe neurons investigated.

no.	Cell type	CB	1	2	3	4	5	1+2+3	1+2+3+4	FE	PAA	E2H	Dendritic branches	Axonal branches
1	PN(?)	n.s.	++	0	0	0	0	n.t.	++	n.t.	+	+	n.s.	n.s.
2	PN	M	0	0	0	0	++	n.t.	0	n.t.	0	0	MGC(a,b)	n.s.
3	PN	M	+	0	0	0	0	n.t.	0	n.t.	0	0	MGC(c)	n.s.
4	PN	M	+	0	0	0	0	n.t.	+	+	0	0	MGC(b,c,d)	MB, ILPR
5	PN	M	0	0	0	0	+++	n.t.	+++	n.t.	+++	0	MGC(a,b,c,d)	MB, ILPR
6	PN	M	+	0	0	+	0	n.t.	n.t.	n.t.	+	+	MGC(b,c)	MB, ILPR
7	PN	M	0	+	0	0	+	n.t.	+	n.t.	+	0	MGC(b,c)	n.s.
8	PN	M	+	0	+	0	0	n.t.	0	n.t.	0	0	MGC(b)	n.s.
9	PN	M	+++	0	+++	0	+	n.t.	n.t.	n.t.	+	++	MGC(b,d)	MB, ILPR
10	PN(?)	n.s.	0	0	++	+	+	n.t.	+++	++	+	0	n.s.	n.s.
11	PN(?)	n.s.	0	+	+	+	0	n.t.	0	+	0	0	n.s.	n.s.
12	PN	M	0	+++	+++	n.t.	n.t.	+++	n.t.	n.t.	n.t.	n.t.	MGC(b)	n.s.
13	PN	n.s.	++	0	+	+	+	n.t.	+++	n.t.	n.t.	n.t.	MGC(b)	n.s.
14	PN(?)	n.s.	+	+	0	+	+	n.t.	n.t.	n.t.	+	+	n.s.	n.s.
15	PN	M	++	+	+	n.t.	n.t.	+++	n.t.	n.t.	n.t.	n.t.	MGC(b)	MB, n.s.
16	PN	M	n.t.	n.t.	n.t.	n.t.	n.t.	+++	n.t.	n.t.	n.t.	n.t.	MGC(b)	MB, ILPR
17	PN	M	+++	+++	++	n.t.	n.t.	+++	n.t.	n.t.	n.t.	n.t.	MGC(c)	n.s.
18	PN	M	++	++	+	+	+	n.t.	n.t.	n.t.	+	0	MGC(b)	n.s.
19	PN	M	++	+	++	+	+	n.t.	0	n.t.	++	+	MGC(c)	MB, ILPR
20	PN(?)	n.s.	++	++	++	++	+	n.t.	n.t.	n.t.	++	+	n.s.	n.s.
21	PN	M	+++	+++	+++	+++	+++	n.t.	+++	n.t.	+++	+++	MGC(b)	MB, ILPR
22	PN	M	+++	+++	+++	+++	+++	n.t.	+++	n.t.	+++	+++	MGC(b,c)	MB, ILPR
23	PN	L	0	0	+	0	0	n.t.	+	n.t.	+	0	G(multi)	LH, MB
24	PN	L	0	n.t.	n.t.	n.t.	n.t.	n.t.	0	0	+++	+++	G(multi)	n.s.
25	PN	L	0	0	0	0	0	n.t.	0	n.t.	0	0	G(single)	n.s.
26	LN	L	0	0	0	0	0	n.t.	+++	n.t.	+++	+++	MGC,G(tot)	0
27	LN	L	0	0	0	0	0	n.t.	0	n.t.	++	0	MGC,G(tot)	0
28	LN	L	+++	0	++	+	++	n.t.	+++	n.t.	+++	+++	MGC,G(tot)	0
29	LN	L	n.t.	n.t.	n.t.	n.t.	+++	n.t.	0	+++	+++	+++	MGC,G(tot)	0
30	RN	A	0	0	0	0	0	n.t.	0	0	0	+++	G(single)	0

The physiological responses are quantified by + (=100–150% of the blank response), ++ (=151–200% of the blank response) and +++ (=>200% of the blank response). Cell body (*CB*) position is indicated by *M* (medial), *L* (lateral) and *A*(antennal). Dendritic arborizations are described by *MGC* (branches in the MGC) and/or *G* (branches in the ordinary glomeruli). In *paranthesis* after MGC is indicated which MGC compartments that are innervated. In *parenthesis* after G is indicated if the innervation is in a single (*single*) glomerulus or in several (*multi*). An innervation of the entire antennal lobe is indicated by (*tot*). The situation of axonal branches is indicated by mushroom body (*MB*), inferior lateral protocerebrum (*ILPR*) and/or the lateral horn of the protocerebrum (*LH*). *n.t.*=not tested, *n.s.*=not stained

in the turnip moth looks very similar to what has been observed in other moth species (Matsumoto and Hildebrand 1981; Christensen and Hildebrand 1987; Koontz and Schneider 1987; Hansson et al. 1991; Christensen et al. 1991). As the AN enters the lobe, the mechanosensory fibers bypass the AL laterally, and the olfactory fibers terminate throughout the AL glomeruli. About 50 olfactory glomeruli surround a coarse, central neuropil. Three clusters of somata can be observed along the surface of the AL: one medial, one lateral and one anterior. As in other species, only the ordinary, sexually isomorphic glomeruli are found in the female, wheras in the male, a prominent MGC is situated at the entrance of the AN (Fig. 2A, B and C). The MGC has been observed in a number of other male moths, but the structural features of the MGC vary considerably in different species (Bretschneider 1924; Christensen and Hildebrand 1987; Hansson et al. 1991; Koontz and Schneider 1987; Christensen et al. 1991).

The morphology of the turnip moth MGC was first reported by Hansson et al. (1992). When sectioned frontally, i.e. at a right angle to the oesophageal canal, the most anterior of the 4 distinct MGC compartments is the first encountered. This compartment is also the smallest, and it lies in a dorso-medial position, deep to the entrance of the AN. This compartment is designated compartment *a*. The next compartment encountered is the largest (*b*), and it is situated just at the entrance of the AN. This compartment has a very complicated double-folded structure, with distinct invaginations from the surface closest to the AN. In more posterior sections, compartment *b* appears to have two parts, but these parts fuse more anteriorly in the AL. Compartment *c* is more caudal than compartment *b*, and it is considerably smaller. Compartment *d* is lateral, and slightly caudal to *c*. These two compartments are usually the same size (Fig. 2A, B and C).

Projection neurons

General physiology and morphology

The PNs encountered in our physiological investigation typically displayed spontaneous activity with evenly

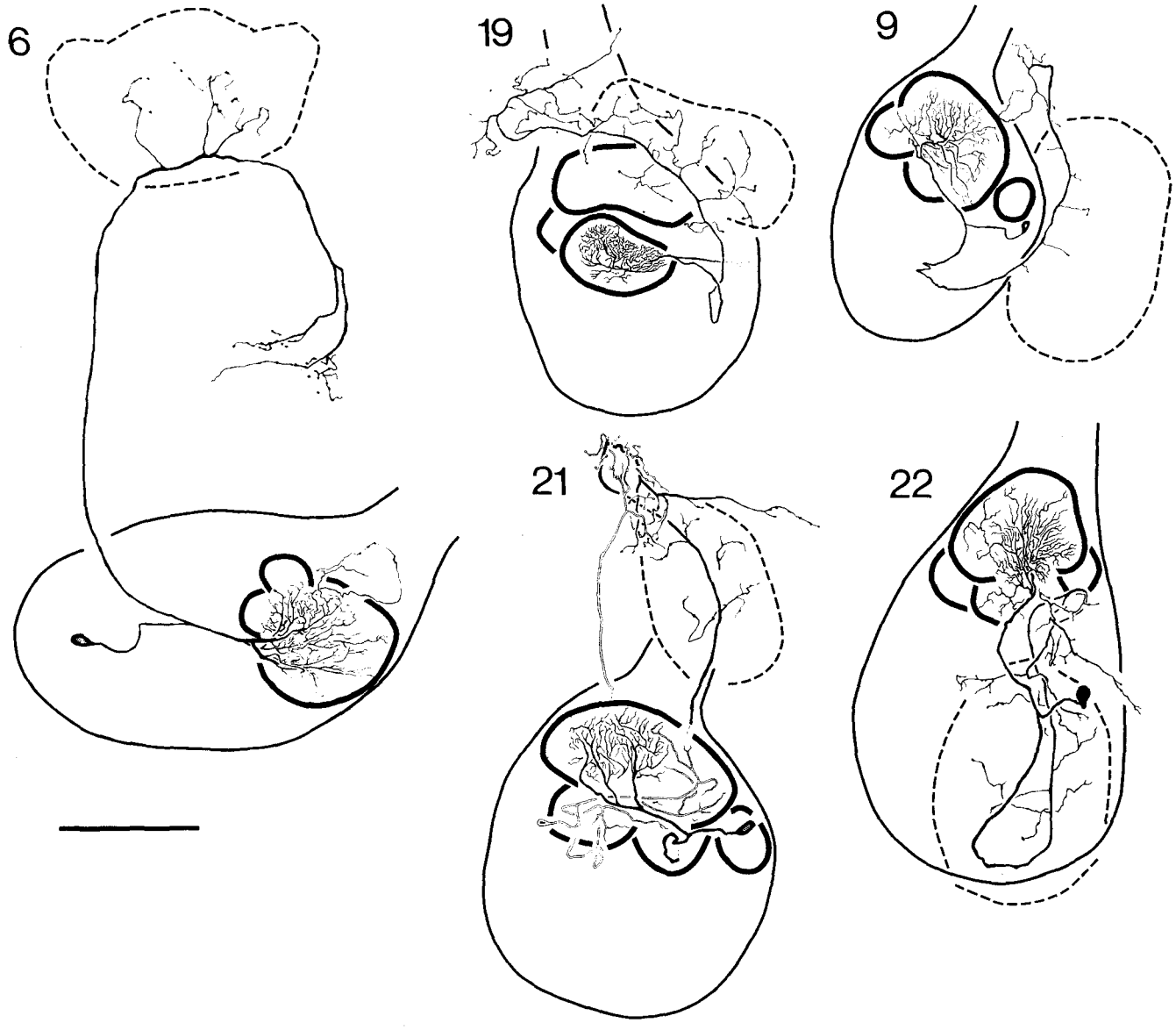


Fig. 4 Reconstructions of five of the PNs showing the axonal pathway (IACT) into the protocerebrum. PN no. 6 is sectioned in a dorsal orientation, and displays dendritic branches in MGC compartments *b* and *c*. One small branch of a dendrite extended into the area of the AN. PN no. 19 is sectioned frontally, but somewhat obliquely. The dendritic arborizations are restricted to MGC compartment *c*. PN no. 9 is sectioned frontally (also somewhat obliquely) and has its dendritic branches in MGC compartments *b* and *d*. PN no. 21 is the neuron from which physiological data was obtained and is shown in *black*. This neuron restricts its dendritic branches to MGC compartment *b*. In addition, the neuron in *white* was contacted very shortly, and was very weakly stained. In this reconstruction a PIa(MGC) neuron (*black*) and a POa(MGC) neuron can be compared. Note that the neurons send axons through different tracts,and that the POa neuron bypasses the calyces of the MB. PN no. 22 is sectioned frontally, and displays dendritic arborizations in MGC compartments *b* and *c*. *Scale bar*=100 μm

spaced action potentials at frequencies between 0.1 and 10 Hz. The action potentials had an amplitude of 10–50 mV, and in a given neuron they were all of the same amplitude. The resting potential ranged from −50 to −60 mV. Intracellular staining revealed that most neurons belonged to the "PIa" class, according to the nomenclature of Homberg et al. (1988). These neurons had dendritic branches in a single spherical AL glomerulus, and an axon which entered the protocerebrum through the inner antennocerebral tract (IACT). One neuron with MGC arborizations had an axon in the outer antennocerebral tract (OACT). Two neurons displayed multiglomerular arborizations in the AL, and one of these neurons had an axon in the OACT.

PIa(MGC) neurons – morphology

Out of 25 PNs investigated physiologically, 22 responded to antennal stimulation with one or several pheromone components (Table 1). It was possible to inject dye into 17 of these pheromone-responsive PNs. Morphological analysis showed that they all belonged to the PIa(MGC) neuron class (Homberg et al. 1988) (Figs. 3, 4, 5 and 7). This type of neuron has dendritic arborizations restricted to the MGC, a cell body in the medial

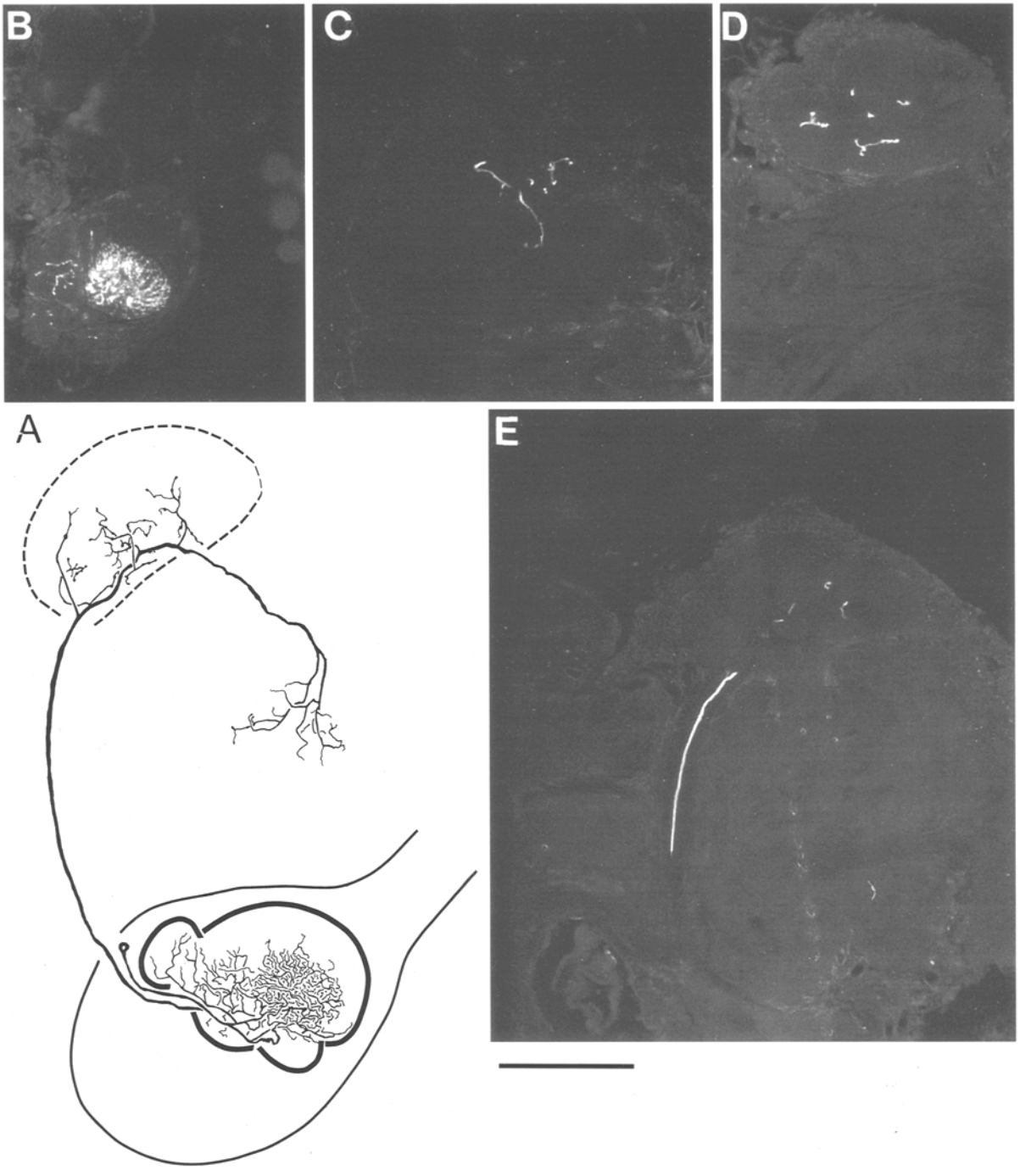


Fig. 5A Reconstruction of PN no. 5, which was sectioned in a dorsal orientation, and extended dendritic arbors into all MGC compartments. **B** Section through the antennal lobe, displaying stained dendritic branches. **C** Axonal terminals in the ILPR. **D** Bouton-like terminals in the calyces of the MB. **E** The axon projecting out of the antennal lobe, through the IACT towards the calyces of the MB, where a few axonal branches can be seen. The physiological responses of this neuron are shown in Fig. 6. *Scale bar*=100 μm

cluster, an axon in the IACT, and blebbed terminal arborizations in the calyces of the mushroom body (MB) and in the inferior lateral protocerebrum (ILPR). The dendritic arborizations varied from neurons with branches in a single compartment to neurons that branched throughout the MGC. Fourteen out of the 17 pheromone-responsive PNs were shown to have branches in the large *b* compartment (all PIa(MGC) neurons except nos. 3, 17 and 19). Out of these, 7 PNs innervated the *b* compartment exclusively (cell nos. 8, 12, 13, 15, 16, 18 and 21 in Figs. 4 and 7), while 7 others also sent branches into other areas of the MGC (nos. 2, 4, 5, 6, 7, 9 and 22 in Figs. 3, 4, 5 and 7).One neuron clearly had arborizations in all 4 compartments (no. 5 in Fig. 5), another neuron had branches in compartments *a*, *b*, and *c* (no. 9 in Fig. 4), and another had branches in compartments *b*, *c*, and *d* (no. 4 in Fig. 3). Three neurons had branches only in compartment *c* (nos. 3, 17 and 19 in Figs. 4 and 7). In nine marking attempts, the axon of the projection neuron

filled all the way to the terminals in the protocerebrum (nos. 4, 5, 6, 9, 15, 16, 19, 21 and 22 in Figs. 3, 4 and 5). All neurons adhered to the PIa(MGC) classification. After leaving the antennal lobe through the IACT, the pheromone-specific PN axons ran just ventro-lateral to the central complex, and turned laterally to pass along the anterior portion of the ipsilateral MB calyces. There it branched into terminal-like processes throughout the lips of the calyces. The axon continued past the calyces laterally, turned anteriorly towards the midline of the brain, and terminated in the ILPR. Terminals in the ILPR resembled those in the calyces.

PIa(MGC) neurons – physiology

All 22 neurons characterized as P(MGC) neurons showed low levels of spontaneous activity typical of PNs in other species (Christensen and Hildebrand 1987; Hansson et al. 1991; Kanzaki et al. 1989). Five neurons were not characterized morphologically (nos. 1, 10, 11, 14 and 20 in Table 1), but were assigned to the P(MGC) class on the basis of physiological evidence alone. Responses were typically biphasic, consisting of a period of depolarization with spiking, followed by a hyperpolarization and complete cessation of activity. In most cases, the P(MGC) neurons fired only for the duration of the stimulus, and were then quieted abruptly by the membrane hyperpolarization. Four neurons were specifically stimulated by a single compound (Table 1). Three of these neurons responded to Z5-10:OAc (nos. 1, 3 and 4) and one responded to the behavioral antagonist Z5-10:OH (no. 2). In other neurons, response patterns were more complicated. One such neuron responded to the behavioural antagonist, but the pheromone blend evoked a much stronger excitatory response followed by a strong membrane hyperpolarization (no. 5 in Figs. 5 and 6). Another neuron responded to the individual components, but not to the blend (no. 19). The remaining neurons were stimulated by different combinations of the pheromone components and also by the behavioral antagonist (Table 1). The threshold level for all tested neurons ranged from below 0.1 ng to 1 ng of the stimulatory pheromone components. Several of the PIa(MGC) neurons were also strongly stimulated by 1 μg of PAA or 1 μg of E2H.

There is no obvious correlation between the dendritic MGC arborization of a PIa(MGC) neuron and its physiological response. Neurons with dendritic arbors in several MGC compartments responsed to a single pheromone component (no. 2, 4 and 5), and neurons with arbors restricted to a single compartment responded to several or all tested odorants (nos. 8, 12, 13, 15, 17, 18, 19, 21).

PIa(G) neuron

A single neuron with a lateral soma displayed uniglomerular arborizations in a glomerulus situated close

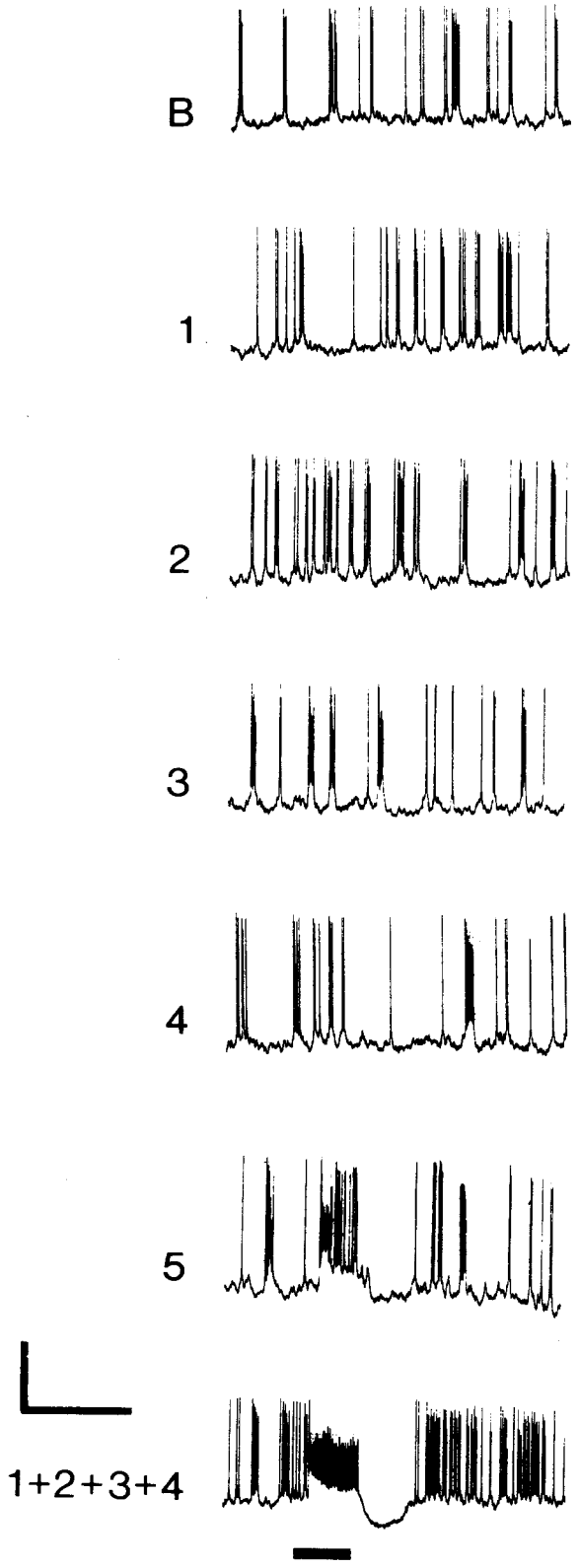


Fig. 6 Response of PN no. 5 to the stimuli tested. Note the zero response to all the single components included in the synthetic pheromone blend, and the very strong response to the mixture of these. A weaker response was also obtained when the antenna was stimulated with the behavioral antagonist (*5*). *Bar* beneath recordings indicate stimulation time. *Horizontal scale bar*=1 s; *vertical scale bar*=20 mV

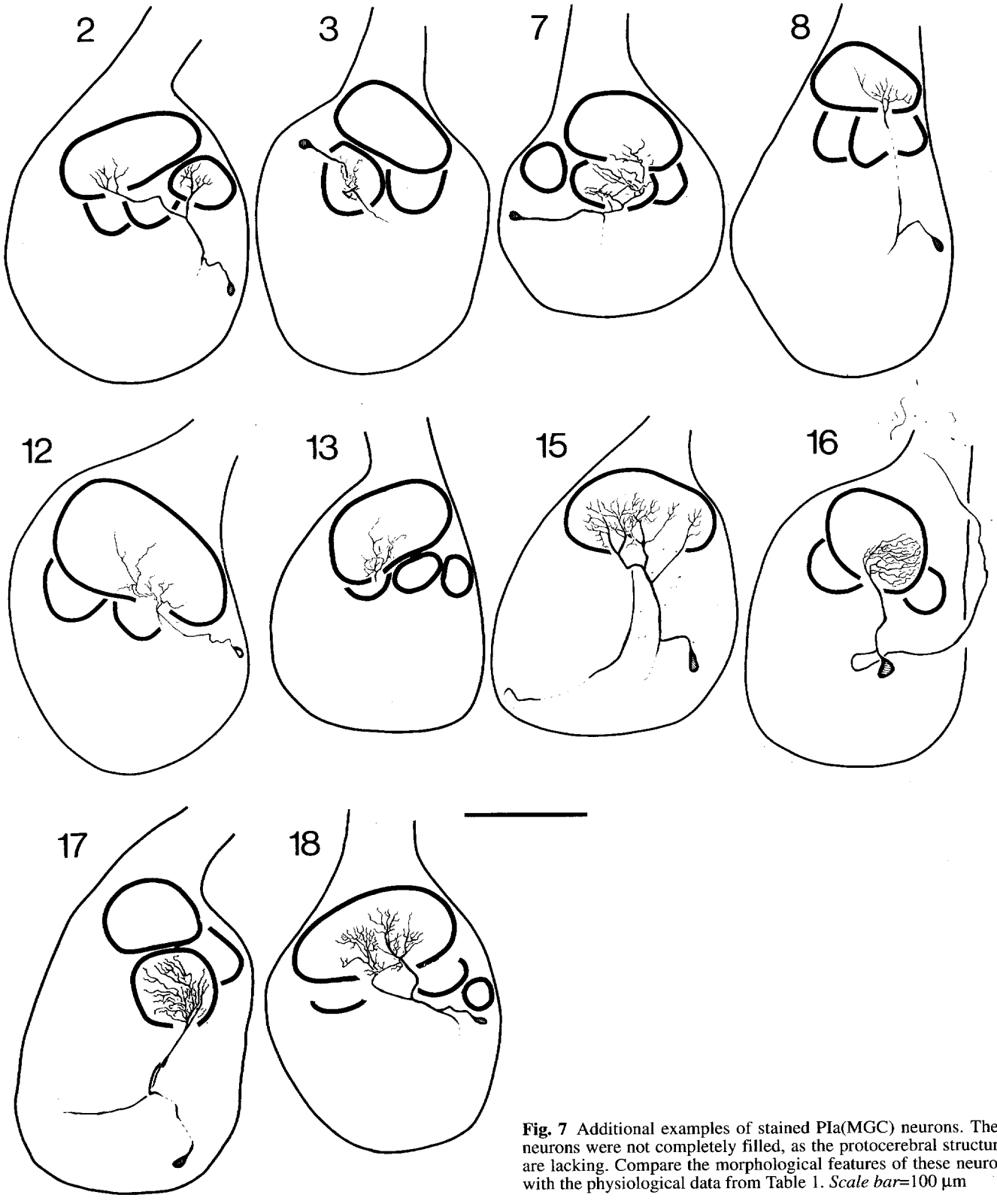


Fig. 7 Additional examples of stained PIa(MGC) neurons. These neurons were not completely filled, as the protocerebral structures are lacking. Compare the morphological features of these neurons with the physiological data from Table 1. *Scale bar*=100 µm

to the exit of the IACT from the AL (no. 25). The protocerebral projection could not be followed, nor did this neuron respond to any of the stimuli tested (Fig. 9).

POa(MGC) neurons

In an attempt to fill a PIa(MGC) neuron in one preparation, a second neuron was filled faintly. This second neuron's morphological characteristics corresponded to the

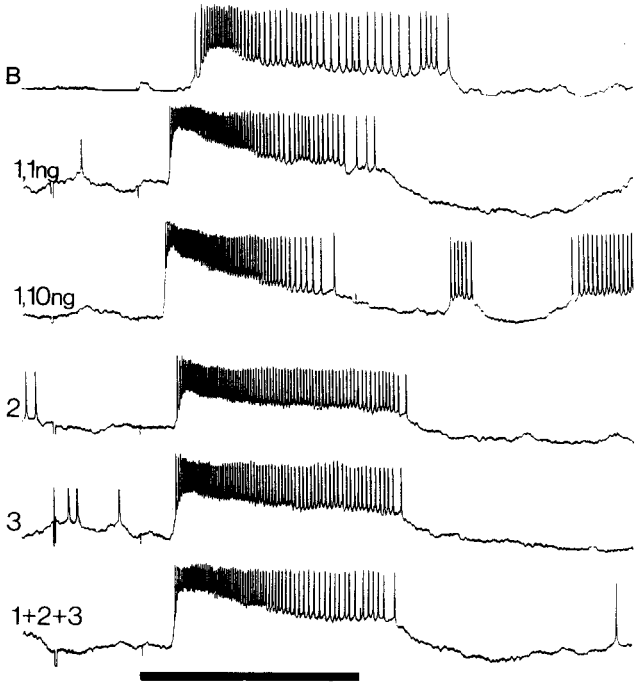


Fig. 8 Physiological responses recorded from PN no. 15. This neuron responded strongly to the three tested pheromone components. Its dendritic branches were, however, restricted to MGC compartment *b* (see Fig. 7). *Bar* beneath recordings indicate 0.5 s stimulation time

POa(MGC) class of neurons classified by Homberg et al. (1988). Incompletely filled branches extended into three MGC compartments, but the axonal projection through the OACT could be followed to the ILPR. This neuron did not have any arborizations in the MB (Fig. 4, no. 21).

POc neurons

One neuron was classified as a POc on the basis of an axonal projection through the OACT, a soma in the lateral cell cluster, and a multiglomerular arborization in an area just below the MGC (no. 23). This neuron differed clearly from the earlier mentioned types because its axon branched first in the lateral horn (LH), and then continued further to terminate within the calyces of the ipsilateral MB. Bouton-like terminals were observed both in the LH and in the MB. This neuron gave a weak response to one pheromone component and to the blend. A second neuron that showed some AL characteristics of a POc neuron responded strongly to both PAA and E2H (no. 24 in Figs. 9 and 10).

Local interneurons

The LNs encountered typically showed higher levels of spontaneous activity with bursts of action potentials (Fig. 12). The range of resting potentials was similar to that of the PNs (−50 to −60 mV). Four physiologically and morphologically investigated LNs resembled the type-IIb

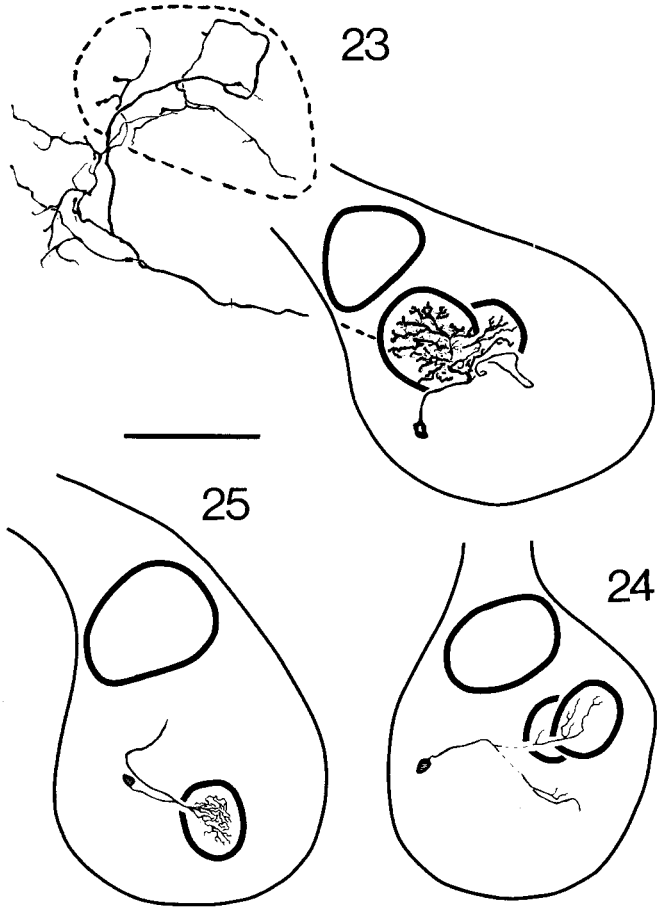


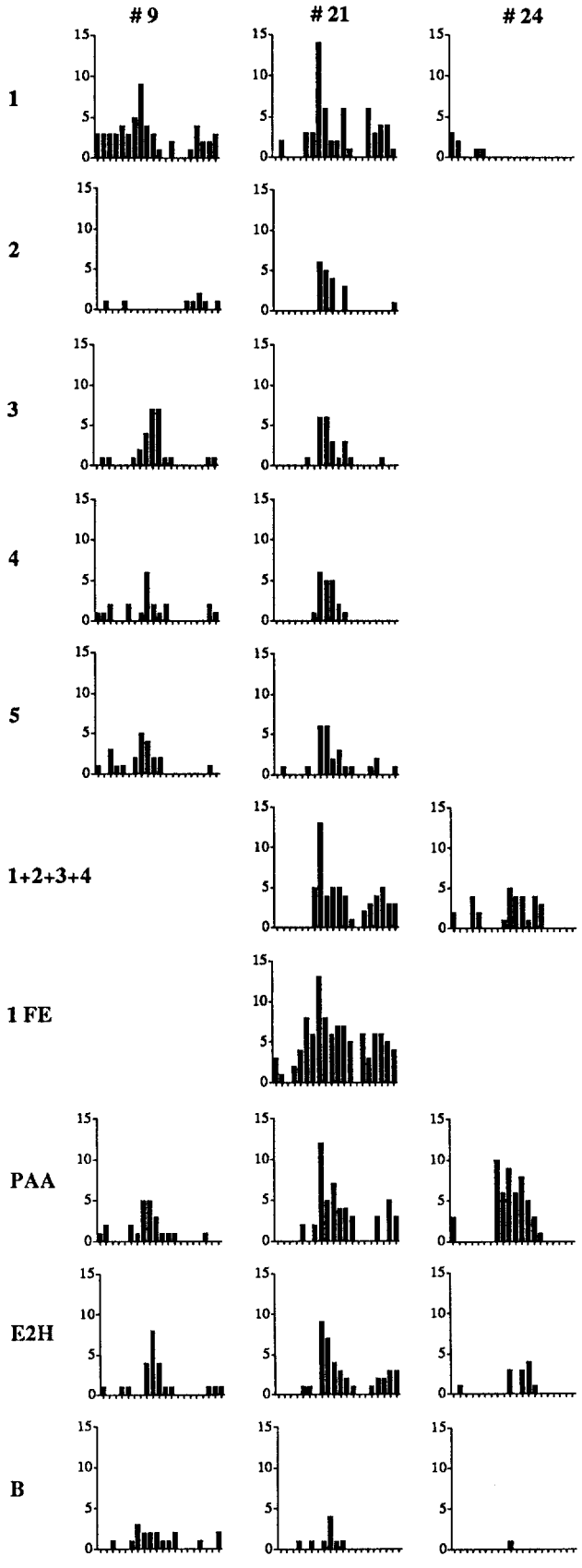
Fig. 9 Three PNs with dendritic arbors in the ordinary glomeruli. PN no. 23 branched in several glomeruli in the AL, was stained into the protocerebrum, displayed initial branches in the LH, and terminal brances in the calyces of the MB. PN no. 24 was of a similar type as no. 23, but was incompletely stained. PN no. 25 had a uniglomerular arborization pattern in the AL, while the protocerebral projection could not be followed. *Scale bar*=100 μm

LNs as classified by Matsumoto and Hildebrand in *M. sexta* (1981). This type has a symmetric arborization pattern in most or all ordinary glomeruli, and in the MGC. All LNs had lateral CBs (Fig. 11).

Three out of the four LNs received input from pheromone-detecting receptor cells (nos. 26, 28 and 29). Two of the LNs responded to several pheromone components, while one neuron was a blend specialist (no. 29). This neuron responded only to the 4-component blend, but not to any of the individual constituents, nor to the behavioral antagonist (Fig. 13). All the pheromone-responsive LNs responded strongly to PAA and E2H at the 1 μg level, while LN no. 27 was specifically stimulated by PAA (Table 1). The threshold value for pheromone components was at 0.1–1 ng stimulus concentration.

Afferent neuron

On a single occasion, an afferent RN was morphologically and physiologically characterized by intracellular



methods (no. 30). The axon could be followed through the AN and extensive axonal arborizations were situated in a single glomerulus near the center of the AL. Bleb-like terminals could be seen on the arbors (Fig. 14A–C).

The afferent neuron was unaffected when stimulated with pheromone components or with PAA. It did, however, respond strongly when stimulated with 1 µg of E2H (Fig. 15).

Discussion

The AL of the male *A. segetum* houses interneurons that detect odors that are involved in the location of a suitable mate and/or potential food sources. Within the AL of the male, distinct functional subdivisions are found. Pheromone-responsive interneurons branch in the MGC, while non-pheromone responsive interneurons branch only in the ordinary spherical glomeruli.

In the sphinx moth *M. sexta*, a clear correlation between physiological response and dendritic branches pattern was observed among the pheromone-specific PIa(MGC)s (Hansson et al. 1991). Neurons that responded only to the principal pheromone component invaded a toroid-shaped compartment of the MGC, while neurons that responded to the second essential pheromone component sent arbors into another distinct compartment, the cumulus. A more recent study of the projection patterns of pheromone-selective RNs situated on the antenna of male *M. sexta* confirms this functional specificity (Christensen et al., unpublished). Moreover, in an investigation of the projections of physiologically-identified RNs in the antenna of male *A. segetum*, a clear pattern of functional projection areas was also seen in the MGC of this noctuid species (Hansson et al. 1992). Neurons that responded specifically to Z5-10:OAc projected to compartment *a*, neurons that responded to Z7-12:OAc projected to compartment *c*, and neurons that responded to the behavioral antagonist Z5-10:OH, projected to compartment *b*. Only a single sensillum containing a Z9-14:OAc specific neuron was filled, and one of the cells in this sensillum projected to compartment *d* (Fig. 2C).

The pheromone-selective PNs in *A. segetum* revealed in this study restrict their dendritic arbors to the male-specific MGC just as in *M. sexta*, but the functional organization of the MGC-PNs in this special area of neuropil appears to be quite different. Although further characterization of these neurons is necessary, we thus far see no clear correlation between the location of dendritic arbors and the physiological responses of MGC-PNs in male *A. segetum*. Most neurons that branched within a single

Fig. 10 Peri-stimulus histograms of PN no. 9, 21 and 24, showing different degrees of specialization. PN no. 9 responds to all tested compounds, except to compound 2. PN no. 21 responds to all tested compounds, but to a different degree. PN no. 24 responds very strongly to PAA. Each bin represents the number of action potentials during 0.1 s

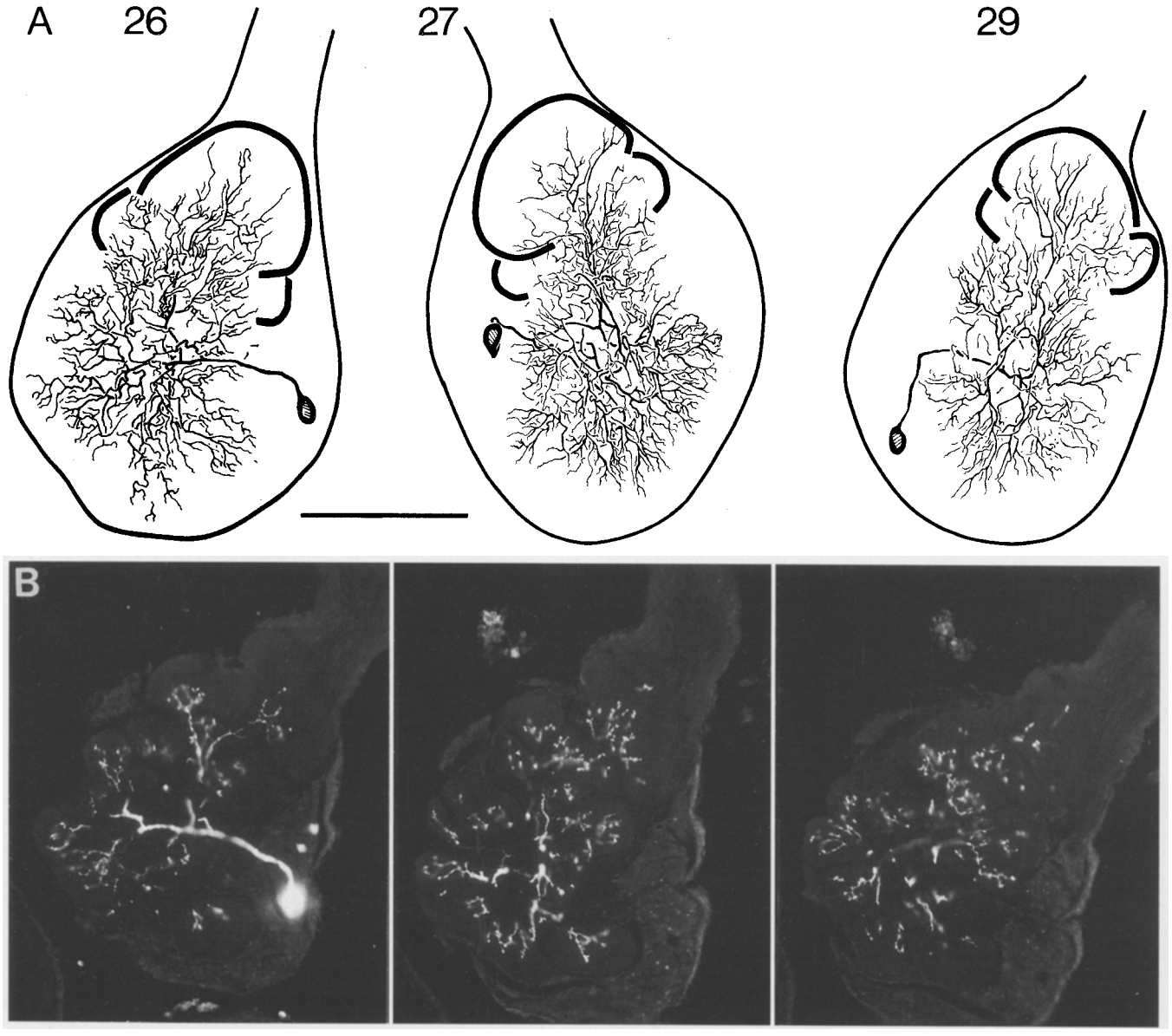
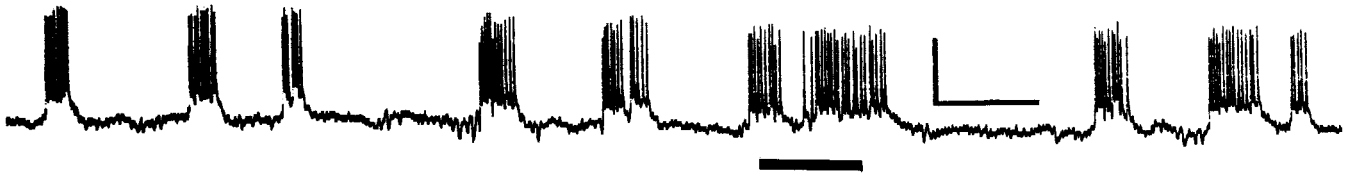
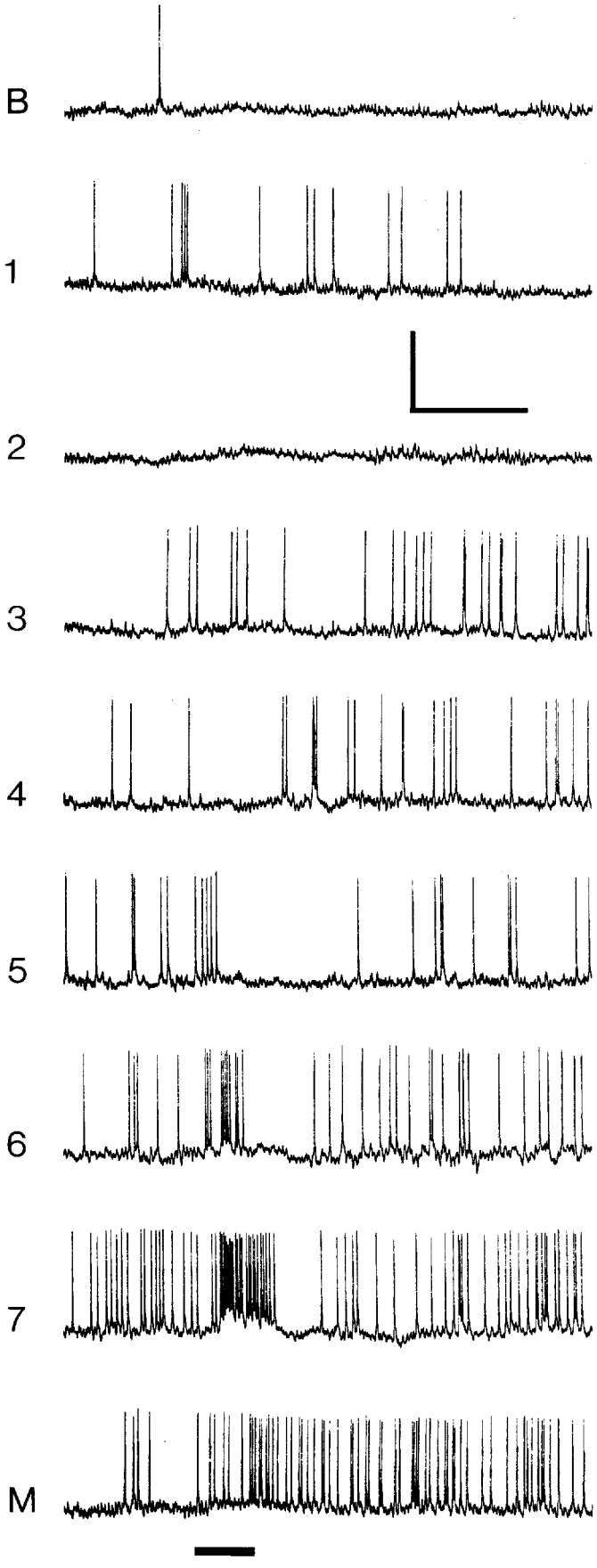


Fig. 11A Reconstructions of three LNs branching throughout the entire AL, including the MGC. **B** Three sections through the AL containing LN no. 26. *Scale bar*=100 μm

Fig. 12 Typical activity of a LN, showing the bursts of spontaneous activity. *Thick, black bar* beneath record indicates a stimulation with PAA, which elicits a reaction. *Horizontal scale bar*=1 s; *vertical scale bar*=20 mV

MGC compartment were restricted to the largest compartment, *b*. These neurons were, however, all responsive to two or more of the pheromone components tested. The MGC-PNs that responded to a single pheromone component generally branched in two or more compartments of the MGC, as did most of the MGC-PNs that responded to only two of the pheromone components (Table 1). This pattern implies that MGC-PNs in *A. segetum* are not simply passive relay elements, but are a functionally-diverse population of neurons that are involved in considerable inter-compartmental processing and integration of information from several input pathways.





Recent results from *M. sexta* (Christensen et al. 1993) and from the cockroach, *Periplaneta americana* (Malun 1991a, b) indicate that the RNs from the antenna very rarely make direct contact with the PNs in the AL. LNs receive monosynaptic input from RNs, and in turn pass this information onto PNs through complex multisynaptic interactions that may also involve other LNs. With these facts as a background, it is easy to envisage how PNs branching in a single compartment of the MGC in *A. segetum* still can integrate information from pheromone-selective afferents that converge on neighboring compartments. The results from different insect species therefore reveal different strategies of olfactory-information coding, i.e. labelled lines and across fibre patterning occur at different levels, in this complex neuropil. While the strength of the correlations between dendritic morphology and function vary greatly from sphingids to noctuids, however, all species studied to date share some functional principles in common: regardless of their morphology, some MGC-PNs represent labelled lines that transmit single-component information to higher brain centers, whereas others participate in more distributed across-fiber coding at this level of the olfactory pathway (Christensen and Hildebrand 1987; Christensen et al. 1989; Hansson et al. 1991; Kanzaki et al. 1989).

It is evident that complex synaptic interactions govern some of the responses observed in the MGC-PNs in *A. segetum*. In one example, no response was recorded when the neuron was stimulated with each of the 4 components of the pheromone blend. However, the same neuron responded strongly to the 4-component blend (Fig. 7). This neuron is similar in many respects to the "pheromone blend specialists" reported previously in *M. sexta* and the noctuid species *Helicoverpa zea* and *Heliothis virescens* (Christensen et al. 1989, 1991). The blend specialist shown in Fig. 7 also showed a strong response to the behavioral antagonist, Z5-10:OH, which was not part of the 4-component pheromone blend. This neuron obviously detects the complete attractive pheromone mixture, but also the behavioral "stop" signal, and thus serves a multiple function in the pheromone information-processing pathway. Another possible "blend specialist" is a neuron responding strongly to all individual components, but not to the complete 4-component blend (no. 19 in Table 1). Such a neuron could convey information about closely related species, using some of the same pheromone components, but not the species-specific blend.

Among the pheromone-stimulated PNs in the turnip moth, several responded also to the two tested plant-produced compounds, PAA and E2H at 1 μg (Table 1). PAA stimulated more neurons than E2H, and none of the neu-

Fig. 13 Physiological response of the blend-specific LN no. 26. Note the long-lasting excitation by the synthetic pheromone blend (*M*), and the low activity of the individual components (*1–4*) and of the blank (*B*). *Thick, black bar* beneath the record indicates stimulation time. *Horizontal bar*=1 s; *vertical bar*=20 mV

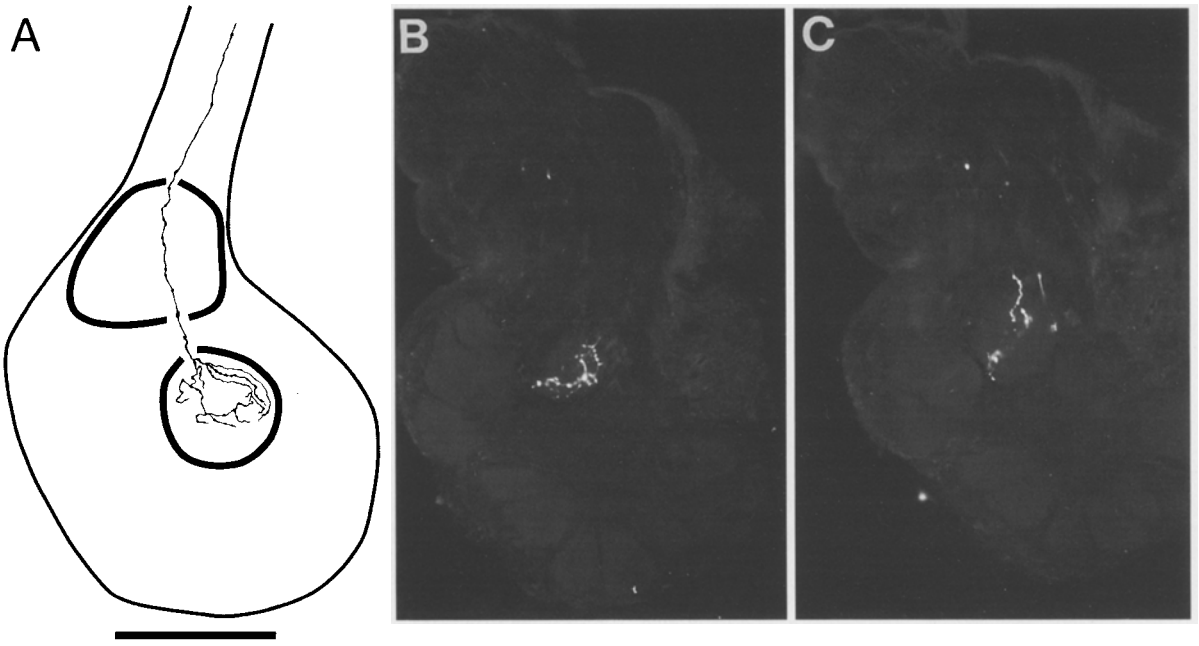


Fig. 14A Reconstruction of RN no. 30, stained intracellularly. Note the varicosities along the axon and on the axonal brances. **B** and **C** Sections displaying the branching of RN no. 30 in the AL. *Scale bar*=100 μm

rons were stimulated only by E2H. It is known that pheromone receptor neurons can also respond to the two plant odors tested, but to release these responses, substantially higher concentrations than 1 μg are needed (Hansson et al. 1989). It is important to note, however, that the responses in MGC-PNs to PAA and to E2H do not necessarily represent a convergence to plant-odor and pheromone-odor information pathways in these neurons. Due to the convergence of many sensory axons onto the dendrites of many fewer interneurons, even a weak response evoked by a plant odor in a large population of receptor neurons that are far more sensitive to pheromones may nevertheless elicit a postsynaptic response in certain MGC-PNs. In the present study, we have tried to use behaviorally relevant concentrations of PAA and E2H (Haynes et al. 1991), but these may still be far more than an insect is likely to encounter in nature. Further investigation is therefore necessary before we can say with confidence if any of the pheromone-processing interneurons in *A. segetum* also integrate information from plant-odor receptor neuron pathways.

When the MGC architecture of *A. segetum* was reexamined, part of it was found to have a more complex structure than reported earlier. The largest compartment, *b*, situated just at the entrance of the AN, is not a homogenous structure like most other glomeruli and MGC compartments. Numerous fingerlike invaginations reach from the edge closest to the AN and into the bulk of the *b* compartment. Several of the species investigated for MGC morphology have been shown to have a large compartment situated close to the entrance of the AN. In *M. sexta* for example, the same type of invaginations were

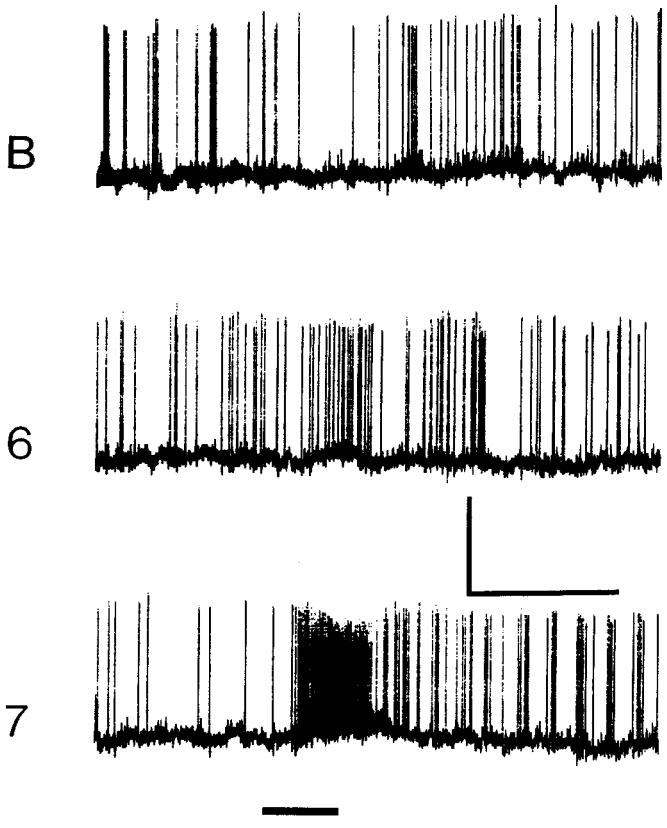


Fig. 15 Physiological response of RN no. 30. A very strong excitation was observed when the antenna was stimulated with E2H. *Bar* beneath record indicates stimulation time. *Horizontal scale bar*=1 s; *vertical scale bar*=10 mV

observed, resulting in the term "cumulus" to describe this compartment (Hansson et al. 1991). In the noctuid *Heliothis virescens*, a similar structure has recently been observed (Hansson, Almaas and Anton, unpubl.). The PNs often extend discrete dendritic branches into the dif-

ferent subcompartments formed by the fingerlike invaginations, suggesting a laminar or columnar organization to the dendritic projection. The functional significance of this complex organization within MGC compartments has yet to be investigated.

The 4 LNs in this study were all of the type that invade the entire AL. Three out of the four LNs responded to some kind of pheromone stimulation, and all responded strongly to the two tested plant compounds. In one LN, a long lasting excitation was elicited by the 4-component blend, while neither the individual pheromone components nor the behavioral antagonist evoked any responses. While it is difficult to draw conclusions from four neurons, it appears that blend specialists, as described earlier for the MGN-PN, may also occur among the LNs in *A. segetum*.

The axons of afferent olfactory neurons are very thin, and previous attempts to record intracellularly from them have failed. During this investigation, we impaled the axon of a single RN. The physiological response to the cell was very distinct, and the subsequent marking procedure worked well. It is thus possible to record intracellularly from RNs. By performing such recordings it will be possible to test if extracellularly recorded amplitude differences between the action potentials elicited in different receptor cells is a physiological phenomenon that affects synaptic release of neuro-active substances at the terminals in the brain (e.g. Hansson et al. 1987)). Will a large amplitude action potential release more of a specific tranmitter substance? Intracellular recordings will also make it possible to corroborate the earlier studies of RN projection patterns in the AL (Hansson et al. 1992).

Besides the PIa(MGC) type of projection neurons, single examples of other types were encountered. All the different types of neurons stained in this study show structural similarities to pheromone-information processing neurons in other Lepidoptera, including other noctuids such as *H. zea* (Christensen et al. 1989, 1991), the silk moth *Bombyx mori* (Kanzaki and Shibuya 1986), and the well-studied sphingid, *M. sexta* (Matsumoto and Hildebrand 1981; Christensen and Hildebrand 1987; Hansson et al. 1991; Kanzaki et al. 1989). All the neuron types encountered in this study could be assigned to types previously described by Homberg et al. (1988).

The basic neural architecture and function of the *A. segetum* antennal lobe neurons adheres very well to findings in other lepidopteran species. One important difference is that only a few neurons in our sample appeared to be labelled lines that carry single-component information to higher centers in the brain. Most AL neurons responded to several pheromone components irrespective of whether they displayed single or multiple dendritic fields in MGC compartments. Future studies will focus on examining these complex synaptic interactions among the diverse neuronal cell types in the AL of *A. segetum*.

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