J Comp Physiol A (1990) 167:309–320

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Olfactory interneurons in the brain of the larval sphinx moth *Manduca sexta*

H. Itagaki* and J.G. Hildebrand**

Arizona Research Laboratories, Division of Neurobiology, 611 Gould-Simpson Bldg., University of Arizona, Tucson, AZ 85721, USA

Accepted April 9, 1990

Summary. 1. The physiology and morphology of olfactory interneurons in the brain of larval *Manduca sexta* were studied using intracellular recording and staining techniques. Antennal olfactory receptors were stimulated with volatile substances from plants and with pure odorants. Neurons responding to the stimuli were investigated further to reveal their response specificities, dose-response characteristics, and morphology.

2. We found no evidence of specific 'labeled-lines' among the odor-responsive interneurons, as none responded exclusively to one plant odor or pure odorant; most olfactory interneurons were broadly tuned in their response spectra. This finding is consistent with an 'across-fiber' pattern of odor coding.

3. Mechanosensory and olfactory information are integrated at early stages of central processing, appearing in the responses of some local interneurons restricted to the primary olfactory nucleus in the brain, the larval antennal center (LAC).

4. The responses of LAC projection neurons and higher-order protocerebral interneurons to a given odor were more consistent than the responses of LAC local interneurons.

5. The LAC appears to be functionally subdivided, as both local and projection neurons had arborizations in specific parts of the LAC, but none had dendrites throughout the LAC.

6. The mushroom bodies and the lateral protocerebrum contain neurons that respond to olfactory stimulation.

Key words: Chemosensory integration – Olfaction – Brain – Larva – Caterpillar – *Manduca sexta* – Lepidoptera

* *Present address:* Dr. Haruhiko Itagaki, Dept. of Biology, Kenyon College, Gambier, OH 43022, USA

** To whom offprint requests should be sent

Abbreviations: *BAL* bombykal; *CNS* central nervous system; *E2H* (*E*)-2-hexenal; *KAc* potassium acetate; *LAC* larval antennal center; *LY* Lucifer Yellow CH; *MGC* macroglomerular complex; *SEM* standard error of the mean

Introduction

Investigations of central mechanisms of olfactory integration in insects have concentrated mainly on the processing of sex-pheromonal information in the brains of adult males (e.g. in moths, *Antheraea* species: Boeckh and Boeckh 1979; *Bombyx mori*: Olberg 1983; Kanzaki and Shibuya 1984, 1986a, b; Light 1986; *Heliothis zea* and *H. virescens*: Christensen et al. 1989; *Manduca sexta*: Matsumoto and Hildebrand 1981; Christensen and Hildebrand 1987a, b, 1988; Kanzaki et al. 1989; and in the cockroach *Periplaneta americana*: Waldow 1977; Burrows et al. 1982; Ernst and Boeckh 1983; Selzer 1984). These investigations have yielded a great deal of information about the physiology and anatomy of central neurons involved in the processing of these specialized olfactory signals. Fewer studies, however, have focused on processing of plant odors and other behaviorally relevant olfactory information (notably, *P. americana*: Selzer 1979; *Apis mellifera*: Homberg 1984; *Leptinotarsa decemlineata*: De Jong and Visser 1988). To our knowledge, there has been no previous physiological investigation of the central olfactory pathways in the brain of a lepidopterous larva. In this report we describe initial findings from a study of olfactory interneurons in the central nervous system (CNS) of the larval sphinx moth *M. sexta*.

The larva is the principal or exclusive feeding stage in the life of a lepidopterous insect. Its feeding behavior is guided primarily by chemosensory information through which the larva discriminates and makes choices among possible food sources (Schoonhoven 1986). Owing to the importance of caterpillars as economic pests, many investigators have studied their feeding behavior and hostplant preferences (e.g. Jermy et al. 1968; Schoonhoven 1969; Saxena and Schoonhoven 1978, 1982; Barbosa et al. 1979; DeBoer and Hanson 1984, 1987) as well as sensory coding in the chemoreceptor neurons that control caterpillar feeding (e.g. Schoonhoven and Dethier 1966; Dethier and Kuch 1971; Ma 1972, 1976; Städler and Hanson 1975; Dethier and Crnjar

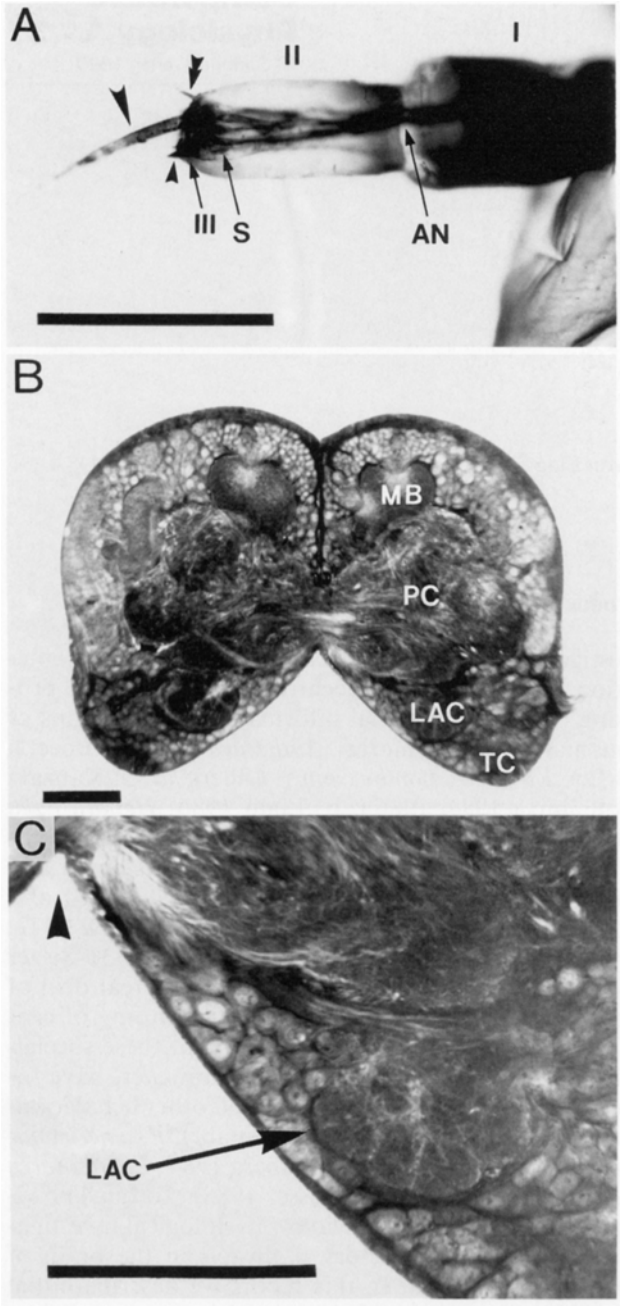

Fig. 1. **A** Anterograde cobalt staining of the antennal nerve in the larval *M. sexta* antenna. *I, II, III* first, second, and third segments of the antenna. The third segment is located at the tip of the second segment. *AN* antennal nerve; *S* somata of the olfactory neurons; *large arrowhead* large tactile hair; *double arrowhead* small tactile hair; *small arrowhead* large *sensillum basiconicum*. Scale bar = 500 μm. **B** Ethyl gallate preparation of a larval *M. sexta* brain, 11 μm frontal section. *MB* mushroom body; *PC* protocerebrum; *LAC* larval antennal center in the deutocerebrum; *TC* tritocerebrum. Dorsal up. Scale bar = 100 μm. **C** Enlarged view of the larval antennal center in the ethyl gallate preparation, 11 μm section. *Arrowhead* midline of the brain. Dorsal up. Scale bar = 100 μm

1982; Visser 1983). Heretofore, however, the CNS has been treated as a 'black box' that decodes the chemosensory afferent information and uses it to produce the observed behaviors.

Lepidopterous larvae are notable among insects for the relatively few olfactory receptors present in their antennae and maxillae, the paired principal chemosensory organs located on the head. Each antenna is innervated by 16 olfactory receptor cells divided among 3 large *sensilla basiconica* (Schoonhoven and Dethier 1966; Dethier and Schoonhoven 1969; Dethier 1980; Schoonhoven 1986) (Fig. 1A). A few other olfactory cells innervate the 3 *sensilla basiconica* located on the maxillary palps (Dethier and Kuch 1971; Hanson and Dethier 1973; Schoonhoven 1986). Ablation studies performed on *M. sexta* have shown that removal of all of the chemosensory structures except one antenna still permits normal olfactory discrimination (Dethier 1967). Discrimination among different foodplants involves a combination of olfactory and gustatory cues, with some plants identifiable through one or the other sensory modality and others discriminable only by means of the combination of both modalities (De Boer and Hanson 1987). Although olfactory and gustatory sensilla are morphologically different, there is good evidence that 'gustatory' receptors can respond to some volatiles from plants' sap at close range (Städler and Hanson 1975).

Behavioral studies have shown that larval olfactory receptor cells are most important for the location and initial discrimination of foodplants (Dethier 1937, 1941; Schoonhoven and Dethier 1966; Hanson and Dethier 1973; Saxena and Schoonhoven 1978, 1982). The coding of chemosensory information by these olfactory receptors has been investigated in several different larval Lepidoptera by means of extracellular recording techniques (Schoonhoven and Dethier 1966; Dethier and Schoonhoven 1969; Dethier 1980). These studies have shown that these olfactory receptors are mostly 'generalists' that respond to a wide variety of plant volatiles with changes in firing rate. Each cell appears to have a characteristic spectrum of responses to different stimuli, with some overlap between different cells. Thus it appears that the relative firing rate of a number of receptors (the 'across-fiber firing pattern') is used by the CNS to differentiate among various chemical stimuli.

In *M. sexta*, the axons of sensory neurons in the larval antenna project to a glomerular neuropil structure in the deutocerebrum of the brain called the larval antennal center (LAC) (Fig. 1B, C). These receptor axons have distinctive 'tufted' terminations in the neuropil of the LAC and also send branches to the protocerebrum and suboesophageal ganglion (Kent and Hildebrand 1987). Very similar projections from the antennae into the brain have been observed in the larval noctuids *H. virescens* and *H. zea* (Randolph and Itagaki 1989; Itagaki, Randolph and Hildebrand, unpublished).

In this study, we have used olfactory stimulation with natural plant odors and synthetic odorants (a single component of a complex odor mixture) to investigate the physiological properties of interneurons in the brain of larval *M. sexta* that respond to olfactory stimulation of the ipsilateral antenna. Whenever possible, the physiologically responsive interneurons were stained with an intracellularly injected dye so that neuronal morphology and physiology could be correlated. Preliminary ac-

counts of some of this work have appeared elsewhere (Itagaki and Hildebrand 1986, 1988).

Materials and methods

Animals. Larval *Manduca sexta* (Lepidoptera: Sphingidae) were reared in laboratory culture on an artificial diet (modified from Bell and Joachim 1976) under a 17L:7D photoperiod regimen at 26 °C and 50–60% relative humidity (Sanes and Hildebrand 1976a) as described previously (Sanes and Hildebrand 1976a; Prescott et al. 1977). Actively feeding female larvae (3.4–10.1 g) in the first half of the fifth instar were chosen for these experiments.

Preparation. We anaesthetized the larva by chilling on ice, isolated the head and pinned it on a wax platform, and pinned the mandibles in place to stabilize the preparation. A rectangular piece of head cuticle including the frons was removed. A platform made by flattening a stainless-steel hypodermic needle was positioned under the brain for support and used to deliver superfusion fluid (Fig. 2). The brain was desheathed and superfused with physiological saline solution (149.9 m*M* NaCl, 3.0 m*M* KCl, 3.0 m*M* $CaCl_2$, 10.0 m*M* TES, 25.0 m*M* sucrose, pH 6.9, modified from Pichon et al. 1972; Christensen and Hildebrand 1987a; Waldrop et al. 1987).

Olfactory stimulation. The preparation was continuously ventilated with charcoal-filtered, humidified air (0.5 l/min) through a glass cartridge (barrel of a Becton-Dickinson 0.5 ml syringe) positioned 1.5–2 cm from the head. A second glass cartridge containing a piece of filter paper (1 × 2 cm, Whatman #1) impregnated with stimulant was positioned at a small angle (approximately 17°) to the first cartridge so that both cartridges were aimed at the right antenna. The odor cartridges could be changed easily during the experiment. We stimulated the antenna by switching the airflow from the first cartridge to the second (odor) cartridge by means of a solenoid-activated valve (General Valve Corp). The solenoid was driven by a physiological stimulator (WPI, 1830 series) or by a computer-based stimulus system (8088-based computer running in DOS and equipped with a Metrabyte PIO12 digital I/O board and software based on ASYST, Keithley Instruments).

Based on the fact that tomato and tobacco leaves are among the preferred hostplants for *M. sexta* larvae (Yamamoto and Fraenkel 1960), we used the following odor stimuli: (1) a piece of crushed tobacco leaf (8 mm dia.); (2) *n*-hexane dilutions of a *n*-hexane extract of tomato leaves; (3) *n*-hexane dilutions of a dried ethanol extract of tomato leaves; and (4) *n*-hexane dilutions of (*E*)-2-hexenal, a common leaf aldehyde. In a few preparations, a *n*-hexane extract of the adult female *M. sexta* sex-pheromone gland (0.5 female equivalent), or *n*-hexane dilutions of (*E*, *Z*)-10, 12-hexadecadienal (bombykal), the major component of the female sex pheromone blend (Starratt et al. 1979; Tumlinson et al. 1989), were used as olfactory stimuli. After application of sample to the filter paper, the solvent was allowed to evaporate completely before the paper was inserted into a cartridge. Odor cartridges were prepared daily, and the order of their presentation in experiments was varied from cell to cell. The responses to 0.5 s or 2 s odor puffs were compared to the responses to a *n*-hexane blank cartridge. Stimuli were separated by at least 30–60 s to avoid receptor adaptation.

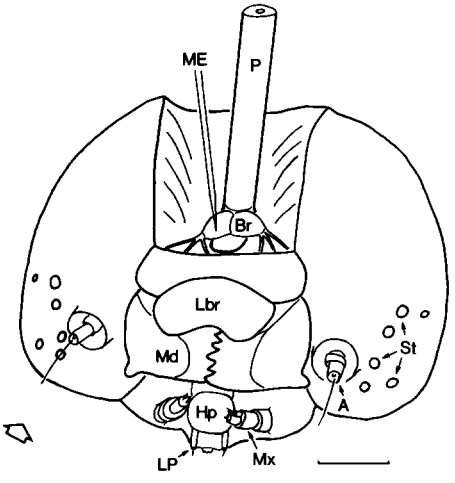

Fig. 2. Schematic drawing of the larval *M. sexta* preparation. *ME* microelectrode; *P* platform; *Br* brain; *Lbr* labrum; *MD* mandible; *Hp* hypopharynx; *LP* labial palp; *Mx* maxilla; *A* antenna; *St* stemmata. The *large arrowhead* indicates the direction from which the olfactory stimulus is delivered. Scale bar = 1 mm

Physiological recordings. Intracellular electrodes pulled on a horizontal puller (Sutter Instruments model P-80 PC) were filled with 2.5 *M* potassium acetate (KAc), 1.5 *M* potassium chloride (KCl), or Lucifer Yellow-CH (LY, Aldrich, 4% solution in 1 *M* LiCl (Stewart 1978)). Typical resistances were 60–80 MΩ for the KAc and KCl electrodes and 160–200 MΩ for LY. LY was iontophoresed into a cell with a DC hyperpolarizing current (10 nA-min). The brains were fixed in 4% paraformaldehyde in 0.1 *M* Sorensen's phosphate buffer (pH 7.4), dehydrated by passage through a graded ethanol series, cleared in methyl salicylate (Aldrich), and viewed in whole mount with an epifluorescence microscope (Marder and Eisen 1984). Drawings of stained cells were made with the aid of a camera lucida attachment.

The signal from the electrode was amplified (Getting model 5A or WPI model 707 DC amplifier), viewed on a storage oscilloscope (Tektronix model 5113), and recorded on magnetic tape (Vetter model B FM tape recorder). We found that we could impale more cells per preparation by modifying the amplifiers to allow a short (2–3 ms) 'buzz' of capacitance overcompensation. The buzz was triggered by a timed pulse from the stimulator, which closed a relay (Magnecraft model 545) to activate the compensation circuit.

Spike-train analysis of taped records was performed either by hand on data transcribed by a pen recorder (Gould model 2200) or by a computer-based data acquisition/analysis system. In the latter case, the signal from the FM recorder was first passed through a signal differentiator circuit (−3 dB at 64 Hz), if the recorded action potentials were small or the baseline was noisy, and then into a window discriminator (Frederick Haer). The output from the window discriminator was stored on a hard disk and analyzed with the aid of software based on ASYST (Keithley Instruments).

Histology. The ethyl gallate method (Wigglesworth 1957; modified by Strausfeld and Seyan 1985) was used to visualize anatomical features of the brain of larval *M. sexta*. After fixation in cacodylate-buffered glutaraldehyde, the brains were treated with OsO_4 followed by ethyl gallate (Fluka Chemical). They were then dehydrated, embedded in Araldite, and sectioned at 11 μm.

Cobalt staining. For anterograde staining of antennal afferents with cobalt, an antenna was excised along with a portion of the antennal nerve from a cold-anaesthetized larva. The cut end of the antennal nerve was draped over the wall of a small petroleum-jelly well constructed in a dissecting dish. More petroleum jelly was added to the walls of the well to isolate the cut end from the rest of the antennal nerve. The well was filled with distilled water, and the cut end of the nerve was allowed to contact the distilled water for 15–30 s to permit hyposmotic shock to open the cut ends of the axons. The water in the well was then replaced with dilute cobalt-lysine solution (Lazar 1978), and the well was closed with more petroleum jelly. The rest of the preparation was flooded with physiological saline solution (Christensen and Hildebrand 1987a) and incubated overnight at 4 °C. The antenna was then washed

with saline solution, the cobalt was precipitated by bubbling H_2S gas through the saline for 15 s, and the preparation was fixed in alcoholic Bouin's solution (Humason 1967). Finally, the tissue was processed by Timm's silver intensification method (Bacon and Altman 1977). The antenna was dehydrated in a graded ethanol series, cleared in methyl salicylate, and embedded in Canada Balsam for viewing in whole mount.

Results

All of the neurons impaled in this study were ipsilateral to the stimulated antenna. Most of the recordings were made from cell bodies (7–15 μm longest dimension) because of the small diameter of the neuronal processes. We therefore observed attenuated action potentials (2–15 mV) with resting membrane potentials in the range of −50 to −60 mV. The few impalements of neurites in the neuropil yielded 40–50 mV action potentials. The background firing rate of the recorded neurons ranged from 0 to 20 impulses/s. We did not attempt to control this background firing by injection of current, as hyperpolarization of the cell would have resulted in intracellular iontophoresis of LY. Owing to the small size of the larval neurons and the associated problem of trying to maintain a stable intracellular impalement during the stimulation and dye injection, the observations from many cells are incomplete, lacking either a part of the physiological data (such as replicates on the dose-response curve) or a full description of the morphology of the neuron.

We studied more than 1700 neurons, of which 530 were odor- and/or wind-sensitive: 74% of those cells responded with excitation, 22% with inhibition, and 4% with mixed excitation and inhibition. Of these 530 odor-responsive neurons, we have enough data on 210 neurons to characterize them based on their cellular morphology and/or their physiological responses: 15 (7%) were LAC local interneurons, 13 (6%) were projection neurons linking the LAC with the higher-order centers of the protocerebrum, 43 (20%) were bilateral neurons, and 139 (66%) were higher-order neurons located either in the mushroom bodies or elsewhere in the protocerebrum. In the following sections, we present observations from the best preparations of neurons that we also believe to be representative of the different types of neurons.

We did not find any odor-sensitive neurons that responded to only one of the odorants used to stimulate the antenna. This was true whether the neuron had arborizations in the LAC, projections to the protocerebrum or to the contralateral hemisphere, or neurites confined to the protocerebrum. The non-odor-responsive neurons may have had no olfactory input or may simply have been unresponsive to the odors tested. The neurons that did respond to odors, however, responded to all of the plant-related odors tested, albeit with different intensities of response to the several odors at the various doses.

The somata of the odor-responsive neurons were found in several regions of the brain (Fig. 3). Among the olfactory interneurons, the cell bodies of the respon-

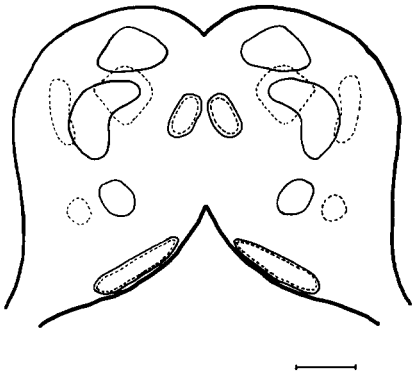

Fig. 3. Schematic drawing of the larval *M. sexta* brain showing regions where the somata of odor-responsive neurons were found. Solid lines outline regions on the rostral surface of the brain, stippled lines outline regions on the caudal surface. Dorsal up. Scale bar = 100 μm

sive neurons with arborizations confined to the LAC (local interneurons) and those with axons leading to the protocerebrum (projection neurons) were located predominantly in the ventral regions of the brain. The cell bodies of the odor-responsive neurons of the mushroom bodies and protocerebrum were divided between the rostral and caudal surfaces in the dorsal regions of the brain.

Figure 4A shows the response of a central neuron (probably a higher-order neuron, judging from the dorsal location of the soma) to 3 different doses of tomato-leaf extract and (*E*)-2-hexenal (E2H), a crushed tobacco leaf, and a *n*-hexane blank cartridge (i.e. clean air). The responses were phasic-tonic and did not outlast the 500-ms stimulus. The responses were also dose-dependent, as shown by Fig. 4B. As often happened, the response to increasing doses of an odor, in this case E2H, rose to a peak and then dropped with higher doses. The response to tomato extract appeared to rise to a peak at the highest dose. The responses to E2H and tomato extract could not be compared directly, however, because of the different initial concentrations and because E2H was a pure odorant and the extract was a mixture. Nevertheless, the responses of this cell to the 3 different odor stimuli did not appear to be qualitatively different. For example, the response to 20% tomato extract (20TOM) and to tobacco leaf were virtually identical in comparisons of individual traces (Fig. 4A, traces 3 and 7) or the mean responses (Fig. 4B). Note also that for a given odor concentration, the responses were quite consistent, as indicated by the small standard errors of the means of the responses (Fig. 4B).

The responses of a cell (probably a LAC local interneuron, judging from the ventro-lateral position of its soma) to different doses of E2H presented randomly are shown in Fig. 5A. The 50-fold concentration range of E2H used for stimulation apparently spanned the response range for this cell (Fig. 5B). The lowest concentration evoked a response similar to that attributable to the blank cartridge. The cell's response increased with increasing doses of E2H before reaching a plateau. There

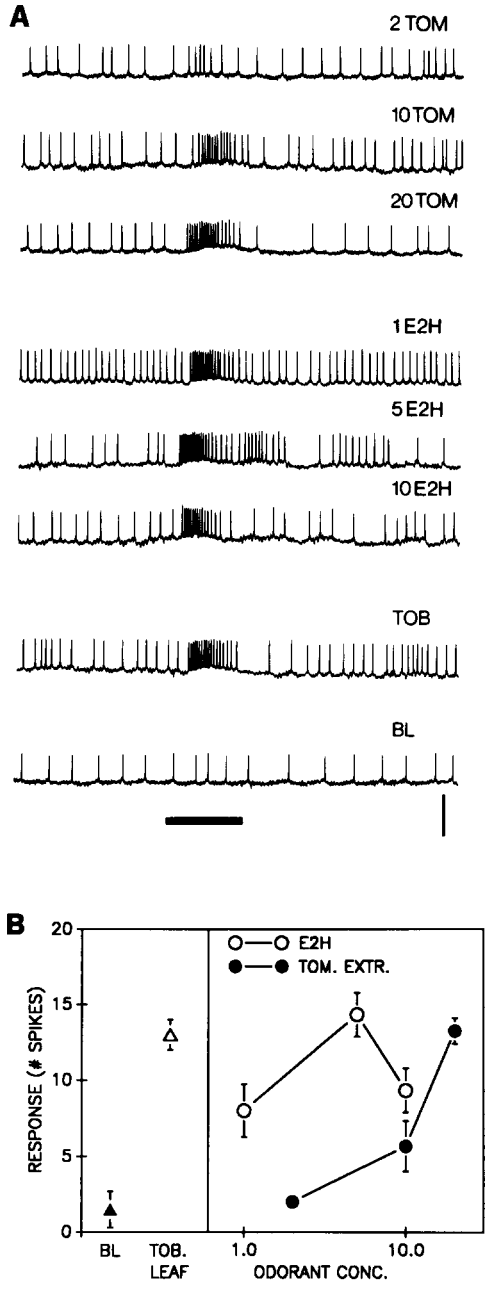

Fig. 4. **A** Responses of a central neuron (probably a higher-order neuron, based on the dorsal cell body position) to different odors. *TOM* tomato leaf extract presented at 2%, 10%, 20% dilutions; *E2H* (*E*)-2-hexenal presented at 1%, 5%, 10% dilutions. All dilutions in *n*-hexane, all 10 µl aliquots. *TOB* small piece of crushed tobacco leaf; *BL* *n*-hexane blank cartridge. Stimulus marker = 500 ms; voltage marker = 4 mV. **B** Dose-response curve of the cell in **A**. Response calculated by subtracting the mean number of impulses per 500 ms before the stimulus (averaged over 2 s) from the number of impulses during the stimulus. Separate baseline number of impulses calculated for each trial. Means of 3 replicates per dose ±SEM

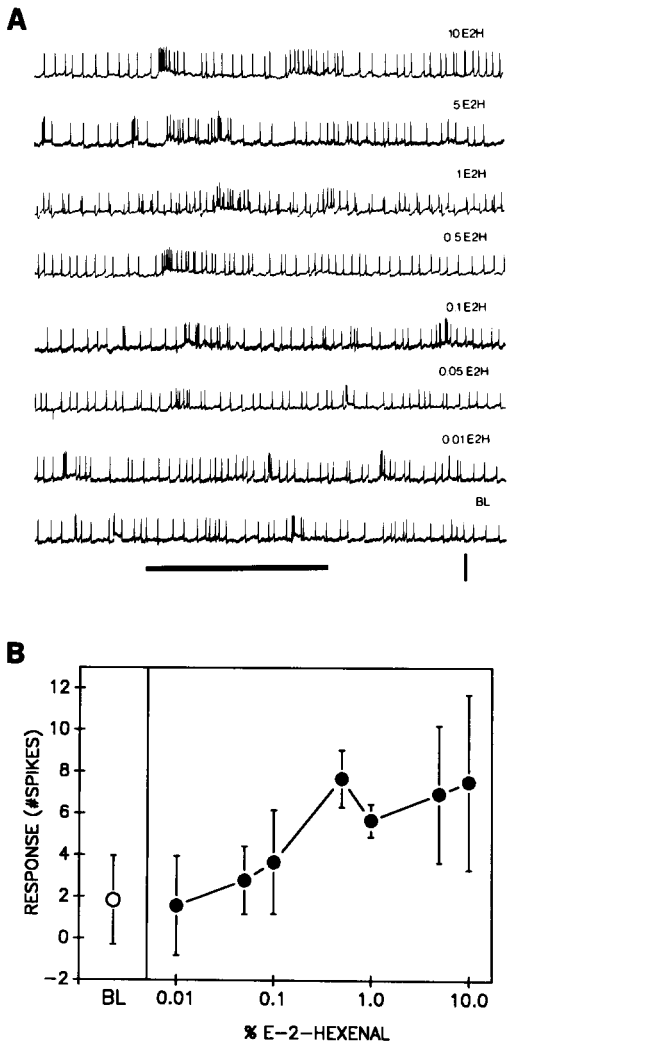

Fig. 5. **A** Responses of a central neuron (probably a LAC local interneuron, based on the ventro-lateral cell body position) to 7 different concentrations of E2H (10%, 5%, 1%, 0.5%, 0.1%, 0.05%, 0.01% – 10 µl each, dilutions in *n*-hexane, and a *n*-hexane blank cartridge. Cartridges presented in random order.) Note variability of responses. Stimulus marker = 2 s, voltage marker = 5 mV. **B** Dose-response curve of the cell in **A**. 3 replicates per concentration. Means calculated as in Fig. 3B. Mean responses ±SEM

was a great deal of variability in the responses of this cell to a given dose of odorant, which can be seen in the large standard errors of the means in the dose-response curve (Fig. 5B, three replicates per dose). The latency of the responses appeared to be shorter at the higher concentrations of stimulus, a finding also seen in adult male *M. sexta* (Kanzaki et al. 1989). Moreover, the responses stopped before the end of the 2-s stimulus, and two spike amplitudes were detectable in some records (e.g. the third trace in Fig. 5A), a phenomenon also found in local interneurons in the antennal lobe of adult *M. sexta* (Matsumoto and Hildebrand 1981).

A local interneuron with neurites confined to the LAC is illustrated in Fig. 6A. The two major processes of this cell converged in a region close to the ventral border of the LAC after branching near the cell body. Figure 6B shows the morphology of another local interneuron that also had branches confined to the LAC. The responses of the cell in Fig. 6A are shown in Fig. 6C. This neuron had a high background firing rate and exhibited action potentials of two amplitudes,

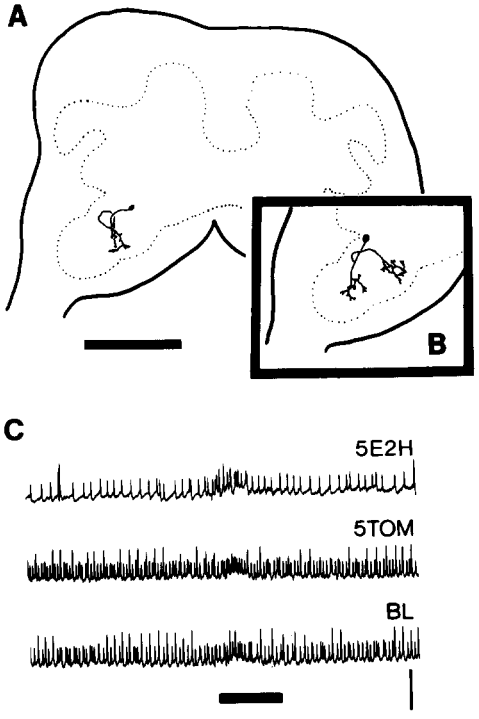

Fig. 6. **A** Morphology of a local neuron with processes confined to the LAC. Anterior view. Scale bar = 250 μm. **B** Morphology of a second local neuron. **C** Responses of the cell in **A** to 5% E2H, 5% tomato leaf extract (both 10 μl, dilutions in *n*-hexane), and a *n*-hexane blank cartridge. Stimulus marker = 500 ms, voltage marker = 4 mV

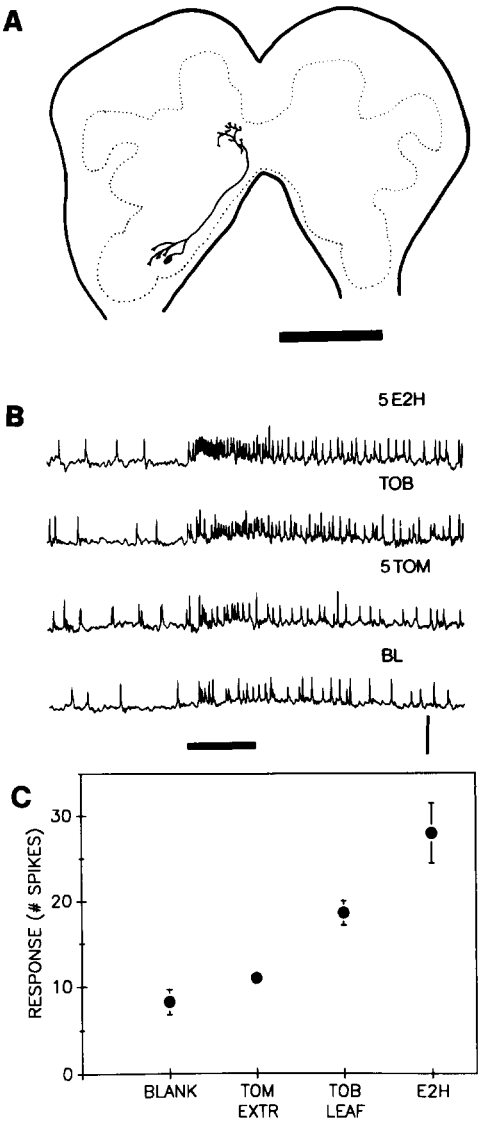

Fig. 7. **A** Morphology of a projection neuron with dendrites in the LAC and projections to the protocerebrum. Anterior view. Scale bar = 250 μm. **B** Responses of the cell in **A** to 5% E2H (10 μl, diluted in *n*-hexane), tobacco leaf, 5% tomato leaf extract (10 μl, diluted in *n*-hexane), and a *n*-hexane blank cartridge. Stimulus marker = 500 ms, voltage marker = 4 mV. **C** Mean (±SEM) responses of the cell in **A** to the different stimuli (3 or 4 replicates each). Means calculated as in Fig. 4B

especially noticeable in the second and third traces. The firing of action potentials of one amplitude did not appear to be tightly correlated with the firing of action potentials of the other amplitude, as the smaller impulses occurred at a much higher frequency than the larger ones. The response of this neuron, evident in the small spikes, occurred at a short latency after the start of the stimulus and appeared to shut off before the end of the stimulus.

A projection neuron (Fig. 7A) had dendritic arborizations in the LAC and an axon that projected to the protocerebrum. As in the local interneurons, the arborizations of this neuron were confined to a part of the LAC. The responses of this neuron (Fig. 7B), which were phasic-tonic and outlasted the stimulus, were especially dramatic because of the cell's relatively low background firing (4–8 Hz) and the observation that the firing rate did not revert to background levels even 5 s after an odor stimulus. Figure 7C shows the mean responses of this neuron to the 4 types of stimuli, averaged over 3 or 4 replicates, and demonstrates that E2H evoked the strongest responses, followed by tobacco leaf and tomato extract.

A higher-order, odor-responsive neuron located in the mushroom body is presented in Fig. 8. It appeared to be a local interneuron with 3 major branches, none of which could be readily identified as an axon (Fig. 8A). The soma of this cell was located at the dorsal margin of the brain. This neuron gave responses to odors and a very small response to the blank cartridge (Fig. 8B, C). The variability of the responses was relatively small except in the case of stimulation with tobacco-leaf volatiles (Fig. 8C).

A second type of higher-order olfactory neuron had processes localized to the lateral part of the protocerebrum (Fig. 9A). Because a small branch of the cell was not stained completely (arrowhead), it is not possible to say whether this neuron had a well-defined axon. The responses of this neuron appeared to be mixed (excitatory-inhibitory), as was most evident in the response to 20% E2H (top trace, Fig. 9B). The responses to E2H and to tomato extract appeared to consist of an extra action potential followed by weak hyperpolarization in

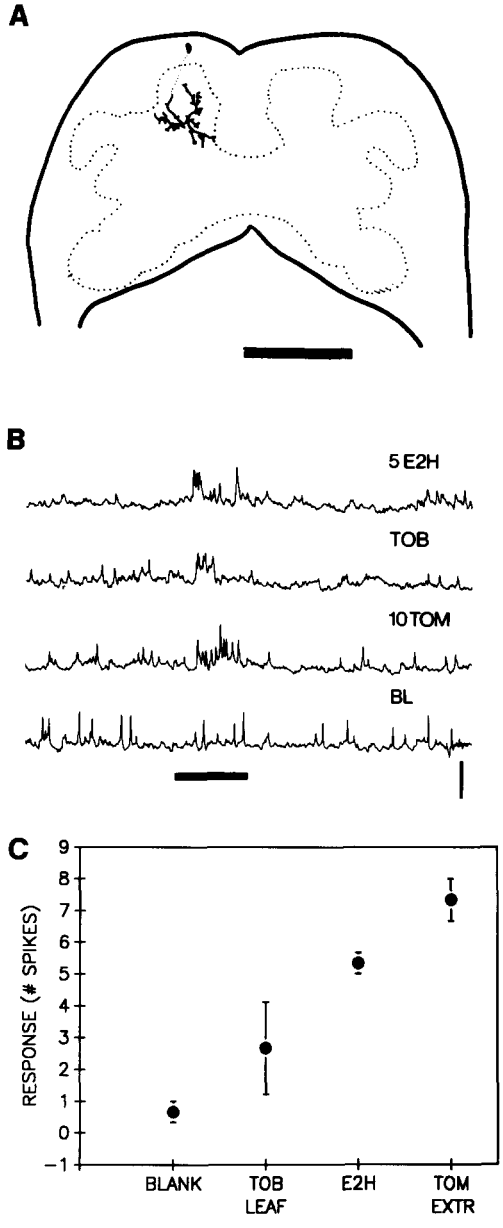

Fig. 8. **A** Morphology of a cell with processes in the mushroom bodies. Anterior view. Scale bar = 250 µm. **B** Responses of the cell in **A** to 5% E2H (10 µl, diluted in *n*-hexane), tobacco leaf, 10% tomato leaf extract (10 µl, diluted in *n*-hexane), and a *n*-hexane blank cartridge. Stimulus marker = 500 ms, voltage marker = 5 mV. **C** Mean (±SEM) responses of the cell in **A** to the different stimuli (3 replicates each). Means calculated as in Fig. 4B

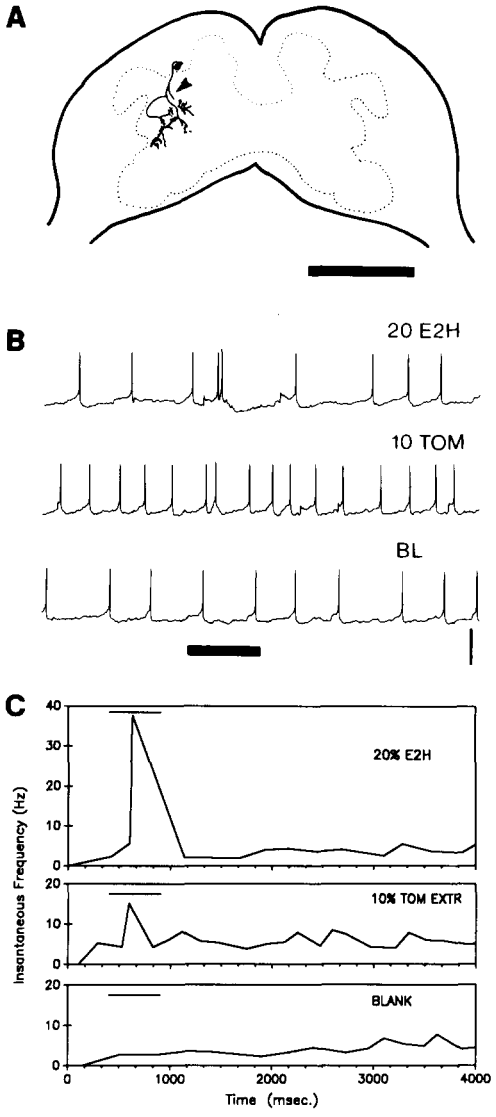

Fig. 9. **A** Morphology of a cell in the lateral part of the protocerebrum. *Arrowhead* points to an unfilled branch. Anterior view. Scale bar = 250 µm. **B** Responses of the cell in **A** to 20% E2H, 10% tomato leaf extract (both 10 µl, diluted in *n*-hexane), and a *n*-hexane blank cartridge. Stimulus marker = 500 ms, voltage marker = 10 mV. **C** Instantaneous frequency plots of the responses shown in **B**

an otherwise slow and steady impulse train. The mean responses of this cell to the different stimuli were not very different from the mean responses to the blank cartridge (not shown). By contrast, the instantaneous frequency plots for the 3 stimuli (Fig. 9C) show a strong peak in the impulse frequency associated with the odorants but not the blank.

The cell in Fig. 10A was one of the few odor-responsive, contralaterally projecting neurons stained in this study. It had sparse arborizations in the lateral protocerebrum and a contralateral projection that was incompletely stained. It is also one of the minority of neurons whose response to odors was inhibitory. Hyperpolarization of the membrane is evident in the responses to 10% and 5% E2H (top two traces, Fig. 10B). The mean responses of this cell (Fig. 10C) show that neither the blank nor the low concentration of E2H had much effect, while the two higher concentrations evoked a significant drop in the basal firing rate. The mean responses to 5% and 10% E2H appeared to be very similar, suggesting that the response of this cell had reached saturation.

Figure 11A and B demonstrates that two different higher-order neurons (identified by dorsal somata positions) responded to sex pheromones from an adult fe-

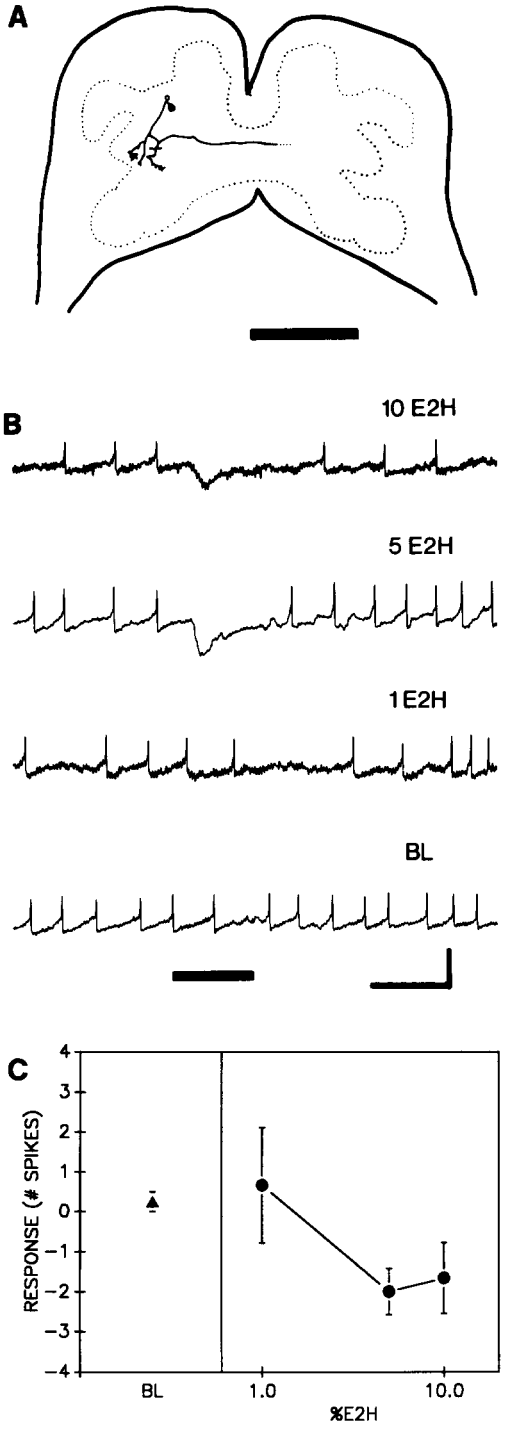

Fig. 10. **A** Morphology of a cell in the protocerebrum with a contralateral projection (incompletely filled). Anterior view. Scale bar = 250 µm. **B** Responses of the cell in **A** to 1%, 5%, and 10% E2H (10 µl, diluted in *n*-hexane) and a *n*-hexane blank cartridge. Stimulus marker = 500 ms, voltage marker = 4 mV for the second and fourth traces, 2 mV for the first and third traces. **C** Mean (±SEM) responses of the cell in **A** to the different stimuli (3 replicates each). Means calculated as in Fig. 4B

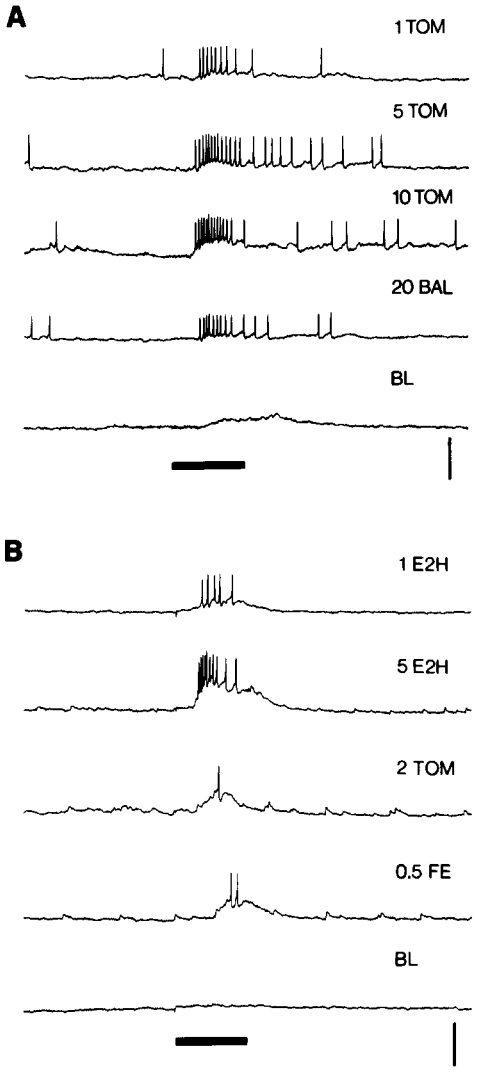

Fig. 11. **A** Responses of a central (probably higher-order) neuron to 1%, 5%, 10% tomato extract (10 µl, diluted in *n*-hexane), 20 ng (*E*, *Z*)-10, 12-hexadecadienal (bombykal or *BAL*), and a blank cartridge. Stimulus marker = 500 ms; voltage marker = 10 mV. **B** Responses of a different (probably higher-order) neuron to 1% and 5% E2H, 2% tomato extract (all 10 µl, diluted in *n*-hexane), 0.5 female equivalent (0.5 *FE*) of the natural adult female pheromone blend (extracted in *n*-hexane), and a *n*-hexane blank cartridge. Stimulus marker = 500 ms; voltage marker = 10 mV

male *M. sexta*. The cell in Fig. 11A responded with a phasic-tonic train of action potentials to antennal stimulation with a cartridge loaded with 20 ng of (*E*, *Z*)-10, 12-hexadecadienal (bombykal or BAL), the major component of the female sex-pheromone blend (Starratt et al. 1979; Tumlinson et al. 1989). The cell gave no response to a *n*-hexane blank cartridge. Figure 11B shows a second cell that responded with excitation to 0.5 female-equivalent of the complete pheromone blend. Both of these cells also responded to E2H and tomato extract with responses that were extremely similar to that elicited by the sex pheromone components. A total of 19 cells were subjected to antennal stimulation with either the pheromone blend or BAL, as well as to one or more non-pheromonal odors: 16 (84%) responded to all odors tested while 3 cells (16%) responded to the non-pheromonal odor(s) but not to BAL or the pheromone blend.

Discussion

Our findings suggest that a *M. sexta* larva assesses the quality of different plant odors by an 'across-fiber' firing pattern of the olfactory interneurons in its central nervous system. We have not encountered any neuron that responded solely to tomato or tobacco odor, or to one of the other stimuli; all cells that responded to one of the olfactory stimuli also responded to some or all of the others. This is in contrast to the situation in the adult male *M. sexta* (Christensen and Hildebrand 1987b, 1988; Kanzaki et al. 1989), in which there is a specific olfactory subsystem that deals almost exclusively with sex pheromones. The neurons of this subsystem respond selectively to the components of the pheromone blend and in some cases, respond to only a single component of the blend (Christensen and Hildebrand 1987b). A similar situation is found in the adult males of *H. zea* and *H. virescens*, two species of noctuid moths (Christensen et al. 1989).

All of the cells in the larval brain that responded to either the adult female's sex-pheromone blend or to BAL, its major component, responded to plant-related odors as well. It is therefore possible that the responses were mediated by the same receptors. Three cells that responded to the plant-related odors, however, did not respond to pheromone, suggesting differences either in their sensitivities or their tuning. Although we have not thoroughly investigated the ability of larvae to perceive pheromone components, preliminary extracellular recordings from olfactory receptors showed that some units responded to a variety of long-chain aldehydes and alcohols (H. Itagaki and J.G. Hildebrand, unpublished observations). Long-chain substances are known to be associated with leaf cuticle (Martin and Juniper 1970; Baker 1982), and a pheromone component may stimulate receptors that are sensitive to these leaf-surface chemicals. In any case, it is clear that the larval receptors that respond to sex-pheromone components are not the same as the adult receptors, because the larval antennal receptor cells degenerate during metamorphosis and adult antennae develop from antennal imaginal disks distinct from the larval antenna (Sanes and Hildebrand 1976a, b).

The fact that a relatively large number of higher-order neurons (in comparison with the other types of neurons) were characterized in this study may be due to the fact that recording from these neurons is easier than recording from LAC local interneurons and projection neurons. We believe that such a sampling bias may skew our sample to some extent because the LAC-associated neurons, especially the projection neurons, have their somata nearer to the ventral margin of the brain, making stable impalements more difficult. Of course, it is also possible that there are more of the higher-order types of neurons than the LAC-associated neurons.

The somata of odor-sensitive neurons in the larval brain seemed to be widely distributed. Although there appeared to be some clustering of the somata of certain types of odor-responsive neurons (see Fig. 6A, B), we could not easily place the cell somata positions on the basis of any external markers on the surface of the brain. Comparisons of positions of somata of odor-responsive neurons from preparation to preparation were hindered by differences in tissue deformation during fixation, which were exacerbated by desheathing. Nevertheless, somata of odor-responsive neurons can be mapped to certain broad regions of the brain surface (Fig. 3).

LAC local interneurons often gave highly variable responses to a given dose of odorant (e.g. Fig. 5), a finding also found in adult male *M. sexta* (TA Christensen, ID Harrow, BR Waldrop, JG Hildebrand, unpublished). This variability was due, in part, to rapid habituation to a given odorant despite a long interstimulus interval. The later responses, in general, were not as robust as the earlier responses. Dose-response curves showed a rough plateau and considerable variability. A possible interpretation is that such local interneurons act as odorant 'threshold detectors', exhibiting a step response to different doses of odorant. If the dose is above threshold, then the interneuron gives a relatively standardized response; if the odor concentration is below threshold, then the response is indistinguishable from a blank response. In the neuron of Fig. 5, this threshold appears to be between 0.1 and 0.5% E2H (Fig. 5B).

The responses of a LAC projection neuron to a given odorant were more tightly clustered about the mean (e.g. Fig. 7C) despite the greater strength of the responses. Habituation was also less of a problem for these neurons. The responses of protocerebral neurons were less variable (Figs. 4A, B; 8B, C; 9B, C) than the responses of the LAC local interneurons, although their responses were less dramatic than those of LAC projection neurons.

The LAC local interneurons stained did not exhibit arborizations throughout the entire LAC. Their processes were confined to certain parts of the LAC, implying the LAC is functionally subdivided. In adult *M. sexta*, the local interneurons in the antennal lobes have arborizations in many or all of the glomeruli, sometimes including the male-specific macroglomerular complex (MGC) (Matsumoto and Hildebrand 1981; Hoskins et al. 1986; Christensen and Hildebrand 1987b).

Dendritic arborizations of projection neurons also appeared to be limited to certain regions of the LAC, as no such neuron was observed to have arborizations throughout the LAC. This is analogous to the situation in the adult, in which the sex-pheromone-specific interneurons all have dendrites confined to the MGC (Christensen and Hildebrand 1987a), and about half of the 'non-MGC' projection neurons appear to have dendrites confined to one glomerulus (Matsumoto and Hildebrand 1981; Christensen and Hildebrand 1987b; Homberg et al. 1988).

Protocerebral neurons in the larval brain gave less dramatic responses to odor stimulation than those recorded from LAC projection interneurons. Compared to LAC local interneurons, these higher-order neurons gave responses that were less variable for a given stimulus. Larval protocerebral neurons were also not very responsive to mechanosensory stimuli (puffs of air deliv-

ered from blank cartridges – Figs. 4A, 8B, 9B, 10B, 11A and B), unlike some of the LAC local and projection interneurons (Figs. 6C, 7B). The subdued responses of the higher-order cells may have been due to (1) a lack of the proper associated stimulus (e.g. light) to maximize the responses of these cells, or (2) a lack of strong mechanosensory inputs. In the latter case, it is possible that some local and projection neurons are multimodal, but that the mechanosensory information is processed by neurons without olfactory inputs in the higher integration centers of the protocerebrum and mushroom bodies. As we did not continue to record from and stain strictly mechanosensory neurons, it is possible that the higher-order mechanosensory neurons were bypassed in the experiments and are therefore not represented here.

Multimodality appears to be common among interneurons of the deutocerebrum in the brains of many insects. Homberg (1981) found all of the deutocerebral output neurons he studied in worker honeybees to be multimodal, while in *P. americana*, Ernst and Boeckh (1983) found some local and projection neurons that were multimodal. In adult *M. sexta*, it appears that a subset of the antennal-lobe projection neurons that do not have arborizations in the MGC are multimodal and respond to mechanosensory as well as olfactory inputs (Kanzaki et al. 1989), while local interneurons are only very weakly stimulated by mechanosensory stimuli (Matsumoto and Hildebrand 1981). Multimodality has also been observed in many pheromone-sensitive deutocerebral cells in *B. mori* (Olberg 1983; Kanzaki and Shibuya 1986). Multimodality of protocerebral neurons has also been demonstrated in many insects (Suzuki et al. 1976; Erber 1978; Homberg 1984; Schildberger 1984; Light 1986). These neurons have more complex responses than deutocerebral neurons, exhibiting after-discharges that alter the cell's subsequent responses and conditional responses that depend on the cell's activity before the stimulus (Erber 1978; Schildberger 1984). It has been suggested that this plasticity may underlie learning and memory in these insects (Erber et al. 1980).

Interhemispheric communication occurs at several levels in the brain of larval *M. sexta*. Olfactory primary afferents do not cross to the contralateral side in larvae (Kent and Hildebrand 1987) or in adults (Camazine and Hildebrand 1979; Kent 1985), although such contralateral primary-afferent projections do exist in Diptera (Boeckh et al. 1970; Strausfeld 1976; Stocker et al. 1983). A pair of bilateral serotonin-immunoreactive cells is also known to exist in larval *M. sexta*, with a cell body in one hemisphere and extensive ramifications throughout the LAC and protocerebrum of the contralateral side (Kent et al. 1987). Interhemispheric communication apparently also occurs at higher levels of the brain, as suggested by the cell depicted in Fig. 10A, which links the lateral protocerebrum with the contralateral hemisphere.

The multiple spike amplitudes recorded from local interneurons (Figs. 5A, 6B) and from some protocerebral neurons (Fig. 8B) may be due to physiological electrical coupling between cells or to artifactual coupling caused by tandem electrode penetrations. Alternatively, the major branches of a cell might fire impulses independently, and these distinct excitable regions might be separated by relatively inexcitable regions with low safety factor, a phenomenon also described in the locust (Heitler and Goodman 1978), leech (Calabrese 1980), crayfish (Calabrese and Kennedy 1974), crab (Sandeman 1969a, b), alligator (Llinás et al. 1968; Llinás and Nicholson 1971), and several other organisms (reviewed in Westerfield et al. 1978). Tandem electrode penetrations and electrical coupling cannot be discounted, as we observed dye-coupling during some of our experiments. Multiple spike amplitudes have also been detected frequently in the local interneurons of the antennal lobes in adult *M. sexta* (Matsumoto and Hildebrand 1981; TA Christensen, ID Harrow, BR Waldrop, JG Hildebrand, unpublished). The major branches of such cells may act as independent units in local circuits within the neuropil of a glomerulus or small groups of glomeruli. This might allow one cell to act as more than one integrating unit, processing different information in different portions of the cell (Matsumoto and Hildebrand 1981).

In insects, the central processing of plant-related odorants has been less intensively studied than the processing of pheromones. In adult *M. sexta*, the results indicate that local interneurons without arborizations in the macroglomerular complex (MGC), the pheromone-processing region of the adult male antennal lobe, respond only to the plant-related odors (e.g. E2H, tobacco leaf, amyl acetate) while the local interneurons with MGC arborizations respond to pheromone components and to plant-related odorants differently (Matsumoto and Hildebrand 1981; reviewed in Christensen and Hildebrand 1987b). The MGC projection interneurons in male *M. sexta* fall into two categories: those that respond only to pheromone components (Christensen and Hildebrand 1987a) and those that show no preference for pheromones. In the latter case, the neurons are excited by pheromone components but inhibited by E2H (Kanzaki et al. 1989).

Physiological work on insects other than *M. sexta* have revealed similar types of odor responses. In *P. americana*, intracellular and extracellular recordings show that there are deutocerebral neurons excited by both food and pheromone odors, neurons that are inhibited by food odors and excited by pheromone components, and neurons that respond only to food odors (Waldow 1977; Selzer 1979; Burrows et al. 1982; reviewed in Boeckh et al. 1984). Two functional classes of neurons have also been found in the deutocerebrum of the phytophagous beetle *L. decemlineata*, where one class is narrowly tuned, responding preferentially to individual odorants in a plant extract, while the other responds preferentially to the complete plant extract, appearing to act as a 'blend' detector (De Jong and Visser 1988).

The present study indicates that olfactory information in larval *M. sexta* is conveyed from the LAC to particular regions of the protocerebrum. The information appears to be refined and made more consistent by the initial processing steps involving neurons associated with the LAC. The limitations imposed by recording from cell somata surely obscure some of the subtlety of the physiological responses (e.g. PSPs), but the physi-

ological mapping approach nevertheless has given us an initial perspective on the olfactory pathway in the larval lepidopteran brain.

Acknowledgments. We thank P. Randolph, D. Sakiestewa, P. Test, and P. Woodfolk for expert technical assistance; C. Hedgcock, LPC, for expert photographic assistance; D. Dixon, L. Johnstone, J. Lawrence, P. Murray, and D. Olson for secretarial assistance; and Drs. J. Buckner and J. Svoboda of the USDA for providing *Manduca sexta* eggs. Dr. B. Waldrop provided the stimulation software as well as help in the data analysis, and Dr. R.C. Bruening of the Department of Chemistry, University of Hawaii, generously supplied different extracts of tomato plants. Dr. N.J. Strausfeld provided help and facilities for the ethyl gallate studies. Drs. T.A. Christensen and B. Waldrop kindly gave us valuable comments on the manuscript. This research was supported by NIH Postdoctoral Fellowship 1-F32-NS07990 (to HI) and by grants from the Monsanto Company and the Cooperative State Research Service, U.S. Department of Agriculture (Agreement No. 87-CRCR-1-2362).

References

Bacon JP, Altman JS (1977) A silver intensification method for cobalt-filled neurons in wholemount preparations. Brain Res 138:359–363

Baker EA (1982) Chemistry and morphology of plant epicuticular waxes. In: Cutler DF, Alvin KF, Price CE (eds) The plant cuticle. Linnaean Society Symposium Series No. 10. Academic Press, New York, pp 139–165

Barbosa P, Greenblatt J, Withers W, Cranshaw W, Harrington EA (1979) Host-plant preferences and their induction in larvae of the gypsy moth, *Lymantria dispar*. Entomol Exp Appl 26:180–188

Bell RA, Joachim FG (1976) Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. Ann Entomol Soc Am 69:365–373

Boeckh J, Boeckh V (1979) Threshold and odor specificity of pheromone-sensitive neurons in the deutocerebrum of *Antheraea pernyi* and *A. polyphemus*. J Comp Physiol 132:235–242

Boeckh J, Sandri C, Akert K (1970) Sensorische Eingänge und synaptische Verbindungen im Zentralnervensystem von Insekten. Z Zellforsch 103:429–446

Boeckh J, Ernst KD, Sass H, Waldow U (1984) Anatomical and physiological characteristics of individual neurones in the central antennal pathways of insects. J Insect Physiol 30:15–26

Burrows M, Boeckh J, Esslen J (1982) Physiological and morphological properties of interneurons in the deutocerebrum of male cockroaches which respond to female pheromone. J Comp Physiol 145:447–457

Calabrese RL (1980) Control of multiple impulse-initiation sites in a leech interneuron. J Neurophysiol 44:878–896

Calabrese RL, Kennedy D (1974) Multiple sites of spike initiation in a single dendritic system. Brain Res 83:316–321

Camazine SM, Hildebrand JG (1979) Central projections of antennal sensory neurons in mature and developing *Manduca sexta*. Soc Neurosci Abstr 5:155

Christensen TA, Hildebrand JG (1987a) Male-specific sex pheromone-selective projection neurons in the antennal lobes of the moth, *Manduca sexta*. J Comp Physiol A 160:553–569

Christensen TA, Hildebrand JG (1987b) Functions, organization, and physiology of the olfactory pathways in the lepidopteran brain. In: Gupta AP (ed) Arthropod brain: its evolution, development, structure, and function. John Wiley and Sons, New York, pp 457–484

Christensen TA, Hildebrand JG (1988) Frequency coding by central olfactory neurons in the sphinx moth *Manduca sexta*. Chem Senses 13:123–130

Christensen TA, Mustaparta H, Hildebrand JG (1989) Discrimination of sex pheromone blends in the olfactory system of the moth. Chem Senses 14:202

De Boer G, Hanson FE (1984) Foodplant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm, *Manduca sexta*. Entomol Exp Appl 35:177–193

De Boer G, Hanson FE (1987) Differentiation of roles of chemosensory organs in food discrimination among host and non-host plants by larvae of the tobacco hornworm. Physiol Entomol 12:387–398

De Jong R, Visser JH (1988) Integration of olfactory information in the Colorado potato beetle brain. Brain Res 447:10–17

Dethier VG (1937) Gustation and olfaction in lepidopterous larvae. Biol Bull 72:7–23

Dethier VG (1941) The function of antennal receptors in lepidopterous larvae. Biol Bull 80:403–414

Dethier VG (1967) Feeding and drinking behavior of invertebrates. In: Code CF (ed) Handbook of physiology, Sec B. Alimentary canal. Am Physiol Assn, Washington DC, vol 1, pp 79–96

Dethier VG (1980) Responses of some olfactory receptors of the eastern tent caterpillar (*Malacosoma americanum*) to leaves. J Chem Ecol 6:213–220

Dethier VG, Crnjar RM (1982) Candidate codes in the gustatory system of caterpillars. J Gen Physiol 79:543–569

Dethier VG, Kuch JH (1971) Electrophysiological studies of gustation in lepidopterous larvae. I. Comparative sensitivity to sugars, amino acids, and glycosides. Z Vergl Physiol 72:343–363

Dethier VG, Schoonhoven LM (1969) Olfactory coding by lepidopterous larvae. Entomol Exp Appl 12:535–543

Erber J (1978) Response characteristics and after-effects of multimodal neurones in the mushroom body area of the honeybee. Physiol Entomol 3:77–89

Erber J, Masuhr T, Menzel R (1980) Localization of short-term memory in the brain of the honeybee *Apis mellifera*. Physiol Entomol 5:343–358

Ernst K-D, Boeckh J (1983) A neuroanatomical study on the organisation of the central antennal pathway in insects. III. Neuroanatomical characterization of physiologically defined response types of deutocerebral neurons in *Periplaneta americana*. Cell Tissue Res 229:1–22

Hanson FE, Dethier VG (1973) Role of gustation and olfaction in food plant discrimination in the tobacco hornworm, *Manduca sexta*. J Insect Physiol 19:1019–1034

Heitler WJ, Goodman CS (1978) Multiple sites of spike initiation in a bifurcating locust neurone. J Exp Biol 76:63–84

Homberg U (1981) Ableitungen und Lucifer Yellow Markierungen von Neuronen des Tractus olfactorio-globularis im Bienengehirn. Verh Dt Zool Ges 74:176

Homberg U (1984) Processing of antennal information in extrinsic mushroom body neurons of the bee brain. J Comp Physiol A 154:825–836

Homberg U, Montague RA, Hildebrand JG (1988) Anatomy of antenno-cerebral pathways in the brain of the sphinx moth *Manduca sexta*. Cell Tissue Res 254:255–281

Hoskins SG, Homberg U, Kingan TG, Hildebrand JG (1986) Immunocytochemistry of GABA in the antennal lobes of the sphinx moth, *Manduca sexta*. Cell Tissue Res 244:243–252

Humason GL (1967) Animal tissue techniques, 2nd ed. Freeman, San Francisco

Itagaki H, Hildebrand JG (1986) Central processing of olfactory information in the brain of the larval tobacco hornworm. Soc Neurosci Abstr 12:857

Itagaki H, Hildebrand JG (1988) Responses of olfactory interneurons in the brain of larval *Manduca sexta*. Soc Neurosci Abstr 14:380

Jermy T, Hanson FE, Dethier VG (1968) Induction of specific food preference in lepidopterous larvae. Entomol Exp Appl 11:211–230

Kanzaki R, Shibuya T (1984) Identification and pheromone responses of the mushroom body neurons in the male silkworm moth, *Bombyx mori*. Zool Sci 1:878

Kanzaki R, Shibuya T (1986a) Identification of deutocerebral neurons responding to the sexual pheromone in the male silkworm moth brain. Zool Sci 3:409–413

Kanzaki R, Shibuya T (1986b) Descending protocerebral neurons related to the mating dance of the male silkworm moth. Brain Res 377:378–382

Kanzaki R, Arbas EA, Strausfeld NJ, Hildebrand JG (1989) Physiology and morphology of projection neurons in the antennal lobe of the male moth *Manduca sexta*. J Comp Physiol A 165:427–453

Kent KS (1985) Metamorphosis of the antennal center and the influence of sensory innervation on the formation of glomeruli in the hawkmoth *Manduca sexta*. PhD Dissertation, Harvard University, Cambridge, USA

Kent KS, Hildebrand JG (1987) Cephalic sensory pathways in the central nervous system of larval *Manduca sexta* (Lepidoptera: Sphingidae). Phil Trans R Soc Lond B 315:1–36

Kent KS, Hoskins SG, Hildebrand JG (1987) A novel serotonin-immunoreactive neuron in the antennal lobe of the sphinx moth *Manduca sexta* persists throughout postembryonic life. J Neurobiol 18:451–465

Lazar G (1978) Application of cobalt-filling technique to retinal projections in the frog. Neuroscience 3:725–736

Light D (1986) Central integration of sensory signals: An exploration of processing of pheromonal and multimodal information in lepidopteran brains. In: Payne TL, Birch MC, Kennedy CEJ (eds) Mechanisms in insect olfaction. Oxford University Press, Oxford, pp 287–301

Llinás R, Nicholson C (1971) Electrophysiological properties of dendrites and somata in alligator Purkinje cells. J Neurophysiol 34:532–551

Llinás R, Nicholson C, Freeman JA, Hillman DE (1968) Dendritic spikes and their inhibition in alligator Purkinje cells. Science 160:1132–1135

Ma WC (1972) Dynamics of feeding responses in *Pieris brassicae* Linn. as a function of chemosensory input: A behavioural, ultrastructural and electrophysiological study. Med Landbouwhogeschool Wag 72-11:1–162

Ma WC (1976) Mouth parts and receptors involved in feeding behaviour and sugar perception in the African armyworm *Spodoptera exempta* (Lepidoptera: Noctuidae). Symp Biol Hung 16:139–151

Marder E, Eisen JS (1984) Transmitter identification of pyloric neurons: Electrically coupled neurons use different transmitters. J Neurophysiol 51:1345–1361

Martin JT, Juniper BE (1970) The cuticle of plants. St Martin's Press, New York, 347 pp

Matsumoto SG, Hildebrand JG (1981) Olfactory mechanisms in the moth *Manduca sexta*: Response characteristics and morphology of central neurons in the antennal lobes. Proc R Soc Lond B 213:249–277

Olberg RM (1983) Interneurons sensitive to female pheromone in the deutocerebrum of the male silkworm moth, *Bombyx mori*. Physiol Entomol 8:419–428

Pichon Y, Satelle DB, Lane NJ (1972) Conduction processes in the nerve cord of the moth *Manduca sexta* in relation to its ultrastructure and haemolymph ionic composition. J Exp Biol 56:717–734

Prescott DJ, Hildebrand JG, Sanes JR, Jewett S (1977) Biochemical and developmental studies of acetylcholine metabolism in the central nervous system of the moth, *Manduca sexta*. Comp Biochem Physiol 56C:77–84

Randolph PW, Itagaki H (1989) Central projections of cephalic sensory neurons in the larvae of *Heliothis* moths. Soc Neurosci Abstr 15:1290

Sandeman DC (1969a) The site of synaptic activity and impulse initiation in an identified motoneurone in the crab brain. J Exp Biol 50:771–784

Sandeman DC (1969b) Integrative properties of a reflex motoneurone in the brain of the crab *Carcinus maenas*. Z Vergl Physiol 64:450–464

Sanes JR, Hildebrand JG (1976a) Structure and development of antennae in a moth, *Manduca sexta*. Dev Biol 151:282–299

Sanes JR, Hildebrand JG (1976b) Origin and morphogenesis of sensory neurons in an insect antenna. Dev Biol 51:300–319

Saxena KN, Schoonhoven LM (1978) Induction of orientational and feeding preferences in *Manduca sexta* larvae for an artificial diet containing citral. Entomol Exp Appl 23:173–180

Saxena KN, Schoonhoven LM (1982) Induction of orientational and feeding preferences in *Manduca sexta* larvae for different food sources. Entomol Exp Appl 32:173–180

Schildberger K (1984) Multimodal interneurons in the cricket brain: Properties of identified extrinsic mushroom body cells. J Comp Physiol A 154:71–79

Schoonhoven LM (1969) Gustation and foodplant selection in some lepidopterous larvae. Entomol Exp Appl 12:555–564

Schoonhoven LM (1986) What makes a caterpillar eat? The sensory code underlying feeding behavior. In: Chapman RF, Bernays EA, Stoffolano JG Jr (eds) Perspectives in chemoreception and behavior. Springer, Berlin Heidelberg New York, pp 79–97

Schoonhoven LM, Dethier VG (1966) Sensory aspects of hostplant discrimination by lepidopterous larvae. Arch Néerl Zool 16:497–530

Selzer LS (1984) Physiology and morphology of the larval sexual pheromone-sensitive neurones in the olfactory lobe of the cockroach, *Periplaneta americana*. J Insect Physiol 30:537–546

Selzer R (1979) Morphological and physiological identification of food odour-specific neurones in the deutocerebrum of *Periplaneta americana*. J Comp Physiol 134:159–163

Städler E, Hanson FE (1975) Olfactory capabilities of the 'gustatory' chemoreceptors of the tobacco hornworm larvae. J Comp Physiol 104:97–102

Starratt AN, Dahm KH, Allen N, Hildebrand JG, Payne TL, Röller H (1979) Bombykal, a sex pheromone of the sphinx moth *Manduca sexta*. Z Naturforsch 34c:9–12

Stewart WW (1978) Functional connections between cells revealed by dye-coupling with a highly fluorescent naphthalimide tracer. Cell 14:741–759

Stocker RF, Singh RN, Schorderet M, Siddiqi O (1983) Projection patterns of different types of antennal sensilla in the antennal glomeruli of *Drosophila melanogaster*. Cell Tissue Res 232:237

Strausfeld NJ (1976) Atlas of an insect brain. Springer, Berlin Heidelberg New York, 214 pp

Strausfeld NJ, Seyan HS (1985) Convergence of visual, haltere and prosternal inputs at weak motor neurons of *Calliphora*. Cell Tissue Res 240:1–16

Suzuki H, Tateda H, Kuwabara M (1976) Activities of antennal and ocellar interneurons in the protocerebrum of the honeybee. J Exp Biol 64:405–418

Tumlinson JH, Brennan MM, Doolittle RE, Mitchell ER, Brabham A, Mazomenos BE, Baumhover AH, Jackson DM (1989) Identification of a pheromone blend attractive to *Manduca sexta* (L.) males in a wind tunnel. Arch Insect Biochem Physiol 10:255–271

Visser JH (1983) Differential sensory perception of plant compounds by insects. In: Hedin PA (ed) Plant resistance to insects. ACS Symposium Series, vol 208, pp 215–230

Waldow U (1977) CNS units in cockroach (*Periplaneta americana*): Specificity of response to pheromones and other odor stimuli. J Comp Physiol 116:1–17

Waldrop B, Christensen TA, Hildebrand JG (1987) GABA-mediated synaptic inhibition of projection neurons in the antennal lobes of the sphinx moth, *Manduca sexta*. J Comp Physiol A 161:23–32

Westerfield M, Joyner RW, Moore JW (1978) Temperature-sensitive conduction failure at axon branch points. J Neurophysiol 41:1–8

Wigglesworth VB (1957) Use of osmium in the fixation and staining of tissues. Proc R Soc Lond B 147:185–199

Yamamoto RT, Fraenkel GS (1960) The specificity of the tobacco hornworm, *Protoparce sexta*, to solanaceous plants. Ann Entomol Soc Am 53:503–507