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Antennal neurones specific for redundant pheromone components in normal and mutant *Trichoplusia ni* males

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Abstract. Recordings were made from the pheromone-sensitive receptor cells within antennal hairs of normal and mutant male cabbage loopers, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), using a cut-sensillum technique. From sampling 136 sensilla on normal males and 123 on mutant males, cells excited by pairs of behaviourally redundant minor pheromone components were discovered: Z9–14:Ac was found to be replaceable with 12:Ac and 11–12:Ac was found to be replaceable with Z5–12:Ac. These cells were not found during previous neurophysiological investigations, but explain most of the associations between mutually replaceable (redundant) pheromone components which had been demonstrated previously to be behaviourally redundant in wind tunnel studies. Our results indicate that the mutant gene in *T. ni* that affects pheromone production does not affect pheromone receptors in males. Using both AC- and DC-coupled recordings from receptor cells, we found that a single minor component could apparently hyperpolarize one cell while depolarizing another cell within the same sensillum, suggesting that noise reduction and other complex signal processing by receptor cells may contribute to odour processing in the macroglomerulus of the antennal lobe.

Key words. Lepidoptera, *Trichoplusia ni*, cabbage looper, electrophysiology, antenna, receptor cell, pheromone, redundancy.

Introduction

Within the Lepidoptera, successful mate location usually involves the use of a female-emitted sex pheromone comprising multiple components (Roelofs, 1980). A six-component blend identified as the sex pheromone of the cabbage looper moth, *Trichoplusia ni* (Hübner), consists of (Z)-7-dodecenyl acetate (Z7–12:Ac), the major component (Berger, 1966), dodecyl acetate (12:Ac) (Bjostad *et al.*, 1980), (Z)-5-dodecenyl acetate (Z5–12:Ac), 11-dodecenyl acetate (11–12:Ac), (Z)-7-tetradecenyl acetate (Z7–14:Ac), and (Z)-9-tetradecenyl acetate (Z9–14:Ac) (Bjostad *et al.*, 1984). These components are emitted by the female in an average blend ratio of 100:4.8:7.2:2.9:0.4:0.3, respectively (Bjostad *et al.*, 1984). Haynes & Hunt (1990a), however, discovered a single gene mutation in females that results in a dramatically different emission of the sex pheromone components. The most obvious differences were the reduction in the emission rate of Z7–12:Ac, the near absence of Z5–12:Ac, and a *c.* 20-fold increase in Z9–14:Ac. These changes in the mutant female emissions were shown to have a dramatic effect on the communication system, with mutant females less effective than normal females at attracting male *T. ni* in the field (Haynes & Hunt, 1990a).

The detection of a receptive conspecific female by a male moth depends ultimately on a coupling between sex pheromone components and olfactory receptor cells located within the trichodeal hairs on the male's antennae. Several studies have indicated that there are two main classes of sensilla on the antennae of male *T. ni*, and that the receptor cells within these sensilla differ in their sensitivity and specificity to various pheromone components, and in their spontaneous rate of background firing (O'Connell *et al.*, 1983; Grant & O'Connell, 1986; Grant *et al.*, 1988). In one class of sensilla, referred to as high-spontaneous-activity sensilla, each hair contains a large-spiking cell (A cell) that responds to low doses of Z7–12:Ac, and a smaller-spiking cell (B cell) that responds to the cor-

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responding alcohol, (Z)-7-dodecenol (Z7−12:OH). In field (Tumlinson *et al.*, 1972) and flight tunnel studies (McLaughlin *et al.*, 1974), Z7−12:OH has been shown to arrest upwind flight when present in the blend. In addition to being an antagonist, Z7−12:OH is apparently not present in most gland extracts (Bjostad *et al.*, 1984), and its presence in volatile emissions is considered an artefact; therefore, its consideration as a pheromone component is in doubt (Haynes & Hunt, 1990b). In the second class of sensilla, referred to as low-spontaneous-activity sensilla, each hair contains an A cell that does not respond to any components or to Z7−12:OH, and a B cell that responds to high doses of Z7−12:Ac (O'Connell *et al.*, 1983; Grant & O'Connell, 1986), and lower doses of Z7−14:Ac (Grant *et al.*, 1988). A third class of sensilla was located recently by Mayer & Mankin (1990), and these sensilla contain an A cell sensitive to extremely low doses of Z9−14:Ac.

Linn *et al.* (1984) demonstrated the behavioural importance of the six pheromone components using flight tunnel bioassays. By conducting a series of subtraction assays, they found that several four-component blends elicited levels of upwind flight and source contact similar to that observed with the complete six-component blend. In particular, they showed that specific minor components could substitute for one another in these incomplete blends, and they characterized these component substitutions as a form of redundancy in the chemical communication channel, perhaps enhancing response specificity and signal recognition in males. They further suggested that these minor component substitutions could reflect interactions at the level of the peripheral receptor system. However, the existing neurophysiological investigations have failed to locate sensilla with receptor cells specific for three minor components, 12:Ac, 11−12:Ac, and Z5−12:Ac, that are important in understanding redundancy in the chemical emission of the cabbage looper.

We report here that by conducting an extensive neurophysiological survey of the sensilla on male *T. ni* antennae, we have located sensilla with receptor cells specific for the behaviourally redundant components Z9−14:Ac and 12:Ac, and others specific for these two components and two additional components, 11−12:Ac and Z5−12:Ac. These cells had not been located in previous studies, but explain most of the behavioural associations between redundant pheromone components observed by Linn *et al.* (1984). We have determined also that males carrying the mutant gene for pheromone production (Haynes & Hunt, 1990a), referred to as mutant males, do not differ significantly from normal males in the proportion of sensilla with cells specific for Z9−14:Ac or for Z9−14:Ac and 12:Ac.

Materials and Methods

Insects. The normal colony originated from insects collected near Riverside, California. The mutant colony was started from a few individuals within the normal colony that expressed an unusual pheromone blend. Selective breeding over successive generations led to a mutant colony in which all females expressed the abnormal pheromone blend, and in which all males carried a gene responsible for this condition. Both colonies were maintained identically following procedures modified from those of Shorey & Hale (1965). Normal and mutant strain males were shipped as pupae from the University of Kentucky to Riverside, California, and stored in an environmental chamber with a LD 14:10 h photoperiod, and a temperature of 24 ± 2°C until adult eclosion 1−3 days after shipment. Antennae were used for electrophysiological recordings when adults were between 1 and 5 days old.

Chemicals. Each of the six acetates and the alcohol was purchased from the pheromone library at the Institute for Pesticide Research, Wageningen, The Netherlands. Serial dilutions of each chemical were prepared in HPLC grade hexane and checked on capillary GLC to ensure purity (>99%). Solutions were stored in 2 dram glass vials at −5°C.

Electrophysiological recordings. To record from the olfactory receptor cells within individual antennal sensilla, we used a cut-sensillum technique developed by Kaissling (Kaissling, 1974; Van der Pers & Den Otter, 1978). Briefly, the right antenna of a moth was excised from the head, and the antennal base was placed in a saline-filled pipette ground electrode. Using a micromanipulator, the antenna was manoeuvred until a single sensillum trichodeum rested on the sharpened blade of a stationary, vertically-positioned glass knife, with its tip hanging over the edge. The sensillar tip was cut off using a mobile glass knife placed in a Leitz joy-stick type micromanipulator that permitted movement in three dimensions, and the cut end was contacted with a saline-filled Ag/AgCl pipette recording electrode. Sensilla were chosen at random from all accessible areas of the antenna, and preparations generally lasted about 2 h, which allowed for sampling from many sensilla on a single antenna.

The antenna was continuously bathed in a stream of purified, humidified air (10 ml/s) that passed through a glass tube (8 mm i.d.) whose outlet was positioned 2 cm from the antenna. Prior to exposure to any of the test compounds, the spontaneous activities of the receptor cells within each sensillum were monitored over a 5 s period. For each of the compounds tested, 10 μg of a diluted solution was pipetted onto a filter paper strip held in a Pasteur pipette glass cartridge. Receptor cells were exposed to 20 ms puffs (Roelofs & Comeau, 1969) of each of the six pheromone components and to Z7−12:OH in random order by manually injecting a 2 ml puff into the airstream through a hole in the glass tube 15 cm from the outlet. Between 10 and 30 s elapsed between puffs.

The electrical responses of the receptor neurones were amplified in both AC and DC, and monitored visually on a Gould 1604 digital storage oscilloscope. They were then recorded onto video tapes for later data analysis. When action potentials (spikes) were elicited by a compound, their frequency was determined for 0.2 s post-stimulation, along with the spike amplitude of the responding receptor cell. Spikes were not visible in DC-coupled recordings due to high frequency filtering. Unless otherwise stated,

monophasic amplitude values (mV) were obtained by measuring the height of a spike from the middle of the baseline upward to its peak.

Results

Sensillar types on normal male antennae

Single-cell recordings were obtained from 136 sensilla on thirty-two antennae of normal male *T.ni* (Fig. 1a). Within each sensillum, two receptor cells often could be distinguished on the basis of spike amplitude. Transmission electron micrographs thus far have shown two or at most three dendrites within a sensillum of male *T.ni* (R. Y. Zacharuk, personal communication), although in sensilla with three dendrites, only two of the neurones are excited by pheromone components, with the third neurone unresponsive to any component or other tested compounds (Grant & O'Connell, 1986). This third dendrite may be from a mechanoreceptive cell, as was found for the redbanded leafroller moth, *Argyrotaenia velutinana* (O'Connell, 1975). To be consistent with previous studies (O'Connell *et al.*, 1983; Grant & O'Connell, 1986), we have designated the larger-spiking cell as A, and the smaller-spiking cell as B. There was no obvious relationship between the background activity of the receptor cells and their specificity for pheromone components (Fig. 1a). In most of the sensilla, both cells fired at variable rates prior to exposure to pheromone components.

The majority of sensilla sampled contained an A cell that responded to Z7−12:Ac and a B cell that responded to Z7−12:OH (Fig. 1a, sensilla 1−27), or else an A cell that responded to Z7−12:Ac and Z7−14:Ac, plus a B cell that responded to Z7−12:OH (Fig. 1a, sensilla 28−75). Within these latter sensilla, the mean (±SE) spike frequency in the first 0.2 s post-stimulation was 156.5 ± 8.6 spikes/s for Z7−12:Ac; 65.2 ± 4.6 spikes/s for Z7−14:Ac; and 143.9 ± 6.4 spikes/s for Z7−12:OH (Fig. 2a). The frequency of the A cell spikes in response to stimulation by Z7−14:Ac was nearly always less than that to Z7−12:Ac. The mean (±SE) spike amplitude of the A cell to Z7−12:Ac and Z7−14:Ac was 5.9 ± 0.3 mV and 6.1 ± 0.3 mV, respectively, and the mean (±SE) spike amplitude of the B cell to Z7−12:OH was 3.7 ± 0.2 mV. These sensilla

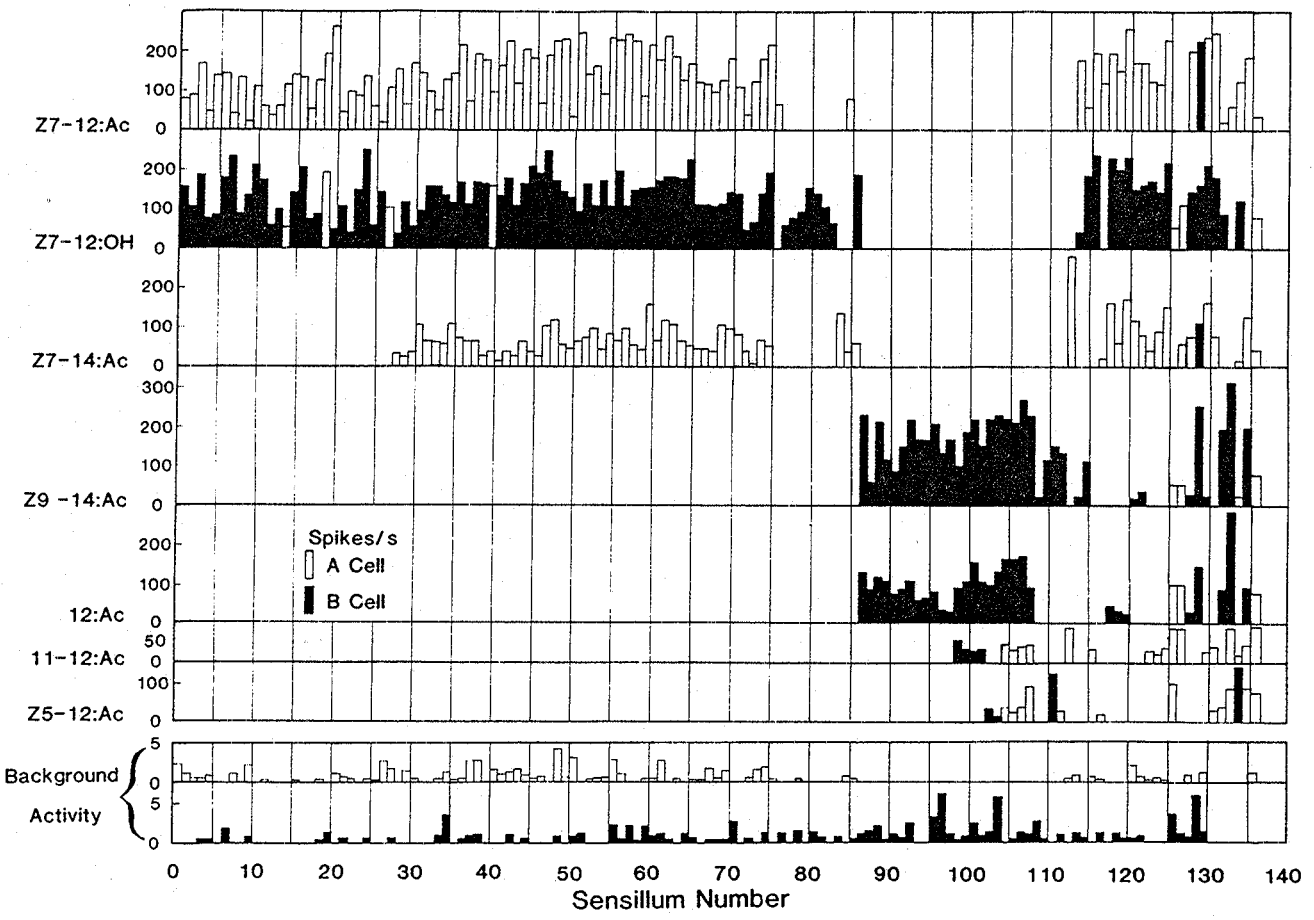

Fig. 1(a). Response spectra of antennal neurones in sensilla of normal male *T.ni* to six pheromone components and to Z7−12:OH. Most sensilla contain two receptor cells, a large-spiking A cell (open bars), and a smaller-spiking B cell (solid bars). The background activity of both cells within each sensillum was determined prior to exposure to test compounds.

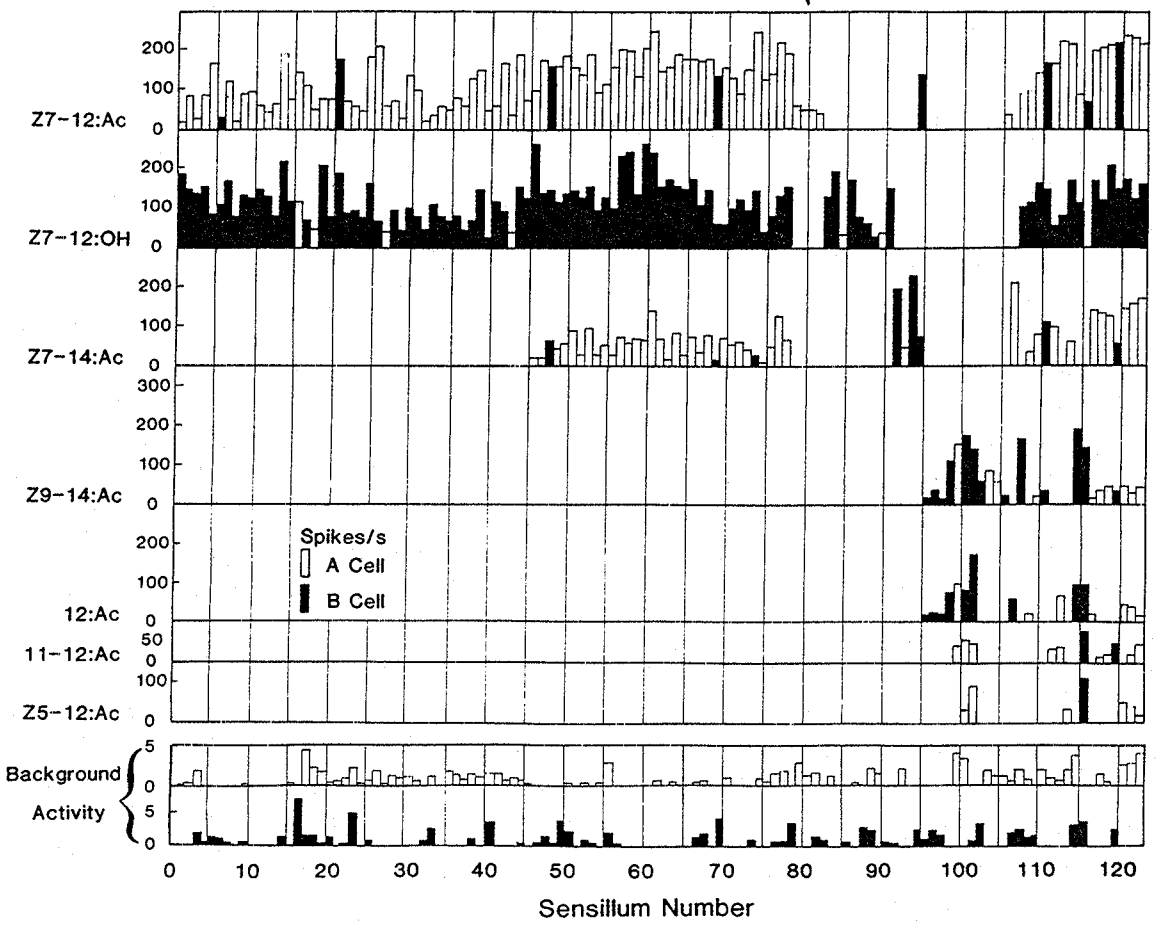

Fig. 1(b). Response spectra of antennal neurones in sensilla of mutant male *T. ni* to six pheromone components and to Z7–12:OH. Most sensilla contain two receptor cells, a large-spiking A cell (open bars) and a smaller-spiking B cell (solid bars). The background activity of both cells within each sensillum was determined prior to exposure to test compounds.

were located on the ventral surface of the antenna along its length, and seemed to occupy no particular position within a subsegment.

Twelve sensilla contained a B cell that responded to Z9–14:Ac and 12:Ac (Fig. 1a, sensilla 87–98). In these sensilla, A cells responding to any pheromone component were not detected (Fig. 1a). The mean (±SE) spike frequency in the first 0.2 s post-stimulation was 157.1 ± 15.4 spikes/s for Z9–14:Ac, and 80.0 ± 8.9 spikes/s for 12:Ac (Fig. 2b). The mean (±SE) spike amplitude of the B cell to Z9–14:Ac was 3.4 ± 0.3 mV and 3.4 ± 0.4 mV to 12:Ac. These sensilla were located in the middle subsegments of the antenna, away from the lateral margins and within a row of hairs. They appeared smaller and more delicate than the sensilla containing cells responding to Z7–12:Ac, Z7–14:Ac and Z7–12:OH. Four sensilla contained an A cell that responded to 11–12:Ac and Z5–12:Ac with predominately biphasic action potentials, plus a B cell that responded to Z9–14:Ac and 12:Ac with mainly monophasic action potentials (Fig. 1a, sensilla 105–108). The mean (±SE) spike frequencies in the first 0.2 s post-stimulation were 48.8 ± 12.8, 35.0 ± 3.5, 227.5 ± 13.1 and 143.7 ± 19.7 spikes/s, respectively (Fig. 2c). The mean (±SE) spike amplitude of the B cell to Z9–14:Ac was 3.4 ± 0.4 mV and 3.9 ± 0.4 mV to 12:Ac. Biphasic measurements of spike amplitude were determined for the A cell to be 6.5 ± 0.9 mV in response to 11–12:Ac and 6.7 ± 0.6 mV in response to Z5–12:Ac. These hairs were located in the same region of the antenna and of a subsegment as Z9–14:Ac/12:Ac hairs.

Two sensilla contained an A cell that responded only to Z7–14:Ac (Fig. 1a, sensilla 84 and 86), and two sensilla contained a B cell that responded only to Z9–14:Ac (Fig. 1a, sensilla 109 and 110). Thus, in normal males we found 13% of the sensilla containing cells with specificity for Z9–14:Ac and 12:Ac, or Z9–14:Ac alone. The remaining sensilla contained receptor cells that responded only to Z7–12:OH (Fig. 1a, sensilla 77–83) or to various combinations of three, four and five components (Fig. 2e), or all six components and Z7–12:OH (Fig. 1a, sensilla 111–136). Because for most of these latter sensilla the cells' cross-reactivities and subsequent relationships to behaviour are quite complex, we did not focus our attention on them.

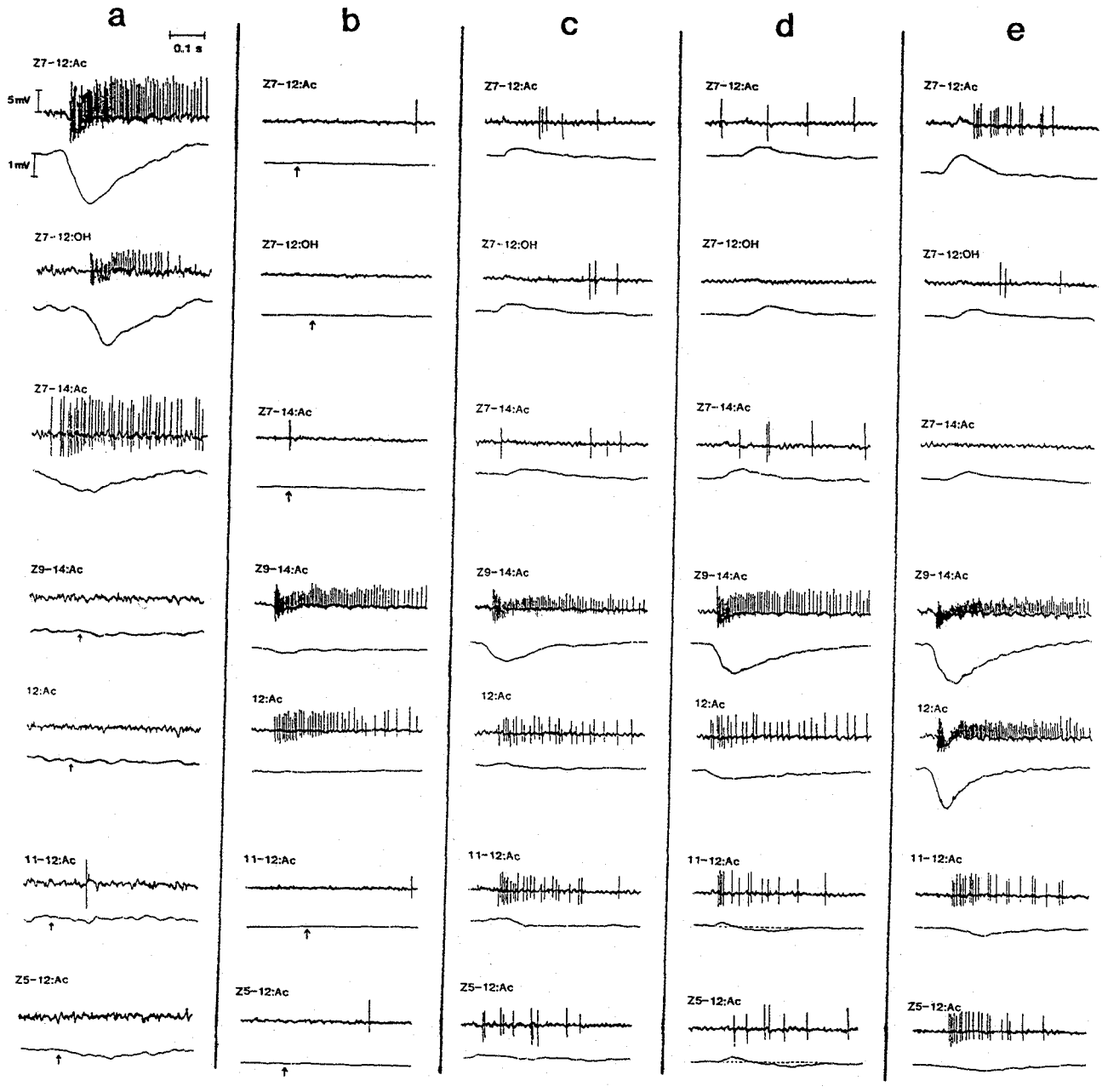

Fig. 2(a–e). Typical AC (upper) and DC (lower) responses of male *T.ni* receptor cells to six pheromone components and to Z7–12:OH. Arrows represent stimulus presentation. (a) Sensillum 75, normal male; (b) sensillum 87, normal male; (c) sensillum 108, normal male; (d) sensillum 101, mutant male; and (e) sensillum 133, normal male. The dotted line in Fig. 2(d) for 11–12:Ac and Z5–12:Ac represents the baseline.

Sensillar types on mutant male antennae

Single-cell recordings were obtained from 123 sensilla on fifty-four antennae of mutant male *T.ni* (Fig. 1b). As with the normal males (Fig. 1a), two receptor cells could be distinguished within most sensilla based on spike amplitude, and there was no clear relationship between the background activity of the receptor cells and their specificity to pheromone components (Fig. 1b). The location of various sensilla along the length of the antenna and within a subsegment was similar to that observed for normal males.

The majority of sensilla contained an A cell that responded to Z7–12:Ac and a B cell that responded to Z7–12:OH (Fig. 1b, sensilla 1–45), or else an A cell that responded to Z7–12:Ac and Z7–14:Ac, plus a B cell that responded to Z7–12:OH (Fig. 1b, sensilla 46–78). Within these latter sensilla, the mean (±SE) spike

frequency in the first 0.2 s post-stimulation was 162.7 ± 6.8 spikes/s for Z7−12:Ac; 56.7 ± 5.3 spikes/s for Z7−14:Ac; and 142.6 ± 9.8 spikes/s for Z7−12:OH. As in normal males, the A cells within these latter sensilla usually responded with lower spike frequency to Z7−14:Ac than to Z7−12:Ac. The mean (±SE) spike amplitude of the A cell to Z7−12:Ac and Z7−14:Ac was 4.9 ± 0.3 mV and 5.5 ± 0.3 mV, respectively, and the mean (±SE) spike amplitude of the B cell to Z7−12:OH was 3.5 ± 0.2 mV.

Four sensilla contained a B cell that responded to Z9−14:Ac and 12:Ac (Fig. 1b, sensilla 96−99). In these sensilla, A cells responding to any pheromone component were not detected. The mean (±SE) spike frequency in the first 0.2 s post-stimulation was 45.1 ± 22.1 spikes/s for Z9−14:Ac, and 37.5 ± 15.9 spikes/s for 12:Ac. The mean (±SE) spike amplitude of the B cell to Z9−14:Ac was 3.9 ± 0.6 mV and 3.6 ± 0.9 mV to 12:Ac. Two sensilla contained an A cell that responded to 11−12:Ac and Z5−12:Ac with predominately biphasic action potentials, plus a B cell that responded to Z9−14:Ac and 12:Ac with predominately monophasic action potentials (Fig. 1b, sensilla 101 and 102). The mean (±SE) spike frequencies for the first 0.2 s post-stimulation were 50.0 ± 5.1, 57.5 ± 32.5, 107.5 ± 32.5 and 127.5 ± 47.5 spikes/s, respectively (Fig. 2d). The mean (±SE) spike amplitude of the B cell to Z9−14:Ac was 4.4 ± 1.4 mV and 4.2 ± 1.5 mV to 12:Ac. Biphasic measurements of spike amplitude were determined for the A cell to be 7.5 ± 2.4 mV in response to 11−12:Ac and 8.0 ± 3.7 mV in response to Z5−12:Ac.

Three sensilla contained a cell, either A or B, that responded only to Z7−14:Ac (Fig. 1b, sensilla 92−94), and three sensilla contained a cell, either A or B, that responded only to Z9−14:Ac (Fig. 1b, sensilla 103−105). The percentage of sensilla in mutant males with cells specific for Z9−14:Ac and 12:Ac, or Z9−14:Ac alone, was 7%, not significantly different ($P > 0.05$; Chi-square 2 × 2 test of independence) from the percentage (13%) in normal males. Most of the remaining sensilla contained cells that responded only to Z7−12:OH (Fig. 1b, sensilla 83−91), or to various combinations of three, four or five components, or to all six components and Z7−12:OH (Fig. 1b, sensilla 109−123).

Hyperpolarization in receptor potentials

Within both mutant and normal male sensilla containing cells responsive to Z9−14:Ac, 12:Ac, 11−12:Ac and Z5−12:Ac, exposure to components that did not elicit spikes (Z7−12:Ac and Z7−14:Ac, or to Z7−12:OH) resulted in significant hyperpolarizations (Figs 2c−e). This unusual result was all the more significant because depolarizations were elicited by some or all of the other components (Figs 2c−e). In some of these sensilla, more complex DC waveforms resulted from exposure to 11−12:Ac and Z5−12:Ac, with slight hyperpolarization followed by depolarization before returning to baseline (see especially Fig. 2d). Even shallow hyperpolarizations such as these were never observed in cells in other sensilla.

Discussion

One important result from our study was the demonstration that the percentage of antennal neurones in mutant males specific for Z9−14:Ac or for Z9−14:Ac and 12:Ac is no greater than the percentage in normal males. The trend in fact was for the percentage of such cells to be lower in mutant males, and for their firing rate to be lower in response to Z9−14:Ac. These results thus indicate that the mutation in the *T. ni* gene that affects the chain-shortening enzyme in the biosynthetic pathway for pheromone production in females (R. Jurenka, personal communication) does not affect pheromone receptors in males in any obvious way. Therefore, *T. ni* genes influencing important steps in pheromone production and reception do not appear to be closely linked, consistent with the communication system of the European corn borer, *Ostrinia nubilalis*; crossing studies performed with two strains of this latter species indicated that no linkage exists between production and reception (Roelofs *et al.*, 1987; Löfstedt *et al.*, 1989).

Another important finding from our study was the discovery of receptor cells in both male strains specific either for Z9−14:Ac and 12:Ac, or also for 11−12:Ac and Z5−12:Ac housed within the same sensillum; such cells had not been discovered in previous studies. Grant & O'Connell (1986), however, did get a response from the B cells in low-spontaneous-activity sensilla when they were stimulated with 12:Ac, but these same cells also responded with greater firing rates to Z7−12:Ac, and Z7−14:Ac, negating their possible contribution to relaying information about 12:Ac when exposed to the natural blend. In our study, the fact that these minor-component-specific cells have virtually equivalent affinities for these two pairs of compounds is particularly striking considering that in wind tunnel tests they were found to be behaviourally important 'redundant' pairs of components (Fig. 3; Linn *et al.*, 1984).

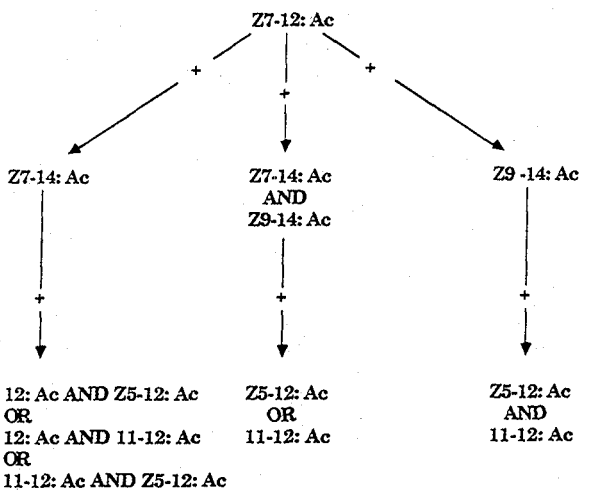

Fig. 3. Flow chart showing the association of four components into blends that elicit responses in male *T. ni* similar to the complete six component blend. Reproduced from Linn *et al.* (1984), *Journal of Chemical Ecology*, **10**, 1635−1658.

Although the sex pheromone of *T.ni* comprises six components, Linn *et al.* (1984) demonstrated in the wind tunnel that several four-component blends elicited peak response levels in males as high as those observed with the complete blend (Fig. 3), and showed that combinations of three minor components could behaviourally compensate for the two missing components as if the latter were extraneous. Only certain pairs of components, however, were mutually replaceable (i.e. redundant) and two such pairs, Z9–14:Ac and 12:Ac, and Z5–12:Ac and 11–12:Ac, correspond to the antennal neurones' affinities found in our study. In other words, the same pairs of minor components found to be mutually replaceable in stimulating antennal neurones within the same sensillum were those found to be mutually replaceable behaviourally. Our current methodology does not allow us to state conclusively that each component pair is stimulating one of the two pheromone-sensitive neurones within a sensillum. For cells having similar spike amplitudes in response to different pheromone components, selective adaptation studies of marked neurones are needed to determine whether the activity can be attributed to the same or to different cells (Kaissling *et al.*, 1989). Even selective adaptation can be equivocal by itself because it does not rule out, in some circumstances, the possibility of different receptor site types residing on the same dendrite. However, based on TEM studies that show two or three dendrites within a sensillum (R. Y. Zachurak, personal communication), and the production of monophasic spikes in response to Z9–14:Ac and 12:Ac and biphasic spikes in response to 11–12:Ac and Z5–12:Ac, our working hypothesis is that each component pair is stimulating a different cell.

Our results, coupled with those of behavioural studies (Linn *et al.*, 1984) raise the possibility that for blends of sex pheromone components to elicit optimal upwind progress and source contacts in male *T.ni*, four major pathways within the macroglomerular complex (Hildebrand *et al.*, 1980) of the deutocerebrum need to receive input from the antennae (Fig. 4). These pathways may or may not form different topographical regions of sub-glomeruli in the macroglomerular complex, as has been found in *Manduca sexta* (Hansson *et al.*, 1991), but for discussion we will visualize them as regions. Because of mutual replaceability, or redundancy, at the receptor level as indicated by our study, there are many different ways to reach these four regions in the macroglomerular complex with different blends of the six pheromone components to evoke optimal behaviour (Fig. 3, Linn *et al.*, 1984).

The proven routes to behaviourally optimal blends (Linn *et al.*, 1984) all begin with Z7–12:Ac (Fig. 3), and the abundant receptor neurones eliciting large-amplitude spikes should project their axons to one region of the macroglomerular complex, which we label as region I (Fig. 4). The next component needed in the variety of four-component blends evoking optimal behaviour is Z7–14:Ac (Fig. 3, left-most and centre pathways), and the few neurones specific for this component should project to a second region of the macroglomerular complex,

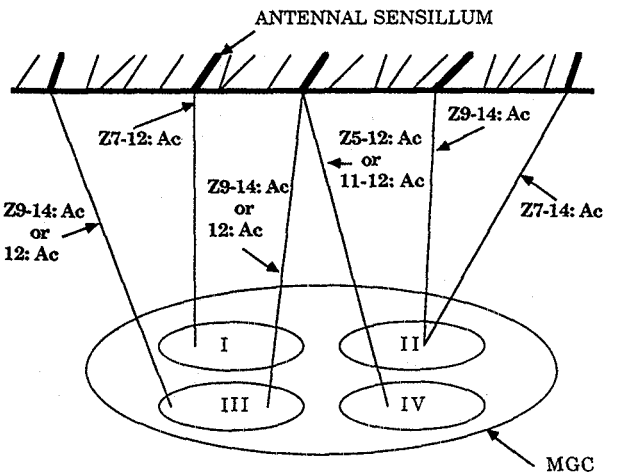

Fig. 4. Schematic representation of pheromone blend processing in male *T.ni* at the peripheral (antennal sensillum) and CNS (macroglomerular complex) levels. Thicker lines represent behaviourally important sensilla, with axons (thin lines) projecting down to specific regions (small ovals) within the macroglomerular complex (large oval, MGC).

region II (Fig. 4). In a different four-component blend, Z9–14:Ac is also a behaviourally necessary component by itself (Fig. 3, right-hand pathway), and we hypothesize that the few neurones specific for Z9–14:Ac alone also project to region II. Even though they are housed in separate sensilla, we propose that these neurones, either Z7–14:Ac-specific or Z9–14:Ac-specific, thus form a mutually replaceable pair with both types of cells synapsing with region II of the macroglomerular complex.

The next behaviourally necessary component in the array of minimal sets is Z9–14:Ac (Fig. 3, centre pathway), but this component can be replaced effectively by 12:Ac (change Z9–14:Ac in the centre pathway to 12:Ac to form all but the lowest entry in the left-hand pathway). Our results indicate that this mutually replaceable pair of components seems to stimulate a subpopulation of antennal receptor cells which project to the hypothetical region III in the macroglomerular complex (Fig. 4). The reason that Z9–14:Ac is not replaceable with 12:Ac in the right-hand pathway (Fig. 3) is that although it *is* mutually replaceable with regard to the input to macroglomerular complex region III, 12:Ac cannot stimulate those few neurones specific for Z9–14:Ac that feed into region II; therefore, the redundancy buffering against a lack of Z7–14:Ac is missing. In the right-hand blend, therefore, we suggest that Z9–14:Ac does double-duty in providing necessary input to regions II and III in the macroglomerular complex, and the former input cannot be replaced by 12:Ac.

Finally, the fourth component needed to complete the behaviourally minimal set is either Z5–12:Ac or 11–12:Ac (Fig. 3, centre pathway and all but the bottom entry in the left-hand and right-hand pathways). The few cells found in our study specific for either of these two components were housed in the same sensilla as Z9–14:Ac/12:Ac-

specific cells (Fig. 1a, sensilla 105–108 in normal males, and Fig. 1b, 101 and 102 in mutant males). We hypothesize that these cells synapse with interneurones in region IV of the macroglomerular complex (Fig. 4), and provide the peripheral redundancy that explains the behavioural redundancy to these two compounds. At the present time, our data and model involving these four needed macroglomerular complex inputs do not seem to provide sensory neuronal explanations for the behavioural requirement that Z5–12:Ac and 11–12:Ac *both* be present in certain four-component optimal blends (Fig. 3, bottom entry in the left and right-hand pathways).

In common with other studies (Grant & O'Connell, 1986; Grant *et al.*, 1988), we located also many sensilla on both normal and mutant male antennae that contained a small-spiking cell that responded only to Z7–12:OH. Because Z7–12:OH is not considered a pheromone component for *T. ni*, the function of a receptor cell that responds only to this compound is not clear. Grant & O'Connell (1986) and Grant *et al.* (1988) suggested that these cells may enable male *T. ni* to recognize a heterospecific female that emits Z7–12:OH as part of her pheromone blend. However, it is suspicious that a Z7–12:OH-sensitive cell is nearly always housed in the same sensillum as a Z7–12:Ac cell (Figs 1a, b). Detectors of heterospecific-emitted Z7–12:OH should not need to be so constrained as to be paired with Z7–12:Ac cells, and to be bathed in the same sensillum liquor. Interestingly, Van der Pers & Löfstedt (1986) also found that a type of abundant sensillum in the turnip moth, *Agrotis segetum*, that contained a cell responsive to the major pheromone component, Z5–10:Ac, nearly always contained a second cell that responded to Z5–10:OH. Again as in *T. ni*, the alcohol corresponding to the major component was found to reduce attraction to the pheromone blend (Löfstedt *et al.*, 1985). Because Z7–12:Ac is emitted by female *T. ni* at up to one hundred times the rate of the other components in the blend, this component is the one most likely to reach excessive amounts for its respective receptor cell. One consequence of exceeding this receptor's capacity would be adaptation of these Z7–12:Ac-specific cells (Baker *et al.*, 1988). In addition, we suggest that excessive amounts of Z7–12:OH may be produced by esterases in the sensillum fluid (Ferkovich *et al.*, 1973; Vogt *et al.*, 1985; Vogt & Riddiford, 1986), and that the binding protein might not be able to function quickly enough to remove the alcohol. By having a cell, in this case an alcohol detector, responsive to the breakdown product of the major component paired within the same sensillum as the cell responsive to the major component, more specific information about concentration-dependent pheromone–receptor site interactions and the kinetics of inactivation of pheromone molecules may be obtained (Vogt & Riddiford, 1986), causing a male *T. ni* to alter his behaviour accordingly.

Our results showing both hyperpolarization and depolarization occurring in response to different components are novel for *T. ni*. Although we did not attempt to measure a reduction in firing during hyperpolarization, if hyperpolarized cells were indeed inhibited, complex interactions may occur within some sensilla when exposed to a filament of the complete pheromone blend that will have significant effects on the integration of odour blend quality. The strong hyperpolarization of at least one cell by Z7–12:Ac, Z7–14:Ac, and Z7–12:OH within the rare sensilla that contain cells that depolarize and fire in response to the four other minor components suggests that selective noise reduction by receptor neurones may be a significant contributor to odour quality processing beginning upstream from the macroglomerular complex. GABA-related postsynaptic inhibition has been the only significant inhibitory force recognized to this point as influencing odour quality integration in moths (Christensen & Hildebrand, 1988; Christensen *et al.*, 1989; Homberg *et al.*, 1987).

It can be envisaged that combinations of reductions in firing of some cells and increases in others may serve to enhance information about the blend that has impinged on a hair in a way similar to that of peripheral cells involved in host volatile detection in lepidopterous larvae (cf. Dethier, 1970). In some *T. ni* sensilla, even a single minor component can apparently hyperpolarize one cell while depolarizing another, as evidenced by complex DC waveforms. Similarly, it appears that the major component, Z7–12:Ac, can sometimes elicit spiking in one cell (larger-spiking) while hyperpolarizing another cell (smaller-spiking) (Fig. 2e). In the future, these sensilla, when discovered, must be presented with blends of the components in order to discern what are the resultant action potentials sent on from the different cells to the macroglomerular complex. In only one other species has hyperpolarization to one pheromone component, and depolarization to another recently been discovered (Hansson *et al.*, 1990), again by using a cut-sensillum technique. Hyperpolarization in response to a plant odour and depolarization to a pheromone component was demonstrated for yponomeutid species (Van der Pers *et al.*, 1980), and for *Antheraea polyphemus* (Kaissling *et al.*, 1989) using a cut-sensillum technique.

By sampling over 100 antennal sensilla on both normal and mutant male *T. ni*, we found receptor cells that responded to the six components in the sex pheromone, either separately or in various combinations (Figs 1a, b). In particular, we have located sensilla with cells excited by 12:Ac, 11–12:Ac and Z5–12:Ac that had not been found in previous studies. Using a tungsten microelectrode recording technique, Mayer (1992) concluded that these three compounds did not have conspecific significance because no neurones had been found that responded to them at naturally-emitted levels. Although we did not try to relate the concentration emitted from our pipettes to that from females, we think that it is significant that when presented with each compound at a particular concentration, only certain cells responded to the behaviourally redundant components. The behavioural importance of 12:Ac, 11–12:Ac and Z5–12:Ac has already been proven unequivocally (Linn *et al.*, 1984); therefore, the conclusion of Mayer (1992) is illogical because it ignores the fact that such specific behavioural effects require specific receptors.

At least five behaviourally important types of sensilla were found during this study, each differentiated by re-

ceptor cell specificities, and not by the spontaneous rate of background firing. Therefore, in contrast to the earlier studies on *T.ni* neurophysiology, we could not rely upon background firing rate as a major criterion for classifying sensilla (O'Connell *et al.*, 1983; Grant & O'Connell, 1986; Grant *et al.*, 1988; Mayer & Mankin, 1990). This discrepancy between our study and the earlier studies may be related to the different recording techniques, with all earlier studies using a tungsten recording technique. Our results are similar to those of earlier studies in that we located also very few sensilla with a receptor cell that was sensitive only to Z9–14:Ac (Mayer & Mankin, 1990), and many sensilla with a large-spiking receptor cell tuned to Z7–12:Ac and a small-spiking receptor cell tuned to Z7–12:OH (O'Connell *et al.*, 1983; Grant & O'Connell, 1986; Grant *et al.*, 1988).

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