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Integration of olfactory information in the Colorado potato beetle brain

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The processing of olfactory information in the Colorado potato beetle, *Leptinotarsa decemlineata* Say, was studied by recording responses of olfactory neurones intracellularly in the deutocerebrum. Response characteristics of neurones in this first relay station of the olfactory pathway were measured when the antennae were stimulated with five general green leaf volatiles, i.e. *cis*-3-hexen-1-ol, *trans*-2-hexenal, *cis*-3-hexenyl acetate, *trans*-2-hexen-1-ol and 1-hexanol. These compounds are part of the so-called green odour of potato, whose defined composition is essential for the beetle's host plant finding. The response spectra of deutocerebral neurones can be divided roughly into two classes: one class containing neurones which are not very specific for the tested compounds, and another class with highly specialized neurones. Their different responses to a potato leaf extract suggest two channels for the processing of olfactory information in the antennal lobe: one channel for the detection of the presence of green leaf odour components, and another one for an evaluation of the component ratios.

INTRODUCTION

Anatomical studies of the olfactory system in insects revealed that axons of antennal receptors terminate in the antennal lobe of the deutocerebrum. This part of the insect brain is the first relay station in the olfactory pathway. Synaptic connections between receptor neurones and deutocerebral neurones are made in glomerular neuropil regions in the deutocerebrum[3,4,11,24]. These glomeruli are innervated by local interneurones and output neurones. Local interneurones remain with their processes within the deutocerebrum, while output neurones have an axon running to the protocerebrum via the tractus olfactorio globularis[3,4,11,18]. The axons of the output neurones terminate in two neuropil structures in the protocerebrum, i.e. in the calyces of the mushroom bodies and in the lobus lateralis protocerebralis[3,4,11].

An important part of the physiological research on this olfactory system has been done with pheromones[2,6,7,14,20,21,31]. Studies at the peripheral level of the nervous system showed that pheromone receptors are extremely narrowly tuned in their sensitivity to chemical compounds, and are specialized in the detection of certain pheromone components[5]. Information from these receptors seems to be processed separately from other olfactory information in several insect species. Deutocerebral neurones sensitive to pheromone components innervate a macroglomerulus[2,4,7,18,20], a neuropil structure which is found exclusively in males.

The narrow tuning of pheromone receptors seems to be different from those of food odour receptors, which generally have broad and overlapping response spectra[27]. A major problem in the investigation of the processing of food odours is that these stimuli usually have a very complex composition[23,26,30], and that it is not clear which of their components are relevant for their identification. Relatively few electrophysiological studies on food odour process-

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ing in the deutocerebrum have been undertaken[1,18,22,31,32]. These studies involved food odours with an undefined chemical composition, like odours of fruit, cheese and bread, and compounds which are sometimes known as potent stimuli for receptors but which have an unknown behavioural significance.

The Colorado potato beetle, *Leptinotarsa decemlineata* Say, is an insect species with a very limited host plant range. Its most important host in Europe is potato, *Solanum tuberosum* L. The beetle can distinguish between a host plant and a non-host plant by differences in their odour composition[29]. In previous work the potato plant odour has been analyzed[26,30] and the beetles' antennae have been tested for their sensitivity to its pure components[16,25,26]. The olfactory receptors are sensitively tuned to the perception of green odour[16], which is composed of C-6 alcohols, aldehydes and the derivative acetate[26]. The upwind locomotory response of the beetle which is induced by potato leaf odour, is prevented by artificial changes in concentration ratios of these green odour volatiles in the potato leaf odour[28]. It was concluded, therefore, that the ratio of these C-6 compounds in the plant odour is decisive in the beetle's host plant finding.

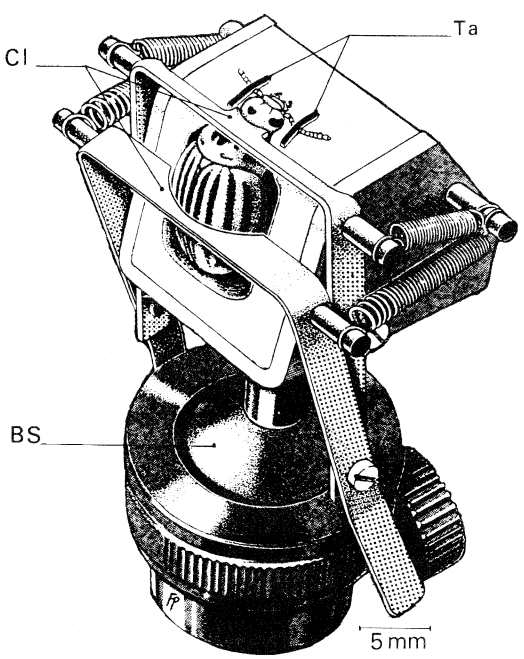

Fig. 1. Stainless-steel holder for mounting the beetles during intracellular recordings of deutocerebral neurones. BS, ball-and-socket joint; Cl, clamps; Ta, tape.

In order to investigate the mechanism of potato leaf odour recognition in the beetle, physiological properties of neurones in the antennal lobe were studied. In this report, responses of deutocerebral neurones are described on stimulation with 5 behaviourally important C-6 compounds, namely *cis*-3-hexen-1-ol, *trans*-2-hexenal, *cis*-3-hexenyl acetate, *trans*-2-hexen-1-ol and 1-hexanol. Responses to the odour of an extract of potato leaves were also recorded. In some cases additional stimulations were performed with an artificial mixture consisting of the 5 C-6 compounds. The results indicate that information concerning stimulus quantity and quality are processed separately.

MATERIALS AND METHODS

One-week-old female beetles from the department stock culture were used in the experiments. An individual beetle was mounted in a stainless steel holder and its antennae were immobilized by tape (Fig. 1). The brain was exposed by removing the part of the head capsule between the eyes. Mouthparts, muscles, fat and the anterior part of the gut were removed. The latter was replaced by a plug of paper tis-

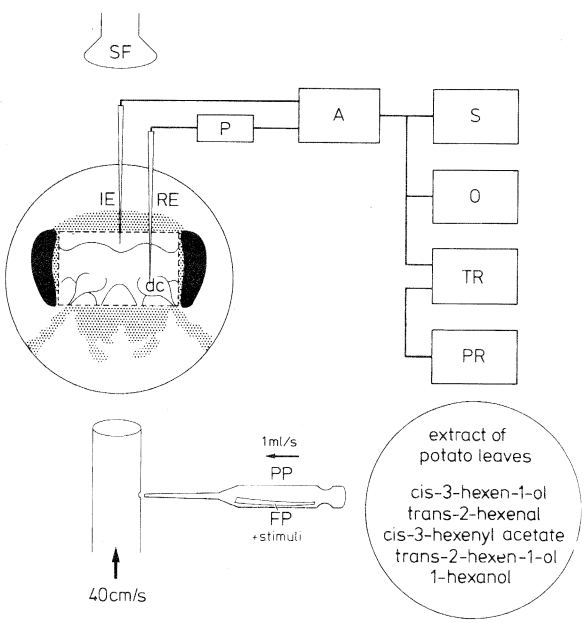

Fig. 2. Schematic representation of the experimental set-up. A, amplifier; dc, deutocerebrum; FP, filter paper; IE, indifferent electrode; O, oscilloscope; P, probe; PP, Pasteur pipette; PR, paper recorder; RE, recording electrode; S, speaker; SF, suction funnel; TR, tape recorder.

sue. An insect pin through the head capsule helped further to stabilize the brain. The brain's tracheal air supply was not interrupted. The brain was constantly immersed in saline solution and the head was surrounded by vaseline to prevent leaking of this solution. The composition of the saline has been described by Khan et al.[15]. The sheath of the brain was treated with a 2% (w/v) solution of pronase (8 DMC-U/mg, Serva) in saline solution for 20 min, in order to enable penetration of the recording electrode. Capillary microelectrodes were made of filament glass. The tip was filled with 5% Lucifer yellow and the shank with 1% lithium chloride. The measured resistance was 120–180 MΩ. Recordings were made with the aid of a Winston Electronics amplifier (Model 1090, with a BR-1 Bridge). After finishing intracellular recording, dye was injected by a direct current of 0.3–1.0 nA.

The animal was placed in a continuous and steady flow of air (40 cm/s, 30 ml/s) (Fig. 2). Antennal receptors were stimulated by injection of odour stimuli (1 ml/s for 2 s) into this airstream from Pasteur pipettes. The time between two stimulations was 30–60 s. The delivery of an odour puff was controlled by an electromagnetic valve. This valve was operated through a timer which also provided a 50-Hz signal as a marker of stimulus application. The Pasteur pipettes were loaded with 6.0 × 0.5 cm strips of filter paper on which a 25-μl paraffin oil solution of the test chemical was pipetted. Initially a dilution of 10^{-4} v/v was used, but this was substituted by a dilution of 10^{-5} v/v to prevent overstimulation.

The test chemicals were obtained from commercial sources (Roth, Koch-Light) and were >97% pure. Five C-6 components of the potato leaf odour complex were used: *cis*-3-hexen-1-ol, *trans*-2-hexenal,

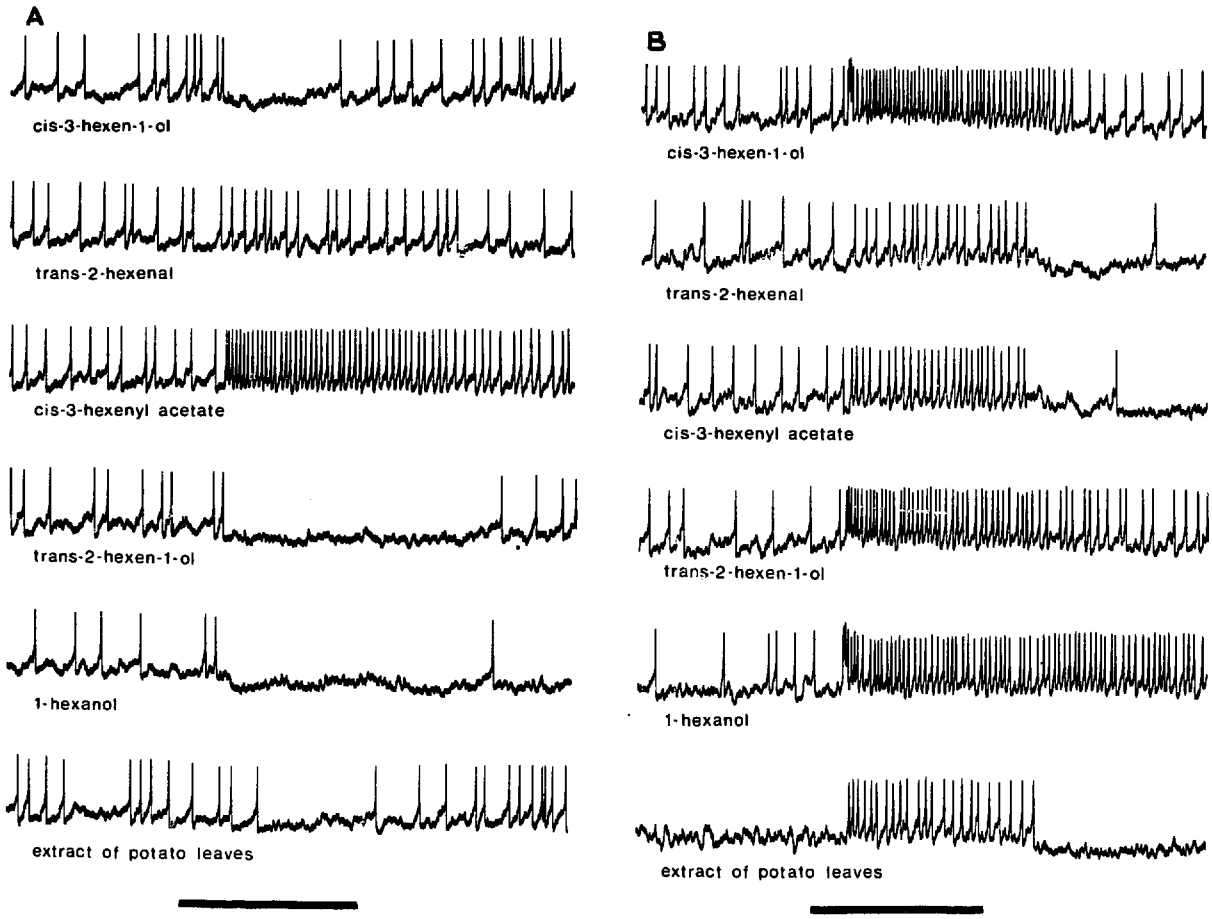

Fig. 3. Responses of 4 different deutocerebral neurones (A–D) to 5 leaf odour components (at a dilution of 10^{-5} v/v) and to a paraffin oil extract of potato leaves. The stimulus duration (2 s) is indicated by bar. A–D correspond with spectra numbers 2, 5, 13 and 21 in Fig. 4.

cis-3-hexenyl acetate, *trans*-2-hexen-1-ol and 1-hexanol. In addition to these test chemicals, the cells were also stimulated with the odour of a paraffin oil extract of potato leaves. This extract was made by blending 40 g potato leaves in the presence of 30 ml water. The product was shaken in 20 ml paraffin oil. The paraffin oil was collected after centrifugation (6000 rpm, 15 min) and stored at 8 °C. This extract contained the potato leaf odour components (Visser, unpublished). Some neurones were additionally tested with an artificial mixture consisting of the 5 test components in an 1:1:1:1:1 ratio (at a dilution of 10^{-5} v/v). Paraffin oil was used as control. The sensitivity of the neurones to a mechanical stimulus was tested by fluttering the airstream.

The number of spikes in the first reaction second (which corresponds with the stimulation time period t = 0.5–1.5 s) was counted and corrected for the spontaneous firing by subtracting the cell's average firing frequency in the 2 s prior to stimulation. The main change in a cell's activity due to a chemical stimulation was set on a 100% level in order to obtain its relative reaction spectrum for the 5 general green leaf volatiles. Inhibition percentages were related to the mean spontaneous activity of the cell.

RESULTS

The activity of 22 neurones showing responses to the test odours used, was recorded from the deutocerebrum of the Colorado potato beetle. Most of these neurones had a background firing of 3–8 spikes/s, but in some cases it was under that level or as high as 25 spikes/s. The amplitude of recorded spikes was 3–7 mV. Most of the cell recordings lasted for 10–15 min. This was too short a period for an additional Lucifer yellow staining to reveal morphological details of a cell. However, since the cell somata were marked, it was possible to identify the recorded neurones as deutocerebral neurones. Two fillings seemed to be

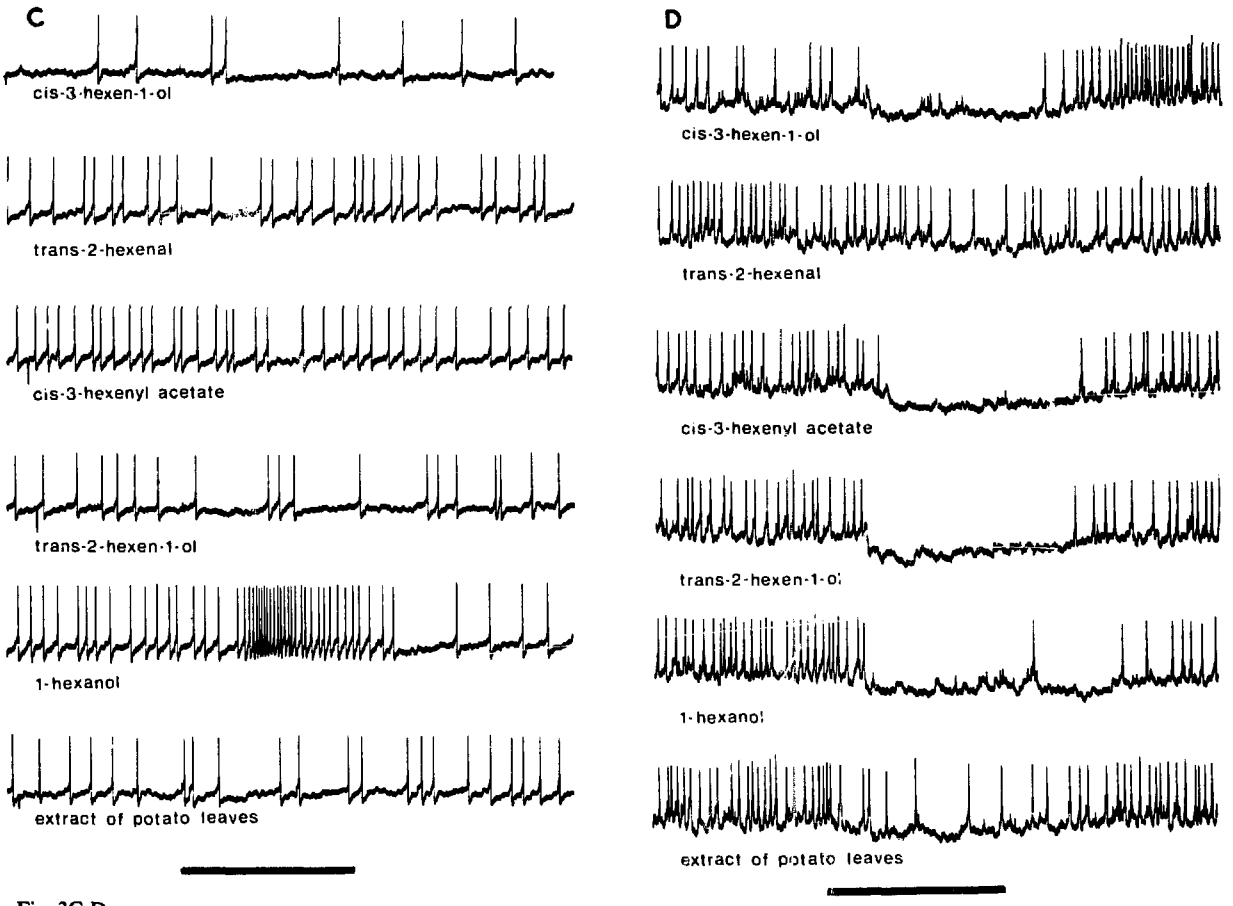

Fig. 3C,D.

complete and showed details of cell innervations. The recordings of these cells lasted for about 45 min.

The recorded neurones differed in their response specificity to the stimuli (Fig. 3). Some neurones were highly specific to one of the tested components (Fig. 3A,C) while other neurones had a more broadly tuned sensitivity (Fig. 3B,D). We classified the neurones (numbers 1–22) in 4 groups, based on their relative response spectra for the five C-6 compounds (Fig. 4).

Group I contains narrowly tuned neurones which were sensitive to *cis*-3-hexenyl acetate. They showed inhibition responses or relatively weak excitation responses to the other volatiles. The spontaneous spike frequency of those neurones hardly changed after a stimulation with the odour of the potato leaf extract. Mechanical stimulation of the antenna had no effect on these neurones.

Neurones of group II were more broadly tuned. They responded in an excitatory manner to most of the volatiles. The tested alcohols elicited the strongest responses in these neurones. All group II neurones showed a clear and excitatory response to the potato leaf extract. Cell number 8 was the only cell in this group which responded (inhibitory) to a mechanical stimulation of the antenna. This cell was identified as an output neurone by Lucifer yellow staining.

Group III neurones were sensitive to 1-hexanol. The other tested leaf odour components, including the other alcohols, elicited relative weak responses. The cells placed in this group showed no reaction, or a weak one, to stimulation with the odour of the potato leaf extract. An exception in this respect was cell number 9 which showed a clear excitatory response to the extract. The cells number 10 and 14 showed an excitatory response after mechanical stimulation of the antenna.

Neurones with an inhibition reaction as the most significant response were placed in group IV. The strongest reactions in group IV neurones were caused by stimulation with the alcohols. These cells showed a clear inhibitory response to stimulation with the odour of the potato leaf extract. Cell number 17 was the only neurone of this group which showed only a very weak inhibition to stimulation with the extract. Cell number 16 was identified as a local interneurone.

The neurones number 4, 5, 6, 13, 16, 18, 19, 20 and 22 were additionally tested with the artificial mixture. The neurones number 4, 5 and 6 of group II showed an excitatory response at levels of 80%, 74% and 50% respectively when compared with the response to their 'best' compound. The neurones number 16, 18, 19, 20 and 22 of group IV responded with a complete inhibition of their spontaneous activity. Neurone number 13 of group III showed an inhibition response to this mixture at a level of 24% when compared with the response to its 'best' compound.

The Colorado potato beetle's deutocerebral neurones can be divided roughly into two physiological classes: one class of neurones which are narrowly tuned and do not respond clearly to the extract of potato leaves, and another class of more broadly tuned neurones which show an evident response to the potato leaf extract.

DISCUSSION

The Colorado potato beetle's receptor cells investigated by Ma and Visser[16] and by De Jong and Visser (in preparation) have been stimulated with higher stimulus concentrations than the neurones at the deutocerebral level in order to elicit clear responses (10^2–10^3 times higher). An increased sensitivity of deutocerebral neurones as compared to receptor neurones is a common feature in insects[2,4,20]. This effect is due to convergence, caused by the connection of a large number of receptors with the same interneurone[4].

Inhibitory responses in interneurones, like the responses of group IV neurones (Fig. 4), have been reported for other insect species as well[1,18,32]. It has been suggested that inhibition responses in the olfactory circuitry will lead to a better signal-to-noise ratio in the processing of olfactory information[1,12,17]. Improvement of the signal-to-noise ratio might be the function of the group IV neurones from which we recorded. Their response spectra are more or less the mirror image of those of group II. Both groups consist of more broadly tuned interneurones. Most of these neurones gave the strongest responses when there was stimulation with an alcohol, and were sensitive to stimulation with the potato leaf extract.

Groups I and III (Fig. 4) contain neurones which

were narrowly tuned to one of the potato leaf odour components. Neurones with similar response spectra as the interneurones of group I have been found recently at the periphery (De Jong and Visser, in preparation). In lobsters highly specialized cells have been found at different neuronal levels[10]. Narrow-spectrum interneurones in lobsters are thought to have an important function in coding, either by dominating the across-fibre pattern for that stimulus or by the formation of labelled lines[8,10]. The highly specific responses of the Colorado potato beetle's group I and III interneurones suggest a similar role for these neurones. They might obtain information about the presence of a particular compound in a mixture.

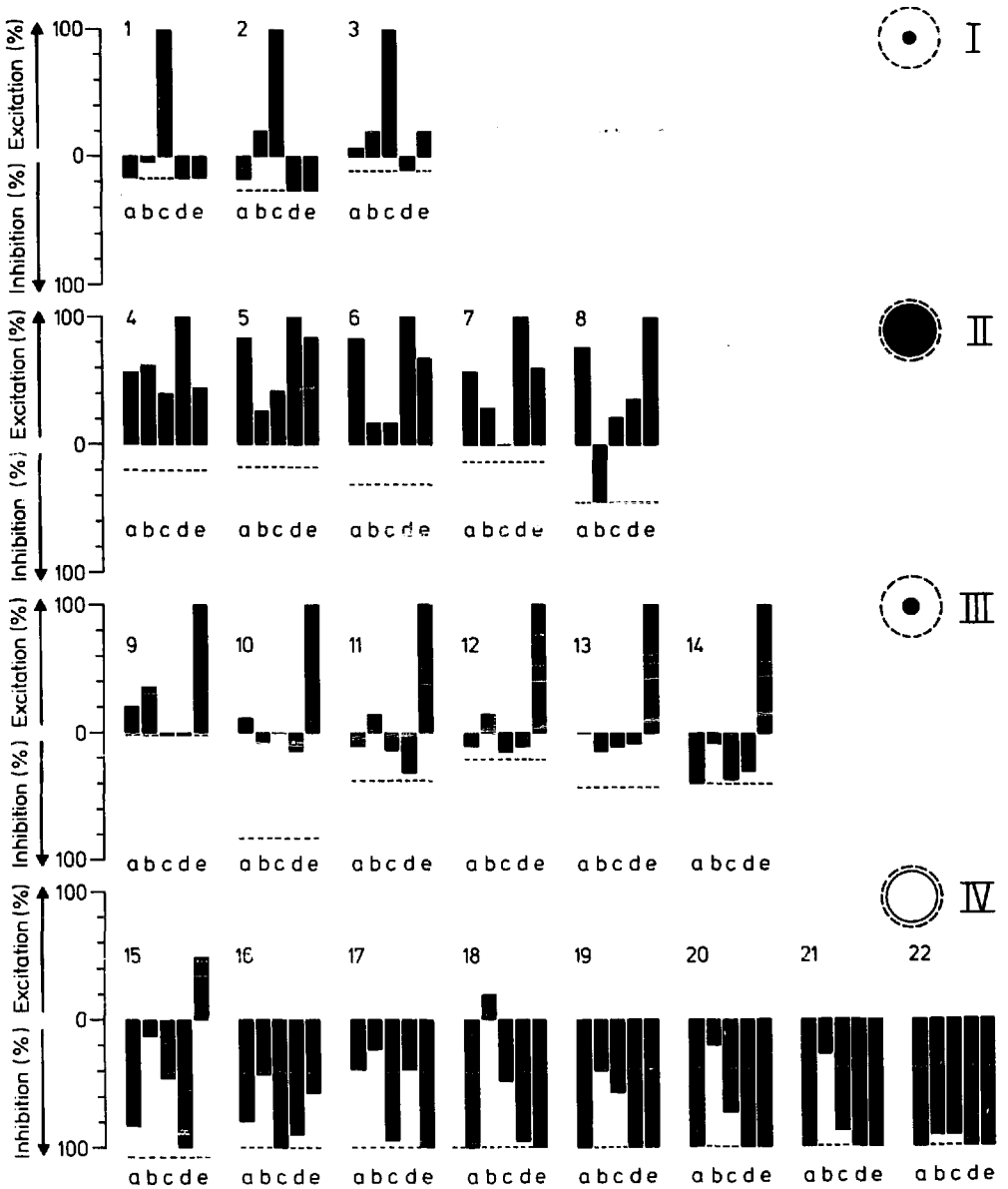

Fig. 4. Relative reaction spectra of 22 neurones of the Colorado potato beetles' deutocerebrum for 5 potato leaf odour components. a, *cis*-3-hexen-1-ol; b, *trans*-2-hexenal; c, *cis*-3-hexenyl acetate; d, *trans*-2-hexen-1-ol; e, 1-hexanol. At the stimulus source filter papers contained the chemicals at a dilution of 10^{-5} v/v, except for spectra number 3, 9, 12, 14, 15 and 17, which were recorded with chemicals at a dilution of 10^{-4} v/v. The broken line indicates the relative level of maximum inhibition (total inhibition of spontaneous activity). Areas of circles indicate the average relative responses for each neurone group to a paraffin oil extract of potato leaves. Filled, open and broken circles indicate excitation, inhibition and the 100%-level respectively.

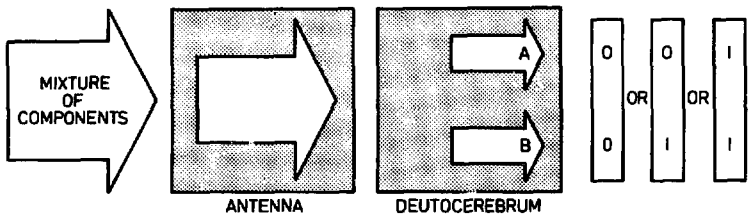

Fig. 5. Hypothetical diagram for the mechanism of host plant odour recognition in the Colorado potato beetle. Channels A and B act independently and show a response (1) or no response (0). In this way information concerning stimulus quality (channel A) and stimulus quantity (channel B) is processed separately (see text for further explanation).

However, since there was no clear response to the potato leaf extract, such a role in the specific detection of components by these deutocerebral neurones is not very probable. Neurone number 13, a specialist for 1-hexanol (Figs. 3 and 4), did not show an excitatory response to the artificial mixture, but was stimulated by 1-hexanol in a pure form. Here the response of the cell to a single compound was apparently also inhibited in the presence of other compounds. Mixture suppression therefore could be an important feature of these narrowly tuned interneurones. Since the level of excitation in the narrowly tuned neurones seems to depend not only on the presence of the stimulus to which they are tuned, but also on the presence of other chemicals, these neurones might process information about the composition of an odour blend.

Our results suggest a warning function for this class of narrowly tuned neurones in the Colorado potato beetle: there is a response if certain stimulus component ratios differ from those in potato leaf odour, and there is no response if these ratios are similar.

A simplified hypothetical diagram for the mechanism of host plant odour recognition in the Colorado potato beetle is presented in Fig. 5. The presence of a mixture of components can be detected by the antennal receptors. These receptors transfer information to the deutocerebrum where two classes of neurones are present: class A neurones which are narrowly tuned and whose response depends on the composition of the stimulus, and class B neurones which respond when certain components are present. There are three possible situations:

(I) Both neurone classes do not respond. In this case important leaf odour components are not present and there is no detection of plant odour.

(II) Only class B neurones respond. In this situation there is a stimulation with green odour components. Since the class A neurones do not respond, these components are in the correct ratio, and the beetle detects the presence of potato leaf odour.

(III) In situation III both classes respond to a stimulus. This stimulus therefore contains green odour components in a ratio which differs from the one in potato leaf odour. This is the situation when there is stimulation from a plant odour other than potato.

Evidence from other insect species also demonstrates the existence of deutocerebral neurones with response levels that depend on the ratio of certain food odour components, rather than on the presence of one specific compound. In the hawk moth *Manduca sexta* and in the locust *Locusta migratoria* deutocerebral neurones have been reported which responded to *trans*-2-hexenal, a common leaf-aldehyde, but not to the odour of tobacco leaf extract and grass, respectively[1,18]. Furthermore, neurones have been found in the antennal lobe of the cockroach *Periplaneta americana* which were sensitive to hexanol, a constituent of lemon oil[23], but not to lemon odour[1].

Mixture interaction in insect neurones has been described previously[13,19,20], and suppression of the response to one chemical by another has been demonstrated to exist at different neuronal levels in the lobster[9]. Such evidence implies an important role of mixture interactions in the coding of chemical cues. The importance of mixture interaction in the Colorado potato beetle's coding mechanism, could lie in the formation of an information channel with a response level depending on the quality of the stimulus. A study of the antennal receptor responses indicates that this mechanism is also present at the peripheral level of the Colorado potato beetle's nervous system (De Jong and Visser, in preparation).

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