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Discrimination among pheromone component blends by interneurons in male antennal lobes of two populations of the turnip moth, *Agrotis segetum*

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ABSTRACT **A difference in female pheromone production and male behavioral response has previously been found in two populations of the turnip moth, *Agrotis segetum*, originating from Sweden and Zimbabwe, respectively. In this study, we investigated the pheromone response of antennal lobe interneurons of males of the two populations by intracellular recordings, stimulating with single pheromone components and various inter- and intra-populational pheromone blends. Three major physiological types of antennal lobe neurons were established in the two populations according to their responses to different stimuli. One type responded broadly to almost all the stimuli tested. The second type responded selectively to some of the single components and blends. The third type did not respond to any single components but did respond to certain blends. Furthermore, some neurons of the second and third type recognized strain specific differences in ratios between pheromone components. Both projection neurons and local interneurons were found among these three types. Two pheromone responding bilateral projection neurons are reported for the first time in this paper.**

The species-specificity of sex pheromones has been well documented in several moth species (1–3). Males of one species are, in most cases, selectively attracted to the complete pheromone blend produced by conspecific females, although sympatric species often share some of the same pheromone components (4, 5). This invites the question of how male moths discriminate among different pheromone blends. In the central nervous system, pheromone sensitive interneurons have been studied in several insect species (ref. 6 and references therein) and the question concerning the blend discrimination by antennal lobe (AL) interneurons has also been investigated in the moths *Antheraea* spp. (7) and *Heliothis* spp. (8, 9). However, the limited number of stimuli used in the previous studies prevents a thorough understanding of pheromone discrimination at the central nervous system level in insects.

The sex pheromone of the turnip moth, *Agrotis segetum*, consists of a mixture of at least four olefinic acetates, *(Z)*-5-decenyl acetate (Z5-10:OAc), *(Z)*-7-dodecenyl acetate (Z7-12:OAc), *(Z)*-9-tetradecenyl acetate (Z9-14:OAc) and *(Z)*-5-dodecenyl acetate (Z5-12:OAc) (10). A distinctive difference in the ratio of the pheromone components has been shown between a Zimbabwean and a Swedish population of *A. segetum*. Females of the Swedish population produce Z5-10:OAc, Z7-12:OAc, Z9-14:OAc, and Z5-12:OAc in a 1:5:2.5:0.1 ratio, whereas the ratio of the four components in the Zimbabwean pheromone blend was 1:0.25:0.03:0.1. Males from each population respond preferentially to their own population's blend in field and flight tunnel tests (ref. 11 and W.W., C. B. Cottrell, B.S.H., and C.L., unpublished work). These two populations therefore provide us with a good model for gaining further insight into how information concerning different inter- and intra-populational blends is processed and integrated in the AL. In the present comparative study, we examined deutocerebral neurons of the two turnip moth populations, stimulating them with four single pheromone components, one behavioral antagonist [*(Z)*-5-decenol (Z5-10:OH)], and mixtures with various combinations of the four components.

MATERIALS AND METHODS

Preparation. The Zimbabwean turnip moth culture was established in our laboratory from pupae shipped from Zimbabwe. The so-called Swedish turnip moths were collected from southern Sweden and Denmark and maintained in the laboratory for more than 10 years. The culture has been rejuvenated on several occasions by addition of field collected insects. A male moth was mounted head up in a plastic pipette (Finnpipette) tip. The cuticle, tracheal sacs, and muscles were carefully removed to expose the brain. The AL was manually desheathed to facilitate microelectrode penetration. A flow system perfused the head cavity with saline (6).

Intracellular Recording and Stimulation. Intracellular recordings were performed according to standard methods (12). A glass microelectrode filled with either 4% Lucifer yellow CH solution or 2 M KCl was used as the recording electrode. Olfactory stimuli used in the experiments were five single compounds at 1 ng, two Swedish blends, and two Zimbabwean blends (Tables 1 and 2). For the Swedish males, blends with different combinations of two or three pheromone components in the ratio found in the Swedish female extracts were also included as stimuli (Table 2). The amount of each component in the blend was assigned according to their amount relative to 1 ng of Z5-10:OAc in the two populational blends, respectively, resembling the natural emission of a female (W.W., C. B. Cottrell, B.S.H., and C.L., unpublished work). All the stimuli were checked by gas chromatography to ensure purity, which was >99% with respect to geometrical isomers, and ratio of components. Each odorant was applied to a piece of filter paper that was then inserted into a Pasteur pipette. A 0.5-sec air pulse was sent through the cartridge containing either clean air or an odorant by means of a stimulation device (Syntech, Hilversum, The Netherlands) (13). The odor stimuli were chosen randomly, interspersed by 10–30 sec. In a first set of experiments, the Zimbabwean four-component blend was not included. The physiological data were stored on a Vetter video recorder and visualized on a Tektronix digital oscilloscope and on a Gould (Cleveland)

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Abbreviations: AL, antennal lobe; MGC, macroglomerular complex; ILPR, inferior lateral protocerebrum; LAL, lateral accessory lobe; PN, projection neuron; LN, local interneuron; Z5-10:OAc, *(Z)*-5-decenyl acetate; Z5-12:OAc, *(Z)*-5-dodecenyl acetate; Z7-12:OAc, *(Z)*-7-dodecenyl acetate; Z9-14:OAc, *(Z)*-9-tetradecenyl acetate; Z5-10:OH, *(Z)*-5-decenol.

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Table 1. Physiological responses of AL interneurons in Zimbabwean male moths

No.	1	2	3	4	5	1+3 +4(Z)	1+2+ 3+4(Z)	1+2+ 4(S)	1+2+ 3+4(S)	Cell type*
						Generalist neurons				
1	+	+	+	+	+	+	nt	+	+	
2	++	+	+	+	+	+	++	++	+	na
3	++	++	+	+	+	+	+	+	+	
4	++	+	+	+	+	+	+	+	+	
5	++	+	+	+	+	+	+	++	++	na
6	+	+	+	+	++	+	++	++	++	
7	++	+	+	+	+	++	++	++	++	na
8	++	+	+	+	+	+	+	+	+	
9	++	+	+	+	++	++	++	+	+	na
10	++	+	+	+	+	+	++	+	+	PN(a)
11	++	+	+	+	+	+	+	+	+	na
12	+	+	+	+	0	+	+	+	+	
						Component-specific neurons				
13	0	+	+	+	+	+	nt	+	+	LN
14	++	0	+	+	+	++	nt	++	+	na
15	+	0	+	+	+	+	++	++	+	na
16	++	0	+	+	+	+	++	+	+	
17	+	+	0	+	0	+	+	+	+	PN(a)
18	+	+	0	+	+	+	nt	+	+	na
19	+	+	0	+	0	++	nt	++	++	PN(a)
20	+	+	0	+	0	+	nt	+	+	
21	++	+	0	+	+	+	nt	++	+	
22	+	+	+	0	+	+	nt	+	+	na
23	++	+	+	0	+	+	++	++	++	PN(a+b)
24	++	+	+	0	+	++	++	++	++	na
25	0	0	+	+	+	+	nt	+	+	na
26	0	0	+	+	0	0	+	0	0	na
27	0	+	0	+	++	0	nt	+	++	
28	+	0	0	+	++	++	nt	++	++	PN(b+c)
29	+	0	0	+	0	0	nt	+	+	PN(a)
30	+	0	0	0	0	+	+	+	+	na
31	++	0	0	0	+	+	+	++	+	PN(b)
32	+	0	0	0	+	0	0	0	0	
33	+	0	0	0	+	+	nt	+	+	LN
34	0	+	0	0	+	0	nt	0	+	
35	0	++	0	0	+	0	nt	0	+	PN(d)
36	0	0	+	0	0	+	nt	+	+	na
37	0	0	+	0	+	+	nt	+	nt	
38	0	0	+	0	+	0	0	++	++	
39	0	0	0	+	+	0	+	0	0	
40	0	0	0	+	+	+	+	+	+	
41	0	0	0	+	+	+	nt	+	+	
42	0	0	0	+	+	0	+	+	0	
43	0	0	0	0	+	0	nt	0	0	na
						Blend-specialist neurons				
44	0	0	0	0	0	0	nt	0	+	na
45	0	0	0	0	0	+	+	+	+	LN
46	0	0	0	0	+	+	nt	0	0	LN
47	0	0	0	0	0	+	++	+	+	PN(a+b+c+d)

The physiological responses are quantified as + (≤ 9 net spikes), ++(= 10–19 net spikes), and +++ (≥ 20 net spikes). Net spikes = (spikes during 300 msec after stimulation) − (spikes occuring 300 msec before stimulation) − blank. 1, Z5-10:OAc; 2, Z5-12:OAc; 3, Z7-12:OAc; 4, Z9-14:OAc; 5, Z5-10:OH. 1+3+4(*Z*) is a blend of components 1, 3, and 4 in the Zimbabwean ratio, 1+3+4(*S*) is a blend of components 1, 3, and 4 in the Swedish ratio. nt = not tested. na = not attempted to fill; LN, local interneuron; PN, projection neuron.
*Arborization.

electrostatic printer. Spikes were counted manually from the storage oscilloscope. Net-spikes (see Tables 1 and 2) were counted within a 300-msec period after the stimulus onset, as inhibition after the initial response was very common, and

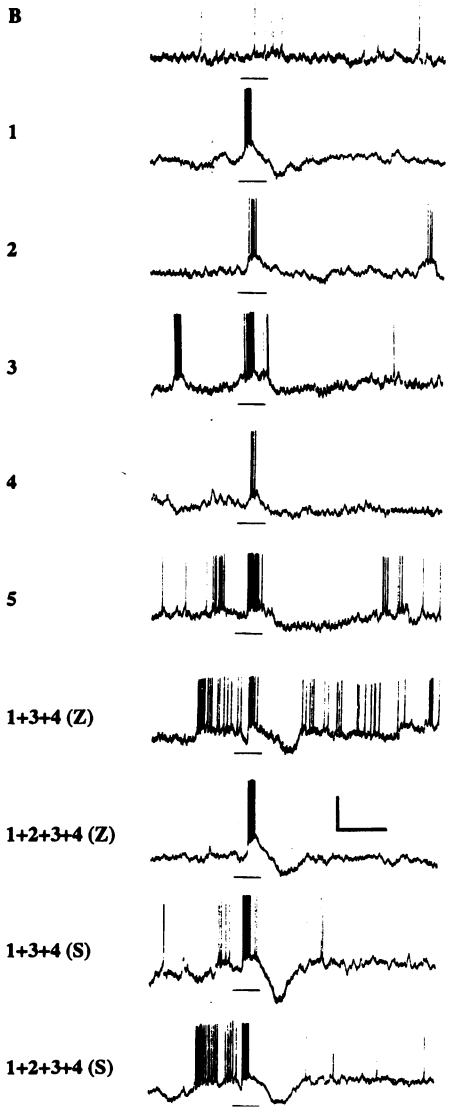

FIG. 1. Physiological responses of a generalist neuron (no. 8) of a Zimbabwean male moth. Note the strong hyperpolarization after the end of stimulation for all stimuli containing Z5-10:OAc (compound 1). The bar beneath the registration indicates onset and duration of the stimulation. B, blank. (Horizontal bar = 1 sec; vertical bar = 10 mV)

obscured excitatory responses over the entire stimulation period. After recording the responses, Lucifer yellow was injected iontophoretically into the neuron. The brain was then dissected out and fixed. After dehydration, the brain was embedded in Spurr's resin and sectioned at 10 μm. Each section was photographed for final reconstruction of the neuron.

RESULTS

The morphology of the AL in Zimbabwean male turnip moths was found to be the same as the AL in Swedish males. The macroglomerular complex (MGC) can be divided into four glomeruli (14). The largest glomerulus *a* is situated just at the entrance of the antennal nerve. Three smaller glomeruli below *a* are called *b* (medio-dorsal), *c* (medial), and *d* (latero-ventral). Both projection neurons (PN) and local interneurons (LN) were found. The LNs all had a lateral cell body and showed multiglomerular arborizations in the entire AL. All the ipsilateral PNs found in this study had uni- or multiglomerular dendritic arborizations within the MGC, sent their axons through the inner antennocerebral tract, and had arborizations in the calyces of the mushroom body and in the inferior lateral

Table 2. Physiological responses of AL neurons in Swedish male moths

No.	1	2	3	4	5	1+3 +4(Z)	1+2+ 3+4(Z)	1+3 +4(S)	1+2+ 3+4(S)	1+3	3+4	1+2	1+4	2+3	2+4	2+3+4	Cell type (arborization)
Generalist neurons																	
1	+	++	++	+	+	+	+	++	++	+++	+++	++	++	++	++	+++	na
2	+	+	+	+	+	+	+	++	++	++	+	++	++	+	++	+	na
3	+	+	+	+	+	+	nt	++	++	+	++	+	+	+	0	+	
4	++	+	+	+	0	+	+	+	+	++	+	+	+	+	++	+	
5	+	+	+	+	0	+	nt	+	+	+	+	nt	nt	nt	nt	nt	PN(b)
6	++	+	+	+	0	+	+	++	++	+	++	++	+	+	++	+	na
7	+++	++	++	+	+	++	nt	+++	++	+++	+	+++	+++	0	+	0	
8	+	+	+	+	+	+	nt	+	+	+	+	+	0	+	+	+	
9	++	+	+	+	+	+	+	++	+++	++	++	++	+++	+	+++	+	na
10	++	+	+	+	+	+	+	+	+	++	+	+	++	+	++	0	PN(a)
Component-specific neurons																	
11	0	+	+	+	++	0	+	+++	+++	+++	++	0	0	+++	+	++	
12	0	+	++	++	++	++	+	+	+	++	++	++	++	++	++	++	
13	+	0	+	+	0	+	nt	+	+	nt	nt	nt	nt	nt	nt	nt	
14	+	+	+	0	+	++	++	++	++	++	nt	++	++	+	+	+	PN(b)
15	+	+	+	0	0	+	0	+	+	+	+	+	+	+	0	+	PN(a)
16	+	+	+	0	+	++	++	+	+	+	++	++	+	+	+	+	
17	+	+	+	0	+	++	+	+	++	+	+	+	++	+	++	+	
18	0	+	+	0	0	nt	+	+	+	+++	++	nt	nt	nt	nt	nt	LN
19	0	+	+	nt	0	+	nt	+	+	+	+	nt	+	nt	0	0	
20	0	0	++	+	++	0	0	++	++	++	++	+	+	+++	+	++	PN(b+c)
21	0	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	
22	++	+	0	0	+	+	+	+	+	++	nt	+	+	+	++	+	PN(a+d)
23	+	+	0	0	0	0	nt	+	+	nt	+	nt	nt	nt	nt	nt	PN(c+d)
24	++	+	0	0	0	+	nt	+	++	nt	nt	nt	nt	nt	nt	nt	LN
25	+	+	0	0	0	+	0	0	++	+	++	+++	0	++	++	+++	na
26	++	0	0	0	+	0	0	+	++	+++	0	++	+	0	+	0	na
27	++	0	0	0	0	0	0	+	+	++	+	+	+	0	+	+	na
28	+	0	0	0	+	+	0	+	+	+	0	0	++	0	+	0	
29	+	0	0	0	+	0	+	0	+	++	0	+	+	0	+	+	na
30	0	+++	0	0	0	0	0	+	++	0	0	++	++	+	++	+	
31	0	++	0	0	0	0	0	0	+	+	++	0	+	0	+++	+	na
Blend-specialist neurons																	
32	0	0	0	0	0	0	0	+	++	+	0	+	+++	+	0	+	na
33	0	0	0	0	0	0	nt	0	+	nt	nt	nt	nt	nt	nt	nt	
34	0	0	0	0	0	0	nt	++	nt	0	+	++	0	+	++	++	
35	0	0	0	0	0	++	nt	0	0	+	++	+	++	0	0	0	
36	0	0	0	0	0	0	+	0	+	0	0	0	0	0	0	0	na

Abbreviations, responses, and labels as in Table 1.

protocerebrum (ILPR). Two bilateral PNs were found that arborized in the lateral accessory lobes (LAL) and the ILPR on both sides (see Fig. 2).

Forty-seven AL interneurons in 31 males of the Zimbabwean population, and 36 interneurons in 28 males of the Swedish population that responded to at least 1 of the stimuli tested, were recorded from. Approximately 30% of the neurons tested did not respond to any of the pheromone stimuli in both populations. According to the responses to the stimuli, three physiological types of AL neurons were distinguished in both populations according to their specificity, namely generalist neurons, component-specific neurons, and blend-specialist neurons.

Type 1 Generalist Neurons, Responding to All the Single Pheromone Components and Most Blends Tested. In the Zimbabwean male ALs, 12 neurons (Table 1, nos. 1–12) responded to all the stimuli tested (e.g., Fig. 1), except one neuron (no. 12) that did not respond to Z5-10:OH. In most cases, the response to Z5-10:OAc was more pronounced than to the other single components.

In the Swedish male ALs, 10 neurons responded to all the single pheromone components and most of the blends (Table 2, nos. 1–10). Six of 10 of these type 1 neurons showed stronger responses to the Swedish blends than to the Zimbabwean blends.

One generalist PN in Zimbabwean and two generalist PNs in Swedish male ALs revealed arborizations in one MGC glomerulus each. Neuron no. 10 in a Zimbabwean AL was identified as an incompletely stained PN, which had a lateral cell body and arborizations in the MGC glomerulus *a*. Neuron no. 5 in a Swedish AL was identified as a PN, with a medial cell body, arborizations in the MGC glomerulus *b*, an axon projecting through the inner antennocerebral tract, and displayed arborizations in the calyces of the mushroom body and the lateral horn of the protocerebrum. Neuron no. 10 in a Swedish AL had a lateral cell body and arborizations in the MGC glomerulus *a*.

Type 2 Component-Specific Neurons, Responding Selectively to Single Compounds and to the Blends Tested. Twelve neurons in the Zimbabwean males responded to three of the four single acetates and all blends tested (Table 1, nos. 13–24). Five neurons (nos. 25–29) responded to two single acetates and some of the blends tested. Fourteen neurons responded only to a single compound and to some of the blends (nos. 30–43). Neuron no. 43 responded only to Z5-10:OH. Neurons no. 26 and no. 39 responded to the Zimbabwean blend, but not to the

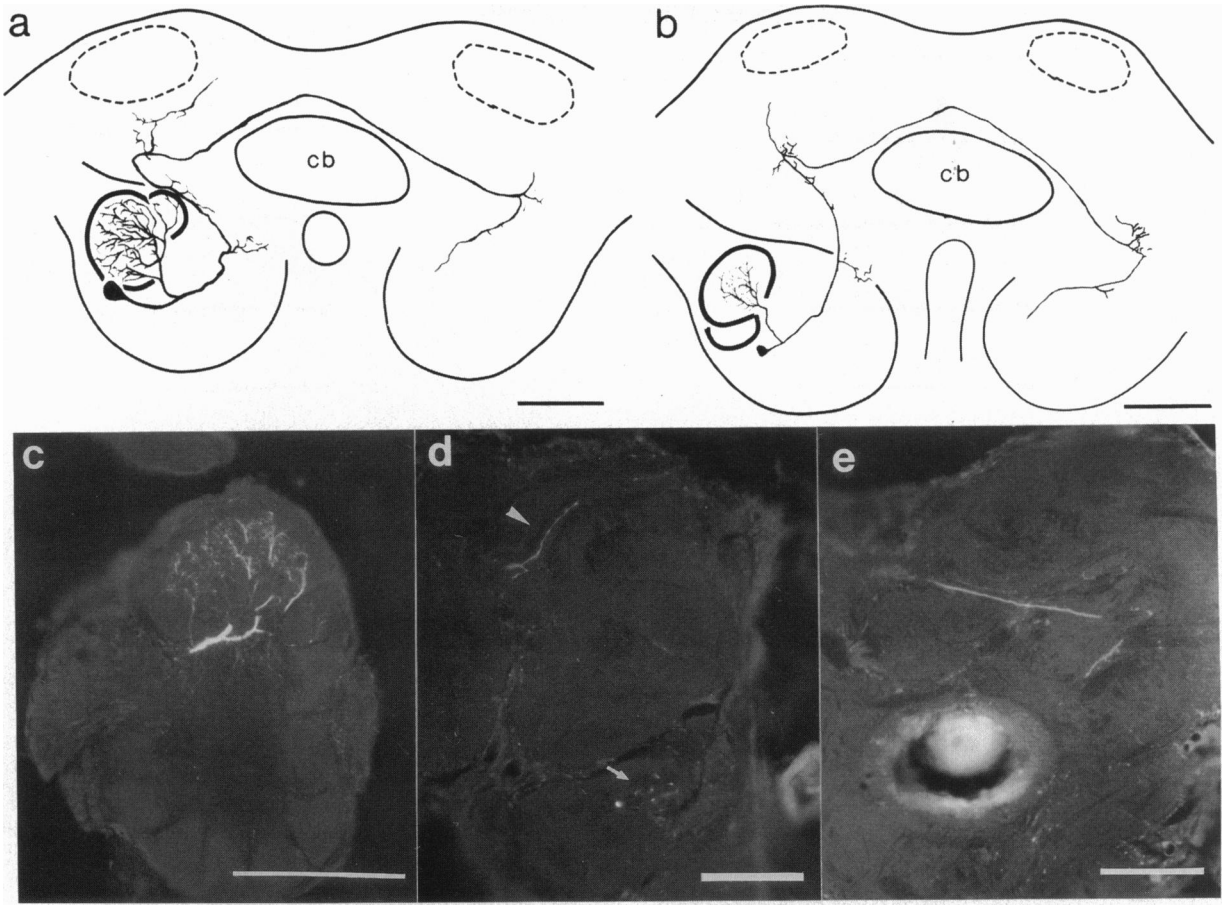

FIG. 2. Reconstructions of two bilateral PNs (no. 23, *a* and no. 17, *b*), showing axons projecting through the outer antennocerebral tract to the ipsilateral and contralateral protocerebrum, with branches in the LAL and the ILPR on both sides. Both are sectioned in a more or less frontal orientation. (*a*) Neuron no. 23 arborizes in MGC glomeruli *a* and *b*. (*b*) Neuron no. 17 arborizes only in MGC glomerulus *a*. (*c–e*) Micrographs of 10-μm sections through preparation no. 23. (*c*) Arborizations within the MGC. (*d*) Arborizations in the ipsilateral LAL (arrow) and a branch leading toward the protocerebrum (arrowhead). (*e*) Contralateral arborizations. (Scale bars = 100 μm.)

Swedish blend, whereas neuron no. 38 showed responses only to the Swedish blend.

Morphologically, two LNs, five ipsilateral PNs, and two bilateral PNs were identified among physiological type 2 neurons. Both LNs (nos. 13 and 33) had a lateral cell body and arborizations all over the AL. The PNs arborized in single or pairs of MGC glomeruli, irrespective of their response characteristics. PNs no. 29 and no. 35 had a lateral cell body, and arborizations in the MGC glomeruli *a* and *d*, respectively. PNs no. 19, no. 28 and no. 31 all had a medial cell body, and displayed arborizations in MGC glomeruli *a*, *b*+*c*, and *b*, respectively. Those PNs sent their axon through the inner antennocerebral tract, arborized in the calyces of the mushroom body and in the ILPR. Neurons no. 17 and no. 23 were identified as bilateral projection neurons. Both neurons had a lateral cell body, arborized in MGC glomeruli *a* and *a*+*b*, respectively. Both sent the axon with branches through the outer antennocerebral tract to the ipsilateral and contralateral protocerebrum of the brain, and arborized in the LAL and ILPR on both sides of the brain (Fig. 2).

Seven neurons in the Swedish males (Table 2, nos. 11–17) responded to three of the four single acetates and some of the blends. Eight neurons (nos. 18–25) responded to two single acetates and some of the blends. Six neurons in the Swedish males (nos. 26–31) responded only to one single compound and some of the blends (e.g., no. 26 in Fig. 3). Specific responses to single pheromone components mostly occurred to Z5-10:OAc. Neurons (nos. 26–31) were found to respond to some partial blends composed of two or three components, but not to these single components. Six neurons (Table 2, nos. 20, 21, 26, 27, 30, and 31) responded only to the Swedish blends, but not to the Zimbabwean blends. Morphologically, neurons no. 18 and no. 24 were identified as LNs. Five neurons (nos. 14, 15, 20, 22, and 23) were identified as PNs, with MGC arborizations in *b*, *a*, *b*+*c*, *a*+*d*, and *c*+*d*, respectively. Again no correlation between the response characteristics and the MGC arborizations was found.

Type 3 Blend-Specialist Neurons only Responding to Certain Blends. In the Zimbabwean male moths, four neurons (Table 1, nos. 44–47) responded only to the blends, but did not respond to any of the single pheromone components. Morphologically, two LNs and one incompletely stained PN (no. 47) were found among neurons belonging to this physiological type. PN (no. 47) had a medial cell body and arborized in the whole MGC (*a*+*b*+*c*+*d*).

In the Swedish males, five neurons (Table 2, nos. 32–36) responded only to the blends, not to any of the single components (e.g., no. 34 in Fig. 4).

DISCUSSION

In this study, the responses of AL interneurons to single pheromone components and to blends of different composition was tested. We asked if these interneurons can discriminate the blends produced by the two populations.

At the peripheral olfactory level, receptor neurons responding exclusively to the three major pheromone components Z5-10:OAc, Z7-12:OAc and Z9-14:OAc, and receptor neurons

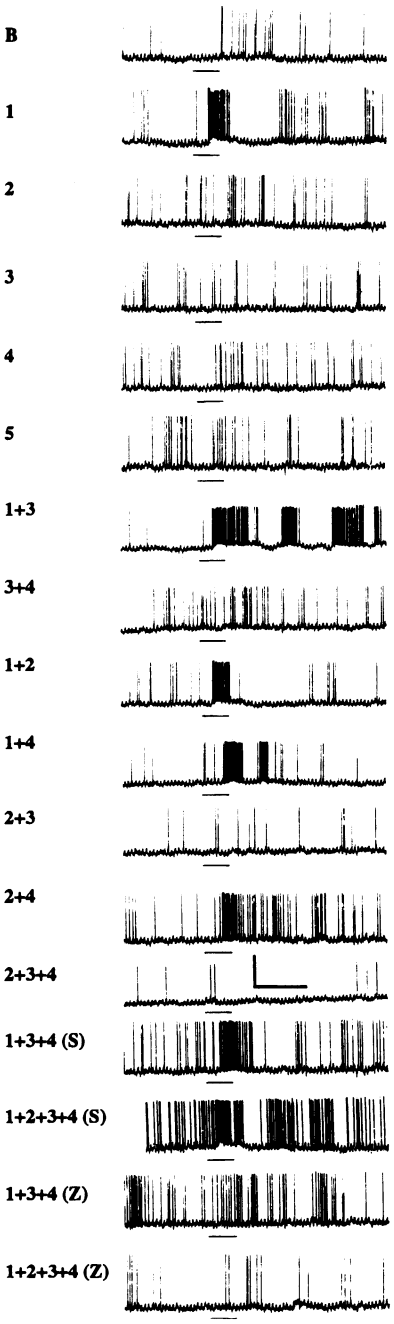

FIG. 3. Physiological responses of a Z5-10:OAc-specific neuron (no. 26) of a Swedish male moth. The neuron responds to all blends containing Z5-10:OAc (compound 1), but also to a mixture of two other compounds (2 and 4), which are not detected when presented alone. The bar beneath the registration indicates onset and duration of the stimulation. (Horizontal bar = 1 sec; vertical bar = 10 mV.)

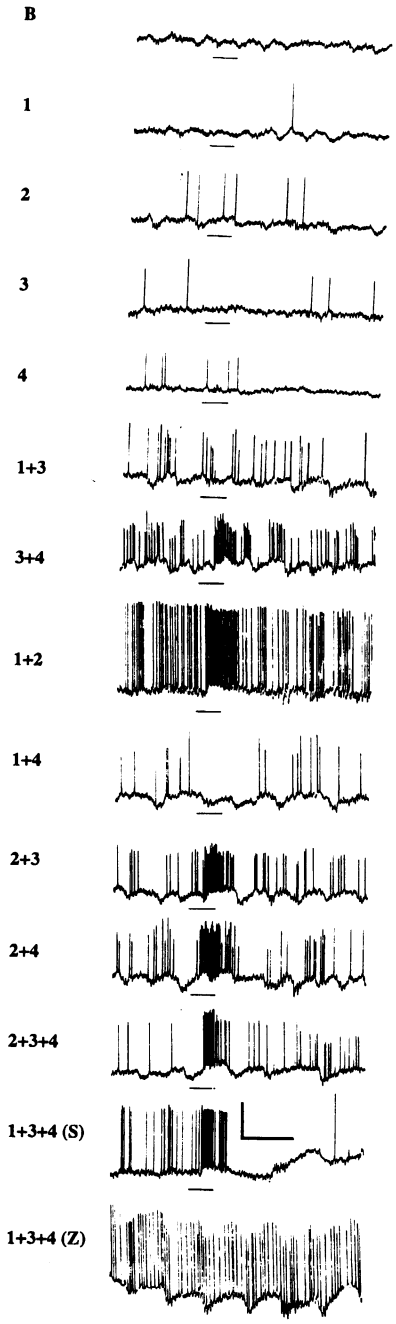

FIG. 4. Physiological responses of a blend specialist neuron (no. 34) in a Swedish male AL. The neuron responds to six of the nine tested blends, but not to any single component. The differences in spike amplitude and spontaneous activity are due to movements of the animal during the long recording time, causing differences in contact quality. The bar beneath the registration indicates onset and duration of the stimulation. (Horizontal bar = 1 sec; vertical bar = 10 mV.)

responding to Z5-12:OAc, in addition to Z5-10:OH were found in both populations (ref. 15 and W.W., C. B. Cottrell, B.S.H., and C.L., unpublished work). The more pronounced difference between the two populations, at the single sensillum level, was that in the Zimbabwean *A. segetum*, the proportion of Z5-10:OAc receptors was significantly higher than in the Swedish population (ref. 10, and W.W., C. B. Cottrell, B.S.H., and C.L., unpublished work). In this investigation, we could identify several physiological and morphological types of interneurons. About 27% of the interneurons in males of both populations acted as generalists, being activated by all of the single pheromone components and most of the blends tested. The remaining interneurons, component-specialists and blend-specialists, were selectivity activated by the different stimuli. Although some of the specific responses could be assigned to dose effects, it had been shown previously that neuron types with different degrees of specificity are present, even if different stimulus quantities are tested (13). The presence of generalist, component-specialist, and blend-specialist neurons shows that both labelled-line and integrative processing regarding pheromone information occur in the AL.

In the Swedish male turnip moth, we found several neurons responding selectively to the Swedish blend. One neuron (no. 38) in the Zimbabwean male AL responded to the Swedish blend, but not to their own blend. A higher response to the

Swedish than to the Zimbabwean blend could be ascribed to the dose effect, since the amount of components in the Swedish blend is higher than that in the Zimbabwean blend. However, we also found neurons that responded preferentially to the Zimbabwean blend, i.e., a higher response to a lower dose, a phenomenon that has to be interpreted as a true blend effect. These blend-specific neurons are interpreted as a neuronal correlate of the behavior of the male moths. Some neurons in both populations also displayed a capability to distinguish a partial blend lacking the recently identified component Z5-12:OAc from a complete blend, including this substance. Such a sensitivity is quite remarkable, as the Z5-12:OAc occurs only at a very low concentration in the blend. Some neurons in the Swedish male moths could also detect some partial blends composed of two or three components but not to these single components. Many of the neurons distinguishing the Swedish and Zimbabwean blends were component-specific neurons, i.e., they did not display the characteristics we have used to term them blend-specific, responding to the blends but not to any of the single components. They did, however, display a blend-specificity in the sense that they could distinguish between the populational blends.

Different levels of specificity were observed among the blend-detecting neurons encountered in this study, based on whether they could differentiate between the different populational blends or between the complete and incomplete blends. Obviously, AL interneurons in male moths not only possess the capability to integrate information concerning the presence of different pheromone components, but they are also able to weigh the input from different receptor neuron types, and make quantitative integrations.

Morphologically, the data collected in the present study support the findings by Hansson *et al.* (13), where it was shown that no general correspondence between physiological response and dendritic arborization pattern within the MGC could be found. However, at the receptor neuron input level, it has been shown that the glomeruli making up the MGC have a specific functional identity, where each physiologically distinct type of receptor neuron projected to a separate glomerulus of the MGC (16).The lack of overlapping arborizations of receptor neurons and PNs responding to the same compounds shows the important role of local interneurons as mediators between the two neuron types (17). The single blend-specialist neuron stained in this study displayed an arborization in the whole MGC area. A similar arborization pattern was observed in one blend-specialist neuron identified by Hansson *et al.* (13). A possible interpretation of this total MGC arborization pattern is that blend-specific neurons need to receive direct inputs from all physiological types of receptor neurons to perform their integrative task.

Pheromone-activated, protocerebral neurons with bilateral arborization patterns in the LAL have been shown in the sphinx moth, *Manduca sexta* (18). Descending interneurons, carrying information from the brain to the thoracic ganglion, have also been shown to dendritically innervate the LAL area (19, 20). In this study, bilaterally projecting interneurons with dendritic arborizations in the AL and axonal branches in the LAL and the ILPR were found. These neurons can provide a direct, bilateral pathway from the AL to the output area of pheromone-derived information in the moth brain. Bypassing protocerebral centers, these neurons could be involved in a reflexive olfactory pathway.

AL interneurons perform an important task in integrating olfactory information supplied by antennal receptor neurons. Some interneurons are able to detect the presence of the full pheromone blend and are also able to distinguish minute changes in the blend. However, much of the pheromone blend specificity in the male moth brain resides in higher order neurons in the protocerebrum, where inputs from other sensory modalities can be integrated to mold the final, decisive output, inducing or not inducing a behavioral response in the animal. Future studies must further elucidate male moth blend specificity in those neural levels above the AL.

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