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AC Recordings of Antennal Responses in The Rice Brown Planthopper to Common Plant Volatile Chemicals

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ABSTRACT Electrophysiological recordings of antennal responses to common plant volatile chemicals in the rice brown planthopper, *Nilaparvata lugens* (Homoptera: Delphasidae), were examined. Volatile plant chemicals were generally credited with a major role in host plant location for food or egg laying by many insects feeding on plants as adults and/or as larvae. An initial examination of extracellular responses has been conducted. Action potentials recorded from the plaque organs were initially positive-going, biphasic spikes and the background firing rate of the cells recorded ranged from 1~22 impulses/sec. A wide range of responses to changes in concentration of the test chemical was observed. The commonest response was a relatively small increase in exitation with increasing concentration between 1 μg and 100 μg on the filter paper in syringe. Acitivity either peaked at 100 μg and remained virtually saturated at 1000 μg or tended to decrease at the highest concentration.

KEY WORDS AC recording, antennal response, plant volatile chemicals, *Nilaparvata lugens*

초 록 식물 휘발성 물질에 대한 벼멸구 촉각의 반응을 전기생리학적인 반응을 관찰하였다. 식물휘발성 물질은 일반적으로 곤충이 그들의 먹이나 산란장소를 찾는데 아주 중요한 요소로 작용하고 있다. 이러한 기주 특이적인 성질을 파악하기 위하여 벼멸구 촉각에 분포하고 있는 화학감각기의 반응을 AC반응을 통하여 기록하였다. 벼멸구의 plaque organ에서 기록된 spike의 모양은 positive-going biphasic형으로 background spike는 초당 1~22개로 다양하였다. 벼멸구 촉각은 실험한 화학물질들에 광범위하게 반응하고 있는 것을 보여 주었으며 농도가 높아짐에 따라 더욱 흥분되지만 일정 수준이상의 농도에서는 더 이상 흥분되지 않거나 억제되는 것을 보여주었다 본 실험에서 검정된 대부분의 식물 휘발성 물질에서 가장 큰 반응을 보인 농도는 단위 용기 안에 100 μg의 물질이 있을 때였고, 가장 큰 반응을 보인 화합물은 hexanal과 acetophenone이었다.

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It is generally agreed that host plant selection consists of a sequence of behavioural activities initiated by various stimuli from the host plant This sequence of activities starts with orientation to the host plant, is followed by host recognition and acceptance. commonly through test biting or sap-sampling, and then by the initiation and maintenance of feeding, terminating with cessation of feeding. Little is known of the role of olfaction during long distance orientation of the Auchenorrhyncha. Indeed the plant played by vision in long-distance orientation is not well understood in many homopterans. Much of the information regarding visual orientation in the Auchenorrhyncha is anecdotal in nature. Verschaffelt (1910) first discovered that mustard oil glucosides influenced feeding behaviour and host selection in *Pieris rapae*. Since then behavioural experiments demonstrating the importance of olfactory stimuli in orientation to the host plant have led to many workers screening for active chemicals using electrophy-

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siological techniques.

Single unit studies from a number of antennae (e.g., male and female of *Dendroctonus pseudotsugae* (Dickens *et al.* 1985); male of *Mamestra suasa* (Lucas & Renou 1989)) have revealed that individual receptors commonly respond to more than one compound. The extensive electrophysiological examination of the antennal responses of a wide range of phytophagous insects over the past 10~15 years has yielded valuable information about the nature of the receptor response to plant volatiles.

Receptors on the antennae of phytophagous insects have generally been reported as 'generalists' with a broad response profile to 'green leaf' compounds. Examining the reaction spectra of the cells on a particular antennae has allowed some authors to separate the 'generalists' on an antennae into a number of receptor types, e.g. the meat- and flower-odour type receptors on the blowfly, *Calliphora vicina* (Kaib 1974), the 7 groups within the sensilla placodea of *Apis mellifera* (Vareschi 1971) and the five receptor types of *Leptinotarsa decemlineata* (Ma & Visser 1978). A small number of cells that respond solely to one class of chemicals has been reported (Kafka 1970, Vareschi 1971).

However, this task must be attempted if we are to understand the mechanism which governs the insects orientation to the plant. There is little information available regarding olfactory orientation in *Nilaparvata lugens*. An initial examination of extracellular responses in *Nilaparvata lugens* has been conducted here to gain some idea of the way in which cells in the plaque organ respond to plant volatile compounds and how these responses relate to the EAG studies (Youn 1992) of the plaque organ.

MATERIALS AND METHODS

The insect was prepared for extracellular spike recordings in the same way as for EAG recordings by Youn (1992). The indifferent electrode was placed at the base of the antennae. The signals from the electrode were fed into an AC headstage, amplified by an AC preamplifier, filtered by a NeuroLog filter and finally passed to an AC-DC NeuroLog amplifier. The signal was then relayed to a storage oscilloscope (Tektronix 5111A), an audio amplifier and a VHS format video cassette recorder through a PCM-1 digital VCR-instrumentation recorder adapter (Medical Systems Corp., New York, U.S.A.). All experiments were stored on tape.

Data analysis was restricted to examining spike frequency before, during and after stimulation. Most spikes were counted on-line by means of a spike counter with four 'windows' which could be set prior to each experiment. The spike frequency for each neuron was then counted for the whole experiment, using peak to trough size as the criterion. This enabled changes over periods longer than one second pre and post stimulus time to be examined if necessary. Spontaneous activity levels throughout the experiment could be monitored. These results were later compared with those obtained by the spike counter and good agreement was found.

Insects were stimulated with a range of 9 compounds from the series of compounds used for the EAG experiments by Youn (1992). Each insect was stimulated three times with each of the compounds. The solvent used for each compound was pentane The insect was given a one second stimulus. Activity was recorded for at least 5 seconds before and after stimulation so that the pre and post stimulus behaviour of the cell could be examined. At the beginning of the experiment a test stimulus of 10 μg of 1-hexanol was given and this was repected at intervals throughout the experiment. If the response to this standard changed markedly then the preparation was abandoned. Individual insects were stimulated with four different concentrations of a range of chemicals. The concentration used were 1, 10, 100 and 1000 μg on filter paper.

RESULTS

Inserting the recording electrode into the plaque organ until spikes were encountered frequently resulted in many spikes of differing heights being recorded, as many as 20 could be countered in some preparations. This is perhaps not surprising since each plaque has 120~150 neurons arranged in groups of 15~30 neurons (Aljunid & Anderson 1983). Since it was impossible to distinguish bet-

ween this number of neurons over the course of a long experiment, either by using the counter or by manually examining photographed traces, such recordings were rejected. Electrode positions were sought in which a few spikes, preferably 2~3, but certainly not more than six, clearly distinguishable in height, were recorded.

Action potentials recorded from the plaque organs were initially positive-going, biphasic spikes. They were relatively small in size with peak to peak amplitudes ranging from 80~350 μV with an average noise level of 50 μV. The background firing rate of the cells recorded ranged from 1~22 impulses/sec. In the results presented here the spontaneous activity rate for each cell did not significantly change ($p<0.01$). In a number of cases where the background activity did fluctuate or increase to very high rates suggesting damage to the cell the preparation was discarded.

The spontaneous activity of these spikes was examined in the interstimulus intervals throughout the experiment and the average response and standard deviation were examined. When examining the response to each chemical compound the average spontaneous activity/sec is subtracted from the response seen in one second of stimulation and this is also the case for the first and for the second of stimulation and this is also the case for the first and for the second sec. after stimulation so that the post stimulus response of the neuron can be examined.

Nine chemicals were tested to the plaque organs with different concentrations. Perhaps small increase in excitation with increasing concentration between 1 and 100 μg on the filter paper (Fig. 1). A number of spike A unit was removed from the total number of spikes With spike B (Fig. 1a), 1-hexenol, hexanal trans-2-hexenal and acetophenone were more responsed than other tested chemicals. Spike B unit, although slightly excited by the compound at the lowest intensity used (1~10 μg), was inhibited at higher intensities (see 1-hexenol, acetophenone and trans-2-hexenal in Fig 1a) With spike C (Fig. 1b) most units slightly excited by the compounds at the increased except 1-hexanol and trans-2-hexenal. As shown in Fig 1c, spike D responses to hexanal and acetophenone were typical excited by the increased intensity from 1 to 1000 μg on filter paper. In the comparison of the total number of spikes, hexanal and acetophenone were higher excited on 100 and 1000 μg on filter paper in syringe.

Figs. 2~3 are spike frequencies before, during and after stimulation by acetophenone and hexanal with different concentration. The spike frequency for each unit was then counter for the whole experiment, using different 3 channels without spike A unit. Figs. 2~3 also showed total spike counts for acetophenone and hexanal with a same range of concentrations. Even although these counts must include neurons that were inhibited by the stimulus or did not change their activity with concentration, many samples showed an overall increase in the activity of the units being sampled in response to increasing concentration. Activity either peaked at 10^2 μg and remained virtually saturated at 10^3 μg, or tended to decrease at the highest concentration. In some cases very little total change was seen in response to changes in concentration.

DISCUSSION

The greatest limitation was imposed by an inability to apply a range of criteria in identifying the activity of a particular cell. Facilities were not available for examing the shape or falling slope of spikes. The only form of analysis readily available was to look at changes in the rate of activity of the cell. A problem in examining the response of plaque organ receptor cell is that, unlike a sensillum containing two or three receptor cells, it is impossible to ensure that the same cell or group of cells is examined repeatedly. This removes an additional way of identifying the activity of an individual cell by building up a wide range of response characteristics for the cell that have to be matched (Frazier & Hanson 1986). However, great care was taken in the examination of spike traces and no recording were accepted unless the preparation under examination yielded results that could be reproduced in two subsequent trials with the same chemicals. Thus, although there are limitations, information has been gained regarding the response characteristics of the plaque

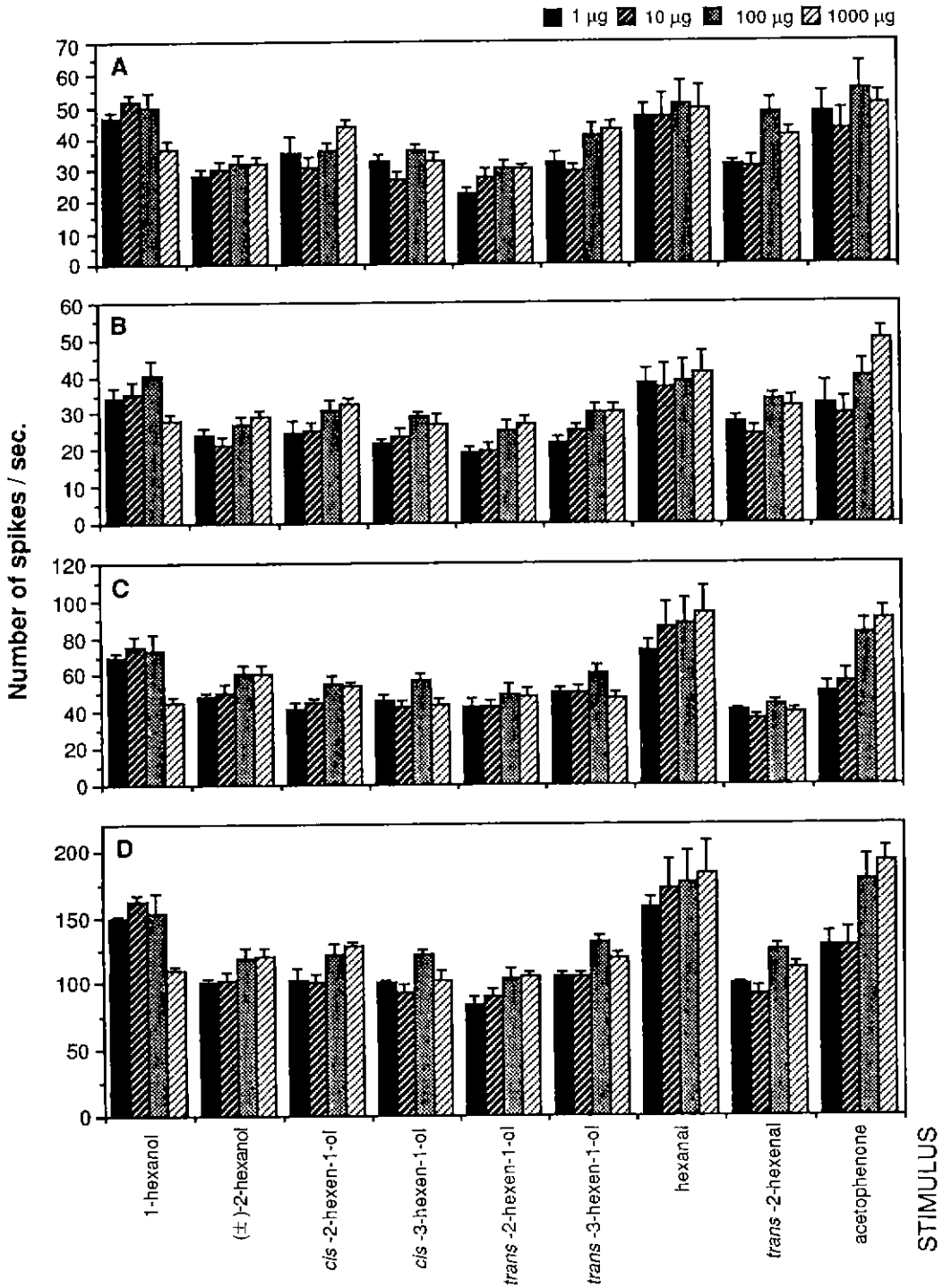

Fig. 1. Diagram shows the antennal responses against 9 different plant volatile chemicals with different concentration from the plaque organs of *N. lugens*. A: spike B, B; spike C, C: spike D; D: total number of spike. Vertical bars represent standard deviations.

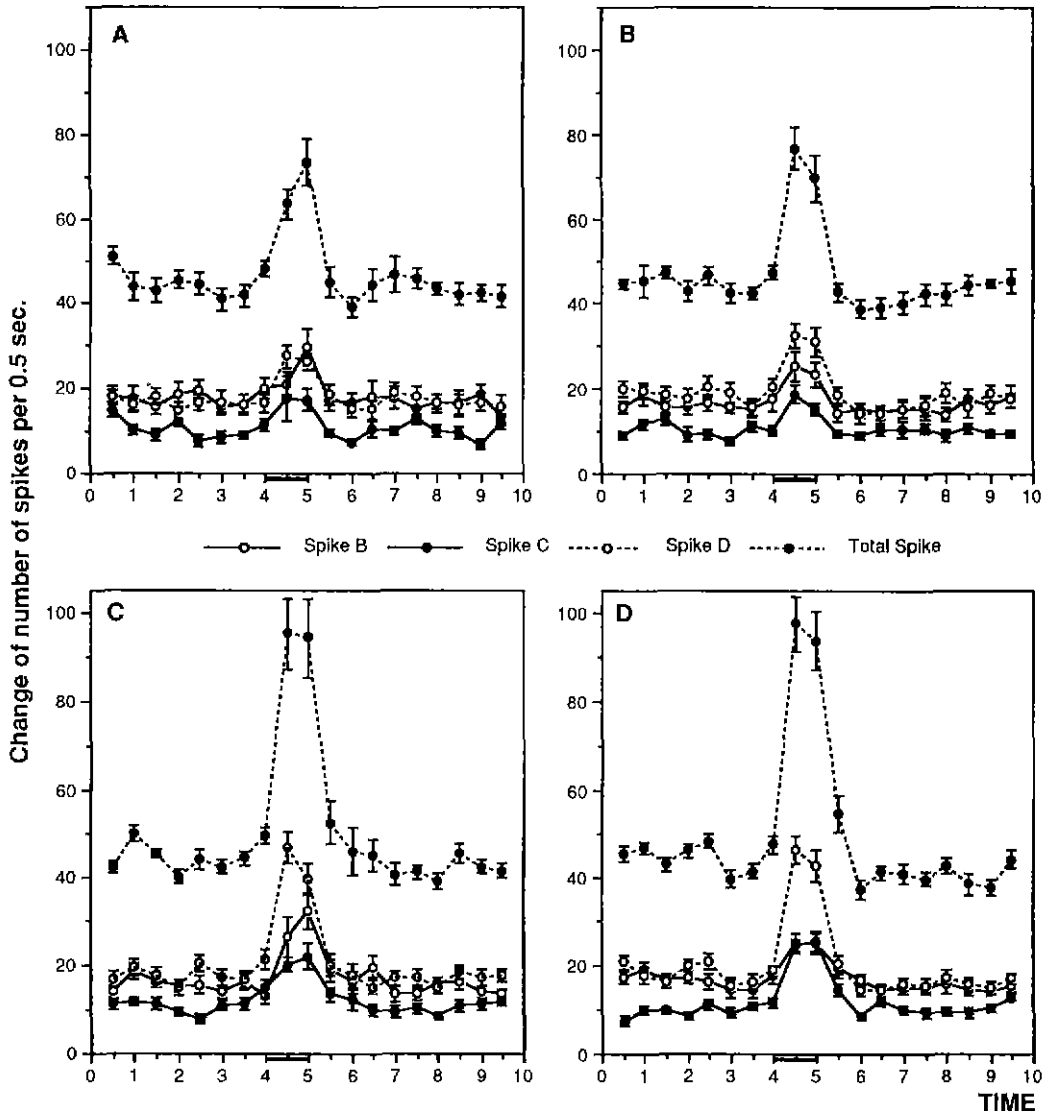

Fig. 2. Diagrams show the antennal responses against acetophenone with 4 different concentrations, 1 μg (A), 10 μg (B), 100 μg (C) and 1000 μg (D), on filter paper from the plaque organs of *N. lugens*. Vertical bars represent standard derviations and bold bars on the x-axis indicate stimulation for 1 second.

receptor cells that may guide a subsequent work.

It is not possible from the evidence gathered in this study to say whether the olfactory system of *Nilaparvata lugens* could be operating in such a way. Certainly there seem to be broadly and more narrowly tuned cells present. There is also a certain amount of evidence from the EAG studies (Youn 1992) that mixtures of compounds can produce a smaller than expected EAG in some cases, suggesting that suppression may be occurring here. Much more single cell work must be carried out before it is possible to speculate fruitfully upon the mechanism of olfactory coding in *N. lugens*. As a first step, coupled GC electrophysiology is necessary to find out which rice plant volatiles are most effective in stimulating the antennae.

In the responses of individual cells the same reduction in response at high intensities can be seen

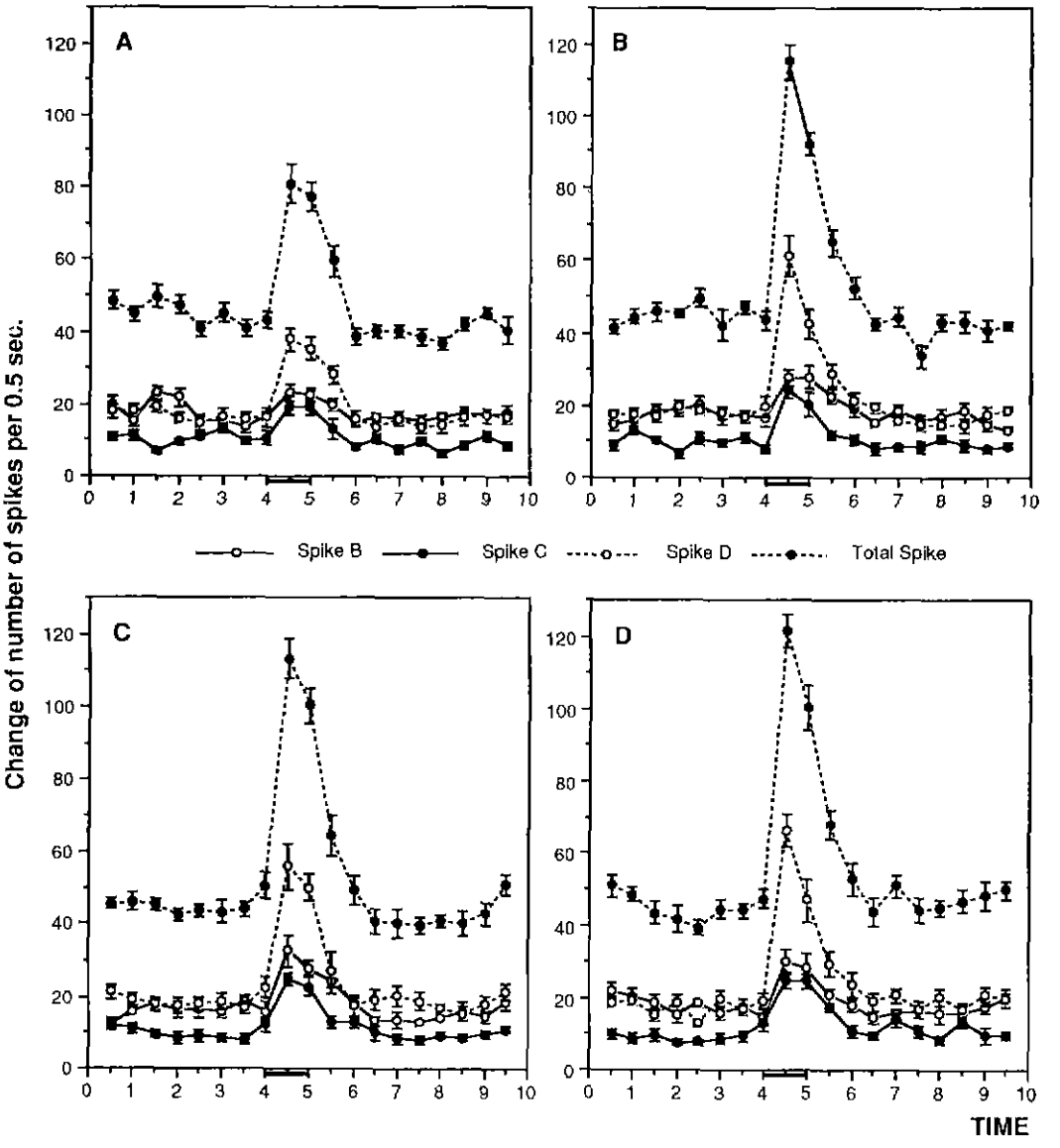

Fig. 3. Diagrams show the antennal responses against hexanal with 4 different concentrations, 1 μg (A), 10 μg (B), 100 μg (C) and 1000 μg (D), on filter paper from the plaque organs of *N. lugens*. Vertical bars represent standard deviations and bold bars on the x-axis indicate stimulation for 1 second.

as was noted for EAG responses (Youn 1992). Here too, we can see that the highest concentrations apparently have less effect in neurons that are inhibited by the compound. Neurons subjected to high concentrations would still respond to lower concentrations with a larger response after the usual interstimulus interval. These observations support those seen in the EAG recordings and show that this effect is a response of the individual cells whose signals comprise the EAG. At present the only explanation that can be proposed that put forward by Selzer (1984) to explain a similar phenomenon seen in cockroach antennal olfactory receptor cells, that high concentrations produce a depolarising block in

receptor cells (Selzer 1984).

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